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Synthesis of Hyaluronic Acid Oligomers using Chemoselective and One-Pot Strategies

Jasper Dinkelaar, Henrik Gold, Herman S. Overkleeft, Jeroen D. C. Codée,* 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

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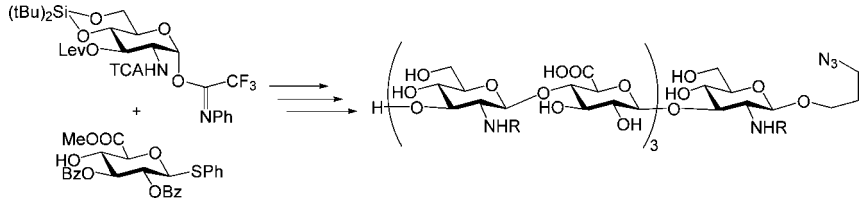
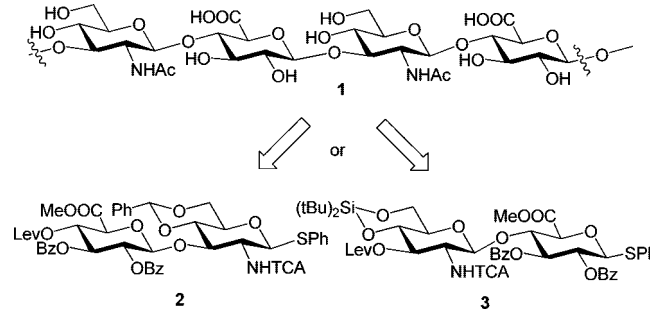


An efficient synthetic strategy toward a hyaluronic acid (HA) tri-, penta-, and heptamer having a glucosamine-reducing end is reported. The synthesis is based on a glucuronate ester thioglycoside and a trifluoro-*N*-phenylimidate glucosamine building block. The HA-fragments are synthesized using an *S*-phenyl GlcN-GluA building block through a combination of chemoselective and one-pot condensation strategies.

Introduction

Hyaluronan (HA, **1**, Scheme 1) is a linear glycosaminoglycan polymer featuring the β-1,3-linked 2-acetamido-2-deoxy-D-glucose-β-(1,4)-D-glucuronic acid disaccharide[1] as repeating unit. HA is involved in a wide variety of biological processes, such as cell-migration, proliferation, adhesion, recognition,[2] tumor invasion[3] and tumor inhibition.[4] Recently, evidence has accumulated that specific activities of HA are related to the length of the carbohydrate chain. For instance, whereas high molecular weight HA polymers are immunosuppressive,[5] small HA oligosaccharides induce complete and irreversible maturation of human dendritic cells through the Toll-like receptor 4 (TLR-4) and thereby activate the innate immune system.[6] In addition, activation of macrophages by HA oligomers, generated by degradation with different types of glycosidases showed different levels of interleukin-12 production, pointing toward the relevance of the nature of the monosaccharide at the reducing end of the HA oligomer (being either *N*-acetyl glucosamine (GlcNAc) or glucuronic acid (GluA)).[7]

SCHEME 1. Retrosynthesis of Hyaluronan



Since the first synthesis of a HA-disaccharide by Jeanloz in 1964,[8] several routes of synthesis have been disclosed for the construction of HA-oligomers having either a GlcA or a GlcNAc at the reducing end.[9,10] For example, Blatter and Jacquinet reported the synthesis of tetra-, hexa- and octameric HA-fragments having a β-*O*-methyl glucuronic acid terminus.[11] Huang and Huang developed an preactivation based iterative one-pot strategy for the synthesis of HA-fragments and could incorporate either a glucuronic acid or a glucosamine moiety at the reducing end.[12] We previously reported the synthesis of

(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) McBride, W. H.; Bard, J. B. *J. Exp. Med.* **1997**, *149*, 507–515.
(6) 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.
(7) Stern, R.; Asari, A. A.; Sugahara, K. N. *Eur. J. Cell. Biol.* **2006**, *85*, 699–715, and references cited therein.
(8) Fowers, H. M.; Jeanloz, R. W. *Biochemistry* **1964**, *3*, 123–125.
(9) For a review on the synthesis of glycosaminoglycans, including HA, see: Yeung, B. K. S.; Chong, P. Y. C.; Peuntilo, P. A. *Carbohydr. Res.* **2002**, *21*, 779–865.

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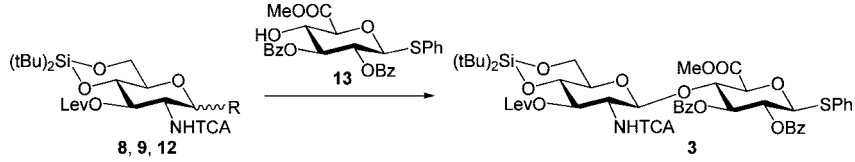
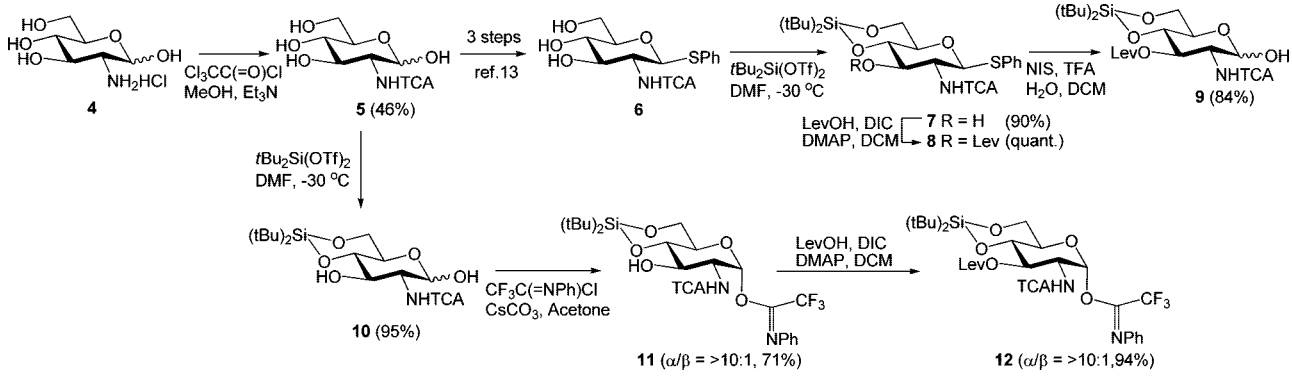
TABLE 1. Glycosylations of 8, 9, and 12 with Acceptor 13



entry	donor	equiv. donor	activator, conditions	yield
1	**8**, R = SPh	1.2	Ph_2SO/Tf_2O, −60 to 0 °C	70%
2	**9**, R = OH	1.2	Ph_2SO /Tf_2O, −60 to 0 °C	63%
3	**9**, R = OH	1.2	BSP/Tf_2O, −60 to 0 °C	56%
5	**12**, R = OC(=NPh)CF_3	1.2	TMSOTf, 0 °C	78%
4	**12**, R = OC(=NPh)CF_3	1.2	TfOH, 0 °C	79%
6	**12**, R = OC(=NPh)CF_3	1.5	TfOH, 0 °C	90%

SCHEME 2. Synthesis of the Monomeric Building Blocks 8, 9 and 12



a HA-pentamer having a glucuronic acid moiety at the reducing end[13] using the glucuronate (GlcA)-glucosamine (GlcN) thiodisaccharide building block **2** (Scheme 1) in combination with the diphenylsulfoxide (Ph_2SO)/trifluoromethanesulfonic anhydride (Tf_2O) activating system.[14,15] In this paper, we present an alternative assembly strategy for the synthesis of the complementary oligomers, having a glucosamine reducing end and ranging in size from three to seven monomers. The HA-fragments are synthesized using GlcN-GlcA building block **3** (Scheme 1) and combines chemoselective and one-pot condensation strategies.

Results and Discussion

In our previous synthesis of HA-oligomers, we found that the acid instability of the benzylidene acetal in **2** necessitated careful tuning of the amount of base (tri*tert*-butylpyrimidine, TTBP[16]) used in the glycosylation reactions.[13] Use of too little base gave unwanted cleavage of the benzylidene group, whereas orthoester/oxazoline formation was observed when excess base was employed.[17] In order to circumvent these drawbacks, we decided to use the more acid stable di-*tert*-butylsilylidene (DTBS) group for the protection of the C4 and C6 hydroxyls of the glucosamine moiety in **3**. As a first research objective, we investigated chemoselective and orthogonal glycosylation strategies to synthesize key building block **3** using 1-thio glucuronate ester **13** in combination with 1-thio, 1-OH, or 1-trifluoro imidate glucosamine donor **8**, **9** or **12** respectively (Table 1). The synthesis of the three glucosamine building blocks started with the introduction of the trichloroacetyl group on the amino function of glucosamine **4**, using trichloroacetylchloride and triethylamine in methanol (Scheme 2).[18] The resulting *N*-TCA glucosamine **5** was then transformed into 1-thio glucosamine **6** in three steps as described by Blatter and Jacquinet.[11] Thus, glucosamine **5** was per-acetylated, after which the anomeric thiophenol function was introduced, and the acetyl groups were removed. Introduction of the di-*tert*-butylsilylene function on **6** was followed by levulinoylation of the C3-OH to give glucosamine donor **8**. Hydrolysis of the thioacetal in **8** using NIS/TFA then provided 1-hydroxy donor **9**.[19] 1-Trifluoroimidate **12** was conveniently prepared in a three step sequence from **5**. Thus, regioselective silylation of the C6-OH and C4-OH in **5** using di-*tert*-butylsilyl bistriflate in DMF at

(10) (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) Yeung, B. K. S.; Hill, D. C.; Janicka, M.; Peuntilo, P. A. *Org. Lett.* **2000**, *2*, 1279–1282. (c) Iyer, S. S.; Rele, S. M.; Baskaran, S.; Chaikof, E. L. *Tetrahedron* **2003**, *59*, 631–638. (d) Lu, X. A.; Chou, C. H.; Wang, C. C.; Hung, S. C. *Synlett* **2003**, *9*, 1364–1366. (e) Palmacci, E. R.; Seeberger, P. H. *Tetrahedron* **2004**, *60*, 7755–7766.

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

(12) Huang, L.; Huang, X. *Chem.−Eur. J.* **2007**, *13*, 529–540.

(13) Dinkelaar, J.; Codée, J. D. C.; Van den Bos, L. J.; Overkleeft, H. S.; Van der Marel, G. A. *J. Org. Chem.* **2007**, *72*, 5737–5742.

(14) (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.

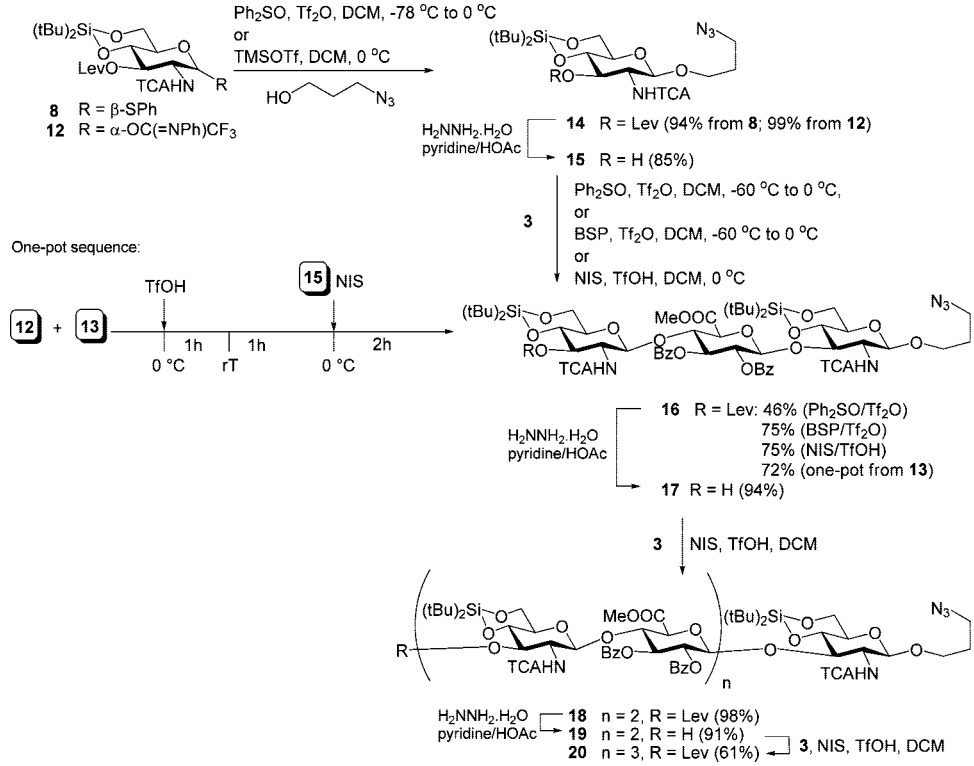
(15) (a) Codée, J. D. C.; Litjens, R. E. J. N.; Den Heeten, R.; Overkleeft, H. S.; Van Boom, J. H.; Van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1519–1522. (b) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020.

(16) Crich, D.; Smith, M.; Yao, Q.; Picione, J. *Synthesis* **2001**, 323–326.

(17) When orthoester or oxazoline formation is not possible in a glycosylation reaction, we generally employ 2.5−3.0 equiv of TTBP with respect to the amount of Tf_2O used. If orthoester or oxazoline formation can occur, the use of base is preferably avoided. In our previous HA-syntheses,[13] we found that the use of 0.95 equiv of TTBP relative to the amount of Tf_2O employed, led to the most productive condenstaion reactions.

(18) The moderate yield (46%) of the isolated 2-deoxy-2-trichloroacetamido-D-glucopyranose is due to the difficult purification and isolation of the product. Preparation of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trichloroacetamido-D-glucopyranose from glucosamine•HCl, following a two step procedure (TCA-introduction and per-acetylation) without purification of the intermediate trichloroacetamido intermediate **5** yielded 77% of the target compound.

SCHEME 3. Assembly of the Protected HA-Oligomers



−30 °C quantitatively yielded 4,6-*O*-di-*tert*-butylsilylidene glucosamine **10**. This diol was regioselectively transformed into the anomeric 1-(*N*-phenyl)trifluoro imidate **11** with the use of *N*-phenyltrifluoroacetimidoyl chloride ($ClC(=NPh)CF_3$) and Cs_2CO_3.[20−22] Levulinoylation of the remaining 3-OH furnished the completely protected imidate donor **12** in 67% yield from **5**. Glucuronate **13** was synthesized from *β*-*S*-phenyl glucopyranose as we described previously. Briefly, benzylidination and ensuing benzoylation of *β*-*S*-phenyl glucopyranose gave the fully protected glucopyranoside, of which the C-4 and C-6 hydroxyls were liberated by acidic hydrolysis of the benzylidene acetal. Subsequent oxidation of the primary alcohol function and formation of the methyl ester gave glucuronate **13**.[23]

The 1-thio (**8**), 1-hydroxy (**9**) and imidate (**12**) donors were then glycosidated with glucuronate **13** as summarized in Table 1.[24] The condensation of 1-thioglucosamine **8** with **13** proceeded rapidly using the Ph_2SO/Tf_2O activator system[14] and yielded disaccharide **3** in 70% (entry 1). No activation of the glucuronate ester (**13**) or thiodisaccharide **3** was found during this glycosylation indicating the reactivity difference of the donor (**8**) and acceptor/product (**13**/**3**).[25] The Ph_2SO/Tf_2O mediated dehydrative condensation of 1-hydroxy glucosamine **9** and thioacceptor **13** proceeded slowly to give dimer **3** (63% yield). The 1-benzenesulfinyl piperidine (BSP)/Tf_2O[15b] reagent combination in this dehydrative condensation appeared less productive than the Ph_2SO/Tf_2O promoter. Finally, imidate donor **12** reacted smoothly with the acceptor using a catalytic amount of trimethylsilyl triflate (TMSOTf) or triflic acid (TfOH) to yield disaccharide **3** in 78 and 79% yield respectively (entries 4 and 5). Thus, all three donor−acceptor combinations provided dimer **3** in satisfactory yields, with imidate **12** performing best. Given the fact that imidate **12** is synthesized in only 4 steps from glucosamine, we tried to improve the yield of the imidate coupling by increasing the amount of donor glycoside. When 1.5 equiv of **12** were used in the triflic acid mediated glycosylation of **13**, dimer **3** was obtained in 90% yield.

With key dimer **3** in hand, we set out to assemble the target HA-oligosaccharides as depicted in Scheme 3. First, the reducing end glucosamine **15** was synthesized by coupling 1-thio glucosamine **8** or imidate **12** with azidopropanol and subsequent delevulinoylation. This reducing-end building block was condensed with dimer **3** using the Ph_2SO/Tf_2O activator system. Although preactivation of the thiodisaccharide proceeded smoothly, the ensuing reaction with acceptor **15** did not go to completion and trisaccharide **16** was isolated in 46% yield. We have observed complete activation and poor condensation yields with uronate ester donors before,[14a] and have previously been able to increase the coupling efficiency by changing from Ph_2SO/Tf_2O to the related BSP/Tf_2O reagent system. Also in the present case, this change in activator system significantly improved the outcome of the glycosylation and we were able

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

(20) Valerio, S.; Pastore, A.; Adinolfi, M.; Iadonisi, A. *J. Org. Chem.* **2008**, *73*, 4496–4503.

(21) The anomeric configuration of the *N*-phenyltrifluoroacetimidate glucosamines **11** and **12** was assigned based on the chemical shift of the anomic proton and anomeric carbon resonances (**11**: $d_{H\text{-}1} = 6.40$ ppm, $d_{C\text{-}1} = 93.0$ ppm; **12** $d_{H\text{-}1} = 6.43$, $d_{C\text{-}1} = 92.6$ ppm).

(22) Attempted selective levulinoyl protection of the C3-OH in diol **10** led to an inseparable mixture of compounds.

(23) 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.

(24) To compare the silylidene functionalized building block **7** with its benzylidene protected counterpart we previously described,13 two trisaccharides were assembled having either a silylidene or a benzylidene protected glucosamine. Glycosylations using the silylidene protected glycosides gave significant higher yields than the corresponding benzylidene protected glycosides. See Supporting Information for experimental details.

(25) Codée, J. D. C.; Van den Bos, L. J.; Litjens, R. E. J. N.; Overkleeft, H. S.; Van Boeckel, C. A. A.; Van Boom, J. H.; Van der Marel, G. A. *Tetrahedron* **2004**, *60*, 1057–1064.

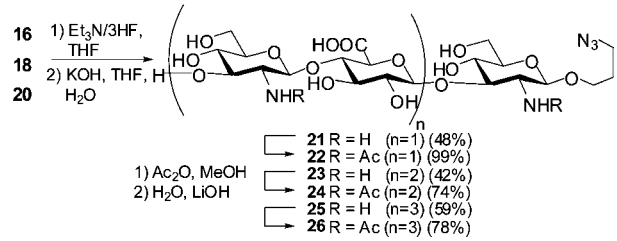
to obtain HA trimer **16** in 75% yield. We also examined *N*-iodosuccinimide (NIS)/TfOH as activator system, since this should provide an opportunity for a one-pot synthesis of trisaccharide **16**, combining imidate chemistry with iodonium ion activation of the thiodisaccharide. Under the agency of NIS/TfOH, dimer **3** and glucosamine **15** were condensed to give trisaccharide **16** in 75% yield. Next, a one-pot procedure was investigated.[26] Accordingly, imidate **12** and 1-thio glucuronate **13** were combined and treated with a catalytic amount of TfOH to produce the 1-thio disaccharide. Subsequently, acceptor **15** and NIS were added to this mixture at 0 °C, leading to the formation of trisaccharide **16**, which was isolated in 72% yield. Interestingly, the NMR spectrum of **16** revealed a rather small homonuclear coupling constant ($J_{H1'-H2'}$) for the anomeric proton of the glucuronate moiety (H-1′) of 4.4 Hz. Upon deprotection of the oligosaccharides (*vide infra*) the coupling constant changes to 8.4 Hz, indicative of the β-glucuronic acid linkage formed. The small coupling constant for the glucuronate anomeric proton indicates that the glucuronate ester takes up a flattened $^4C_1$-chair conformation, when positioned in between two 4,6-*O*-di-*tert*-butylsilylidene glucosamine residues.[27]

To elongate trisaccharide **16**, the C3″-OLev was deprotected and the resulting alcohol **17** was condensed with dimer **3**. To this end, the 1-thiodisaccharide was activated by BSP/Tf_2O and treated with acceptor **17**. We observed that, while the activated disaccharide decomposed in time, little to no glycosylation took place, and we recovered unreacted acceptor. We therefore switched to a NIS-TfOH glycosylation protocol which does not require preactivation of the donor glycoside at low temperature. In addition, iodonium activated glycosylations are more easily executed at higher concentration than preactivation based sulfonium promoted coupling reactions. Thus, donor **3**, acceptor **17** and NIS were dissolved in dichloromethane (0.1 M in acceptor) and cooled to 0 °C before addition of a catalytic amount of TfOH. This time, complete consumption of trisaccharide **17** was observed and pentamer **18** was obtained in 98% yield. Ensuing delevulinoylation of **18** gave alcohol **19** which was elongated in a subsequent NIS/TfOH mediated glycosylation with building block **3**. This reaction proved to be difficult to monitor, because of the similar polarities of the reaction partners and product as well as the rather viscous nature of the reaction mixture. Nonetheless, heptamer **20** was easily separated from the smaller products in the reaction mixture by size-exclusion chromatography on Sephadex LH-20, and isolated in 61% yield.

The synthesis of the HA-fragments was completed by global deprotection of the oligomers **16**, **18** and **20** as depicted in Scheme 4. The silylidene groups were removed with Et_3N/3HF, and subsequent saponification of the ester and amide functionalities using KOH in THF/H_2O yielded the zwitterionic tri-, penta-, and heptamer **21**, **23**, and **25**. Finally, *N*-acetylation with Ac_2O in MeOH and subsequent basic treatment in H_2O provided the anionic HA-fragments **22**, **24** and **26**.

In conclusion, we have described the highly efficient synthesis of a set of HA oligosaccharides combining chemoselective and one-pot glycosylation strategies. The 4,6-silylidene function is a valuable alternative to the benzylidene functionality we previously employed in our HA syntheses, since the former is

SCHEME 4. Deprotection of the HA Oligomers



stable under acidic conditions. The synthesis of the oligomers builds on the chemoselective condensation of glucosamine *N*-phenyltrifluoroimidate **12** and *S*-phenyl glucuronate ester **13**, which are both accessible using short, high yielding synthetic routes. Monomers **12** and **13** are condensed to give the key 1-thio disaccharide building block **3** or combined in a one-pot glycosylation sequence with azidopropanol glucosamine **15** to produce the reducing end trimer **16**. For the synthesis of the higher HA-oligomers, iodonium ion activation of dimer building block **3** proved to be more effective than the sulfonium based activator systems.[28]

Experimental Section

2-Deoxy-2-trichloroacetamido-D-glucopyranose (5). To a mixture of D-glucosamine-HCl (53.9 g, 250 mmol) in MeOH (625 mL) and Et_3N (70 mL, 500 mmol) trichloroacetyl chloride (TCACl) (28 mL, 250 mmol) was added dropwise at 0 °C. After 5 days, the mixture was filtered and concentrated *in vacuo*. Purification by column chromatography (EtOAc, MeOH) yielded **5** as an off-white solid (37.8 g, 46%). Analytical data were identical to those described in literature previously.[29]

Phenyl 4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-β-thio-D-glucopyranoside (7). To a solution of phenyl 2-deoxy-2-trichloroacetamido-β-thio-D-glucopyranoside (**6**) (6.95 g, 16.8 mmol) in DMF (80 mL) at −30 °C was added di-*tert*-butylsilylidene bistriflate (5.42 mL, 16.8 mmol). The reaction was warmed to −10 °C in 1 h after which pyridine (4.0 mL, 50 mmol) was added and subsequently, the reaction was diluted with Et_2O and washed with H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **7** as a white amorphous solid (8.44 g, 90%). $[\alpha]_D^{22} = -18$ (c = 0.1, DCM); IR (neat): 818, 1063, 1528, 1687, 2359, 2887, 2931, 3335 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 0.94 (s, 9H, *t*Bu), 1.04 (s, 9H, *t*Bu), 2.99 (d, 1H, J = 2.0 Hz, OH), 3.48 (dt, 1H, J = 5.2 Hz, 9.6 Hz, H-5), 3.69−3.76 (m, 2H, H-2, H-4), 3.91 (t, 1H, J = 10.0 Hz, H-6), 3.99 (dt, 1H, J = 1.6 Hz, 8.4 Hz, H-3), 4.19 (dd, 1H, J = 4.8 Hz, 5.2 Hz, H-6), 5.11 (d, 1H, J = 10.4 Hz), 7.28−7.33 (m, 4H, NH, H Arom), 7.45−7.48 (m, 2H, H Arom); ^{13}C NMR (100 MHz): δ = 19.8 (C_q *t*Bu), 22.5 (C_q *t*Bu), 26.8 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 56.4 (C-2), 65.9 (C-6), 74.2 (C-5), 74.3 (C-3), 77.3 (C-4), 86.1 (C-1), 92.3 (C_q CCl_3), 128.2 (CH Arom), 128.9 (CH Arom), 132.0 (C_q Arom), 132.9 (CH Arom), 162.0 (C═O TCA); HRMS: $C_{22}H_{32}Cl_3NO_5SSi + H^+$ requires 556.0909, found 556.0907.

Phenyl 4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-4-*O*-levulinoyl-2-trichloroacetamido-β-thio-D-glucopyranoside (8). To a solution of **7** (8.44 g, 15.2 mmol) in DCM (40 mL) at 0 °C was added levulinic acid (LevOH) (3.87 g, 33.3 mmol), di-*iso*-propylcarbodiimine (DIC) (2.58 mL, 16.7 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP) were added. The mixture was stirred for four hours and allowed to warm to RT. Filtration over Celite and purification by column chromatography (PE, EtOAc) yielded **8** as

(26) 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.

(27) A similar coupling constant was observed for the glucuronate ester anomeric protons in pentamer **18** and heptamer **20**. The anomeric proton of the glucuronate moiety in dimer **3** has a regular *trans*-diaxial coupling constant of 10.0 Hz.

(28) Crich, D.; Wu, B.; Jayalath, P. *J. Org. Chem.* **2007**, *72*, 6806–6815.

(29) Blatter, G.; Beau, J.-M.; Jacquinet, J.-C. *Carbohydr. Res.* **1994**, *260*, 189–202.

a white amorphous solid (10.19 g, quant.). $[\alpha]_D^{22} = -26$ ($c = 0.1$, DCM); IR (neat): 825, 1070, 1166, 1525, 1701, 2341, 2360, 2860, 2933, 3315 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.95$ (s, 9H, *t*Bu), 1.04 (s, 9H, *t*Bu), 2.15 (s, 3H, CH_3 Lev), 2.57−2.61 (m, 2H, CH_2 Lev), 2.70−2.73 (m, 2H, CH_2 Lev), 3.54 (dt, 1H, $J = 5.2$ Hz, 10.0 Hz, H-5), 3.88−3.98 (m, 3H, H-2, H-4, H-6), 4.24 (dd, 1H, $J = 4.8$ Hz, 5.2 Hz, H-6), 4.92 (d, 1H, $J = 10.4$ Hz, H-1), 5.18 (dd, 1H, $J = 9.2$ Hz, 9.2 Hz, H-3), 6.88 (d, 1H, $J = 9.2$ Hz, NH), 7.31−7.34 (m, 3H, H Arom), 7.46−7.48 (m, 2H, H Arom);^{13}C NMR (100 MHz): δ =19.8 (C_q *t*Bu), 22.6 (C_q *t*Bu), 26.8 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 28.0 (CH_2 Lev), 29.7 (CH_3 Lev), 38.0 (CH_2 Lev), 54.7 (C-2), 66.0 (C-6), 74.6, (C-5), 74.9 (C-4), 75.1 (C-3), 87.3 (C-1), 92.3 (C_q CCl_3), 128.5 (CH Arom), 129.1 (CH Arom), 132.0 (C_q Arom), 133.0 (CH Arom), 161.7 (C═O TCA), 172.5 (C═O COO Lev), 205.8 (C═O CO Lev); HRMS: $C_{27}H_{38}Cl_3NO_7SSi + H^+$ requires 654.1277, found 654.1278.

4,6-*O*-Di-*tert*-butylsilylidene-2-deoxy-4-*O*-levulinoyl-2-trichloroacetamido-α/*β*-D-glucopyranose (9). To a solution of **8** (0.655 g, 1.00 mmol) in DCM (10 mL) and H_2O (1 mL) at 0 °C was added NIS (0.225 g, 1.00 mmol) and TFA (77 μL, 1.0 mmol). After 30 min a second equivalent of NIS (0.225 g, 1.00 mmol) was added and the reaction was stirred for an additional 30 min. The reaction was quenched by addition of Et_3N and washed with $Na_2S_2O_3$. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*, purification by column chromatography (PE, EtOAc) yielded **9** as a colorless oil (0.838 g, 84%). IR (neat): 765, 817, 1064, 1092, 1533, 1701, 1747, 2337, 2931 cm^{-1}; NMR assignment of major isomer (α) ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.98$ (s, 9H, *t*Bu), 1.05 (s, 9H, *t*Bu), 2.17 (s, 3H, CH_3 Lev), 2.60−2.64 (m, 2H, CH_2 Lev), 2.72−2.75 (m, 2H, CH_2 Lev), 3.34 (bs, 1H, OH), 3.89 (d, 1H, $J = 9.6$ Hz, H-6), 3.93 (t, 1H, $J = 9.2$ Hz, H-4), 4.06 (t, 1H, $J = 4.8$ Hz, H-5), 4.08−4.18 (m, 2H, H-2, H-6), 5.29 (dd, 1H, $J = 9.2$ Hz, 10.4 Hz, H-3), 5.32 (d, 1H, $J = 3.2$ Hz, H-1), 7.06 (d, 1H, $J = 8.8$ Hz, NH);^{13}C NMR (100 MHz): $\delta = 19.9$ (C_q *t*Bu), 22.7 (C_q *t*Bu), 26.8 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 28.1 (CH_2 Lev), 29.8 (CH_3 Lev), 38.0 (CH_2 Lev), 54.0 (C-2), 66.4 (C-6), 66.8, (C-5), 72.5 (C-3), 74.9 (C-4), 87.3 (C-1), 91.3 (C-1), 162.1 (C═O TCA), 173.0 (C═O COO Lev), 205.9 (C═O CO Lev); HRMS: $C_{21}H_{34}Cl_3NO_8Si + H^+$ requires 562.1192, found 562.1192.

4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-α/*β*-D-glucopyranose (10). To a solution of **5** (13.9 g, 43.0 mmol) in DMF (215 mL) at −30 °C was added di-*tert*-butylsilylidene bistriflate (13.6 mL, 42.0 mmol). The reaction was warmed to −10 °C in 1 h after which pyridine (10.9 mL, 129 mmol) was added and subsequently the reaction was diluted with Et_2O and washed with H_2O. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to afford **10** as white amorphous solid (18.5 g, 95%). IR (neat): 765, 817, 1064, 1092, 1533, 1683, 2337, 2931 cm^{-1}; NMR assignment of major isomer (α), ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.99$, (s, 9H, *t*Bu), 1.06 (s, 9H, *t*Bu), 3.30 (bs, 1H, OH), 3.74−3.82 (m, 1H), 3.87−3.94 (m, 2H), 3.96−4.13 (m, 3H, H-2, H-6), 5.34 (d, 1H, $J = 3.2$ Hz, H-1), 6.98 (d, 1H, $J = 8.4$ Hz, NH);^{13}C NMR (100 MHz): $\delta = 19.7$ (C_q *t*Bu), 22.7 (C_q *t*Bu), 26.9 (CH_3 *t*Bu), 27.4 (CH_3 *t*Bu), 54.4 (C-2), 66.3 (C-6), 66.4 (C-5), 71.9 (C-3), 77.7 (C-4), 91.7 (C-1), 162.3 (C═O TCA). HRMS: $C_{16}H_{28}Cl_3NO_6Si + H^+$ requires 464.0824, found 464.0823.

(*N*-Phenyl)-2,2,2-trifluoroacetimidate 4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-α/*β*-D-glucopyranoside (11). To a solution of **10** (10.4 g, 22.4 mmol) in acetone (80 mL) at 0 °C was added Cs_2CO_3 (8.0 g, 24.6 mmol) and ClC(═NPh)CF_3 (6.8 mL, 44.8 mmol). The reaction was warmed to RT, when TLC analysis showed complete consumption of starting material the mixture was filtered over Celite and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **11** as a colorless oil (α/*β* >10:1, 10.1 g, 71%).[221] ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.01$ (s, 9H, *t*Bu), 1.07 (s, 9H, *t*Bu), 2.84 (bs, 1H, OH), 3.84−3.93 (m, 3H, H-4, H-5, H-6), 3.98 (t, 1H, $J = 8.8$ Hz, H-3), 4.16−4.20 (m, 2H, H-2, H-6), 6.40 (bs, 1H, H-1), 6.78 (d, 2H, $J = 7.6$ Hz, H Arom), 6.84 (d, 1H, $J = 7.2$ Hz, NH), 7.11 (t, 1H, $J = 7.2$ Hz, H Arom), 7.27 (m, 2H, H Arom);^{13}C NMR (100 MHz): $\delta = 19.9$ (C_q *t*Bu), 22.7 (C_q *t*Bu), 26.8 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 54.3 (C-2), 66.0 (C-6), 68.5 (C-5), 71.3 (C-3), 77.0 (C-4), 92.0 (C_q CCl_3), 93.0 (C-1), 119.2 (CH Arom), 124.7 (CH Arom), 128.8 (CH Arom), 142.8 (C_q Arom), 162.2 (C═O TCA). HRMS: $C_{24}H_{32}Cl_3F_3N_2O_6Si + H^+$ requires 635.1120, found 635.1120.

(*N*-Phenyl)-2,2,2-trifluoroacetimidate 4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-4-*O*-levulinoyl-2-trichloroacetamido-α/*β*-D-glucopyranoside (12). Imidate **11** (6.64 g, 10.0 mmol) was dissolved in DCM (40 mL) and after cooling to 0 °C LevOH (3.28 g, 28.3 mmol), DIC (2.2 mL, 14.2 mmol) and a catalytic amount of DMAP were added. The mixture was stirred for four hours and allowed to warm to RT. Filtration over Celite and purification by column chromatography (PE, Et_2O) yielded **12** as a colorless oil (α/*β* >10:1, 6.90 g, 94%).[221] ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.99$ (s, 9H, *t*Bu), 1.07 (s, 9H, *t*Bu), 2.13 (s, 3H, CH_3 Lev), 2.62−2.65 (m, 2H, CH_2 Lev), 2.70−2.75 (m, 2H, CH_2 Lev), 3.88−3.99 (m, 2H, H-5, H-6), 3.98 (t, 1H, $J = 9.2$ Hz, H-4), 4.14−4.19 (m, 1H, H-6), 4.26−4.28 (m, 1H, H-2), 5.27 (t, 1H, $J = 10.0$ Hz, H-3), 6.43 (bs, 1H, H-1), 6.78 (d, 2H, $J = 8.0$ Hz, H Arom), 7.11 (t, 1H, $J = 7.6$ Hz, H Arom), 6.84 (d, 1H, $J = 7.6$ Hz, NH), 7.27 (t, 2H, $J = 7.6$ Hz, H Arom);^{13}C NMR (100 MHz): $\delta = 19.7$ (C_q *t*Bu), 22.4 (C_q *t*Bu), 26.6 (CH_3 *t*Bu), 27.1 (CH_3 *t*Bu), 27.7 (CH_2 Lev), 29.4 (CH_3 Lev), 37.7 (CH_2 Lev), 53.5 (C-2), 65.9 (C-6), 68.8 (C-5), 71.8 (C-3), 73.8 (C-4), 91.6 (C_q CCl_3), 92.6 (C-1), 119.4 (CH Arom), 124.6 (CH Arom), 129.0 (CH Arom), 142.5 (C_q Arom), 162.0 (C═O TCA), 173.5 (C═O COO Lev), 205.2 (C═O CO Lev). HRMS: $C_{29}H_{38}Cl_3F_3N_2O_8Si + Na^+$ requires 755.1307, found 755.1310.

Methyl (phenyl 2,3-Di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranoside)-*β*-D-glucopyranosyl) uronate (3). Method A. A mixture of 1-thio donor **8** (0.157 g, 0.24 mmol) and Ph_2SO (0.057 g, 0.28 mmol) was coevaporated with toluene two times to remove traces of water, dissolved in DCM (5 mL) and stirred over activated 3 Å molsieves for 30 min. At −60 °C, Tf_2O (40 μL, 0.24 mmol) was added and after 15 min at −60 °C, a solution of acceptor **13** (0.102 g, 0.2 mmol) in DCM (2 mL) was slowly added and the reaction mixture was allowed to warm to 0 °C in 3 h. Dry Et_3N (0.13 mL) was added and the reaction mixture was diluted with DCM and washed with $NaHCO_{3\,(aq)}$. The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **3** as a white foam (0.147 g, 70%).

Method B. A mixture of 1-hydroxy donor **9** (0.135 g, 0.24 mmol) and Ph_2SO (0.097 g, 0.48 mmol) was coevaporated with toluene two times to remove traces of water, dissolved in DCM (5.6 mL) and stirred over activated 3 Å molsieves for 30 min. At −60 °C, Tf_2O (42 μL, 0.25 mmol) was added and the temperature was raised to −40 °C. After 1 h, a solution of acceptor **13** (0.102 g, 0.2 mmol) in DCM (2 mL) was slowly added and the reaction mixture was allowed to warm to 0 °C in 2 h. Dry Et_3N (0.13 mL) was added and the reaction mixture was diluted with DCM and washed with $NaHCO_{3\,(aq)}$. The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **3** as a white foam (0.126 g, 63%).

Method C. A mixture of 1-hydroxy donor **9** (0.135 g, 0.24 mmol) and BSP (0.10 g, 0.48 mmol) was coevaporated with toluene two times to remove traces of water, dissolved in DCM (5.6 mL) and stirred over activated 3 Å molsieves for 30 min. At −60 °C, Tf_2O (42 μL, 0.25 mmol) was added and the temperature was raised to −40 °C. After 1 h, a solution of acceptor **13** (0.102 g, 0.2 mmol) in DCM (2 mL) was slowly added and the reaction mixture was allowed to warm to 0 °C in 2 h. Dry Et_3N (0.13 mL) was added and the reaction mixture was diluted with DCM and washed with $NaHCO_{3\,(aq)}$. The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **3** as a white foam (0.118 g, 56%).

Method D. Imidate donor **12** (0.176 g, 0.24 mmol) and acceptor **13** (0.102 g, 0.20 mmol) in DCM (4 mL) were stirred over activated 3 Å molsieves for 30 min. The mixture was cooled to 0 °C before a catalytic amount of TfOH (1 μL, 0.01 mmol) was added. The mixture was allowed to warm to RT. After TLC analysis showed complete consumption of starting material (1 h), the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $NaHCO_{3\ (aq)}$. The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **3** as a white foam (0.164 g, 78%). $[\alpha]_D^{22} = -18$ ($c = 0.1$, DCM); IR (neat): 826, 1074, 1533, 1697, 2098, 2341, 2361, 2860, 2933, 3315 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta =$ 0.878 (s, 18H, 2 x *t*Bu), 2.14 (s, 3H, CH_3 Lev), 2.52−2.59 (m, 3H, H-6′, CH_2 Lev), 2.64−2.70 (m, 2H, CH_2 Lev), 3.24 (dt, 1H, $J =$ 4.8 Hz, 10.0 Hz, H-5′), 3.45 (dd, 1H, $J = 4.8$ Hz, 5.6 Hz, H-6′), 3.55 (t, 1H, $J = 9.2$ Hz, H-4′), 3.80−3.86 (m, 4H, H-2′, CH_3 COOMe), 4.14 (d, 1H, $J = 10.0$ Hz, H-5), 4.24 (t, 1H, $J = 9.6$ Hz, H-4), 4.92 (d, 1H, $J = 8.4$ Hz, H-1′), 4.99 (d, 1H, $J = 10.0$ Hz, H-1), 5.02 (t, 1H, $J = 10.4$ Hz, H-3′), 5.38 (t, 1H, $J = 10.0$ Hz, H-2), 5.65 (t, 1H, $J = 9.2$ Hz, H-3), 6.84 (d, 1H, $J = 9.2$ Hz, NH), 7.28−7.53 (m, 11H, H Arom), 7.90−7.94 (m, 4H, H Arom);^{13}C NMR (100 MHz): $\delta =$19.6 (C_q *t*Bu), 22.3 (C_q *t*Bu), 26.6 (CH_3 *t*Bu), 27.2 (CH_3 *t*Bu), 27.9 (CH_2 Lev), 29.6 (CH_3 Lev), 37.9 (CH_2 Lev), 53.1 (CH_3 COOMe), 55.6 (C-2′), 64.7 (C-6′), 69.5 (C-2), 70.4, (C-5′), 73.6 (C-3), 74.1 (C-3′), 74.3 (C-4′), 76.3 (C-4), 76.9 (C-5), 86.7 (C-1), 92.3 (C_q CCl_3), 100.3 (C-1′), 128.3−129.6 (CH Arom), 129.8 (C_q Arom), 131.5 (C_q Arom), 132.6−133.3 (CH Arom), 161.5 (C═O TCA), 164.9 (C═O Bz), 165.0 (C═O Bz), 168.4 (C═O COOMe) 172.5 (C═O COO Lev), 205.8 (C═O CO Lev); HRMS: $C_{48}H_{56}Cl_3NO_{15}SSi + Na^+$ requires 1074.2098, found 1074.2112.

3-Azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranoside (14). Method A. A mixture of thio donor **8** (0.655 g, 1.0 mmol) and Ph_2SO (0.233 g, 1.1 mmol) was coevaporated with toluene two times to remove traces of water, dissolved in DCM (20 mL) and stirred over activated 3 Å molsieves for 30 min. The mixture was cooled to −78 °C before Tf_2O (0.176 μL, 1.05 mmol) was added. The mixture was stirred for 10 min at −78 °C followed by addition of 3-azidopropanol (0.303 g, 3.0 mmol) in DCM (6 mL). The reaction mixture was allowed to warm to 0 °C in 4 h and Et_3N (0.15 mL) was added. The reaction mixture was diluted with DCM and washed with $NaHCO_{3\ (aq)}$. The water layer was extracted twice with DCM after which the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **14** as a white amorphous solid (0.606 g, 94%).

Method B. Imidate donor **12** (0.176 g, 0.24 mmol) and 3-azidopropanol (0.073 g, 0.72 mmol) in DCM (4.8 mL) were stirred over activated 3 Å molsieves for 30 min. The mixture was cooled to 0 °C before a catalytic amount of TfOH (1 μL, 0.01 mmol) was added, then the mixture was allowed to warm to RT. After TLC analysis showed complete consumption of starting material (1 h) the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $NaHCO_{3\ (aq)}$. The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **14** as a white amorphous solid (0.143 g, 99%). $[\alpha]_D^{22} = -30$ ($c = 0.1$, DCM); IR (neat): 827, 1078, 1558, 1699, 1716, 2098, 2341, 2361, 2860, 2933 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.96$ (s, 9H, *t*Bu), 1.05 (s, 9H, *t*Bu), 1.76−1.84 (m, 2H, CH_2 $C_3H_6N_3$), 2.15 (s, 3H, CH_3 Lev), 2.58−2.61 (m, 2H, CH_2 Lev), 2.71−2.75 (m, 2H, CH_2 Lev), 3.36 (t, 2H, $J = 6.8$ Hz, CH_2 $C_3H_6N_3$), 3.48−3.60 (m, 2H, H-5, CH_2 $C_3H_6N_3$), 3.89−4.02 (m, 4H, H-2, H-4, H-6, CH_2 $C_3H_6N_3$), 4.20 (dd, 1H, $J = 4.8$ Hz, 5.2 Hz, H-6), 4.80 (d, 1H, $J =$ 8.4 Hz, H-1), 5.28 (t, 1H, $J = 10.0$ Hz, H-3), 7.72 (d, 1H, $J = 8.8$ Hz, NH);^{13}C NMR (100 MHz): $\delta =$19.5 (C_q *t*Bu), 22.3 (C_q *t*Bu), 26.5 (CH_3 *t*Bu), 27.0 (CH_3 *t*Bu), 27.8 (CH_2 Lev), 28.6 (CH_2 $C_3H_6N_3$), 29.4 (CH_3 Lev), 37.7 (CH_2 Lev), 47.5 (CH_2 $C_3H_6N_3$), 55.4 (C-2), 65.8 (C-6), 66.1 (CH_2 $C_3H_6N_3$), 70.3, (C-5), 73.5 (C-3), 74.7 (C-4), 92.2 (C_q CCl_3), 100.7 (C-1), 162.0 (C═O TCA), 171.8 (C═O COO Lev), 205.8 (C═O CO Lev); HRMS: $C_{24}H_{39}Cl_3N_4O_8Si + H^+$ requires 645.1676, found 645.1677.

3-Azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-*β*-D-glucopyranoside (15). Glucosamine **14** (0.579 g, 0.896 mmol) was dissolved in a mixture of pyridine (4 mL) and AcOH (1 mL), after which hydrazine monohydrate (0.22 mL, 4.5 mmol) was added. The mixture was stirred for 15 min and diluted with EtOAc (20 mL), 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 (PE, EtOAc) yielded **15** as a white amorphous solid (0.765 g, 85%).; IR (neat): 826, 1074, 1533, 1697, 2098, 2341, 2360, 2860, 2933, 3315 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.99$ (s, 9H, *t*Bu), 1.06 (s, 9H, *t*Bu), 1.79−1.86 (m, 2H, CH_2 $C_3H_6N_3$), 3.02 (s, 1H, OH), 3.37 (t, 2H, $J = 6.8$ Hz, CH_2 $C_3H_6N_3$), 3.44−3.60 (m, 3H, H-2, H-5, CH_2 $C_3H_6N_3$), 3.70 (t, 1H, $J = 9.2$ Hz, H-4), 3.89−3.94 (m, 2H, H-6, CH_2 $C_3H_6N_3$), 4.02 (t, 1H, $J = 9.6$ Hz, H-3), 4.19 (dd, 1H, $J = 4.8$ Hz, 5.2 Hz, H-6), 4.88 (d, 1H, $J = 8.4$ Hz, H-1), 7.11 (d, 1H, $J = 6.7$ Hz, NH);^{13}C NMR (100 MHz): $\delta =$ 19.8 (C_q *t*Bu), 22.6 (C_q *t*Bu), 26.8 (CH_3 *t*Bu), 27.3 (CH_3 *t*Bu), 28.9 (CH_2 $C_3H_6N_3$), 47.9 (CH_2 $C_3H_6N_3$), 58.1 (C-2), 65.9 (C-6), 66.5 (CH_2 $C_3H_6N_3$), 70.2, (C-5), 72.6 (C-3), 77.7 (C-4), 92.4 (C_q CCl_3), 99.8 (C-1), 162.1 (C═O TCA); HRMS: $C_{19}H_{33}Cl_3N_4O_6Si + H^+$ requires 547.1308, found 547.1306.

3-Azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranoside (16). Method A. Thio dimer **3** (0.211 g, 0.2 mmol) and Ph_2SO (0.045 g, 0.22 mmol) were coevaporated with toluene two times to remove traces of water, dissolved in DCM (4 mL) and stirred over activated 3 Å molsieves for 30 min. At −60 °C, Tf_2O (37 μL, 0.22 mmol) was added and after 15 min at −60 °C a solution of acceptor **15** (0.132 g, 0.24 mmol) in DCM (2.4 mL) was slowly added and the reaction mixture was allowed to warm to 0 °C in 3 h. Dry Et_3N 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 size exclusion and column chromatography (PE, EtOAc) yielded **16** as a off white foam (0.137 g, 46%).

Method B. Thio dimer **3** (0.90 g, 0.86 mmol) and BSP (0.198 g, 0.946 mmol) were coevaporated with toluene two times to remove traces of water, dissolved in DCM (17 mL) and stirred over activated 3 Å molsieves for 30 min. At −60 °C, Tf_2O (0.152 mL, 0.903 mmol) was added and after 15 min at −60 °C a solution of acceptor **15** (0.564 g, 1.03 mmol) in DCM (10 mL) was slowly added and the reaction mixture was allowed to warm to 0 °C in 3 h. Dry Et_3N (0.57 mL) 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 size exclusion and column chromatography (PE, EtOAc) yielded **16** as a off white foam (0.956 g, 75%).

Method C. Thio dimer **3** (0.211 g, 0.2 mmol) and acceptor **15** (0.132 g, 0.24 mmol) were coevaporated with toluene two times to remove traces of water and dissolved in DCM (4 mL). NIS (0.054 g, 0.24 mmol) was added and the mixture was stirred over activated 3 Å molsieves for 30 min. The mixture was cooled to 0 °C before a catalytic amount of TfOH (1 μL, 0.01 mmol) was added. After TLC analysis showed complete consumption of thio dimer (1.5 h) the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $Na_2S_2O_{3\ (aq)}$ and $NaHCO_{3\ (aq)}$. The water layers were extracted twice with DCM and the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography (PE, EtOAc) yielded **17** as an off white foam (0.221 g, 75%).

Method D (1-Pot Procedure). Imidate donor **12** (1.36 g, 1.80 mmol) and acceptor **13** (0.610 g, 1.20 mmol) in DCM (18 mL) were stirred over activated 3 Å molsieves for 30 min. The mixture was cooled to 0 °C before a catalytic amount of TfOH (8 μL, 0.09

mmol) was added, then the mixture was allowed to warm to RT over approximately 1 h. After TLC analysis showed complete consumption of thio acceptor (1 h), the mixture was cooled to 0 °C. Then, a mixture of glucosamine acceptor **15** (0.99 g, 1.60 mmol) and NIS (0.32 g, 1.44 mmol) (dried over activated molecular sieves) in DCM (18 mL) was added. After 2 h at 0 °C, TLC analysis showed complete consumption of thio dimer and the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $NaHCO_{3\ (aq)}$. The water layer was extracted twice with DCM, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography (PE, EtOAc) yielded **16** as an off white foam (1.29 g, 72%). $[\alpha]_D^{22} = -26$ ($c = 0.1$, DCM); IR (neat): 827, 1070, 1521, 1716, 2098, 2341, 2359, 2860, 2933 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.84$ (s, 9H, *t*Bu), 0.88 (s, 9H, *t*Bu), 0.90 (s, 9H, *t*Bu), 1.03 (s, 9H, *t*Bu), 1.79−1.82 (m, 2H, CH_2 $C_3H_6N_3$), 2.14 (s, 3H, CH_3 Lev), 2.54−2.57 (m, 2H, CH_2 Lev), 2.67−2.71 (m, 3H, H-6″, CH_2 Lev), 3.28 (dd, 1H, $J = 4.8$ Hz, 9.6 Hz H-5″), 3.34 (t, 2H, $J = 6.4$ Hz, CH_2 $C_3H_6N_3$), 3.46 (dd, 1H, $J = 4.8$ Hz, 9.6 Hz, H-5), 3.54−3.62 (m, 4H, H-2, H-4″, H-6″, CH_2 $C_3H_6N_3$), 3.84 (s, 3H, CH_3 COOMe), 3.87−3.91 (m, 4H, H-4, H-6, H-2″, CH_2 $C_3H_6N_3$), 4.16−4.19 (m, 2H, H-6, H-5′), 4.70 (t, 1H, $J = 9.2$ Hz, H-3), 4.33 (t, 1H, $J = 9.2$ Hz, H-4′), 4.81 (d, 1H, $J = 8.0$ Hz, H-1), 4.97 (d, 1H, $J = 8.4$ Hz, H-1″), 5.02 (t, 1H, $J = 9.6$ Hz, H-3″), 5.21 (dd, 1H, $J = 4.4$ Hz, 8.8 Hz, H-2′), 5.39 (d, 1H, $J = 4.4$ Hz, H-1′) 5.62 (t, 1H, $J = 9.2$ Hz, H-3′), 6.91 (d, 1H, $J = 8.8$ Hz, NH), 7.01 (d, 1H, $J = 8.4$ Hz, NH), 7.34−7.43 (m, 4H, H Arom), 7.49−7.54 (m, 2H, H Arom), 7.91−7.93 (m, 4H, H Arom);^{13}C NMR (100 MHz): $\delta = 19.6$ (C_q *t*Bu), 19.7 (C_q *t*Bu), 22.3 (C_q *t*Bu), 22.5 (C_q *t*Bu), 26.6 (CH_3 *t*Bu), 26.6 (CH_3 *t*Bu), 27.1 (2 x CH_3 *t*Bu), 27.8 (CH_2 Lev), 28.8 (CH_2 $C_3H_6N_3$), 29.6 (CH_3 Lev), 37.9 (CH_2 Lev), 47.7 (CH_2 $C_3H_6N_3$), 52.9 (CH_3 COOMe), 55.5 (C-2″), 57.3 (C-2), 64.9 (C-6″), 65.9 (C-6), 66.4 (CH_2 $C_3H_6N_3$), 70.2 (C-5), 70.5 (C-5″), 71.3 (C-3′), 74.0, 74.2, 74.3, 74.4 (C2′, C-5′, C-3″, C-4″), 76.0 (C-4), 76.5 (C-4′), 77.8 (C-3), 92.3 (C_q CCl_3), 92.5 (C_q CCl_3), 99.6 (C-1′), 99.9 (C-1), 100.9 (C-1″), 128.2−128.7 (CH Arom, C_q Arom), 129.5−129.8 (CH Arom, C_q Arom), 133.0−133.4 (CH Arom), 161.5 (C═O TCA), 161.7 (C═O TCA), 165.0 (C═O Bz), 165.6 (C═O Bz), 170.2 (C═O COOMe), 172.0 (C═O COO Lev), 205.9 (C═O CO Lev); HRMS: $C_{61}H_{83}Cl_6N_5O_{21}Si_2 + H^+$ requires 1488.3323, found 1488.3330.

3-Azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranoside (17). Trimer **16** (1.08 g, 0.72 mmol) was dissolved in a mixture of pyridine (6.4 mL) and AcOH (1.6 mL), after which hydrazine monohydrate (0.18 mL, 3.6 mmol) was added. The mixture was stirred for 15 min and diluted with EtOAc (20 mL), washed with 1 M $HCl_{(aq)}$, NaHCO3 (aq), and brine. The organic layer was dried over MgSO4 and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **17** as a white foam (0.942 g, 94%). [α]D22 = −26 ($c = 0.1$, DCM); IR (neat): 827, 1072, 1527, 1724, 2100, 2860, 2933 cm−1; 1H NMR (400 MHz, CDCl3): $\delta = 0.85$ (s, 9H, *t*Bu), 0.90 (s, 18H, *t*Bu), 1.03 (s, 9H, *t*Bu), 1.78−1.80 (m, 2H, CH2 C3H6N3), 2.65 (t, 1H, $J = 12.0$ Hz, H-6″), 2.93 (bs, 1H, OH), 3.22 (dd, 1H, $J = 4.8$ Hz, 9.6 Hz H-5″), 3.31−3.40 (m, 3H, H-3″, CH2 C3H6N3), 3.45−3.58 (m, 5H, H-2, H-5, H-2″, H-6″, CH2 C3H6N3), 3.74 (t, 1H, $J = 9.6$ Hz, H-4″), 3.84 (s, 3H, CH3 COOMe), 3.86−3.97 (m, 3H, H-4, H-6, CH2 C3H6N3), 4.11−4.20 (m, 2H, H-6, H-5′), 4.26−4.36 (m, 2H, H-3, H-4′), 4.82 (d, 1H, $J = 8.4$ Hz, H-1), 4.98 (d, 1H, $J = 8.4$ Hz, H-1″), 5.24 (dd, 1H, $J = 4.4$ Hz, 8.8 Hz, H-2′), 5.36 (d, 1H, $J = 4.4$ Hz, H-1′) 5.61 (t, 1H, $J = 9.2$ Hz, H-3′), 6.97 (d, 1H, $J = 8.0$ Hz, NH), 7.04 (d, 1H, $J = 7.6$ Hz, NH), 7.34−7.42 (m, 4H, H Arom), 7.49−7.55 (m, 2H, H Arom), 7.91−7.93 (m, 4H, H Arom);13C NMR (100 MHz): $\delta = 19.7$ (Cq *t*Bu), 19.8 (Cq *t*Bu), 22.4 (Cq *t*Bu), 22.7 (Cq *t*Bu), 26.6 (CH3 *t*Bu), 26.8 (CH3 *t*Bu), 27.2 (CH3 *t*Bu), 27.3 (CH3 *t*Bu), 28.9 (CH2 C3H6N3), 47.8 (CH2 C3H6N3), 53.0 (CH3 COOMe), 57.6 (C-2″), 57.9 (C-2), 65.0 (C-6″), 66.0 (C-6), 66.5 (CH2 C3H6N3), 70.1 (C-5″), 70.3 (C-5), 71.5 (C-3′), 73.9 (C-2′), 74.1 (C-5′), 74.4 (C-4″), 76.0 (C-4), 76.2 (C-4′), 77.3 (C-3″), 77.9 (C-3), 92.5 (Cq CCl3), 92.6 (Cq CCl3), 99.8 (C-1′), 99.9 (C-1″), 100.0 (C-1), 128.3−128.8 (CH Arom, Cq Arom), 129.6−129.8 (CH Arom, Cq Arom), 133.0−133.4 (CH Arom), 161.8 (C═O TCA), 162.2 (C═O TCA), 165.2 (C═O Bz), 165.7 (C═O Bz), 170.2 (C═O COOMe); HRMS: C56H77Cl6N5O19Si2 + H+ requires 1390.2955, found 1390.2975.

3-Azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranoside (18). Thio dimer **3** (1.132 g, 1.074 mmol) and trimer acceptor **17** (0.998 g, 0.716 mmol) were coevaporated with toluene two times to remove traces of water then dissolved in DCM (10 mL). NIS (0.242 g, 1.074 mmol) was added and the mixture was stirred over activated 3 Å molsieves for 30 min. The mixture was cooled to 0 °C before a catalytic amount of TfOH (4 μL, 0.05 mmol) was added, then the mixture was allowed to warm to RT. After TLC analysis showed complete consumption of starting material (2 h) the reaction was quenched with Et3N. The reaction mixture was diluted with DCM and washed with NaHCO3 (aq). The water layer was extracted twice with DCM and the collected organic layers were dried over MgSO4 and concentrated *in vacuo*. Purification by size exclusion and column chromatography (PE, EtOAc) yielded **18** as a white foam (1.64 g, 98%). IR (neat): 825, 1069, 1527, 1724, 2099, 2860, 2933 cm−1; 1H NMR (400 MHz, CDCl3): $\delta = 0.77$ (s, 9H, *t*Bu), 0.85 (s, 9H, *t*Bu), 0.89 (s, 27H, *t*Bu), 1.03 (s, 9H, *t*Bu), 1.78−1.80 (m, 2H, CH2 C3H6N3), 2.13 (s, 3H, CH3 Lev), 2.54−2.57 (m, 2H, CH2 Lev), 2.67−2.71 (m, 4H, H-6‴, H-6″), 3.19−3.21 (m, 1H), 3.29−3.34 (m, 3H), 3.38−3.44 (m, 2H), 3.45−3.63 (m, 9H), 3.81 (s, 3H, CH3 COOMe), 3.83 (s, 3H, CH3 COOMe), 3.86−3.96 (m, 5H), 4.06−4.19 (m, 5H), 4.26−4.37 (m, 3H), 4.82 (d, 1H, $J = 8.0$ Hz), 4.96−5.04 (m, 3H), 5.09−5.12 (m, 1H), 5.22−5.25 (m, 1H), 5.31−5.38 (m, 2H), 5.54−5.59 (m, 2H), 6.87−6.95 (m, 3H, NH), 7.35−7.42 (m, 8H, H Arom), 7.48−7.55 (m, 4H, H Arom), 7.88−7.92 (m, 8H, H Arom);13C NMR (100 MHz): $\delta = 19.5$ (Cq *t*Bu), 19.7 (Cq *t*Bu), 22.4 (Cq *t*Bu), 22.6 (Cq *t*Bu), 26.6−26.7 (CH3 *t*Bu), 27.1−27.2 (CH3 *t*Bu), 28.0 (CH2 Lev), 28.9 (CH2 C3H6N3), 29.6 (CH3 Lev), 38.1 (CH2 Lev), 47.9 (CH2 C3H6N3), 52.8 (CH3 COOMe), 53.0 (CH3 COOMe), 55.6, 58.2, 58.7 (C-2, C-2″, C-2‴), 65.1, 65.2, 66.1, (C-6, C-6″, C-6‴), 66.6 (CH2 C3H6N3), 69.9, 70.4, 70.6, 71.3, 71.6, 73.8, 74.4, 74.4, 74.7, 74.8, 75.8, 75.9, 77.9, 78.3, (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-3″, C-4″, C-5″, C-2‴, C-3‴, C-4‴, C-5‴, C-3⁗, C-4⁗, C-5⁗), 92.5 (Cq CCl3), 92.5 (Cq CCl3), 92.7 (Cq CCl3), 99.5, 99.7, 99.9, 99.9, 101.1 (C-1, C-1′, C-1″, C-1‴, C-1⁗), 128.2−128.9 (CH Arom, Cq Arom), 129.5−130.0 (CH Arom, Cq Arom), 132.9−133.5 (CH Arom), 161.4 (C═O TCA), 161.6 (C═O TCA), 161.8 (C═O TCA), 165.1 (C═O Bz), 165.2 (C═O Bz), 165.6 (C═O Bz), 165.7 (C═O Bz), 169.7 (C═O COOMe), 170.7 (C═O COOMe), 172.1 (C═O COO Lev), *205* (C═O CO Lev); HRMS: C98H127Cl9N6O34Si3 + 2H+ requires 1166.2522, found 1166.2526.

Azidopropyl (4,6-O-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-O-(methyl (2,3-di-O-benzoyl-4-O-(4,6-O-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-O-(methyl (2,3-di-O-benzoyl-4-O-(4,6-O-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranoside (19). Pentamer **18** (1.635 g, 0.70 mmol) was dissolved in a mixture of pyridine (8 mL) and AcOH (2 mL), after which hydrazine monohydrate (35 μL, 3.58 mmol) was added. The mixture was stirred for 30 min and diluted with EtOAc (20 mL), washed with 1 M HCl (aq), NaHCO3 (aq), and brine. The organic layer was dried over MgSO4 and concentrated *in vacuo*. Purification by column chromatography (PE, EtOAc) yielded **19** as a white foam

(1.42 g, 91%). IR (neat): 828, 1072, 1527, 1719, 2098, 2829, 2933 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.78$ (s, 9H, *t*Bu), 0.86 (s, 9H, *t*Bu), 0.89 (m, 18H, *t*Bu), 0.91 (s, 9H, *t*Bu), 1.04 (s, 9H, *t*Bu), 1.78−1.80 (m, 2H, CH_2 $C_3H_6N_3$), 2.65−2.67 (m, 2H, H-6‴, H-6″), 2.93 (bs, OH), 3.20−3.25 (m, 2H), 3.33 (t, 2H, $J = 6.8$ Hz, CH_2 $C_3H_6N_3$), 3.37−3.70 (m, 11H), 3.78−3.79 (m, 1H), 3.82 (s, 3H, CH_3 COOMe), 3.83 (s, 3H, CH_3 COOMe), 3.86−3.97 (m, 5H), 4.06−4.19 (m, 5H), 4.26−4.41 (m, 4H), 4.82 (d, 1H, $J = 8.0$ Hz), 4.96−5.04 (m, 3H), 5.09−5.12 (m, 1H), 5.22−5.25 (m, 1H), 5.33−5.41 (m, 2H), 5.53−5.60 (m, 2H), 6.88−6.93 (m, 2H, NH), 7.14 (d, 1H, $J = 7.6$ Hz, NH), 7.33−7.42 (m, 8H, H Arom), 7.48−7.54 (m, 4H, H Arom), 7.88−7.92 (m, 8H, H Arom);^{13}C NMR (100 MHz): $\delta = 19.5$ (C_q *t*Bu), 19.6 (C_q *t*Bu), 22.4 (C_q *t*Bu), 22.5 (C_q *t*Bu), 26.6−26.8 (CH_3 *t*Bu), 27.0−27.3 (CH_3 *t*Bu), 28.9 (CH_2 $C_3H_6N_3$), 47.8 (CH_2 $C_3H_6N_3$), 52.7 (CH_3 COOMe), 52.9 (CH_3 COOMe), 57.1, 57.7, 57.7 (C-2, C-2″, C-2‴), 65.1, 65.1, 66.0, (C-6, C-6″, C-6‴), 66.5 (CH_2 $C_3H_6N_3$), 69.8, 70.1, 70.3, 71.3, 71.5, 73.7, 74.3, 74.4, 74.7, 74.9, 75.8, 75.9, 76.3, 77.0, 77.8, 78.4, (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-3″, C-4″, C-5″, C-2‴, C-3‴, C-4‴, C-5‴, C-3⁗, C-4⁗, C-5⁗), 92.5 (C_q CCl_3), 92.6 (C_q CCl_3), 92.6 (C_q CCl_3), 99.5, 99.6, 99.8, 99.8, 100.3 (C-1, C-1′, C-1″, C-1‴, C-1⁗), 128.2−128.9 (CH Arom, C_q Arom), 129.5−129.9 (CH Arom, C_q Arom), 132.7−133.4 (CH Arom), 161.4 (C═O TCA), 161.7 (C═O TCA), 162.3 (C═O TCA), 165.0 (C═O Bz), 165.2 (C═O Bz), 165.6 (C═O Bz), 165.7 (C═O Bz), 169.7 (C═O COOMe), 170.9 (C═O COOMe); HRMS: $C_{93}H_{121}Cl_9N_6O_{32}Si_3 + 2K^+$ requires 1136.2117, found 1136.2108.

Azidopropyl (4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-di-*tert*-butylsilylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-glucopyranosyl)-β-D-glucopyranosyl) uronate)-β-D-glucopyranosyl)-β-D-glucopyranosyl) uronate)-β-D-glucopyranosyl)-β-D-glucopyranosyl) uronate)-β-D-glucopyranoside (20). Thio dimer **3** (0.542 g, 0.515 mmol) and pentamer acceptor **19** (0.922 g, 0.412 mmol) were coevaporated with toluene two times to remove traces of water then dissolved in DCM (4.1 mL). NIS (0.116 g, 0.515 mmol) was added and the mixture was stirred over activated 3 Å molsieves for 30 min. The mixture was cooled to 0 °C before a catalytic amount of TfOH (2 μL, 0.025 mmol) was added, then the mixture was allowed to warm to RT. TLC analysis was complicated because of the viscosity of the mixture as well as the similar polarities of donor and acceptor. After allowing the reaction to run for approximately 4 h (this reaction time was estimated based on the construction of the pentamer, which took approximately 2 h), the reaction was quenched with Et_3N. The reaction mixture was diluted with DCM and washed with $NaHCO_{3\,(aq)}$. The water layer was extracted twice with DCM and the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by size exclusion and column chromatography (PE, EtOAc) yielded **20** as a white foam (0.76 g, 61%). IR (neat): 826, 1028, 1068, 1707, 2098, 2860, 2934 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.73$ (s, 9H, *t*Bu), 0.74 (s, 9H, *t*Bu), 0.85 (s, 9H, *t*Bu), 0.89 (s, 36H, *t*Bu), 1.03 (s, 9H, *t*Bu), 1.78−1.81 (m, 2H, CH_2 $C_3H_6N_3$), 2.13 (s, 3H, CH_3 Lev), 2.54−2.57 (m, 2H, CH_2 Lev), 2.64−2.71 (m, 5H, H-6″, H-6‴, H-6⁗⁗), 3.19−3.22 (m, 2H), 3.23−3.35 (m, 3H), 3.38−3.63 (m, 12H), 3.81 (s, 6H, CH_3 COOMe), 3.83 (s, 3H, CH_3 COOMe), 3.85−3.96 (m, 5H), 4.07−4.18 (m, 7H), 4.28−4.36 (m, 4H), 4.82 (d, 1H, $J = 8.0$ Hz), 4.96−5.04 (m, 4H), 5.09−5.13 (m, 2H), 5.22−5.25 (m, 1H), 5.31−5.39 (m, 3H), 5.51−5.59 (m, 3H), 6.88−6.98 (m, 4H, NH), 7.33−7.42 (m, 12H, H Arom), 7.48−7.55 (m, 6H, H Arom), 7.87−7.93 (m, 12H, H Arom);^{13}C NMR (100 MHz): $\delta = 19.6$ (C_q *t*Bu), 19.7 (C_q *t*Bu), 22.4 (C_q *t*Bu), 22.6 (C_q *t*Bu), 26.6−26.7 (CH_3 *t*Bu), 27.1−27.2 (CH_3 *t*Bu), 28.0 (CH_2 Lev), 28.9 (CH_2 $C_3H_6N_3$), 29.6 (CH_3 Lev), 38.1 (CH_2 Lev), 47.9 (CH_2 $C_3H_6N_3$), 52.8 (CH_3 COOMe), 52.8 (CH_3 COOMe), 53.0 (CH_3 COOMe), 55.6, 57.0, 57.3, 57.8 (C-2, C-2″, C-2‴, C-2⁗⁗), 65.1, 65.1, 65.2, 66.1, (C-6, C-6″, C-6‴, C-6⁗⁗), 66.6 (CH_2 $C_3H_6N_3$), 69.9, 70.4, 70.6, 71.4, 71.5, 71.6, 73.8, 74.4, 74.4, 74.4, 74.7, 74.8, 75.8, 75.9, 77.8, 78.2, (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-3″, C-4″, C-5″, C-2‴, C-3‴, C-4‴, C-5‴, C-3⁗, C-4⁗, C-5⁗, C-2⁗⁗, C-3⁗⁗, C-4⁗⁗, C-5⁗⁗, C-3⁗⁗⁗, C-4⁗⁗⁗, C-5⁗⁗⁗), 92.5 (C_q CCl_3), 99.5, 99.6, 99.9, 100.0, 101.2 (C-1, C-1′, C-1″, C-1‴, C-1⁗, C-1⁗⁗, C-1⁗⁗⁗), 128.3−128.9 (CH Arom, C_q Arom), 129.5−130.0 (CH Arom, C_q Arom), 133.0−133.5 (CH Arom), 161.4 (C═O TCA), 161.4 (C═O TCA), 161.6 (C═O TCA), 161.8 (C═O TCA), 165.1 (C═O Bz), 165.1 (C═O Bz), 165.2 (C═O Bz), 165.6 (C═O Bz), 165.7 (C═O Bz), 165.7 (C═O Bz), 169.7 (C═O COOMe), 170.3 (C═O COOMe), 170.8 (C═O COOMe), 172.1 (C═O COO Lev); HRMS: $C_{135}H_{171}Cl_{12}N_7O_{47}Si_4 + 2H^+$ requires 1587.8349, found 1587.8345.

3-Azidopropyl (2-deoxy-2-amino-3-O-(4-*O*-(2-deoxy-2-amino-β-D-glucopyranosyl)-β-D-glucopyranosyl) uronate)-β-D-glucopyranoside (21). Fully protected trimer **16** (0.173 g, 0.116 mmol) was dissolved in THF (2.3 mL) and Et_3N/3HF (0.11 mL, 0.696 mmol) was added. After 2 h the mixture was diluted with EtOAc and washed with $NaHCO_{3(aq)}$. The water layer was extracted twice with EtOAc and the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was then dissolved in THF (2 mL) and H_2O (2 mL) and a 0.5 M solution of KOH in H_2O (1.62 mL, 0.812 mmol) was added stepwise (per 1 equiv.) over a period of 10 h. The reaction mixture was stirred for 4 days after which it was quenched with AcOH and concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **21** as a white amorphous solid (28 mg, 48%). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.87$−1.92 (m, 2H, CH_2 $C_3H_6N_3$), 3.04 (dd, 1H, $J = 8.4$, 10.2 Hz, H-2 or H-2″), 3.08 (t, 1H, $J = 9$ Hz, H-2 or H-2″), 3.43 (t, 2H, $J = 6.6$ Hz, CH_2 $C_3H_6N_3$), 3.45−3.50 (m, 4H), 3.61−3.67 (m, 4H), 3.69−3.76 (m, 4H), 3.79−3.82 (m, 1H, H-5′), 3.88−3.93 (m, 3H), 3.97−4.01 (m, 1H, CH_2 $C_3H_6N_3$), 4.63 (d, 1H, $J = 8.4$ Hz, H-1 or H-1′ or H-1″), 4.67 (d, 1H, $J = 8.4$ Hz, H-1 or H-1′ or H-1″), 4.74 (d, 1H, $J = 8.4$ Hz, H-1 or H-1′ or H-1″);^{13}C NMR (150 MHz): $\delta = 28.2$ (CH_2 $C_3H_6N_3$), 48.0 (CH_2 $C_3H_6N_3$), 55.1, 55.7 (C-2, C-2″), 60.3, 60.4 (C-6, C-6″), 67.6 (CH_2 $C_3H_6N_3$), 68.0, 69.4, 72.2, 72.7, 74.1, 74.6, 75.8, 76.3, 79.9 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-3″, C-4″, C-5″), 82.8 (C-5′), 98.9, 99.8, 101.9 (C-1, C-1′, C-1″), 174.9 (COOH); HRMS: $C_{21}H_{37}N_5O_{15} + H^+$ requires 600.2359, found 600.2378.

3-Azidopropyl (2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-β-D-glucopyranosyl)-β-D-glucopyranosyl) uronate)-β-D-glucopyranoside (22). Zwitterionic HA-trisaccharide **21** (17 mg, 0.028 mmol) was dissolved in MeOH (5 mL) and Ac_2O (0.5 mL) was added. After 4 h, this mixture was coevaporated three times with toluene and concentrated *in vacuo*. When NMR revealed an additional methylester signal, the residue was dissolved in H_2O and LiOH (0.1 mL, 0.5 M) was added. The mixture was stirred for 2 h and quenched with AcOH until neutral and concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **22** as a white amorphous solid (19 mg, 99%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.79$−1.84 (m, 2H, CH_2 $C_3H_6N_3$), 2.01 (s, 3H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac), 3.32−3.36 (m, 3H), 3.40−3.57 (m, 7H), 3.62−3.75 (m, 7H), 3.78−3.83 (m, 1H), 3.88−3.98 (m, 3H), 4.45 (d, 1H, $J = 6.7$ Hz, H-1 or H-1′ or H-1″), 4.50 (d, 1H, $J = 8.4$ Hz, H-1 or H-1′ or H-1″), 4.51 (d, 1H, $J = 8.4$ Hz, H-1 or H-1′ or H-1″);^{13}C NMR (100 MHz): $\delta = 22.2$ (CH_3 Ac), 22.4 (CH_3 Ac), 28.1 (CH_2 $C_3H_6N_3$), 47.7 (CH_2 $C_3H_6N_3$), 54.5, 55.3 (C-2, C-2″), 60.5, 60.6 (C-6, C-6″), 67.1 (CH_2 $C_3H_6N_3$), 68.5, 69.6, 72.4, 73.5, 73.8, 75.3, 75.8, 76.3, 79.8, 82.2 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-3″, C-4″, C-5′, C-5″), 100.7, 100.9, 103.1 (C-1, C-1′, C-1″), 174.2, 174.6, 174.8 (C═O Ac, COOH); HRMS: $C_{25}H_{41}N_5O_{17} + H^+$ requires 684.2570, found 684.2573.

Azidopropyl (2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amido-β-D-glucopyranosyl)-β-D-glucopyranosyl) uronate)-β-D-glucopyranosyl)-β-D-glucopyranosyl) uronate)-β-D-glucopyranoside (23). Fully protected HA pentamer **18** (0.289 g, 0.124 mmol) was dissolved in THF (3 mL) and Et_3N/3HF (0.121

mL, 0.741 mmol) was added. After 6 h, the mixture was diluted with EtOAc and was washed with $NaHCO_{3(aq)}$. The water layer was extracted twice with EtOAc, the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was then dissolved in THF (3 mL) and H_2O (3 mL) and a 0.5 M solution of KOH in H_2O (2.73 mL, 1.36 mmol) was added stepwise (per 1 equiv) over a period of 72 h. The reaction mixture was stirred for 4 days after which it was quenched with AcOH and concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **23** as a white amorphous solid (51 mg, 42%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.86−1.91 (m, 2H, CH_2 $C_3H_6N_3$), 3.01−3.06 (m, 2H, H-2 or H-2″ or H-2‴), 3.14−3.18 (m, 1H, H-2 or H-2″ or H-2‴), 3.41−3.50 (m, 8H), 3.60 (t, 1H, J = 9 Hz), 3.63−3.67 (m, 4H), 3.69−3.78 (m, 7H), 3.86−3.93 (m, 6H), 3.96−4.00 (m, 1H, CH_2 $C_3H_6N_3$), 4.59 (d, 1H, J = 8.4 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗), 4.65 (d, 1H, J = 8.4 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗), 4.66 (d, 1H, J = 8.4 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗), 4.70 (d, 1H, J = 9.0 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗), 4.73 (d, 1H, J = 8.4 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗);^{13}C NMR (150 MHz): δ = 28.2 (CH_2 $C_3H_6N_3$), 48.0 (CH_2 $C_3H_6N_3$), 55.0, 55.2, 55.7 (C-2, C-2″, C-2⁗), 60.3, 60.3, 60.4 (C-6, C-6″, C-6⁗), 67.6 (CH_2 $C_3H_6N_3$), 67.8, 68.0, 69.5, 72.2, 72.6, 72.7, 74.1, 74.5, 74.7, 75.8, 75.9, 76.2, 80.0, 80.1 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-3″, C-4″, C-5″, C-2‴, C-3‴, C-4‴, C-3⁗, C-4⁗, C-5⁗), 82.4, 83.3 (C-5′, C-5⁗), 99.1, 99.5, 100.2, 101.8, 102.1 (C-1, C-1′, C-1″, C-1‴, C-1⁗), 174.8, 174.8 (COOH); HRMS: $C_{33}H_{56}N_6O_{25}$ + H^+ requires 937.3368, found 937.3376.

Azidopropyl (2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranoside (24). Zwitterionic HA-pentasaccharide **23** (12 mg, 0.013 mmol) was dissolved in MeOH (5 mL) and Ac_2O (0.5 mL) was added. After 4 h, this mixture was coevaporated three times with toluene and concentrated *in vacuo*. When NMR revealed additional methyl ester signals, the residue was dissolved in H_2O and LiOH (0.1 mL, 0.5 M) was added. The mixture was stirred for 2 h and quenched with AcOH until neutral and concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **24** as a white amorphous solid (10 mg, 74%).^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.84−1.88 (m, 2H, CH_2 $C_3H_6N_3$), 2.05 (s, 6H, CH_3 Ac), 2.07 (s, 3H, CH_3 Ac), 3.37−3.40 (m, 4H), 3.47−3.62 (m, 10H), 3.69−3.86 (m, 14H), 3.92−4.12 (m, 4H), 4.48 (d, 1H, J = 7.6 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗), 4.49 (d, 1H, J = 8.0 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗), 4.54 (d, 1H, J = 8.4 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗), 4.55 (d, 1H, J = 9.6 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗), 4.57 (d, 1H, J = 8.8 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗);^{13}C NMR (100 MHz): δ = 22.2 (CH_3 Ac), 22.4 (CH_3 Ac), 22.5 (CH_3 Ac), 28.1 (CH_2 $C_3H_6N_3$), 47.8 (CH_2 $C_3H_6N_3$), 54.3, 54.6, 55.4 (C-2, C-2″, C-2⁗), 60.5, 60.5, 60.7 (C-6, C-6″, C-6⁗), 67.2 (CH_2 $C_3H_6N_3$), 68.4, 68.6, 69.7, 72.5, 72.5, 73.6, 73.9, 75.4, 75.9, 76.3, 76.4, 79.8, 80.0, 82.2, 82.6 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-3″, C-4″, C-5″, C-2‴, C-3‴, C-4‴, C-3⁗, C-4⁗, C-5⁗), 100.6, 100.7, 101.0, 103.1, 103.1 (C-1, C-1′, C-1″, C-1‴, C-1⁗), 174.1, 174.2, 174.6, 174.9, 174.9 (C═O Ac, COOH); HRMS: $C_{39}H_{62}N_6O_{28}$ + H^+ requires 1063.3685, found 1063.3694.

Azidopropyl (2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amino-3-*O*-(4-*O*-(2-deoxy-2-amino-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranoside (25). Fully protected HA heptamer **20** (0.261 g, 0.082 mmol) was dissolved in THF (1.6 mL) and Et_3N/3HF (0.107 mL, 0.656 mmol) was added. After 2 h the mixture was diluted with EtOAc and was washed with $NaHCO_{3(aq)}$. The water layer was extracted twice with EtOAc the collected organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting syrup was then dissolved in THF (1.6 mL) and H_2O (1.6 mL) and a 0.5 M solution of KOH in H_2O (0.164 mL, 1.23 mmol) was added stepwise (per 1 equiv) over a period of 10 h. The reaction mixture was stirred for 4 days, after which it was quenched with AcOH and concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **25** as a white amorphous solid (64 mg, 59%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.86−1.90 (m, 2H, CH_2 $C_3H_6N_3$), 2.93 (t, 1H, J = 9.6 Hz, H-2 or H-2″ or H-2‴ or H-2⁗), 2.98 (t, 1H, J = 10.2 Hz, H-2 or H-2″ or H-2‴ or H-2⁗), 3.08 (t, 2H, J = 9.6 Hz, H-2 or H-2″ or H-2‴ or H-2⁗), 3.41−3.49 (m, 11H), 3.55 (t, 1H, J = 9.6 Hz), 3.59−3.80 (m, 18H), 3.87−3.91 (m, 7H), 3.96−3.99 (m, 1H, CH_2 $C_3H_6N_3$), 4.50 (d, 1H, J = 8.4 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗ or H-1⁗′, or h-1⁗″), 4.62−4.63 (M, 4H, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗ or H-1⁗′, or h-1⁗″), 4.64 (d, 1H, J = 8.4 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗ or H-1⁗′, or h-1⁗″), 4.68 (d, 1H, J = 8.4 Hz, H-1 or H-1′ or H-1″ or H-1‴ or H-1⁗ or H-1⁗′, or h-1⁗″); ^{13}C NMR (150 MHz): δ = 28.2 (CH_2 $C_3H_6N_3$), 48.0 (CH_2 $C_3H_6N_3$), 55.3, 55.3, 55.5, 55.8 (C-2, C-2″, C-2‴, C-2⁗″), 60.3, 60.3, 60.4 (C-6, C-6″, C-6‴, C-6⁗″), 67.5 (CH_2 $C_3H_6N_3$), 67.9, 67.9, 68.1, 69.5, 72.6, 72.8, 74.2, 74.2, 74.7, 74.9, 75.0, 75.8, 75.8, 76.2, 80.1, 80.2 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-3″, C-4″, C-5″, C-2‴, C-3‴, C-4‴, C-3⁗, C-4⁗, C-5⁗, C-2⁗′, C-3⁗′, C-4⁗′, C-3⁗″, C-4⁗″, C-5⁗″, C-2⁗‴, C-3⁗‴, C-4⁗‴, C-5⁗‴), 83.4, 83.5, 84.7 (C-5′, C-5‴, C-5⁗″), 99.6, 99.6, 100.3, 101.2, 102.2, 102.2, 102.5 (C-1, C-1′, C-1″, C-1‴, C-1⁗, C-1⁗′, C-1⁗″, C-1⁗‴), 174.7, 174.7, 174.8 (COOH); HRMS: $C_{45}H_{75}N_7O_{35}$ + H^+ requires 1274.4377, found 1274.4395.

Azidopropyl (2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-3-*O*-(4-*O*-(2-deoxy-2-acetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranoside (26). Zwitterionic HA-heptasaccharide **25** (11 mg, 0.0089 mmol) was dissolved in MeOH (5 mL) and Ac_2O (0.5 mL) was added. After 4 h, this mixture was coevaporated three times with toluene and concentrated *in vacuo*. When NMR revealed additional methyl ester signals, the residue was dissolved in H_2O and LiOH (0.1 mL, 0.5 M) was added. The mixture was stirred for 2 h and quenched with AcOH until neutral and concentrated *in vacuo*. The remaining solid was subsequently purified by gel filtration and lyophilized 3 times yielding **26** as a white amorphous solid (10 mg, 78%).^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.80−1.84 (m, 2H, CH_2 $C_3H_6N_3$), 2.00 (s, 6H, CH_3 Ac), 2.01 (s, 6H, CH_3 Ac), 2.03 (s, 3H, CH_3 Ac), 3.30−3.37 (m, 5H), 3.43−3.58 (m, 13H), 3.60−3.72 (m, 17H), 3.74−3.84 (m, 3H), 3.88−3.95 (m, 4H), 3.96−3.98 (m, 1H), 4.43−4.54 (m, 7H, H-1, H-1′, H-1″, H-1‴, H-1⁗, H-1⁗′, H-1⁗″); ^{13}C NMR (100 MHz): δ = 22.2 (CH_3 Ac), 22.4 (CH_3 Ac), 22.4 (CH_3 Ac), 28.0 (CH_2 $C_3H_6N_3$), 47.7 (CH_2 $C_3H_6N_3$), 54.3, 54.3, 54.5, 55.3 (C-2, C-2″, C-2⁗, C-2⁗″), 60.5, 60.6 (C-6, C-6″, C-6⁗, C-6⁗″), 67.1 (CH_2 $C_3H_6N_3$), 68.4, 68.5, 69.6, 72.4, 73.5, 73.8, 75.3, 75.8, 76.2, 76.4, 79.7, 79.9 (C-3, C-4, C-5, C-2′, C-3′, C-4′, C-3″, C-4″, C-5″, C-2‴, C-3‴, C-4‴, C-3⁗, C-4⁗, C-5⁗, C-2⁗′, C-3⁗′, C-4⁗′, C-3⁗″, C-4⁗″, C-5⁗″), 82.1, 82.5 (C-5′, C-5‴, C-5⁗″), 100.6, 100.9, 103.1 (C-1, C-1′, C-1″, C-1‴, C-1⁗, C-1⁗′, C-1⁗″, C-1⁗‴), 174.1, 174.2, 174.6, 174.8, 174.9 (C═O Ac, COOH); HRMS: $C_{53}H_{83}N_7O_{39}$ + H^+ requires 1442.4799, found 1442.4819.

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

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