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Synthesis of ^{13}C-labeled and functionalized Hyaluronan derivatives for biophysical studies and surface modifications

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ABSTRACT

A convergent synthesis of a tetrasaccharide partial sequence of ^{13}C-labeled Hyaluronan is presented. This tetrasaccharide can be used for biophysical studies as well as for surface modifications. Furthermore, tetrasaccharide **7** can be employed for the synthesis of additionally labeled higher oligomers of Hyaluronan on the basis of the presented methodology.

1. Introduction

For a long time the extracellular matrix (ECM) was only considered as a type of static glue that helps to connect the cells with each other. This view has completely changed during last years, because it was found that there is a plethora of specific interactions between extracellular molecules as well as between ECM molecules and imbedded cells.[1] Today the sum of all macromolecules that can be found outside of the plasma membrane of cells are count as constituents of the ECM. Mainly, they can be divided into fibrous proteins and glycosaminoglycans (GAGs).[2] GAGs are built out of disaccharide repeating units consisting of a hexose or a hexuronic acid connected with a hexosamine. Examples for these are: Heparan sulfate, Chondroitin sulfate and Keratan sulfate which can also form proteoglycans and Hyaluronan (HA) as an example for a non-proteoglycan forming GAG. Hyaluronan is an unbranched, exclusively non-sulfated polysaccharide that appears as a polyanion under physiological pH values. HA is built out of disaccharide repeating units of 2-acetamido-2-deoxy-D-glucose and D-glucuronic acid. Compared with other GAGs HA is different: it is non-sulfated, is neither produced in the Golgi apparatus nor in the endoplasmic reticulum but by integral membrane proteins—the HA synthases (HAS1, HAS2, HAS3). Also the size is different: HAs molecular weight can reach 10^5–10^7 Da and this is two orders of magnitudes higher compared with other GAGs.[3] Altogether, HA plays an important role as part of the ECM: so it acts as water storage, it affects cell proliferation, migration and differentiation[4] and also serves as ligand for different receptors such as RHAMM, ICAM-1 and CD44. The latter is a cell-surface glycoprotein involved in cell–cell and cell–ECM interactions, cell trafficking and lymph node homing and cancer (stem) cell proliferation.[4] HA is also important in tissue injury and repair.[5] Here, the function is correlated to the size of HA fragments. While short HA chains are immunostimulatory longer sequences cause the opposite effect. For all these reasons it is not surprising, that extracellular matrix molecules represent important targets in pharmacotherapy.[6]

Well defined HA subunits of different length are required for such investigations[7] and substantial work can already been found on that field. Flowers and Jeanloz as well as Takanashi et al. succeeded with the first chemical synthesis of HA disaccharides in 1962.[8,9] After this pioneering work also longer fragments were synthesized.[10–12] Usage of different protective groups is serving the specific needs to suit different reaction conditions. The synthesized fragments, if not bearing a free reducing end, mainly exhibit a non-functionalized aglycon (e.g., methyl, aryl) or a mono-functionalized moiety like an alkyl-azide.[13] Recently van der Marel and Codée reported an impressive automated solid phase synthesis of hepta- to pentadecameric *O*-1-allyl substituted HA sequences.[14]

Here, we describe a convergent synthesis of a ^{13}C-labeled HA tetramer for ongoing biophysical studies.

2. Results and discussion

Reaction of glycosyl donor **1**[15] with derivative **2**[16,17] using TMSOTf as promoter, afforded disaccharide **4** in 90% yield. Similarly, disaccharide **5** was obtained after coupling of trichloracetimidate **1** with monosaccharide **3**[18] and subsequent *O*-TBS group cleavage with TBAF in modest yield. In both cases only the desired β-anomers were obtained. Next, both disaccharides were linked by activating thioglycoside **4** with NIS and trifluoromethanesulfonic acid, to afford protected tetrasaccharide **6** in a 59% yield. Then,

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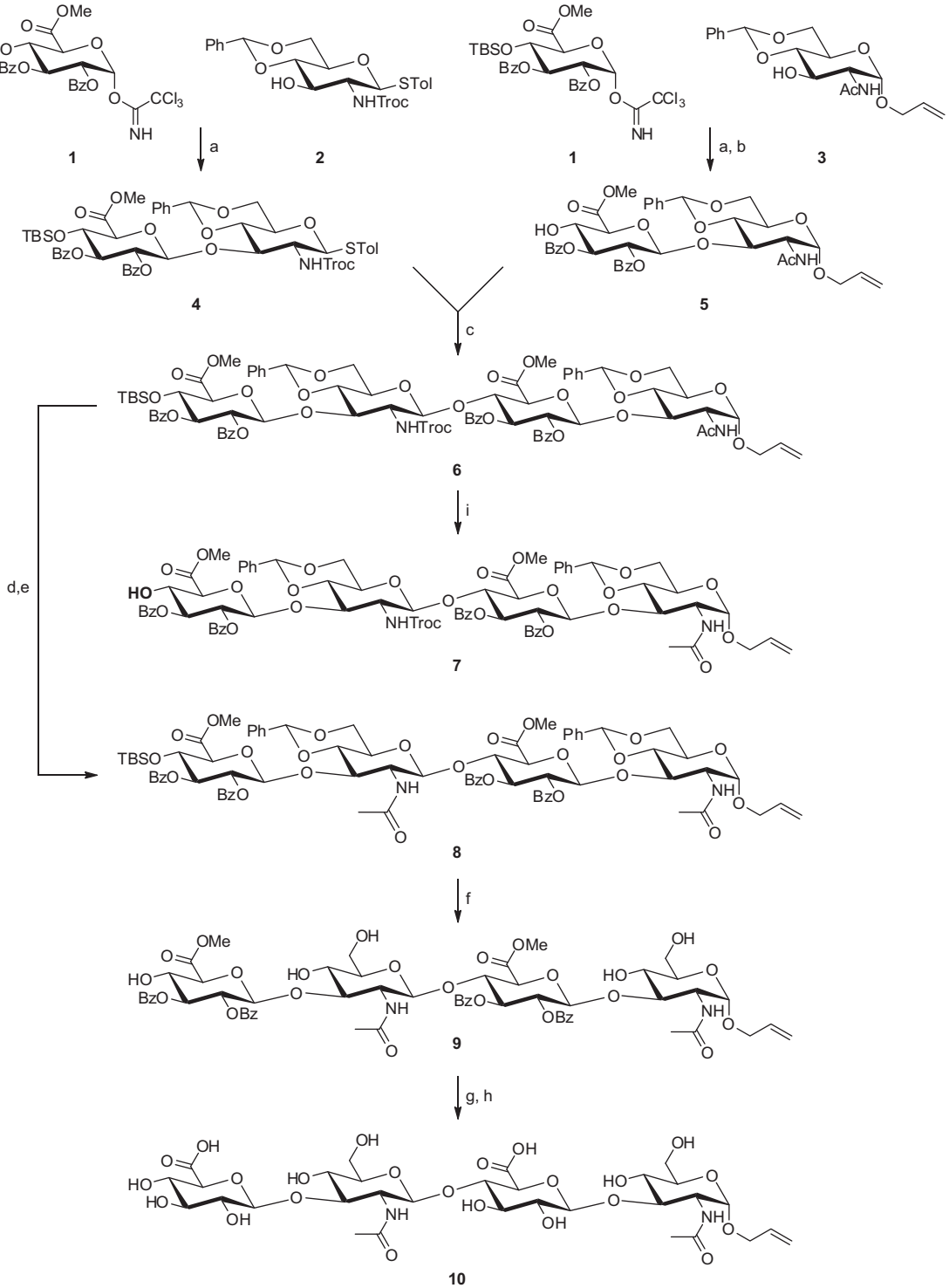
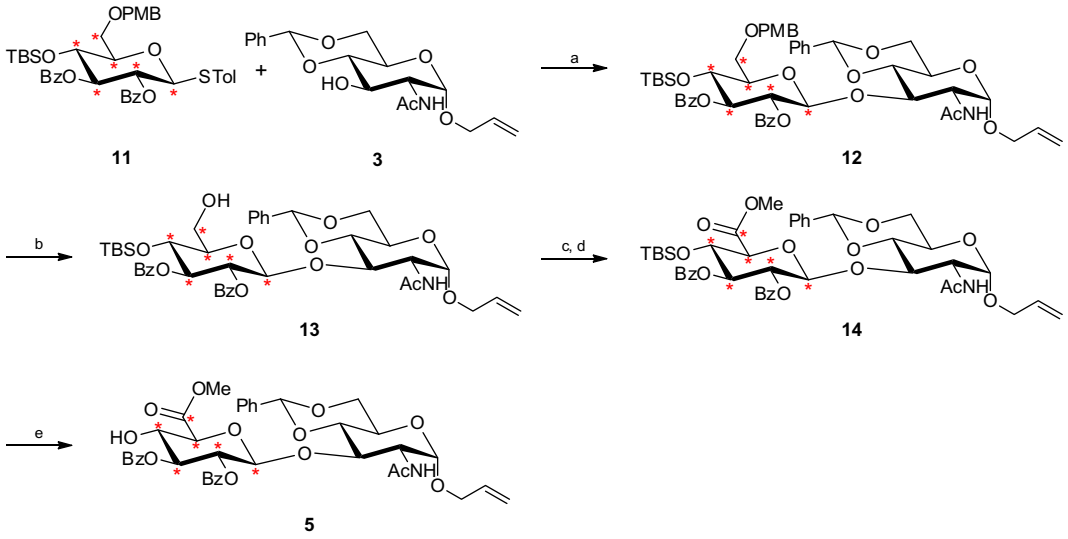
the *N*-Troc group was cleaved under mild reducing conditions (Zn, AcOH)[19] and subsequently the generated amino group was acetylated to obtain tetrasaccharide **8**. The removal of the *O*-TBS as well as 4,6-benzylidene groups was achieved by treatment with Olah's reagent. This reaction was slow and required four days. Last, Zemplén conditions were applied to cleave all benzoates before the ester groups of the glucuronic acid moieties were hydrolyzed by sodium hydroxide to furnish *O*-1-allyl Hyaluronan tetramer **10**. Although, derivative **10** can be obtained in one step by alkaline hydrolysis of **9** we experienced difficulties to separate it from benzoic acid and therefore we used the described two-step procedure.

For future studies we also prepared the glycosyl acceptor **7** by removal of the TBS group at *O*-4‴. Since TBAF was not working for cleavage of this protective group we applied again Olah's reagent adding an additional amount of pyridine to avoid benzylidene group cleavage. Derivative **7** was finally obtained in 78% yield.

Synthesis of the ^{13}C-labeled derivative **5** (label on glucuronic acid moiety) was accomplished analogously with small variations



Scheme 1. Reagents and conditions: Synthesis of free tetrasaccharide **10**. (a) TMSOTf, −20 °C→rt, 90% (for **4**), 62% (for coupling of **1**+**3**); (b) TBAF, THF, 35%; (c) NIS, TfOH, 59%; (d) AcOH, Zn; (e) Ac_2O, pyridine, 43% (over 2 steps); (f) HF-Py, 46%; (g) NaOMe, MeOH, Amberlite 120; (h) NaOH, H_2O, Dowex 50, 63% (over two steps); (i) HF-Py, Py, 78%.



Scheme 2. Reagents and conditions: Synthesis of ^{13}C-labeled disaccharide **5**. (a) NIS, AgOTf, 80%; (b) DDQ, CH_2Cl_2, $NaHCO_{3\ sat}$, 67%; (c) BAIB, TEMPO, CH_2Cl_2, H_2O; (d) MeI, K_2CO_3, 90% (over 2 steps); (e) TBAF, THF, 35%.

(Scheme 2). Here, disaccharide **12** was prepared from the corresponding thioglycoside **11** and monosaccharide **3**. Then, the PMB group was cleaved by treatment with DDQ and subsequently the generated primary alcohol moiety was converted to the carboxylic acid using the TEMPO/BAIB methodology.[20] Next, the carboxy group of the glucuronic acid was protected as methyl ester by treatment MeI and potassium carbonate. Last, TBS deprotection using TBAF furnished disaccharide **5**. This derivative was used for the synthesis of the labeled tetrasaccharide **10** on the basis of the above described methodology (Scheme 1).

3. Conclusion

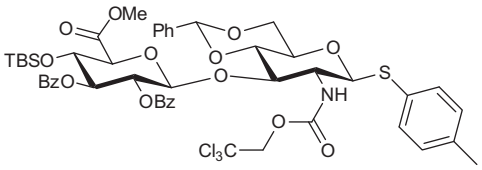
In summary, here we reported the first synthesis of a ^{13}C-labeled tetrasaccharide sequence of Hyaluronan. In addition the corresponding non-labeled derivative **10** was obtained. Whereas the labeled tetrasaccharide can be employed for NMR spectroscopic investigations (interaction with Interleukin-8 or growth factors)[21] the non-labeled derivative can be used for surface modifications.[14] Finally, derivative **7** can be used in the context of other oligosaccharide synthesis. The selective removal of the *N*-Troc group (transformation from **6** to **8**) opens the possibility for the introduction of different groups covalently attached to the amino function of the subterminal D-glucosamine moiety of the HA tetrasaccharide.

4. Experimental section

4.1. General experimental methods

All reactions were run under an atmosphere of argon unless otherwise indicated. Room temperature refers to 22 °C, ambient pressure to 1013 hPa. Reagents and anhydrous solvents were transferred via oven-dried syringe or cannula. Flasks were flame-dried under vacuum and cooled under a constant stream of argon. Tetrahydrofuran (THF) was distilled under argon from potassium, dichloromethane from SICAPENT (phosphorus pentoxide on solid support with indicator). Acetone, acetonitrile and pyridine were purchased from Acros or Aldrich (anhydrous over molecular sieves). All other chemicals were purchased from ABCR, Acros, Aldrich, Alfa Aesar, Fluorochem, TCI Europe and VWR at highest commercially available purity and used as such. Reactions were monitored by thin layer chromatography using Merck silica gel 60 F_{254} TLC aluminium sheets and visualized under an UV lamp and/or with ceric ammonium molybdate, potassium permanganate or vanillin staining solution. Chromatographic purification was performed as flash chromatography on Acros silica gel 35–70, 60 Å, using a forced flow of eluent (method of Still) or as preparative TLC on Merck silica gel 60 F_{254} glass plates with concentration zone. Concentration under reduced pressure was performed by rotary evaporation at 40 °C at the appropriate pressure. NMR spectra were recorded on a Varian Mercury plus 300 (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C acquisitions), a Varian Mercury plus 400 (operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C), and a Bruker Avance-700 (operating at 700 MHz for ^{1}H, 175 MHz for ^{13}C). Chemical shifts δ are reported in ppm with the solvent resonance as internal standard (chloroform-d_1: 7.26 (^{1}H NMR), 77.16 (^{13}C NMR); methanol-d_4: 3.31 (^{1}H NMR), 49.00 (^{13}C NMR). Coupling constants J are given in Hertz (Hz). Multiplicities are classified by the following abbreviations: s = singlet, d = doublet, t = triplet and combinations thereof, or m = multiplet or br = broad signal. High resolution mass spectra were obtained on a Bruker Daltonics ESI-FT-ICR-MS APEX II [7 T]. IR spectra were obtained on an ATI/MATTSON Genesis FT-IR as thin film or KBr disk. Absorbance frequencies are reported in reciprocal centimeters (cm^{-1}). Melting points were measured with a Büchi 'Melting Point B-540' and are uncorrected. Optical rotation data was obtained with a Schmidt + Haensch Polartronic MHZ-8 at the sodium-D line (589 nm) using a 50 mm pathlength cell in the solvent and concentration indicated.

4.1.1. 4-Methylphenyl 4,6-*O*-benzylidene-2-deoxy-3-*O*-{2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl-(dimethyl)silyl]-6-methyl-β-D-glucopyranuronosyl}-1-thio-2-{[(2,2,2-trichloroethoxy)-carbonyl]amino}-β-D-glucopyranoside 4

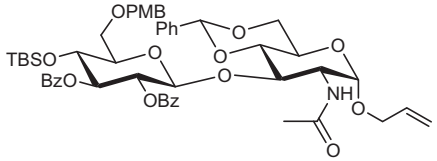


Methyl 2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-1-*O*-(2,2,2-trichloroethanimidoyl)-α-D-glucopyranuronate **1** (3.00 g, 4.44 mmol, 1.1 equiv) and 4-methylphenyl 4,6-*O*-benzylidene-2-deoxy-1-thio-2-{[(2,2,2-trichloroethoxy)carbonyl]amino}-β-D-glucopyranoside **2** (2.22 g, 4.04 mmol, 1.0 equiv) were co-evaporated twice with

toluene (15 ml) and then dried at high vacuum for 16 h. This mixture was dissolved in dichloromethane (60 ml) and transferred to a flask containing activated powdered 4 Å molecular sieves (5.50 g). After stirring at rt for 1 h it was cooled to −20 °C and TMSOTf (0.146 ml, 0.180 g, 0.808 mmol, 0.2 equiv) was added. Next, the reaction was allowed to slowly warm up to rt during 15 h. Then triethylamine (0.150 ml) was added to quench the reaction. After removal of solvent and purification by column chromatography disaccharide **4** (3.86 g, 3.64 mmol, 90%) was obtained as white solid.

Rf: 0.34 (n-hexane/ethyl acetate = 4:1 v/v).
^{1}H NMR: (400 MHz, CDCl3) δ [ppm] −0.23 (s, 3H, Si-CH3), −0.08 (s, 3H, Si-CH3), 0.70 (s, 9H, Si-C(CH3)3), 2.32 (s, 3H, –CH3), 2.91 (dt, J = 9.8, 7.5 Hz, 1H, H-2), 3.51 (td, J = 9.5, 4.7 Hz, 1H, H-5), 3.56–3.61 (m, 1H, H-4), 3.63 (s, 3H, –O-CH3), 3.74 (t, J = 10.2 Hz, 1H, H-6a), 3.86 (d, J = 9.0 Hz, 1H, H-5′), 4.11 (d, J = 12.1 Hz, 1H, –CHaHb–CCl3), 4.26 (t, J = 8.8 Hz, 1H, H-4′), 4.34 (dd, J = 10.4, 4.8 Hz, 1H, H-6b), 4.42 (t, J = 9.2 Hz, 1H, H-3), 4.55 (d, J = 12.1 Hz, 1H, –CHaHb–CCl3), 4.90 (d, J = 7.8 Hz, 1H, H-1′), 5.01 (d, J = 7.2 Hz, 1H, NH), 5.17 (d, J = 10.3 Hz, 1H, H-1), 5.34 (dd, J = 9.4, 8.0 Hz, 1H, H-2′), 5.46 (t, J = 9.1 Hz, 1H, H-3′), 5.51 (s, 1H, Ph-CH), 7.02 (d, J = 7.8 Hz, 2H, Har), 7.20–7.25 (m, 2H, Har), 7.30–7.37 (m, 3H, Har), 7.37–7.44 (m, 4H, Har), 7.44–7.51 (m, 3H, Har), 7.51–7.56 (m, 1H, Har), 7.84–7.88 (m, 2H, Har), 7.92–7.96 (m, 2H, Har).
^{13}C NMR: (100 MHz, CDCl3) δ [ppm] −5.1 (Si-CH3), −4.3 (Si-CH3), 17.8 (Si-C(CH3)3), 21.3 (–CH3), 25.5 (Si-C(CH3)3), 52.5 (–O-CH3), 56.7 (C-2), 68.7 (C-6), 70.5 (C-5), 70.9 (C-4′), 72.5 (C-2′), 74.0 (CH2-CCl3), 75.2 (C-3′), 76.6 (C-5′), 78.7 (C-3), 80.1 (C-4), 84.9 (C-1), 95.5 (CCl3), 101.2 (Ph-CH, C-1′), 126.2 (Car), 127.2 (Car), 128.4 (Car), 128.5 (Car), 128.7 (Car), 129.1 (Car), 129.2 (Car), 129.6 (Car), 129.8 (Car), 129.9 (Car), 130.0 (Car), 133.2 (Car), 133.5 (Car), 133.9 (Car), 137.3 (Car), 138.9 (Car), 153.5 (NH-CO), 165.0 (CO), 165.6 (CO), 168.3 (C-6′).
HR-MS: (ESI positive, MeOH) Calcd for [C50H56Cl3NO14SSi-Na]+: [M+Na]+ 1082.21486. Found: 1082.21395.
IR: (KBr) νmax = 3437 cm−1, 2953, 2928, 2857, 1735, 1272, 1094, 840, 710.
Optical rotation: $[\alpha]_D^{23}$(deg · cm^3 · g^{-1} · dm^{-1}) +38.1 (c 1.0, CHCl3).
Melting point: 146–148 °C.

4.1.2. Prop-2-en-1-yl 2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-3-*O*-{2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-6-*O*-(4-methoxybenzyl)-β-D-glucopyranosyl}-α-D-glucopyranoside and prop-2-en-1-yl 2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-3-*O*-{2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-6-*O*-(4-methoxybenzyl)-β-D-(^{13}C$_6$)glucopyranosyl}-α-D-glucopyranoside 12



4-Methylphenyl 2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-6-*O*-(4-methoxybenzyl)-1-thio-β-D-glucopyranoside **11** (0.729 g, 1.00 mmol, 1.0 equiv) and prop-2-en-1-yl 2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside **2** (0.349 g, 1.00 mmol, 1.0 equiv) were co-evaporated three times with toluene (5 ml) and then dried at high vacuum for 1 h. This mixture was dissolved in dichloromethane (20 ml) and transferred to a flask containing activated powdered 4 Å molecular sieves (150 mg). After cooling the reaction mixture to 0 °C NIS (0.0247 g, 1.10 mmol, 1.1 equiv) and AgOTf (0.0514 g, 0.200 mmol, 0.2 equiv) was added and the reaction was allowed to slowly warm up to rt and stirred for 1 h. Then triethylamine (100 μl) was added to quench the reaction. After removal of solvent and purification by column chromatography disaccharide **12** (0.762 g, 0.799 mmol, 80%) was obtained as white solid.

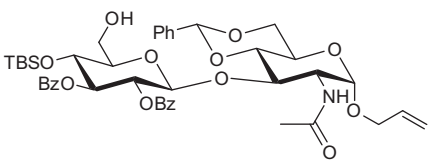
4.1.2.1. Non-labeled.

R_f: 0.25 (*n*-hexane/ethyl acetate = 1:1 v/v).
^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm] −0.27 (s, 3H, Si-CH_3), −0.06 (s, 3H, Si-CH_3), 0.68 (s, 9H, Si-C(CH_3)$_3$), 1.70 (s, 3H, CO-CH_3), 3.23–3.27 (m, 1H), 3.39–3.42 (m, 2H), 3.68–3.73 (m, 2H), 3.95–4.00 (m, 2H), 4.03–4.10 (m, 2H), 4.18–4.26 (m, 2H), 3.81 (s, 3H, O-CH_3), 4.34 (d, J = 12.0 Hz, *p*MP-CH_aH$_b$), 4.53 (d, J = 12.0 Hz, *p*MP-CH$_a$$H_b$), 4.86 (d, J = 4.0 Hz, **H-1**), 4.99 (d, J = 8.0 Hz, **H-1′**), 5.12 (dd, J = 7.1, 7.1 Hz), 5.11–5.23 (m, 2H), 5.40 (s, 1H, Ph-C*H*), 5.41–5.46 (m, 2H), 5.70–5.80 (m, 1H, CH=CH_2), 6.89 (d, J = 8.6 Hz 2H, **H$_{ar}$**), 7.25–7.27 (m, 2H, **H$_{ar}$**), 7.30–7.36 (m, 7H, **H$_{ar}$**), 7.42–7.51(m, 4H, **H$_{ar}$**), 7.85–7.88 (m, 4H, **H$_{ar}$**), 8.77 (br, 1H, N*H*).
^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] −4.7 (Si-*C*H$_3$), −4.0 (Si-*C*H$_3$), 17.9 (Si-*C*(CH$_3$)$_3$), 25.7, 29.7, 52.7 (**C-2**), 55.4 (–O-*C*H$_3$), 62.9, 67.9, 68.7, 69.0, 69.2, 72.9, 74.1, 75.7, 76.3, 76.5, 81.5, 97.1 (**C-1**), 100.1 (**C-1′**), 101.6 (Ph-*C*H), 113.9, 117.8 (CH=*C*H$_2$), 126.3, 128.3, 128.6, 129.2, 129.4, 129.6, 129.82, 129.85, 129.91, 130.4, 133.1, 133.4, 133.5, 137.4, 159.3, 165.3, 166.0, 170.3, 177.6.
HR-MS: (ESI positive, MeOH) Calcd for [C$_{52}$H$_{63}$NO$_{14}$SiNa]$^+$: [M+Na]$^+$ 976.39100. Found: 976.39087.
IR: (KBr) ν_{max} = 3439 cm^{-1}, 2951, 2930, 2858, 1713, 1514, 1275, 1109, 1093, 711.
Optical rotation: $[\alpha]_D^{23}$(deg · cm^3 · g^{-1} · dm^{-1}) + 96.3 (*c* 1.0, CHCl$_3$).
Melting point: 67–70 °C.

4.1.2.2. ^{13}C-labeled.

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm] −0.27 (s, 3H), −0.06 (s, 3H), 0.68 (s, 9H), 1.70 (s, 3H), 3.24 (d, J = 144 Hz, 1H), 3.41 (d, J = 140 Hz, 2H), 3.68–3.73 (m, 2H), 3.81 (s, 3H), 3.99–4.03 (m,1H), 4.05–4.26 (m, 4H), 4.34 (d, J = 2.8,12 Hz, 1H), 4.53 (dd, J = 4.8, 12 Hz, 1H), 4.86 (d, J = 4 Hz, 1H), 4.99 (dd, J = 160 Hz, 1H), 5.11–5.20 (m, 2H), 5.40 (s, 1H), 5.43 (d, J = 149 Hz, 2H), 5.70–5.79 (m, 1H), 6.89 (d, 2H), 7.25 (d, 2H), 7.25–7.27 (m, 2H), 7.30–7.36 (m, 7H), 7.42–7.51(m, 4H), 7.85–7.88 (m, 4H), 8.77 (br, 1H).
^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] −4.8, −4.1, 17.9, 22.9, 25.7, 52.7, 55.4, 62.9, 67.9 (d, J = 45 Hz), 68.7, 69.0, 69.2 (dd, J = 41.4, 41.4 Hz), 73.0, 74.1 (dd, J = 48.5, 39.5 Hz), 76.0–75.8 (m), 81.6, 97.1, 100.2 (d, J = 47 Hz), 101.6, 113.9, 117.8, 126.3, 128.3, 128.6, 129.2, 129.4, 129.6, 129.8, 129.87, 129.92, 130.4, 133.1, 133.4, 137.4, 159.3, 165.4, 166.0, 170.2, 177.4.
HR-MS: (ESI positive, MeOH) Calcd for [C$_{46}$^{13}C$_6$H$_{63}$NO$_{14}$SiNa]$^+$: [M+Na]$^+$ 982.41168. Found: 982.41101

4.1.3. Prop-2-en-1-yl 2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-3-*O*-{2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-β-D-glucopyranosyl}-α-D-glucopyranoside and prop-2-en-1-yl 2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-3-*O*-{2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-β-D-(^{13}C$_6$) glucopyranosyl}-α-D-glucopyranoside 13



To an aluminum foil covered flask containing a solution of disaccharide **12** (0.670 g, 0.702 mmol, 1.0 equiv) in dichloromethane (7.00 ml) was added sat. sodium bicarbonate solution

(0.350 ml), followed by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.207 g, 0.913 mmol, 1.3 equiv). The mixture was stirred at rt for 5 h, then washed with sat. sodium bicarbonate solution. The aqueous phase was extracted with dichloromethane for three times. The combined organic phases were washed with brine and dried over sodium sulfate. After removal of solvent and purification by column chromatography disaccharide **13** (0.392 g, 0.471 mmol, 67%) was obtained as white solid.

4.1.3.1. Non-labeled.

R_f: 0.10 (dichloromethane/ethyl acetate = 9:1 v/v).

^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] −0.23 (s, 3H, Si-CH_3), 0.05 (s, 3H, Si-CH_3), 0.74 (s, 9H, Si-C(CH_3)$_3$), 1.79 (s, 3H, CO-CH_3), 3.03 (br, 1H), 3.36–3.41 (m, 1H), 3.54–3.58 (m, 1H), 3.76–3.97 (m, 6H), 4.04–4.13 (m, 2H), 4.24–4.27 (m, 1H), 4.45–4.51 (m, 1H), 4.79 (d, J = 3.6 Hz, 1H, **H-1**), 5.02 (d, J = 8.0 Hz, 1H), 5.15–5.26 (m, 3H), 5.50–5.55 (t, J = 9.4 Hz, 2H), 5.59 (s, 1H, Ph-C*H*), 5.74–5.83 (m, 1H, CH=CH_2), 7.22–7.26 (m, 2H), 7.29–7.36 (m, 5H), 7.42–7.47(m, 4H), 7.78–7.87 (m, 2H).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] −4.6 (Si-CH_3), −4.1 (Si-**C**H_3), 17.9 (Si-*C*(CH_3)$_3$), 23.3, 25.7, 52.9 (**C-2**), 61.3, 63.4, 68.7, 68.9, 69.0, 75.8, 76.0, 79.3, 97.6, 98.9, 100.0 (**C-1**), 101.6 (Ph-*C*H), 104.9 (**C-1′**), 114.5, 118.0 (CH=*C*H$_2$), 126.1, 128.39, 128.45, 129.3, 129.5, 129.8, 129.9, 132.1, 133.1, 133.2, 133.4, 137.1, 165.6 (*C*O), 166.0 (*C*O), 170.3 (*C*O).

HR-MS: (ESI positive, MeOH) Calcd for $[C_{44}H_{55}NO_{13}SiNa]^+$: $[M+Na]^+$ 856.33369. Found: 856.33313, Calcd for $[C_{44}H_{55}NO_{13}SiK]^+$: $[M+K]^+$ 872.30782. Found: 872.30673.

IR: (KBr) ν_{max} = 3437 cm^{-1}, 2952, 2929, 2858, 1732, 1665, 1275, 1093, 854, 839, 711.

Optical rotation: $[\alpha]_D^{23}$(deg · cm^3 · g^{-1} · dm^{-1}) + 100.1 (*c* 1.0, $CHCl_3$).

Melting point: 209–211 °C.

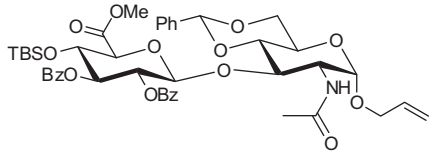
4.1.3.2. ^{13}C-labeled.

^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] −0.23 (s, 3H), 0.05 (s, 3H), 0.74 (s, 9H), 1.79 (s, 3H), 3.14 (br, 1H), 3.34 (m, 1H), 3.54 (m, 1H), 3.76–4.22 (m, 8H), 4.26 (m, 1H), 4.48 (m, 1H), 4.79 (d, J = 3.6 Hz, 1H), 5.02 (d, J = 127 Hz, 1H), 5.15–5.26 (m, 3H), 5.52 (t, J = 9.4 Hz, 2H), 5.59 (s, 1H), 5.78 (m, 1H), 7.24 (m, 2H), 7.31 (m, 5H), 7.45 (m, 4H), 7.83 (m, 2H),

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] −4.6, −4.1, 17.9, 23.3, 25.7, 51.8, 52.9, 61.6 (dd, J = 48, 48 Hz), 62.8, 63.4, 68.4–69.8 (m), 72.0–73.8 (m), 75.2–78.2 (m), 79.3, 97.5, 100.0 (dd, J = 64, 151 Hz), 101.6, 104.9, 114.5, 118.0, 118.3, 126.1, 128.4, 128.4, 129.3, 129.5, 129.6, 129.8, 129.9, 130.0, 133.1, 133.2, 133.3, 133.5, 133.4, 137.1, 165.6, 166.0, 169.9, 170.3.

HR-MS: (ESI positive, MeOH) Calcd for $[C_{38}{}^{13}C_6H_{55}NO_{13}SiNa]^+$: $[M+Na]^+$ 862.35381. Found: 862.35324, Calcd for $[C_{38}{}^{13}C_6H_{55}NO_{13}SiK]^+$: $[M+K]^+$ 878.31810. Found: 878.32545

4.1.4. Prop-2-en-1-yl 2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-3-*O*-{2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-6-methyl-β-D-glucopyranosyl}-α-D-glucopyranoside and prop-2-en-1-yl 2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-3-*O*-{2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-6-methyl-β-D-($^{13}C_6$) glucopyranosyl}-α-D-glucopyranoside 14



Method A: To a solution of disaccharide **13** (0.388 g, 0.464 mmol, 1.0 equiv) in dichloromethane (17.0 ml) water (8.50 ml) was added, followed by bis(acetoxy)iodobenzene (0.374 g, 1.16 mmol, 2.5 equiv) and (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (0.0145 g, 0.0928 mmol, 0.2 equiv). The mixture was stirred at rt for 7 h and quenched by addition of sat. sodium thiosulfate solution. The aqueous phase was extracted with dichloromethane for three times. The combined organic phases were washed with brine and dried over sodium sulfate. After removal of solvent, the residue was dissolved in DMF (10.0 ml) followed by the addition of methyl iodide (0.260 ml, 0.593 g, 4.176 mmol, 9.0 equiv) and potassium carbonate (0.641 g, 4.64 mmol, 10.0 equiv). The mixture was stirred at rt for 2 h. Excess potassium carbonate was removed by filtration. After solvent removal under reduced pressure and purification by column chromatography disaccharide **14** (0.361 g, 0.419 mmol, 90%) was obtained as white solid.

Method B: Methyl 2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)-silyl]-1-*O*-(2,2,2-trichloroethanimidoyl)-α-D-glucopyranuronate **1** (2.42 g, 3.58 mmol, 1.0 equiv) and prop-2-en-1-yl 2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside **3** (1.50 g, 4.29 mmol, 1.2 equiv) were co-evaporated twice with toluene (15 ml) and then dried at high vacuum for 16 h. This mixture was dissolved in dichloromethane (50 ml) and transferred to a flask containing activated powdered 4 Å molecular sieves (4.00 g). After stirring at rt for 1 h it was cooled to −20 °C and TMSOTf (0.130 ml, 0.159 g, 0.716 mmol, 0.2 equiv) was added. Next, the reaction was allowed to slowly warm up to rt during 15 h. Then triethylamine (0.140 ml) was added to quench the reaction. After removal of solvent and purification by column chromatography disaccharide **14** (1.91 g, 2.22 mmol, 62%) was obtained as white solid.

4.1.4.1. Non-labeled.

R_f: 0.30 (*n*-hexane/ethyl acetate = 1:1 v/v).

^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] −0.23 (s, 3H, Si-CH_3), −0.07 (s, 3H, Si-CH_3), 0.70 (s, 9H, Si-C(CH_3)$_3$), 1.68 (s, 3H, CO-CH_3), 3.62–3.65 (m, 1H), 3.70 (s, 3H, CO-O-CH_3), 3.74–4.30 (m, 8H), 4.81 (d, J = 3.6 Hz, 1H), 5.09–5.14 (m, 2H), 5.23–5.31 (m, 2H), 5.47 (t, J = 9.1 Hz, 1H), 5.58 (s, 1H), 5.63–5.78 (m, 1H, CH=CH_2), 7.32–7.53 (m, 11H), 7.87–7.90 (m, 4H).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] −5.1 (Si-CH_3), −4.3 (Si-CH_3), 17.9 (Si-C(CH_3)$_3$), 22.9, 25.5, 52.4, 52.6, 63.0, 68.7, 69.0, 70.7, 73.9, 75.3, 76.7, 76.9, 81.7, 97.2 (**C-1**), 100.9, 101.4, 117.8, 126.1, 126.2, 128.4, 128.5, 128.7, 129.0, 129.19, 129.22, 129.6, 129.8, 129.9, 133.3, 133.4, 133.6, 137.3, 165.3, 165.8, 168.2, 170.0.

HR-MS: (ESI positive, MeOH) Calcd for $[C_{45}H_{55}NO_{14}SiNa]^+$: $[M+Na]^+$ 884.32895. Found: 884.32832.

IR: (KBr) ν_{max} = 3438 cm^{-1}, 2953, 2929, 2858, 1734, 1271, 1112, 1092, 1029, 841, 711.

Optical rotation: $[\alpha]_D^{23}$(deg · cm^3 · g^{-1} · dm^{-1}) + 90.4 (*c* 1.0, $CHCl_3$).

Melting point: 193–194 °C.

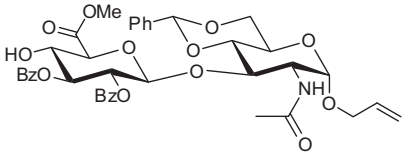
4.1.4.2. ^{13}C-labeled.

^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] −0.23 (s, 3H), −0.07 (s, 3H), 0.70 (s, 9H), 1.68 (s, 3H), 3.60–3.67 (m, 1H), 3.70 (d, J = 3.6 Hz, 3H), 3.74–4.30 (m, 8H), 4.81 (d, J = 3.6 Hz, 1H), 4.84–5.38 (m, 6H), 5.37 (d, J = 7.5 Hz, 1H), 5.58 (s, 1H), 5.65–5.78 (m, 1H), 7.32–7.53 (m, 11H), 7.87–7.89 (m, 4H).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] −5.1, −4.3, 17.9, 22.9, 25.5, 52.4, 52.6, 63.0, 68.7, 68.9, 70.7 (m), 73.9 (m), 75.3 (m), 76.9 (m), 81.7, 97.2, 100.9 (dt, J = 46.8, 6.0 Hz), 101.4, 117.8, 126.1,

128.4, 128.5, 128.7, 129.0, 129.18, 129.22, 129.6, 129.8, 129.9, 133.3, 133.4, 133.6, 137.3, 168.2 (m), 170.0.
HR-MS: (ESI positive, MeOH) Calcd for $[C_{39}{}^{13}C_6H_{55}NO_{14}SiNa]^+$: $[M+Na]^+$ 890.34873. Found: 890.34822.

4.1.5. Prop-2-en-1-yl 2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3-di-*O*-benzoyl-6-methyl-β-D-glucopyranosyl}-α-D-glucopyranoside and prop-2-en-1-yl 2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-3-*O*-(2,3-di-*O*-benzoyl-6-methyl-β-D-($^{13}C_6$)glucopyranosyl}-α-D-glucopyranoside 5



To a solution of disaccharide **14** (2.16 g, 2.51 mmol, 1 equiv) in THF (100 ml) was added a 1 m solution of TBAF in THF (2.51 ml, 0.656 g, 2.51 mmol, 1 equiv) at rt. Then the reaction was stirred at rt for 2 h. Removal of solvent and purification by column chromatography furnished disaccharide **5** (0.656 g, 0.878 mmol, 35%) as white solid.

4.1.5.1. Non-labeled.

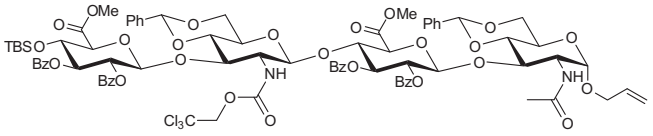
R_f: 0.30 (*n*-hexane/ethyl acetate = 2:3 v/v).
^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm] 1.66 (s, 3H, CO-CH_3), 3.41 (br, 1H, -O***H***), 3.76 (s, 3H, CO-OCH_3), 3.78–3.83 (m, 1H, H-5), 3.83–3.91 (m, 3H, -O-CH_aH$_b$-, **H-4**, **H-6a**), 4.02 (dd, *J* = 10.1, 8.6 Hz, 1H, **H-3**), 4.05–4.15 (m, 2H, -O-CH$_a$$H_b$-, **H-4′**), 4.21–4.30 (m, 2H, **H-2**, **H-6b**), 4.82 (d, *J* = 3.6 Hz, 1H, **H-1**), 5.04 (d, *J* = 7.6 Hz, 1H, **H-1′**),5.11–5.14 (m, 1H, CH=CH_aH$_b$), 5.17 (ddd, *J* = 7.9, 1.5, 1.4 Hz, 1H, CH=CH$_a$$H_b$), 5.23 (d, *J* = 8.6 Hz, 1H, N*H*), 5.32 (dd, *J* = 9.6, 7.7 Hz, 1H, **H-2′**), 5.41 (dd, *J* = 9.4, 9.4 Hz, 1H, **H-3′**), 5.60 (s, 1H, Ph-C*H*), 5.75 (dddd, *J* = 16.7, 10.5, 6.0, 5.5 Hz, 1H, CH_2-CH=CH_2), 7.31–7.45 (m, 7H, $\mathbf{H_{ar}}$), 7.45–7.51 (m, 3H, $\mathbf{H_{ar}}$), 7.51–7.59 (m, 1H, $\mathbf{H_{ar}}$), 7.90–8.00 (m, 4H, $\mathbf{H_{ar}}$).
^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] 22.9 (CO-CH_3), 52.3 (**C-2**), 52.9 (CO-OCH_3), 62.9 (**C-5**), 68.7 (-O-CH_2-), 69.1 (**C-6**), 70.4 (**C-4′**), 72.9 (**C-2′**), 74.6 (**C-5′**), 75.1 (**C-3′**), 76.9 (**C-3**), 81.8 (**C-4**), 97.2 (**C-1**), 100.9 (**C-1′**), 101.8 (Ph-**C**H), 117.9 (CH=CH_2), 126.0 (2 × $\mathbf{C_{ar}}$), 128.48 (2 × $\mathbf{C_{ar}}$), 128.51 (2 × $\mathbf{C_{ar}}$), 128.8 (2 × $\mathbf{C_{ar}}$), 129.17 ($\mathbf{C_q}$), 129.18 ($\mathbf{C_q}$), 129.4 ($\mathbf{C_{ar}}$), 129.9 (2 × $\mathbf{C_{ar}}$), 130.0 (2 × $\mathbf{C_{ar}}$), 133.4 (CH=CH_2), 133.5 ($\mathbf{C_{ar}}$), 133.7 ($\mathbf{C_{ar}}$), 137.3 ($\mathbf{C_q}$), 165.1 (*c* O), 166.5 (*c* O), 169.1 (**C-6′**), 170.0 (-NH-**C**O-).
HR-MS: (ESI positive, MeOH) Calcd for $[C_{39}H_{41}NO_{14}Na]^+$: $[M+Na]^+$ 770.24193. Found: 770.24193, Calcd for $[C_{78}H_{82}N_2O_{28}Na]^+$: $[2\,M+Na]^+$ 1517.49518. Found: 1517.49246.
IR: (KBr) ν_{max} = 3434 cm^{-1}, 2917, 2868, 1731, 1383, 1279, 1122, 1092, 1029, 712.
Optical rotation: $[\alpha]_D^{22}$ ($deg \cdot cm^3 \cdot g^{-1} \cdot dm^{-1}$) + 109.5 (*c* 1.0, $CHCl_3$).
Melting point: 137–139 °C.

4.1.5.2. ^{13}C-labeled.

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm] 1.66 (s, 3H, CO-CH_3), 3.29 (br, 1H, –O*H*), 3.56 (br, 0, 5H, ^{13}C*H*) * 3.76 (d, *J* = 3.8 Hz, 3H, CO-OCH_3), 3.79–4.11 (m, 6.5H) *, 4.23–4.29 (m, 2H, **H-2**, **H-6b**), 4.82 (d, *J* = 3.6 Hz, 1H, **H-1**), 5.13–5.25 (m 4H), 5.60 (s, 1H, Ph-C*H*), 5.75 (dddd, *J* = 16.7, 10.5, 6.0, 5.5 Hz, 1H, CH_2–CH=CH_2), 7.31–7.45 (m, 7H, $\mathbf{H_{ar}}$), 7.45–7.51 (m, 3H, $\mathbf{H_{ar}}$), 7.51–7.59 (m, 1H, $\mathbf{H_{ar}}$), 7.90–8.00 (m, 4H, $\mathbf{H_{ar}}$). *both multiplets contain one half of a doublet representing one proton.

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] 22.9 (CO-CH_3), 52.2 (**C-2**), 52.9 (CO-OCH_3), 62.9 (**C-5**), 68.7 (-O-CH_2-), 69.1 (**C-6**), 70.0–70.8 (m, **C-4′**), 72.4–73.3 (m, **C-2′**), 74.0–74.7 (m, **C-5′**), 74.7–75.5 (m, **C-3′**), 76.9 (**C-3**), 81.8 (**C-4**), 97.2 (**C-1**), 100.6–101.8 (m, **C-1′**), 101.8 (Ph-CH), 117.9 (CH=CH_2), 126.0 (2 × $\mathbf{C_{ar}}$), 128.48 (2 × $\mathbf{C_{ar}}$), 128.51 (2 × $\mathbf{C_{ar}}$), 128.8 (2 × $\mathbf{C_{ar}}$), 129.17 ($\mathbf{C_q}$), 129.18 ($\mathbf{C_q}$), 129.4 ($\mathbf{C_{ar}}$), 129.9 (2 × $\mathbf{C_{ar}}$), 130.0 (2 × $\mathbf{C_{ar}}$), 133.4 (CH=CH_2), 133.5 ($\mathbf{C_{ar}}$), 133.7 ($\mathbf{C_{ar}}$), 137.3 ($\mathbf{C_q}$), 165.1 (*C* = O), 166.5 (*C* = O), 168.8–170.0 (m, **C-6′**), 170.0 (-NH-*C*O-).
HR-MS: (ESI positive, MeOH) Calcd for $[C_{33}{}^{13}C_6H_{41}NO_{14}Na]^+$: $[M+Na]^+$ 776.26211. Found: 776.26107.

4.1.6. Prop-2-en-1-yl 2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-6-methyl-β-D-glucopyranuronosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-{[(2,2,2-trichloroethoxy) carbonyl]-amino}-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-methyl-β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside and prop-2-en-1-yl 2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-6-methyl-β-D-glucopyranuronosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-{[(2,2,2-trichloroethoxy)carbonyl]-amino}-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-methyl-β-D-($^{13}C_6$) glucopyranuronosyl-(1→3)-2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside 6



Thioglycoside **4** (0.515 g, 0.485 mmol, 1.5 equiv) and alcohol **5** (0.242 g, 0.323 mmol, 1.0 equiv) were co-evaporated twice with toluene (5 ml) and then dried at high vacuum for 16 h. This mixture was dissolved in dichloromethane (10 ml) and transferred to a flask containing activated powdered 4 Å molecular sieves (0.900 g). After stirring at rt for 1 h NIS (0.116 g, 0.517 mmol, 1.6 equiv) and TfOH (0.0114 ml, 0.0194 g, 0.129 mmol, 0.4 equiv) were added. Then it was stirred for 16 h at rt before the reaction was quenched with triethylamine (0.050 ml). After removal of solvent and purification by column chromatography tetrasaccharide **6** (0.321 g, 0.191 mmol, 59%) was obtained as white solid.

4.1.6.1. Non-labeled.

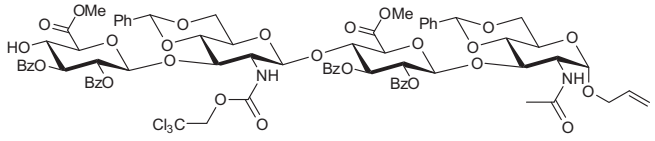
R_f: 0.35 (*n*-hexane/ethyl acetate = 1:1 v/v).
^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm] −0.25 (s, 3H, Si-CH_3), −0.09 (s, 3H, Si-CH_3), 0.69 (s, 9H, Si-C(CH_3)$_3$), 1.71 (s, 3H, CO-CH_3), 2.25–2.38 (m, 1H), 2.48–2.60 (m, 1H), 3.10–3.19 (m, 1H), 3.24–3.34 (m, 2H), 3.54 (br, 1H, N*H*), 3.62 (s, 3H, –O-CH_3), 3.64 (s, 3H, –O-CH_3), 3.69–3.93 (m, 8H), 3.93–4.09 (m, 2H), 4.16–4.30 (m, 5H), 4.45 (d, *J* = 11.7 Hz, 1H), 4.61 (br, 1H, N*H*), 4.67–4.91 (m, 3H, **H-1**), 5.02 (d, *J* = 7.2 Hz, 1H), 5.13 (d, *J* = 10.3 Hz, 1H), 5.16 (s, 1H, Ph-C*H*), 5.22–5.47 (m, 4H), 5.56 (s, 1H, Ph-C*H*), 5.67–5.80 (m, 1H, CH=CH_2), 7.28–7.43 (m, 15H, $\mathbf{H_{ar}}$), 7.43–7.55 (m, 7H, $\mathbf{H_{ar}}$), 7.81–7.99 (m, 8H, $\mathbf{H_{ar}}$).
^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] −5.1 (Si-CH_3), −4.3 (Si-CH_3), 17.8 (Si-C(CH$_3$)$_3$), 23.0 (CO-CH_3), 25.5 (Si-C(CH_3)$_3$), 52.3, 52.5, 53.1, 58.3, 62.9, 66.0, 67.7, 68.7, 68.9, 70.8, 72.4, 72.5, 73.2, 74.57, 74.63, 75.3, 76.60, 76.64, 77.4, 79.4, 81.4, 95.6 (*C*Cl_3), 97.3 (**C-1**), 100.0, 100.8, 100.9, 101.1, 101.3, 118.0 (CH=CH_2), 126.0, 126.2, 128.3, 128.4, 128.47, 128.52, 128.6, 128.7, 128.9, 129.1, 129.2, 129.3, 129.7, 129.82, 129.89, 129.92, 130.0, 133.2, 133.33, 133.37, 133.41, 133.7, 137.2,

137.3, 153.9 (CO_{Troc}), 165.1 (*C*O), 165.2 (*C*O), 165.6 (*C*O), 167.9 (*C*O), 168.3 (*C*O), 170.0 (*C*O), 177.4 (*C*O).
HR-MS: (ESI positive, $CHCl_3$/MeOH) Calcd for $[C_{82}H_{89}Cl_3N_2O_{28}SiNa]^+$: $[M+Na]^+$ 1705.43289 found 1705.43255.
IR: (KBr) ν_{max} = 3433 cm^{-1}, 2957, 2927, 1732, 1384, 1279, 1178, 1094, 1029, 713.
Optical rotation: $[\alpha]_D^{23}(deg \cdot cm^3 \cdot g^{-1} \cdot dm^{-1})$ + 50.8 (*c* 1.0, $CHCl_3$).
Melting point: 144–145 °C.

4.1.6.2. ^{13}C-labeled.

^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm] −0.26 (s, 3H, Si-CH_3), −0.10 (s, 3H, Si-CH_3), 0.69 (s, 9H, Si-C(CH_3)$_3$), 1.72 (s, 3H, CO-CH_3), 2.25–2.38 (m, 0.5H) *, 2.48–2.57 (m, 1H), 3.10–3.19 (m, 1.5H) *, 3.24–3.34 (m, 2H), 3.54 (br, 1H, N*H*), 3.62 (s, 3H, –O-CH_3), 3.64 (d, *J* = 3.8 Hz, 3H, –O-CH_3), 3.69–4.30 (m, 13H), 4.45 (d, *J* = 11.7 Hz, 1H), 4.67–4.91 (m, 3H), 5.13 (m, 1H), 5.16 (s, 1H, Ph-C*H*), 5.22–5.47 (m, 4H), 5.56 (s, 1H, Ph-C*H*), 5.67–5.80 (m, 1H, CH=CH_2), 7.28–7.43 (m, 15H, **H_{ar}**), 7.43–7.55 (m, 7H, **H_{ar}**), 7.81–7.99 (m, 8H, **H_{ar}**). *both multiplets contain one half of a doublet representing one proton.
^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] −5.1 (Si-CH_3), −4.3 (Si-CH_3), 17.8 (Si-*C*(CH_3)$_3$), 23.0 (CO-CH_3), 25.5 (Si-C(CH_3)$_3$), 52.3, 52.5, 53.1, 58.3, 62.9, 66.0, 67.7, 68.7, 68.9, 70.8, 72.1–73.7 (m, ^{13}C), 74.0–75.1 (m, ^{13}C), 76.5–77.4 (m, ^{13}C), 81.8, 96.0 (CCl_3), 97.3 (**C-1**), 100.3–101.7 (m, ^{13}C), 118.0 (CH=CH_2), 126.0, 126.1, 128.36, 128.48, 128.52, 128.6, 128.7, 128.9, 129.1, 129.2, 129.3, 129.7, 129.82, 129.89, 129.92, 130.0, 133.2, 133.37, 133.43, 133.47, 133.7, 137.2, 137.3, 153.8 (CO_{Troc}), 165.0 (*C*O), 165.2 (*C*O), 165.3 (***C***O), 166.6, 167.3–168.8 (m, ^{13}C), 169.0, 170.0.
HR-MS: (ESI positive, MeOH) Calcd for $[C_{76}{}^{13}C_6H_{89}Cl_3N_2O_{28}SiNa_2]^{2+}$: $[M+2Na]^{2+}$ 867.22167. Found: 867.22180.

4.1.7. Prop-2-en-1-yl 2,3-di-*O*-benzoyl-6-methyl-β-D-glucopyranuronosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-{[(2,2,2-trichloroethoxy)carbonyl]amino}-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-methyl-β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside 7

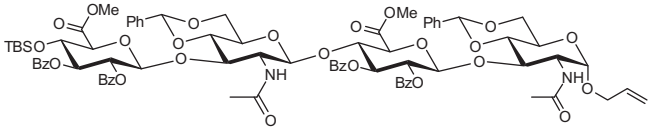


To a solution of fully protected tetrasaccharide **6** (101 mg, 0.0600 mmol, 1 equiv) in THF (404 μl) was subsequently added Olah's reagent (202 μl, 184 mg) and pyridine (404 μl) before the reaction was stirred for 12 h at rt. Then it was diluted with dichloromethane quenched by carful addition of sat. $NaHCO_3$ solution, washed twice with sat. cupric sulfate solution and brine. The organic layer was dried over $MgSO_4$, concentrated and the crude product was subjected to column chromatography for purification to obtain tetrasaccharide **7** (73.5 mg, 0.0468 mmol, 78%) as white solid.

4.1.7.1. Non-labeled.

R_f: 0.25 (*n*-hexane/ethyl acetate = 1:1 v/v).
^{1}H NMR: (300 MHz, CD_3OD) δ [ppm] 1.65 (s, 3H, CO-CH_3), 2.15–2.23 (m, 1H), 2.64 (t, *J* = 10.3 Hz, 1H), 3.08–3.19 (m, 1H), 3.40–3.50 (m, 2H), 3.65 (s, 3H, CO-O-CH_3), 3.67 (s, 3H, CO-O-CH_3), 3.76–3.82 (m, 2H), 3.87–4.11(m, 9H), 4.11–4.20 (m, 2H), 4.26 (t, *J* = 9.0 Hz, 1H), 4.42 (d, *J* = 10.8 Hz, 2H), 4.46–4.55 (m, 2H), 4.74 (d, *J* = 2.3 Hz, 1H, **H-1**), 5.03 (d, *J* = 7.9 Hz, 1H), 5.09–5.35 (m, 7H, Ph-C*H*), 5.35–5.43 (m, 1H), 5.55–5.64 (m, 2H, Ph-C*H*), 5.81–5.97 (m, 1H, CH=CH_2), 7.27–7.41 (m, 14H), 7.41–7.61 (m, 8H), 7.78–7.94 (m, 8H).
^{13}C NMR: (75 MHz, CD_3OD) δ [ppm] 22.5 (CO-CH_3), 52.9, 53.7, 54.5, 58.7, 64.4, 67.0, 68.9, 69.65, 69.73, 71.1, 73.58, 73.61, 73.64, 74.5, 75.3, 75.6, 76.5, 76.7, 77.1, 78.48, 78.50, 78.52, 78.6, 80.1, 81.2, 96.8 (CCl_3), 98.2 (**C-1**), 101.5, 101.6, 102.0, 102.2, 103.5, 118.2 (CH=CH_2), 127.3, 129.06, 129.14, 129.40, 129.42, 129.49, 129.6, 129.7, 130.61, 130.69, 130.78, 130.80, 130.9, 131.0, 131.1, 134.35, 134.37, 134.47, 134.54, 135.1, 138.8, 139.2, 156.1 (NH-CO-O–), 166.70 (*C*O), 166.73 (*C*O), 167.0 (*C*O), 167.3 (*C*O), 169.5 (*C*O), 170.1 (*C*O), 173.0 (*C*O).
HR-MS: (ESI positive, EtOAc/MeOH) Calcd for $[C_{76}H_{75}Cl_3N_2O_{28}Na]^+$: $[M+Na]^+$ 1591.34641. Found: 1591.34759.
IR: (KBr) ν_{max} = 3434 cm^{-1}, 2954, 2926, 1735, 1643, 1384, 1278, 1093, 712.
Optical rotation: $[\alpha]_D^{23}(deg \cdot cm^3 \cdot g^{-1} \cdot dm^{-1})$ + 6.9 (*c* 1.0, CH_3OH).
Melting point: 182–184 °C.

4.1.8. Prop-2-en-1-yl 2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-6-methyl-β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-methyl-β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside and prop-2-en-1-yl 2,3-di-*O*-benzoyl-4-*O*-[*tert*-butyl(dimethyl)silyl]-6-methyl-β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-methyl-β-D-(**$^{13}C_6$**)glucopyranuronosyl-(1→3)-2-(acetylamino)-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside 8



To a solution of Troc-containing tetrasaccharide **6** (195 mg, 0.116 mmol, 1 equiv) in acetic acid (3.00 ml) was added zinc powder (300 mg) and the mixture was allowed to stir at rt for 3 h. After excess zinc was filtered off and solvent removal under diminished pressure the residue was dried at high vacuum for 1 h. The crude product was dissolved in pyridine (6.00 ml) and acetic anhydride (3.00 ml) was added dropwise to the reaction before it was allowed to stir for 16 h at rt. Then it was quenched with sat. $NaHCO_3$ solution, washed twice with sat. $CuSO_4$ solution and finally with brine. The organic phase was dried over $MgSO_4$, all volatiles were removed under reduced pressure and the crude product was purified by column chromatography to yield derivative **8** (77.3 mg, 0.0498 mmol, 43% over two steps) as white solid.

4.1.8.1. Non-labeled.

R_f: 0.36 (*n*-hexane/ethyl acetate = 3:7 v/v).
^{1}H NMR: (400 MHz, $CDCl_3$) δ [ppm] −0.25 (s, 3H, Si-CH_3), −0.11 (d, *J* = 6.6 Hz, 3H, Si-CH_3), 0.68 (s, 9H,, Si-C(CH_3)$_3$), 1.68 (s, 3H, CO-CH_3), 2.35 (s, 3H, CO-CH_3), 2.49 (t, *J* = 10.2 Hz, 1H), 3.14–3.27 (m, 2H), 3.54 (s, 3H, CO-O-CH_3), 3.56–3.63 (m, 2H), 3.65 (s, 3H, CO-O-CH_3), 3.75–3.89 (m, 3H), 3.93–4.10 (m, 3H), 4.14–4.29 (m, 5H), 4.47–4.56 (m, 1H), 4.76–4.83 (m, 3H), 4.83–4.91 (m, 2H), 4.98–5.20 (m, 4H), 5.20–5.49 (m, 5H),

5.51–5.61 (m, 2H), 5.66–5.90 (m, 2H, CH=CH_2), 7.11–7.58 (m, 22H), 7.80–7.99 (m, 8H).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] −5.1 (Si-CH_3), −4.4 (Si-CH_3), 17.8 (Si-$C(CH_3)_3$), 22.9 (CO-CH_3), 23.0 (CO-CH_3), 25.5 (Si-$C(CH_3)_3$), 52.2, 52.3, 53.0, 62.9, 65.6, 68.6, 70.8, 72.7, 72.8, 72.9, 74.9, 75.3, 76.35, 76.38, 76.7, 77.0, 77.4, 80.2, 81.6, 97.2, 98.8, 100.76, 100.80, 101.0, 101.4, 117.8 (CH=CH_2), 125.4, 126.0, 126.12, 126.17, 128.3, 128.38, 128.49, 128.53, 128.6, 128.8, 129.0, 129.09, 129.12, 129.2, 129.3, 129.6, 129.8, 129.9, 133.2, 133.4, 133.46, 133.54, 133.7, 137.3, 165.06 (*C*O), 165.09 (*C*O), 165.3 (*C*O), 165.7 (*C*O), 167.3 (*C*O), 168.4 (*C*O), 170.0 (*C*O), 170.9 (*C*O).

HR-MS: (ESI positive, $CHCl_3$/MeOH) Calcd for $[C_{14}H_{18}O_6Na]^+$: $[M+Na]^+$ 1573.53924. Found: 1573.53976.

IR: (KBr) ν_{max} = 3434 cm^{-1}, 2953, 2929, 2859, 1734, 1697, 1384, 1273, 1093, 1029, 712.

Optical rotation: $[\alpha]_D^{23}$(deg · cm^3 · g^{-1} · dm^{-1}) + 67.3 (*c* 1.0, $CHCl_3$).

Melting point: 172–174 °C.

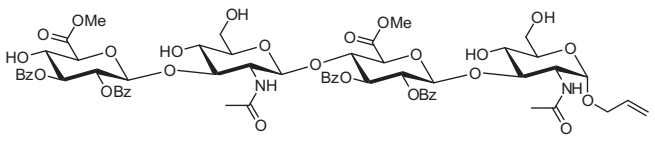
4.1.8.2. ^{13}C-labeled.

^{1}H NMR: (300 MHz, $CDCl_3$) δ [ppm] −0.25 (s, 3H, Si-CH_3), −0.12 (s, 3H, Si-CH_3), 0.68 (s, 9H,, Si-$C(CH_3)_3$), 1.40 (s, 3H, CO-CH_3), 1.68 (s, 3H, CO-CH_3), 2.49 (t, *J* = 10.2 Hz, 1H),2.69–2.80 (m, 1H) 3.14–3.31 (m, 2H), 3.54 (s, 3H, CO-O-CH_3), 3.56–3.63 (m, 2H), 3.65 (d, *J* = 3.8 Hz, 3H, CO-O-CH_3), 3.75–4.29 (m, 11H), 4.49–4.56 (m, 1H), 4.76–4.83 (m, 3H), 4.83–4.91 (m, 2H), 4.98–5.53 (m, 9H), 5.54 (s, 1H), 5.65–5.78 (dddd, 1H, CH=CH_2), 7.11–7.58 (m, 22H), 7.80–7.99 (m, 8H).

^{13}C NMR: (100 MHz, $CDCl_3$) δ [ppm] −5.1 (Si-CH_3), −4.4 (Si-CH_3), 17.8 (Si-$C(CH_3)_3$), 22.9 (CO-CH_3), 23.0 (CO-CH_3), 25.5 (Si-$C(CH_3)_3$), 52.2, 52.3, 53.0, 62.9, 65.6, 68.6, 70.8, 72.5–77.6 (m, ^{13}C), 80.2, 81.6, 97.2, 98.8, 100.5–101.2 (m, ^{13}C), 101.4, 117.8 (CH=CH_2), 125.4, 126.0, 126.12, 126.17, 128.3, 128.38, 128.49, 128.53, 128.6, 128.8, 129.0, 129.09, 129.12, 129.2, 129.3, 129.6, 129.8, 129.9, 133.2, 133.4, 133.46, 133.54, 133.7, 137.3, 165.06 (*C*O), 165.09 (***C***O), 165.3 (*C*O), 165.7 (*C*O), 166.8–167.7 (m, ^{13}CO), 168.4 (*C*O), 170.0 (*C*O), 170.9 (*C*O).

HR-MS: (ESI positive, $CDCl_3$/MeOH) Calcd for $[C_{75}{}^{13}C_6H_{90}N_2O_{27}SiNa]^+$: $[M+Na]^+$ 1579.55937. Found: 1579.65094

4.1.9. Prop-2-en-1-yl 2,3-di-*O*-benzoyl-6-methyl-β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-methyl-β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-2-deoxy-α-D-glucopyranoside and prop-2-en-1-yl 2,3-di-*O*-benzoyl-6-methyl-β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-methyl-β-D-(^{13}C$_6$) glucopyranuronosyl-(1→3)-2-(acetylamino)-2-deoxy-α-D-glucopyranoside 9



To a solution of tetrasaccharide **8** (69.9 mg, 0.0451 mmol, 1 equiv) in THF (2.10 ml) was added Olah's reagent (1.05 ml, 953 mg) before the reaction was stirred for 4 d at rt. Then it was diluted with dichloromethane quenched by carful addition of sat. $NaHCO_3$ solution, washed twice with sat. cupric sulfate solution and brine. The organic layer was dried over $MgSO_4$, concentrated and the crude product was subjected to column chromatography for purification to obtain tetrasaccharide **9** (26.1 mg, 0.0207 mmol, 46%) as white solid.

4.1.9.1. Non-labeled.

R_f: 0.10 (ethyl acetate).

^{1}H NMR: (400 MHz, CD_3OD) δ [ppm] 1.38 (s, 3H, CO-CH_3), 1.48 (s, 3H, CO-CH_3), 3.05–3.12 (m, 2H), 3.41–3.50 (m, 2H), 3.50–3.62 (m, 3H), 3.64–3.72 (m, 2H), 3.85–3.72 (m, 8H, 2 × O-CH_3), 3.87–3.98 (m, 2H), 4.03 (t, *J* = 9.5 Hz, 1H), 4.08–4.23 (m, 2H), 4.29 (d, *J* = 7.8 Hz, 1H), 4.46 (t, *J* = 7.8 Hz, 1H), 4.50 (d, *J* = 8.2 Hz, 1H), 4.71 (d, *J* = 3.5 Hz, 1H, **H-1**), 5.00 (d, *J* = 7.8 Hz, 1H), 5.07 (d, *J* = 7.5 Hz, 1H), 5.15 (ddd, *J* = 10.4, 2.8, 1.2 Hz, 1H, CH=CH_aH$_b$), 5.22–5.31 (m, 3H, CH=CH$_a$$H_b$), 5.55 (t, *J* = 9.4 Hz, 1H), 5.60 (t, *J* = 7.7 Hz, 1H), 5.88 (dddd, *J* = 15.6, 10.4, 6.2, 5.2 Hz, 1H, CH=CH_2), 7.31–7.46 (m, 8H, **H$_{ar}$**), 7.48–7.62 (m, 4H, **H$_{ar}$**), 7.82–7.96 (m, 8H, **H$_{ar}$**).

^{13}C NMR: (100 MHz, CD_3OD) δ [ppm] 22.2 (CO–CH_3), 23.0 (CO–CH_3), 53.2, 53.5, 54.0, 56.1, 62.6, 62.6, 69.2, 70.4, 70.5, 71.0, 73.5, 73.6, 74.4, 75.8, 76.2, 76.4, 76.6, 77.5, 82.6, 84.5, 97.4 (**C-1**), 101.9, 102.1, 102.2, 117.9 (CH=CH_2), 129.45, 129.54, 130.60, 130.64, 130.67, 130.73, 130.79, 130.9, 131.00, 131.01, 134.42, 134.48, 134.54, 134.59, 135.3, 166.52 (*C*O), 166.566 (*C*O), 166.573 (*C*O), 166.8 (*C*O), 167.3 (*C*O), 169.6 (*C*O), 170.1 (*C*O), 173.1 (*C*O), some signals of aromatic carbons are overlapping.

HR-MS: (ESI positive, $CDCl_3$/MeOH) Calcd for $[C_{61}H_{69}N_2O_{27}]^+$: $[M+Na]^+$ 1283.39017. Found: 1283.39095.

IR: (KBr) ν_{max} = 3454 cm^{-1}, 2954, 2846, 1730, 1651, 1384, 821.

Optical rotation: $[\alpha]_D^{23}$(deg · cm^3 · g^{-1} · dm^{-1}) + 23.2 (*c* 1.0, MeOH).

Melting point: 205–207 °C (decomp).

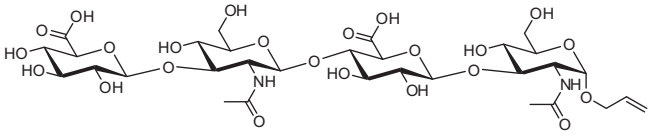
4.1.9.2. ^{13}C-labeled.

^{1}H NMR: (400 MHz, CD_3OD) δ [ppm] 1.38 (s, 3H, CO-CH_3), 1.48 (s, 3H, CO-CH_3), 3.07–3.09 (m, 2H), 3.45–3.70 (m, 7H), 3.42–3.79 (m, 8H, 2 × O-CH_3), 3.87–4.29 (m, 6H), 4.5–4.71 (m, 3H), 5.00 (d, *J* = 7.8 Hz, 1H), 5.07–5.60 (m, 7H), 5.88 (dddd, *J* = 15.6, 10.4, 6.2, 5.2 Hz, 1H, CH=CH_2), 7.31–7.46 (m, 8H, **H$_{ar}$**), 7.48–7.62 (m, 4H, **H$_{ar}$**), 7.82–7.96 (m, 8H, **H$_{ar}$**).

^{13}C NMR: (100 MHz, CD_3OD) δ [ppm] 22.2 (CO-CH_3), 23.0 (CO-CH_3), 53.2, 53.5, 54.0, 56.1, 62.6, 62.6, 69.2, 70.4, 70.5, 71.0, 73.1–76.6 (m, ^{13}C), 77.5, 82.6, 84.5, 97.4 (**C-1**), 101.6–102.1(m, ^{13}C), 102.2, 117.9 (CH=CH_2), 129.45, 129.54, 130.60, 130.64, 130.67, 130.73, 130.79, 130.9, 131.00, 131.01, 134.42, 134.48, 134.54, 134.59, 135.3, 166.52 (*C*O), 166.566 (*C*O), 166.573 (*C*O), 166.8 (*C*O), 167.3 (*C*O), 169.3–169.9 (*C*O), 170.1 (*C*O), 173.1 (*C*O).

HR-MS: (ESI positive, EtOAc/MeOH) Calcd for $[C_{55}{}^{13}C_6H_{68}N_2O_{27}Na]^+$: $[M+Na]^+$ 1289.41030 found 1289.41046.

4.1.10. Prop-2-en-1-yl β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl-(1→4)-β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-2-deoxy-α-D-glucopyranoside and prop-2-en-1-yl β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-2-deoxy-β-D-(^{13}C$_6$)glucopyranosyl-(1→4)-β-D-glucopyranuronosyl-(1→3)-2-(acetylamino)-2-deoxy-α-D-glucopyranoside 10



To a solution of tetrasaccharide **9** (10.6 mg, 0.00837 mmol, 1 equiv) in methanol (1.00 ml) was added a 0.05 m solution of sodium methoxide in methanol (1.00 ml) and the reaction mixture was stirred for 14 h at rt. Then Amberlite 120 (H^+ form) was added to neutralize the solution. After removal of the ion exchange resin by filtration all volatiles were removed under reduced pressure and the crude product was partly purified by passage through a bed of RP silica gel (eluent: water). After lyophilization the residue was dissolved in a 0.1 m NaOH solution (0.50 ml) before the reaction is allowed to stir for 2 h at rt. Afterwards Dowex 50 (H^+ form) was added to neutralize the solution and the solvent was removed by lyophilization. Finally, purification was achieved by HPLC (column: Nucleosil 120-7 C4, eluent: water) to furnish tetrasaccharide **10** (4.30 mg, 0.00527 mmol, 63%) as white solid.

4.1.10.1. Non-labeled.

R_f: 0.30 (dichloromethane/methanol/acetic acid = 3:7:1 v/v/v).

^{1}H NMR: (400 MHz, D_2O) δ [ppm] 1.90 (s, 3H, CO-CH_3), 1.92 (s, 3H, CO-CH_3), 3.18–3.28 (m, 2H), 3.36–3.49 (m, 5H), 3.59–3.77 (m, 8H), 3.78–3.83 (m, 2H), 3.83–3.85 (m, 1H), 3.91 (dd, J = 12.9, 6.2 Hz, 1H), 3.97 (dd, J = 10.5, 3.5 Hz, 1H), 4.06–4.13 (m, 1H), 4.36 (t, J = 8.1 Hz, 2H), 4.45 (d, J = 8.4 Hz, 1H), 4.54 (d, J = 1.6 Hz, 1H, **H-1**), 4.75–4.78 (m, 2H), 5.10–5.18 (m, 1H, CH=CH_aH$_b$), 5.19–5.27 (m, 1H, CH=CH$_a$$H_b$), 5.85 (dddd, J = 10.8, 10.1, 5.6, 2.7 Hz, 1H, CH=CH$_2$).

^{13}C NMR: (175 MHz, D_2O) δ [ppm] 22.24, 22.25, 52.1, 54.5, 60.5, 61.0, 68.4, 68.5, 68.6, 71.6, 71.7, 71.9, 72.7, 75.3, 75.4, 76.8, 79.5, 80.6, 82.8, 96.0, 100.3, 101.3, 102.8, 117.7, 133.5, 173.8, 174.8, both amide carbons are not visible and some carbohydrate backbone carbons are overlapping.

HR-MS: (ESI negative, MeOH) Calcd for $[C_{31}H_{46}N_2O_{23}]^{2-}$: $[M-2H]^{2-}$ 407.12512. Found: 407.12503.

IR: (KBr) ν_{max} = 3442 cm^{-1}, 2958, 2925, 2853, 1635, 1385, 1056, 712.

Optical rotation: $[\alpha]_D^{25}(\mathrm{deg}\cdot\mathrm{cm}^3\cdot\mathrm{g}^{-1}\cdot\mathrm{dm}^{-1})$ + 29.5 (c 0.14, H_2O).

Melting point: 288–290 °C (decomp.).

4.1.10.2. ^{13}C-labeled.

^{1}H NMR: (400 MHz, D_2O) δ [ppm] 2.08 (s, 3H), 2.11 (s, 3H), 3.23–3.66 (m, 6H), 3.68–4.13 (m, 10H), 4.24 (dd, J = 13.1, 5.1 Hz, 1H), 4.35 (d, J = 6.2 Hz, 1H), 4.51 (d, J = 7.6 Hz, 1H), 5.66–5.39 (m, 2H), 5.98 (dddd, J = 17.3, 10.6, 5.9, 5.3 Hz, 1H).

^{13}C NMR: (100 MHz, D_2O) δ [ppm] 22.5, 52.3, 54.8, 60.8, 61.2, 68.6, 68.7, 68.8, 72.0 (dd, J = 38, 38 Hz), 72.9, 74.9–76.5 (m), 77.0 (dd, J = 37, 37 Hz), 80.8, 83.0, 96.2, 100.5 (d, J = 48 Hz), 101.5, 103.0, 105.0, 117.9, 133.8, 174.6–177.2 (m).

HR-MS: (ESI positive, D_2O/MeOH) Calcd for $[C_{25}{}^{13}C_6H_{49}N_2O_{23}]^+$: $[M+H]^+$ 823.29219. Found: 823.29191, Calcd for $[C_{25}{}^{13}C_6H_{48}N_2O_{23}Na]^+$: $[M+Na]^+$ 845.27414. Found: 845.27396

Acknowledgments

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Supplementary data

Supplementary data (copies of ^{1}H and ^{13}C NMR spectra of all new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.11.025.

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