Oxacarbenium ion intermediates in the stereoselective synthesis of anionic oligosaccharides

Dinkelaar, J.

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Chapter 3

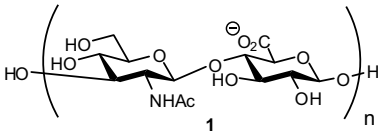
Synthesis of Hyaluronic Acid Oligomers using Ph_2SO/Tf_2O Mediated Glycosylations[1]

Introduction

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

activity.[8] For the understanding of the role of HA at a molecular level, the development of an efficient synthetic approach to sufficient quantities of well-defined oligomers and derivatives thereof is crucial.

Figure 1



1

Repeating unit of hyaluronan oligosaccharides.

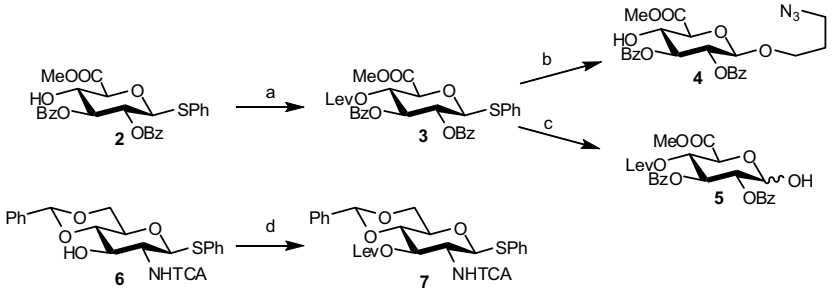
Since the pioneering work of Jeanloz[9] in 1964, several research groups have studied the synthesis of HA oligomers.[10] Compared to other glycosaminoglycan family members such as heparin sulfate, HA has received little attention from the synthetic carbohydrate community. In this chapter the synthesis of a HA trimer, tetramer and pentamer, each with a glucuronic acid moiety as the reducing end sugar is described.

Results and discussion

The synthetic strategy presented here, is based on the finding of Codée *et al.*[11] that donor 1-hydroxysugars can be chemoselectively condensed with acceptor 1-thioglycosides under the agency of Gin's activator system for dehydrative glycosylations (diphenylsulfoxide / trifluoromethanesulfonic anhydride),[12] resulting in the formation of 1-thiodisaccharides amenable for elongation at both reducing end and non-reducing end.[13] The reducing glucuronide in the target HA oligomers is masked as the 3-azido-1-propanol glycoside, with the dual advantage of locking the anomeric configuration and enabling functionalization of the azide moiety for conjugation studies.

The syntheses of functionalized monosaccharides **4**, **5**, **6** and **7** that were required for executing the selected strategy are summarized in Scheme 1. Partially protected 1-phenylthioglucuronide **2**, prepared following a previously reported procedure,[14] was transformed into the corresponding 4-*O*-levulinoyl derivative **3**. Glycosylation with 3-azidopropanol (Ph_2SO, Tf_2O) followed by deblocking of the 4'-hydroxyl function gave reducing end building block **4**. The procedure described in Chapter 2 was used to hydrolyze the thioacetal funtion in **3** providing 1-hydroxy donor **5**.[15] Partially protected glucosamine derivative **6** was prepared as described by Blatter and Jacquinet,[16] and levulinoylated to give donor thioglycoside **7**.

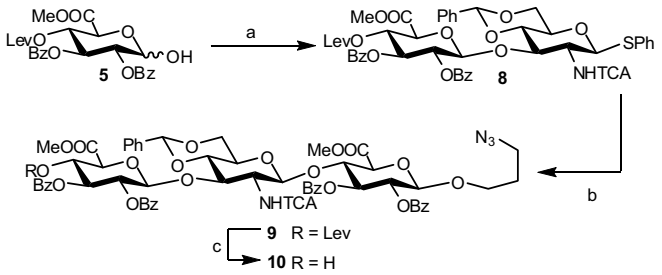
Scheme 1



Synthesis of monomer HA building blocks. Reagents and conditions: a) Lev_2O, dioxane, pyridine (86%); b) Ph_2SO, DCM, -60 °C, then Tf_2O, 15 min., then $HO(CH_2)_3N_3$, -60 °C to rT; *ii*. Pyridine, AcOH, hydrazine, (64% over 2 steps); c) TFA, NIS, DCM, H_2O (82%); d) Lev_2O, dioxane, pyridine (87%).

The synthesis of the fully protected HA trimer **9** is depicted in Scheme 2 and commenced with the Ph_2SO/Tf_2O/TTBP mediated condensation of donor glycoside **5** and acceptor glycoside **6** to give **8** in 56% yield. Thiodisaccharide **8** was condensed with acceptor glucuronide **4** under the same conditions to provide trisaccharide **9** (47%). Deprotection of the levulinoyl group in **9** afforded trisaccharide **10** (hydrazine, pyridine/AcOH). It is of interest to note that replacement of the *N*-trichloroacetyl group in glucosamine acceptor **6** by either a *N*-acetyl or *N*-phthaloyl protective group resulted in a dramatic drop in coupling efficiency.

Scheme 2

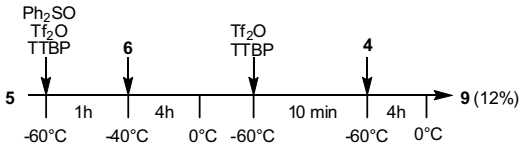


Sequential glycosylation strategy. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -60 °C, then Tf_2O, to -40 °C then add acceptor **6**, -40 °C to 0 °C (56%); b) Ph_2SO, TTBP, DCM, -60 °C, Tf_2O, 10 min, then add acceptor **4**, to 0 °C (47%); c) Pyridine, AcOH, hydrazine (96%).

At this stage, the efficiency in preparing trisaccharide **9** following a one-pot procedure was investigated.[17] Accordingly, the reaction mixture containing disaccharide **8**, formed after Tf_2O/Ph_2SO mediated condensation of 1-hydroxydonor **5** with thioglycoside **6**, was cooled and activated with an additional equivalent of Tf_2O and TTBP (0.95 equiv. with respect to

Tf_2O) for 10 minutes, followed by addition of acceptor glycoside **4** (1 equiv.). Following this protocol (Scheme 3), trisaccharide **9** could be prepared, but the yield (12%) proved to be considerably lower than the overall yield (26%) of the two separate glycosylation steps.

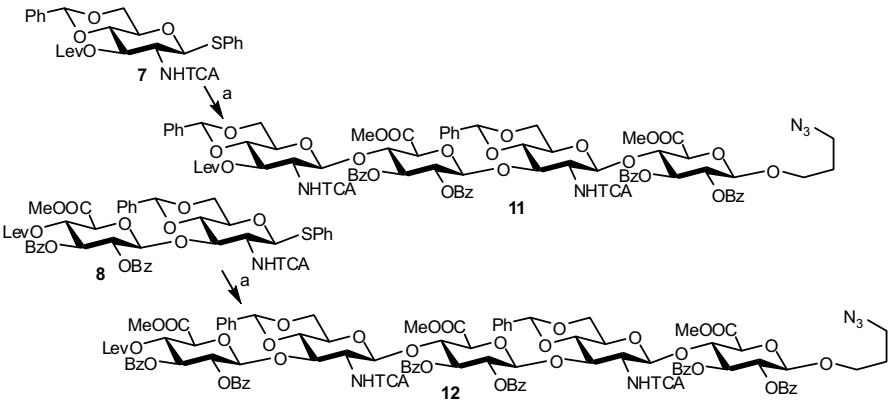
Scheme 3



One pot glycosylation strategy towards trisaccharide **9**.

The difficulty of this reaction sequence is that base (TTBP) has 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 the dehydrative glycosylation and oxazolidine formation upon activation of the thioglucosamine donor is avoided. The efficiency of the one-pot procedure could not be enhanced by varying the amount of TTBP. On the basis of these results, it was concluded that trisaccharide **9** is best prepared via two individual glycosylation steps, and that the optimal amount of based used in both steps is 0.95 equivalents with respect to Tf_2O.

Scheme 4



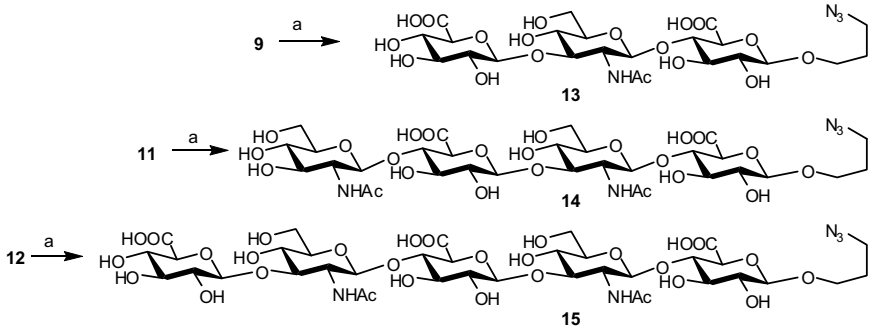
HA tetramer and pentamer synthesis. Reagents and conditions: a) Ph_2SO, TTBP, DCM, -60 °C, then Tf_2O, 10 min., then **10**, to -15 °C (**11** 62%, **12** 48%).

Fully protected HA tetramer **11** and pentamer **12** were prepared starting from trimer **10** as follows (Scheme 4). Activation of thioglucosamine **7** using the protocol described above (Ph_2SO, Tf_2O, TTBP) followed by addition of acceptor trisaccharide **10** 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 **8** as the donor, fully protected pentasaccharide **12** (42% yield) was prepared.

Finally, the fully deprotected target tri-, tetra- and pentamers **13**, **14** and **15** were obtained (Scheme 5) by acid cleavage of the benzylidene group followed by saponification of the ester and amide functionalities under the agency of KOH, *N*-acetylation in MeOH with Ac_2O and purification by gel filtration.

Scheme 5

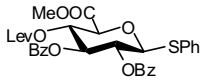


HA deprotection. Reagents and conditions: a) *i.* MeOH, *p*-TsOH; *ii.* H_2O, THF, KOH; *iii.* Ac_2O, MeOH (**13** 58%, **14** 54%, **15** 48%).

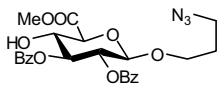
In conclusion, HA oligomers that are suitably functionalized for future biological studies can be conveniently prepared by making use of thioglycosides and 1-hydroxyglycosides, in combination with the Ph_2SO/Tf_2O/TTBP activating system. The yields in the glycosidic bond formations are moderate, and the combination of the presence of acid-labile benzylidene protective groups and the propensity of orthoester formation makes that the glycosylations have to be monitored with care, especially with respect to the amount of base used. However, the general strategy is convenient, in that useful quantities can be prepared from readily available building blocks. By making use of glucuronic acid building blocks, post-glycosylation manipulations can be kept to a minimum. The compounds 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

General: Dichloromethane was refluxed with P_2O_5 and distilled before use. Trifluoromethanesulfonic anhydride was distilled from P_2O_5. Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by co-evaporation with toluene. All other chemicals (Acros, Fluka, Merck, Schleicher & Schue) were used as received. Column chromatography was performed on Merck silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminum sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H_2SO_4 in ethanol or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ 25 g/L, $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ 10 g/L, 10% H_2SO_4 in H_2O followed by charring at +/- 140 °C. ^1H and ^{13}C NMR spectra were recorded with a Bruker AV 400 (400 and 100 MHz respectively), AV 500 (500 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in $CDCl_3$ with chemical shift (δ) relative to tetramethylsilane unless stated otherwise. Optical rotations were measured on a Propol automatic polarimeter. High resolution mass spectra were recorded on a LTQ-orbitrap (thermo electron). IR spectra were recorded on a Shimadzu FTIR-8300 and are reported in cm^{-1}.

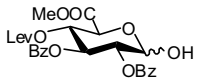


Methyl (phenyl 2,3-di-*O*-benzoyl-4-*O*-levulinoyl-1-thio-*β*-D-glucopyranoside) uronate (3). To a solution of **2** (3.81 g, 7.49 mmol) in pyridine (75 ml) was added a solution of Lev_2O in dioxane (0.5 M, 37.5 ml, 18.7 mmol). After 18 h the mixture was diluted with EtOAc (200 ml), washed with 1M HCl (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 (3.91 g, 86%). $[\alpha]_D^{22}$: +63 (c = 1, $CHCl_3$); 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 x 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, H Arom), 7.87 (d, 2H, *J* = 7.2 Hz, H Arom), 7.93 (d, 2H, *J* = 7.2 Hz, H Arom); ^{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 (CH 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.

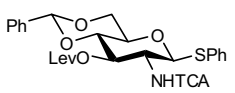


3-azidopropyl (methyl (2,3-di-*O*-benzoyl-*β*-D-glucopyranoside) uronate) (4). A mixture of **3** (1.21 g, 2.00 mmol) and Ph_2SO (0.485 g, 2.40 mmol) were co-evaporated with toluene two times to remove traces of water, dissolved in DCM (40 ml) and further dried by stirring over molsieves 3Å for 15 min. 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. and diluted with EtOAc (50 ml), washed with

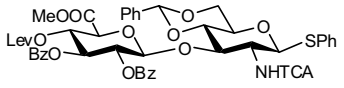
1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **4** as a colorless oil (0.639 g, 64%). $[\alpha]_D^{22}$: +56 (c = 1, $CHCl_3$); IR (neat): 709, 1067, 1252, 1717, 2103, 2930 cm^{-1}; ^1H NMR (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, H Arom), 7.51 (m, 2H, H Arom), 7.97 (m, 4H, H Arom); ^{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 Arom), 128.4 (C_q Bz), 128.9 (C_q Bz), 129.7 (CH Arom), 129.9 (CH Arom), 133.4 (CH Arom), 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.



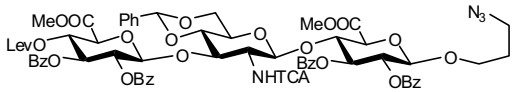
Methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-D-glucopyranose) uronate (5). To a vigorously stirred solution of **3** (0.30 g, 0.50 mmol) in CH_2Cl_2 (5 ml) and H_2O (0.5 ml) was added at 0 °C NIS (112 mg, 0.50 mmol) and TFA (39 µl, 0.50 mmol). After TLC analysis showed complete consumption of starting 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 **5** 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, H Arom) , 7.48 (m, 2H, H Arom), 7.94 (m, 4H, H Arom); ^{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 (CH Arom), 128.7 (C_q Bz), 129.6 (CH Arom), 133.3 (CH Arom), 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 (7). To a solution of **6** (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, washed with 1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **7** 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, H Arom), 7.46 (m, 4H, H 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.

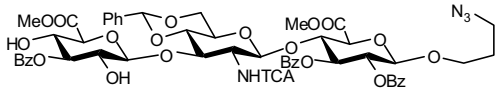


Phenyl (4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyl) uronate)-1-thio-*β*-D-glucopyranoside (8). A mixture of 1-hydroxy donor **5** (0.514 g, 1.00 mmol), Ph_2SO (0.485 g, 2.40 mmol) and TTBP (0.248 g, 1.00 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (20 ml) and further dried by stirring over molsieves 3Å 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 **6** (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 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 **8** 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, H Arom), 7.83 (m, 4H, H 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.

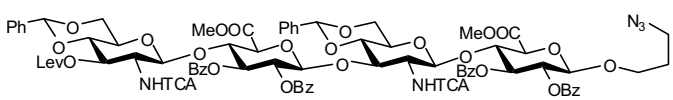


3-azidopropyl (methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside) uronate (9). A mixture of 1-thio donor **8** (0.314 g, 0.314 mmol), Ph_2SO (0.070 g, 0.345 mmol) and TTBP (0.078 g, 0.314 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (6 ml) and further dried by stirring over molsieves 3Å 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 **4** (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 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 **9** 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, 10.8Hz, 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, H Arom),

7.85 (d, 2H, *J* = 7.2 Hz, H Arom), 7.93 (d, 2H, *J* = 7.2 Hz, H Arom), 7.99 (d, 2H, *J* = 7.2 Hz, H Arom); ^{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 (CHPh), 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.

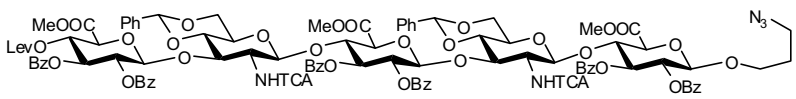


3-azidopropyl (methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside) uronate) (10). HA-trimer (**9**) (204 mg, 0.147 mmol) was dissolved in a mixture of pyridine (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), washed with 1M HCl (aq), $NaHCO_3$ (aq), and brine. The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography yielded **10** as a white solid (182 mg, 96%). IR (neat): 705, 1027, 1091, 1264, 1711, 1734, 2098, 2343, 2360, 2890, 3374, 3503 cm^{-1}; ^{1}H 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, H Arom), 7.88 (m, 4H, H Arom), 7.93 (m, 2H, H Arom), 7.99 (m, 2H, H Arom); ^{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.



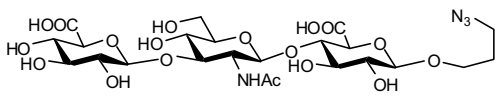
3-azidopropyl (methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside) uronate) (11). A mixture of 1-thio donor **7** (0.081 g, 0.135 mmol), Ph_2SO (0.030 g, 0.148 mmol) and TTBP (0.034 g, 0.135 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (2.7 ml) and further dried by stirring

over molsieves 3Å 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 **10** (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}; ^1H 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, H Arom), 7.91 (m, 4H, H Arom), 7.99 (d, 2H, *J* = 7.2 Hz, H Arom); ^{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.

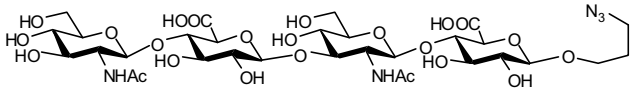


3-azidopropyl (methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-(4,6-*O*-benzylidene-2-deoxy-2-trichloroacetamido-3-*O*-(methyl (2,3-di-*O*-benzoyl-4-*O*-levulinoyl-β-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl) uronate)-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside) uronate) (12). A mixture of 1-thio donor **8** (0.164 g, 0.164 mmol), Ph_2SO (0.036 g, 0.177 mmol) and TTBP (0.039 g, 0.157 mmol) was co-evaporated with toluene two times to remove traces of water, dissolved in DCM (3.3 ml) and further dried by stirring over molsieves 3Å 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 **10** (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 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 **12** as a colorless oil (0.116 g, 48%). IR (neat): 709, 1027, 1069, 1267, 1728, 2100, 2342, 2360, 2926 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = ^1H NMR (400 MHz, $CDCl_3$): δ = 1.75 (m, 2H, CH_2 $C_3H_6N_3$), 2.01 (s, 3H, CH_3 Lev), 2.45 (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),

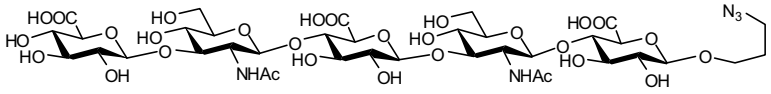
2.51 (m, 3H, CH_2 Lev), 3.25 (m, 5H, CH_2 $C_3H_6N_3$, H-5', H-5''', H-2'''), 3.39 (m, 3H, H-2', H-4', H-4'''), 3.59 (m, 1H, CH_2 $C_3H_6N_3$), 3.62 (s, 3H, CH_3 COOMe), 3.63 (s, 3H, CH_3 COOMe), 3.67 (m, 2H, H-6', H-6'''), 3.78 (m, 2H, H-5, H-5''''), 3.80 (s, 3H, CH_3 COOMe), 3.85 (m, 1H, CH_2 $C_3H_6N_3$), 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, H Arom), 7.78-7.98 (m, 12H, H Arom); ^{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 (13). Trisaccharide **9** (44 mg, 0.032 mmol) was dissolved in MeOH (5 ml) and a catalytic amount of *p*-toluene sulfonic acid was added. The reaction mixture was stirred for 15 h were it was 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 hours this mixture was co-evaporated three times with MeOH and toluene (1/1 v/v) and concentrated *in vacuo*. Purification by gel filtration (LH-20) and lyophilization yielded **13** 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-glucopyranosyl)-*β*-D- glucopyranuronic acid)-*β*-D-glucopyranosyl)-*β*-D- glucopyranuronic acid (14). Tetrasaccharide **11** (80 mg, 0.045 mmol) was dissolved in MeOH (5 ml) and a catalytic amount of *p*-toluene sulfonic acid was added. The reaction mixture was stirred for 15 h where it was 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 hours this mixture was co-evaporated three times with toluene and concentrated *in vacuo*. Purification by gel filtration (LH-20) and lyophilization yielded **14** 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_2-O) 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 (15). Pentasaccharide **12** (53 mg, 0.024 mmol) was dissolved in MeOH (5 ml) and a catalytic amount of *p*-toluene sulfonic acid was added. The reaction mixture was stirred for 15 h where it was 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 hours this mixture was co-evaporated three times with toluene and concentrated *in vacuo*. Purification by gel filtration (LH-20) and lyophilization yielded **15** 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.

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