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An Expedient Synthesis of the Repeating Unit of the Acidic Polysaccharide of the Bacteriolytic Complex of Lysoamidase**

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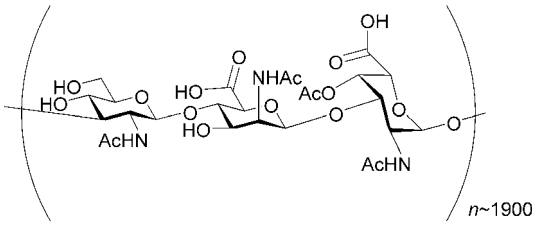
In memoriam Jacques van Boom

Abstract: The first synthesis of the trisaccharide repeating unit of the acidic polysaccharide of the bacteriolytic complex of lysoamidase is presented. The construction is based on a linear glycosylation strategy that starts from the reducing end and employs thio- and selenoglycosides in a highly stereoselective manner by a single set of activation conditions. The thus-formed trisaccharide is selectively deprotected and oxidised, after which a final deprotection step furnishes the desired repeating unit.

Keywords: carbohydrates · glycosylation · lysoamidase · oligosaccharides · uronic acids

Lysoamidase is a bacteriolytic complex isolated from bacteria of the genus *Xanthomonas* and contains, next to several proteins, a high molecular mass (1300 kDa) acidic polysaccharide.[1] This glycan is built up from trisaccharide repeating units comprised of an *N*-acetyl-D-glucosamine β(1→4) linked to *N*-acetyl-D-mannosaminuronic acid which in turn is β(1→3) linked to 4-*O*-acetyl-*N*-acetyl-L-galactosaminuronic acid. The trisaccharide repeats are connected through an α(1→3) linkage (Scheme 1).[2] The ability of lysoamidase to combat external infectious diseases caused by Gram-positive bacteria is based on the presence of several hydrolytic enzymes in the complex, including a glycyl-glycine endopeptidase, an *N*-acetylmuramyl-L-alanine amidase and an endoacetylglycosidase which cleaves *N*-acetylglucosaminyl-*N*-acetylmuramic acid linkages.[3–5] In the few reports discussing the biological activity of lysoamidase, several functions of the acidic polysaccharide are indicated. Interaction of the hydrolytic enzymes with the polysaccharide appears to be required to attain a stable bacteriolytic complex. Furthermore, interaction with the glycan influences the kinetic parameters of the enzymes with respect to their action on specific substrates.[6] Interesting biological properties aside, the synthesis of the substructures of the lysoamidase polysaccharide itself represents a scientific challenge. Indeed, to date, there are no literature reports describing the chemical synthesis of the repeating trisaccharide.



Scheme 1. Structure of the acidic polysaccharide in lysoamidase.

Inspection of the repeating unit **1** (Scheme 2) reveals a number of synthetic hurdles. These include the presence of

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[**] This article is dedicated to the memory of our colleague Jacques van Boom, who passed away on July 31, 2004, at the age of 67.

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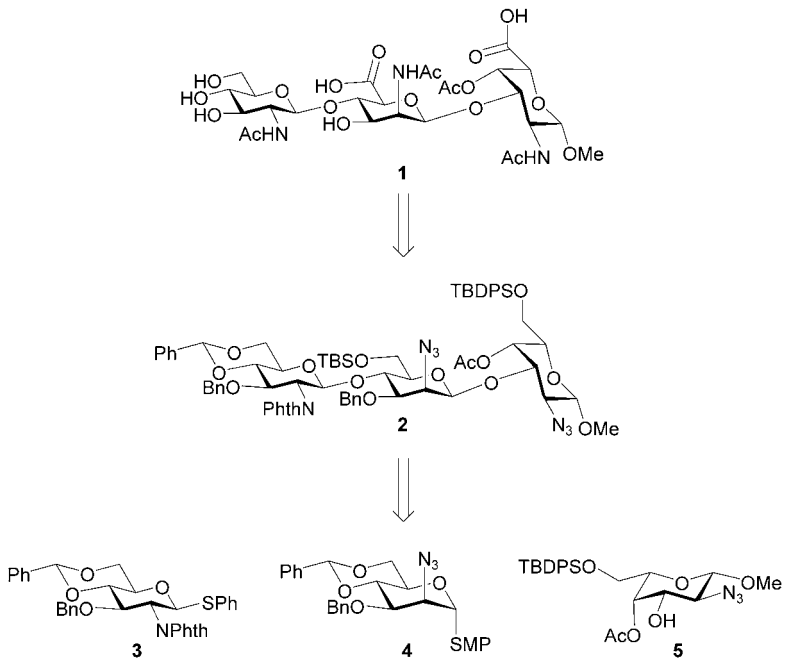
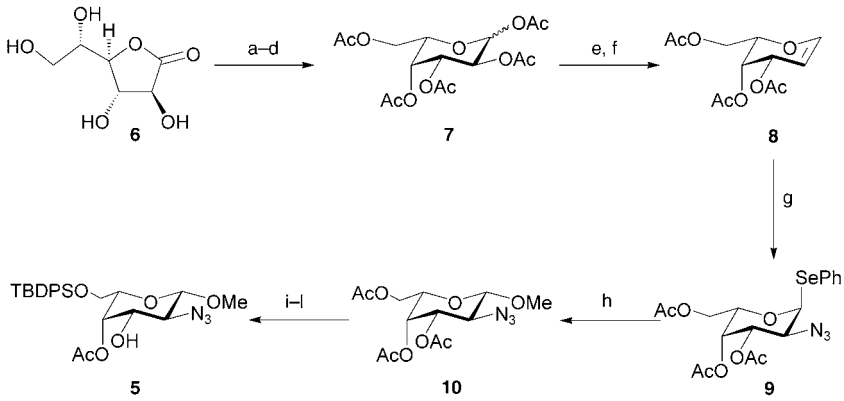


Scheme 2. Retrosynthetic analysis for the repeating unit of lysoamidase polysaccharide. Bn = benzyl, MP = *p*-methoxyphenyl, NPhth = phthalimido, TBDPS = *tert*-butyldiphenylsilyl, TBS = *tert*-butyldimethylsilyl.

the rare L-aminogalacturonic acid residue and the β linkage connecting the galactosaminuronic and mannosaminuronic acid residues. A further complication is the presence of the 4′-*O*-acetate in the trisaccharide, which excludes any basic *O* deprotection at the end of the synthetic sequence.

In devising a synthetic route for the preparation of uronic acid containing oligosaccharides, two general strategies are normally considered. The first—and most applied—strategy entails the construction of orthogonally protected oligosaccharides, followed by liberation and oxidation of those primary alcohol functions occupying carboxylate positions in the ultimate acidic oligosaccharide.[7a–d] In the second strategy, suitably protected monosaccharide and uronic acid building blocks are prepared from which a fully protected uronic acid containing oligosaccharide is assembled.[8a–d] Since the presence of the acetate in the target compound precludes the use of a number of carboxylate protecting groups, we elected to pursue a synthetic route following the first general strategy. Retrosynthetically, it follows that target compound **1** can be prepared from orthogonally protected trimer **2** after a deprotection–oxidation–deprotection sequence. Precursor trimer **2** is in turn assembled through a linear condensation procedure from orthogonally protected monosaccharide building blocks **3**–**5**. A key consideration in the synthetic strategy is our recent finding that *p*-methoxyphenyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-mannopyranoside (**4**)[9,10] is a suitable donor to attain β-selective condensations by using the *S*-(4-methoxyphenyl)benzenethiosulfinate/trifluoromethanesulfonic anhydride (MPBT/Tf_2O) or the more powerful 1-benzenesulfinyl piperidine (BSP)/Tf_2O sulfonium-activator systems developed by Crich and Smith.[11,12]

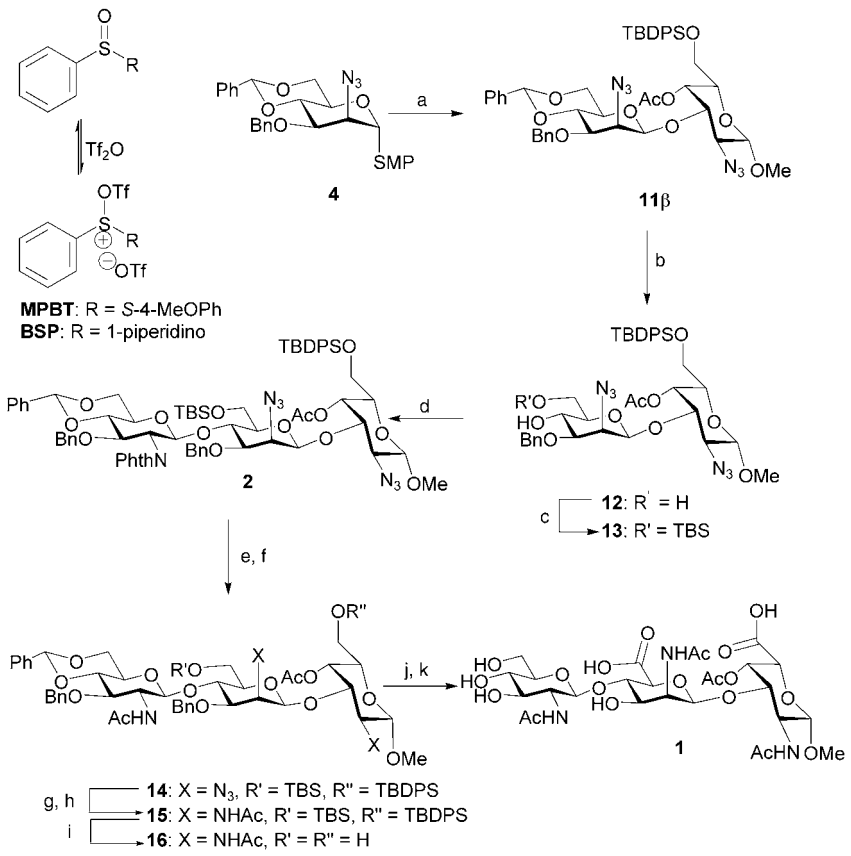
The construction of target trimer **1** commences with the synthesis of the monomeric building blocks **3**–**5**. Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**3**)[13] and *p*-methoxyphenyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-1-thio-α-D-mannopyranoside (**4**)[9] were prepared following well-established literature procedures. For the preparation of L-galactosamine acceptor **5**, the following efficient 12-step synthetic route was developed (Scheme 3). Silylation of the free hydroxy groups in the commercially available L-galactono-1,4-lactone **6**[14] (trimethylsilylchloride, imidazole, pyridine) was followed by low-temperature DIBAL-H reduction (−78 °C) to furnish the corresponding silylated lactol. Subsequent desilylation and ensuing acid-catalysed acetylation afforded the



Scheme 3. a) TMSCl, imidazole, pyr; b) DIBAL-H, Et_2O, −78 °C; c) 80 % HOAc/H_2O; d) Ac_2O, $HClO_4$ (cat.), 84 % over 4 steps; e) 37 % HBr in AcOH; f) Zn, $CuSO_4$, NaOAc, HOAc, H_2O, DCM, 92 % over two steps; g) PhSeSePh, NaN_3, BAIB, DCM, 61 %; h) BSP/Tf_2O, TTBP, DCM, −60 °C, 5 min, then MeOH, 92 %; i) KO*t*Bu (cat.), MeOH; j) TBDPSCl, pyr; k) $H_3CC(OMe)_3$, *p*TsOH (cat.), DMF; l) 80 % HOAc/H_2O, 80 % over four steps. BAIB = bisacetoxyiodobenene, BSP = 1-benzenesulfinyl piperidine, DCM = dichloromethane, DIBAL-H = diisobutylaluminium hydride, DMF = *N*,*N*-dimethylformamide, *p*Ts = toluene-4-sulfonyl, pyr = pyridine, Tf_2O = trifluoromethanesulfonic anhydride, TMS = trimethylsilyl, TTBP = tri-*tert*-butylpyrimidine.

known[15] 1,2,3,4,6-penta-*O*-acetyl-α,β-L-galactose (**7**) in 84% yield over the four steps.

At this stage the amine functionality was introduced by the following sequence of reactions. The anomeric acetate group in compound **7** was substituted with a bromide functionality by HBr/AcOH treatment. A subsequent reduction with Zn/$CuSO_4$ gave 3,4,6-tri-*O*-acetyl-L-galactal (**8**) in 92% yield over two steps. Azido–phenylselenylation[16] of the enol ether in **8** afforded the crystalline 2-azido-2-deoxy-α-L-selenogalactoside **9** in 61% yield.[17] Selenogalactoside **9** was condensed with MeOH under the influence of BSP/Tf_2O to give the methyl 2-azido-2-deoxy-β-L-galactoside **10** in 91% yield. Interestingly, while 4-*O*-acyl functionalities in galactopyranosides have been shown to be α-directing, presumably through remote neighbouring-group participation,[18] we found that only the β-methylgalactoside was formed. It should be noted that this stereochemical outcome is in line with a previously reported unusual stereoselectivity in glycosylations employing MeOH as acceptor.[19] To continue the synthetic scheme, the acetyl groups in **10** were removed and the TBDPS group was selectively installed on the primary hydroxy functionality. The thus-obtained diol was treated with trimethyl orthoacetate and the resulting ortho ester was opened regiospecifically under acidic conditions to afford the desired acceptor **5** in an overall yield of 13% over the 12 steps.



Scheme 4. a) BSP/Tf_2O, TTBP, −60°C, 10 min, then **5** in DCM, 76%, α:β = 1:4.5; b) CSA (cat.), MeOH, 62%; c) TBSCl, imidazole (cat.), pyr, 72%; d) **3**, BSP/Tf_2O, TTBP, −60°C, DCM, 71%; e) EDA, *n*BuOH, 90°C; f) Ac_2O, pyr, 88% over two steps; g) Me_3P, THF/H_2O; h) Ac_2O, pyr, 50% over two steps; i) HF·pyr, THF, 75%; j) TEMPO (cat.), NaOCl, KBr, nBu_4NBr, $NaHCO_3$, NaCl, DCM, H_2O; k) Pd/C (10 mol%), H_2, HCl, *t*BuOH/H_2O, 37% over two steps. CSA = camphorsulfonic acid, EDA = ethylenediamine, TEMPO = 2,2,6,6-tetramethyl-1-piperidinoxyl.

With the desired building blocks **3**–**5** in hand, their connection was undertaken by the application of appropriate glycosylation protocols (Scheme 4). In the first instance, attention was focussed on the stereoselective introduction of the β-mannosaminic linkage.

Based on our recent findings concerning the β-selective coupling of mannosazides, we selected the BSP/Tf_2O protocol to effect condensation of mannosazide donor **4** and L-galactosazide acceptor **5**.[10,20] The expected dimer **11** was isolated in good yield (76%) with a satisfactory α:β ratio of 1:4.5. The anomeric configuration of the formed glycosidic bonds was firmly established by ^{13}C-gated NMR spectroscopy experiments on the chromatographically separated anomers ($^1J_{CH,\alpha} = 170.9$ Hz, $^1J_{CH,\beta} = 159.0$ Hz).[21] To enable the next glycosylation event the benzylidene acetal in **11β** was removed with catalytic CSA in MeOH to give diol **12** (62%), the primary hydroxy group of which was selectively silylated with TBSCl to give disaccharide **13** in 72% yield. BSP/Tf_2O-mediated activation of thiodonor **3** and addition of dimer **13** at low temperature furnished the fully protected trisaccharide **2**, with the expected β configuration[22] for the newly introduced glycosidic linkage, in a rewarding 71% yield.

The stage was now set for the completing deprotection–oxidation–deprotection sequence. First, the phthaloyl group in **2** was removed with ethylenediamine (EDA) under anhydrous conditions. Acetylation afforded **14** in 88% over the two steps. Subsequently, the conversion of the two azide moieties in **14** into the *N*-acetates was undertaken. The reaction of **14** with thiolacetic acid (AcSH) in pyridine afforded **15** in a moderate yield of 44%. Alternatively, treatment of **14** with Me_3P in THF/H_2O followed by acetylation of the generated free amines afforded **15** in a slightly improved yield (50%). Subsequent cleavage of the silyl groups with hydrogen fluoride/pyridine complex proceeded without acetyl migration to furnish diol **16** in 75% yield. Finally, the liberated hydroxy groups in **16** were transformed into the

corresponding carboxylic acids by using TEMPO/NaOCl oxidation conditions and the benzylidene and benzyl functions were removed by hydrogenolysis to give the desired acidic trisaccharide **1** in 37% yield.

In conclusion, the first synthesis of an unprotected repeating trisaccharide unit of the acidic polysaccharide from the bacteriolytic lysoamidase complex has been accomplished in an efficient and highly stereoselective manner. The synthetic trimer, as is the case with the naturally occurring repeating unit, is provided with a 4-*O*-acetyl substituent on the L-galactosaminuronic acid residue. The synthetic approach to trisaccharide **1** described here is an asset for future syntheses of longer fragments of the acidic polysaccharide component of the antibiotic agent lysoamidase; these developments will ultimately enable an in-depth study of the interaction of the lysoamidase enzymes with well-defined parts of the glycan.

Experimental Section

General methods: Dichloromethane was refluxed with P_2O_5 and distilled before use. BSP[12] and TTBP[23] were synthesised as described by Crich et al.[23] Trifluoromethanesulfonic anhydride (Aldrich) was stirred for 3 h on P_2O_5 and subsequently distilled. All other chemicals (Fluka, Acros, Merck, Aldrich, Sigma) were used as received. Reactions were performed under an inert atmosphere and under strictly anhydrous conditions. Traces of water from reagents used in reactions that require anhydrous conditions were removed by coevaporation with toluene and dichloroethane (DCE). Molecular sieves (3 Å) were flame dried before use. Column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). TLC analysis was conducted on DC-fertigfolien (Schleicher & Schuell, F1500, LS254) or HPTLC aluminium sheets (Merck, silica gel 60, F254). Compounds were visualised by UV absorption (254 nm), by spraying with 20% H_2SO_4 in ethanol, with a solution of ninhydrin 0.4 g in EtOH (100 mL) containing acetic acid (3 mL) or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g L^{-1}), followed by charring at ≈ 140 °C. ^{1}H and ^{13}C NMR spectra were recorded with a Jeol JNM-FX-200 (200 and 50 MHz), a Bruker DPX 300 (300 and 75 MHz), a Bruker AV 400 (400 and 100 MHz) or a Bruker DMX 600 (600 and 125 MHz) spectrometer. NMR spectra were recorded in $CDCl_3$ with chemical shifts (δ) relative to tetramethylsilane unless otherwise stated. Mass spectra were recorded on a Perkin–Elmer SCIEX API 165 spectrometer equipped with electrospray interface (Perkin–Elmer) or LTQ-FT (Thermo Electron) apparatus. Optical rotations were recorded on a Propol automatic polarimeter. IR spectra were recorded on a Shimadzu FTIR-8300 spectrometer and are reported in cm^{-1}. Melting points were measured on a Büchi Schmeltzpunkt Bestimmungs apparatus.

Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (3):[13] ^{1}H NMR: δ = 7.39 (m, 10H; H-arom.), 6.87 (m, 4H; H-arom.), 5.69 (d, J = 10.4 Hz, 1H; H-1), 5.59 (s, 1H; C*H*-benzylidene), 4.75 (d, J = 12.4 Hz, 1H; C*H*Ph), 4.47 (d, J = 12.4 Hz, 1H; C*H*Ph), 4.41 (m, 2H; H-2, H-6), 4.29 (t, J = 10.0 Hz, 1H; H-3), 3.79 (m, 2H; H-4, H-6), 3.66 (m, 2H; H-5) ppm; ^{13}C NMR: δ = 167.7, 167.2, 140.9, 137.5, 137.2, 133.9, 132.6, 131.5, 128.9, 128.8, 128.4, 128.2, 128.1, 128.0, 127.9, 127.5, 127.4, 126.9, 126.0, 123.3, 101.2, 84.0, 82.6, 75.3, 70.2, 86.5, 65.1, 54.6 ppm; ESI-MS: 602.2 $[M+Na]^+$.

3,4,6-Tri-*O*-acetyl-L-galactal (8): Compound **7** (4.86 g, 12.5 mmol) was dissolved in DCE (50 mL) and HBr (18.5 mL, 37% in AcOH) was added. The reaction vessel was tightly stoppered and stirred at 0 °C for 3 h, after which the mixture was concentrated and coconcentrated with toluene (3×) and Et_2O (3×). The resulting oil was dissolved in DCM and slowly added to a mixture of $CuSO_4$ (0.89 g, 5.6 mmol), NaOAc (12.26 g, 149.5 mmol), AcOH (20 mL) and Zn dust (9.77 g, 149.5 mmol) in H_2O (20 mL). After the reaction mixture had been vigorously stirred for 3 h at −10 °C, TLC analysis (ethyl acetate) showed the reaction was complete. The mixture was filtered over Hyflo and extracted with DCM (3×), then the combined organic extracts were washed with saturated aqueous $NaHCO_3$. The organic layer was dried ($MgSO_4$), filtered and concentrated. Purification by column chromatography afforded galactal **8** (3.12 g, 11.4 mmol, 92%) as a colourless oil: $[\alpha]_D^{25}$ +17.3 (c = 1.0, $CHCl_3$); IR (thin film): $\tilde{\nu}$ = 1743, 1652, 1430, 1223, 1038 cm^{-1}; ^{1}H NMR: δ = 6.47 (dd, J = 6.2, 1.8 Hz, 1H; H-1), 5.56 (m, 1H; H-4), 5.43 (m, 1H; H-2), 4.74 (m, 1H; H-3), 4.24 (m, 3H; H-3, 2×H-6), 2.13 (s, 3H; O(CO)CH_3), 2.11 (s, 3H; O(CO)CH_3), 2.03 (s, 3H; O(CO)CH_3) ppm; ^{13}C NMR: δ = 170.1, 169.9, 169.4, 145.0, 98.6, 72.5, 63.6, 63.4, 61.6, 20.4 ppm; ESI-HRMS: calcd for $C_{12}H_{16}O_7$: 290.1234; found: 290.1234.

Phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-seleno-α-L-galactopyranoside (9): Galactal **8** (1.63 g, 6.0 mmol) was dissolved in DCM (150 mL). Diphenyl diselenide (3.30 g, 10.5 mmol), NaN_3 (1.35 g, 21.0 mmol) and BAIB (4.2 g, 13.2 mmol) were added. After 18 h, TLC analysis (ethyl acetate/light petroleum 1:2) showed complete consumption of the starting compound. The reaction mixture was poured into saturated aqueous $NaHCO_3$, the phases were separated and the aqueous phase was extracted with DCM (2×). The combined organic phases were dried ($MgSO_4$), filtered and concentrated. Column chromatography (ethyl acetate/light petroleum 1:20→1/6) afforded a mixture of products with the similar R_f values (0.54 in ethyl acetate/light petroleum 1:2). Pure compound **9** (1.72 g, 3.66 mmol, 61%) was obtained by crystallisation from ethyl acetate/light petroleum to give ivory crystals: m.p. 163 °C; $[\alpha]_D^{25}$ −25.4 (c = 0.1, $CHCl_3$); IR (thin film): $\tilde{\nu}$ = 2120, 1750, 1351, 1220 cm^{-1}; ^{1}H NMR: δ = 7.60 (m, 3H; H-arom.), 7.30 (m, 2H; H-arom.), 6.01 (d, J = 4.9 Hz, 1H; H-1), 5.47 (d, J = 2.8 Hz, 1H; H-4), 5.12 (dd, J = 11.0, 2.8 Hz, 1H; H-3), 4.67 (t, J = 6.2 Hz, 1H; H-5), 4.27 (dd, J = 11.0, 4.9 Hz, 1H; H-2), 4.05 (m, 2H; 2×H-6), 2.16 (s, 3H; O(CO)CH_3), 2.07 (s, 3H; O(CO)CH_3), 1.98 (s, 3H; O(CO)CH_3) ppm; ^{13}C NMR: δ = 169.7, 169.4, 168.9, 134.3, 132.6, 131.8, 127.6, 83.5, 70.6, 68.5, 66.7, 61.1, 58.1, 20.0 ppm; ESI-HRMS: calcd for $C_{18}H_{21}N_3O_7Se$ $[M+NH_4]$: 489.0883; found: 489.0881.

Methyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-L-galactopyranoside (10): Tf_2O (555 μL, 3.3 mmol) was added to a solution of **9** (1.40 g, 3.0 mmol), BSP (690 mg, 3.3 mmol) and TTBP (1.49 g, 6.0 mmol) in DCM (50 mL) containing 3-Å molecular sieves (≈500 mg) at −60 °C. The mixture was stirred at this temperature for 10 min, after which MeOH (1.2 mL, 30 mmol) was added. The mixture was allowed to warm to room temperature gradually; this was followed by addition of Et_3N (2 mL). After filtration, the organic phase was washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), filtered, concentrated in vacuo and applied on a silica gel column (ethyl acetate/light petroleum 1:20→1:4) to give compound **10** (600 mg, 2.74 mmol, 91%) as the pure β isomer: $[\alpha]_D^{25}$ +6.1 (c = 1, $CHCl_3$); IR (thin film): $\tilde{\nu}$ = 2976, 2110, 1675, 1250, 1045 cm^{-1}; ^{1}H NMR: δ = 5.34 (d, J = 3.3 Hz, 1H; H-4), 4.80 (dd, J = 10.6, 3.3 Hz, 1H; H-3), 4.28 (d, J = 8.0 Hz, 1H; H-1), 4.15 (m, 2H; 2×H-6), 3.85 (t, J = 6.8 Hz, 1H; H-5), 3.67 (dd, J = 10.6, 8.0 Hz, 1H; H-2), 3.61 (s, 3H; OMe), 2.15 (s, 3H; O(CO)CH_3), 2.06 (s, 3H; O(CO)CH_3), 2.05 (s, 3H; O(CO)CH_3) ppm; ^{13}C NMR: δ = 169.9, 169.7, 169.5, 103.0, 71.0, 70.5, 66.3, 60.8, 60.7, 57.3, 20.5 ppm; ESI-HRMS: calcd for $C_{13}H_{19}N_3O_8$ $[M+H]$: 346.1245; found: 346.1243.

Methyl 4-*O*-acetyl-2-azido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-L-galactopyranoside (5): Compound **10** (600 mg, 2.74 mmol) was dissolved in MeOH and KO*t*Bu (cat.) was added. After stirring for 1 h, the reaction mixture was neutralised by addition of Dowex-H$^+$, filtered and concentrated under reduced pressure. The resulting oil was dissolved in pyridine (15 mL) and TBDPSCl (784 μL, 3.01 mmol) was added. After TLC analysis showed full consumption of the starting material (4 h), MeOH was added and the mixture was concentrated in vacuo. The resulting oil was taken up in ethyl acetate and washed with brine, then the organic layer was dried ($MgSO_4$), filtered and concentrated. Column chromatography of the residue gave the intermediate *cis*-diol (1.00 g, 2.19 mmol, 80% over two steps) as a colourless oil: $[\alpha]_D^{25}$ −11.1 (c = 1, $CHCl_3$); ^{1}H NMR: δ = 7.70 (m, 4H; H-arom.), 7.47 (m, 6H; H-arom.), 4.14 (d, J = 7.6 Hz, 1H; H-1), 4.07 (d, J = 2.7 Hz, 1H; H-4), 3.94 (m, 2H; 2× H-6), 3.55 (s, 3H; OMe), 3.53 (t, J = 8.8 Hz, 1H; H-2), 3.45 (m, 2H; H-3, H-5), 1.07 (s, 9H; CH_3-*t*Bu) ppm; ^{13}C NMR: δ = 135.9, 132.8, 132.6, 127.8, 103.1, 73.8,

72.6, 68.6, 64.3, 63.3, 56.9, 22.7, 19.1 ppm; ESI-HRMS: calcd for $C_{23}H_{31}N_3O_5Si$ [M+H]: 458.2106; found: 458.2126. The diol was dissolved in DMF (7 mL) and trimethyl orthoacetate (640 μL, 3.29 mmol) and CSA (cat.) were added. After stirring for 1 h, the mixture was neutralised with Et_3N, taken up in Et_2O and washed with brine. The organic layer was dried ($MgSO_4$), filtered and concentrated. The resulting oil was dissolved in AcOH/H_2O (20 mL, 4:1) and allowed to react for 15 min. The mixture was concentrated under reduced pressure after which column chromatography (ethyl acetate/light petroleum 1:20→1:8) afforded **5** (943 mg, 1.89 mmol, 86% over 2 steps) as a colourless syrup: $[\alpha]_D^{25}$ −23.4 (c=1, $CHCl_3$); IR (thin film): $\tilde{\nu}$=2980, 2077, 1746, 1381, 1247, 1043 cm^{-1}; ^{1}H NMR: δ=7.64 (m, 4H; H-arom.), 7.37 (m, 6H; H-arom.), 5.43 (d, J=3.3 Hz, 1H; H-4), 4.19 (d, J=8.0 Hz, 1H; H-1), 3.76 (m, 1H; H-5), 3.70 (m, 3H; H-3, 2×H-6), 3.53 (s, 3H; OMe), 3.47 (dd, J=10.3, 8.0 Hz, 1H; H-2), 2.96 (brs, 1H; OH), 2.03 (s, 3H; $O(CO)CH_3$), 1.06 (s, 9H; CH_3-tBu) ppm; ^{13}C NMR: δ=171.1, 135.3, 132.8, 132.7, 132.5, 132.1, 129.4, 127.6, 102.9, 73.3, 71.2, 68.7, 63.8, 61.3, 56.8, 26.5, 20.5, 18.8 ppm; ESI-HRMS: calcd for $C_{25}H_{33}N_3O_6Si$ [M+H]: 500.2211; found: 500.2211.

Methyl 4-*O*-acetyl-2-azido-3-*O*-(2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranosyl)-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-L-galactopyranoside (11β): Tf_2O (410 μL, 2.44 mmol) was added dropwise to a mixture of **4** (1.14 g, 2.26 mmol), BSP (510 mg, 2.44 mmol), TTBP (1.12 g; 4.52 mmol) and 3-Å molecular sieves (≈500 mg) in DCM (50 mL) at −60 °C. After stirring at the same temperature for 10 min, **5** (939 mg, 1.88 mmol) in DCM (5 mL) was added dropwise. The mixture was allowed to warm to RT then Et_3N (2 mL) was added. The reaction mixture was filtered and washed with saturated aqueous $NaHCO_3$,then the organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residual oil was purified by column chromatography (light petroleum→ethyl acetate/light petroleum 1:10) to give the pure α isomer (200 mg, 0.23 mmol, 12%) as a colourless oil and the pure β isomer (858 mg, 0.99 mmol, 53%) as a white foam: **11α**: $[\alpha]_D^{25}$ +6.2 (c=1, $CHCl_3$); IR (thin film): $\tilde{\nu}$=2985, 2976, 2076, 1746, 1381, 1247, 1076, 1043 cm^{-1}; ^{1}H NMR: δ=7.61 (m, 4H; H-arom.), 7.38 (m, 16H; H-arom.), 5.60 (s, 1H; C*H*-benzylidene), 5.40 (d, J=1.6 Hz, 1H; H-4), 5.03 (s, 1H; H-1′), 4.89 (d, J=12.4 Hz, 1H; C*H*Ph), 4.72 (d, J=12.4 Hz, 1H; C*H*Ph), 4.23 (dd, J=10.2, 4.8 Hz, 1H; H-6′), 4.18 (d, 1H; H-1, 7.6 Hz), 4.10 (t, J=9.2 Hz, 1H; H-4′), 4.00 (m, 3H; H-2′, H-6′, H-3′), 3.81 (m, 2H; H-6, H-3), 3,74 (m, 1H; H-5′), 3.66 (t, J=6.8 Hz, 1H; H-6), 3.62 (m, 1H; H-5), 3.56 (m, 4H; OMe, H-2), 1.93 (s, 3H; $O(CO)CH_3$), 1.05 (s, 9H; CH_3-tBu) ppm; ^{13}C NMR: δ=169.3, 138.3, 138.1, 135.5, 133.1, 132.85, 129.9, 129.8, 129.4, 128.2, 128.1, 127.8, 127.7, 127.6, 127.5, 103.3, 101.7, 100.8, 78.9, 75.9, 75.0, 73.9, 73.4, 73.3, 68.4, 68.0, 64.6, 63.3, 62.7, 61.5, 57.2, 26.7, 20.5, 19.1 ppm; ESI-HRMS: calcd for $C_{45}H_{52}N_6O_{10}Si$ [M+NH_4]: 882.3852; found: 882.3879. **11β**: $[\alpha]_D^{25}$ −44.2 (c=1, $CHCl_3$); IR (thin film): $\tilde{\nu}$=2986, 2976, 2078, 1746, 1380, 1247, 1074, 1047 ppm; ^{1}H NMR: δ=7.63 (m, 4H; H-arom.), 7.39 (m, 16H; H-arom.), 5.63 (s, 1H; C*H*-benzylidene), 5.48 (d, J=3.2 Hz, 1H; H-4), 4.89 (d, J=12.0 Hz, 1H; C*H*Ph), 4.84 (d, J=1.2 Hz, 1H; H-1′), 4.75 (d, J=12.0 Hz, 1H; C*H*Ph), 4.34 (dd, J=10.8, 5.2 Hz, 1H; H-6′), 4.16 (d, J=8.0 Hz, 1H; H-1), 4.02 (t, 1H; H-4′, J=9.6 Hz), 3.92 (m, 2H; H-2′, H-6′), 3.81 (m, 3H; H-3, H-6, H-3′), 3.69 (t, 1H; H-6, 8.0 Hz), 3.65 (m, 1H; H-5), 3.55 (m, 4H; OMe, H-2), 3.38 (m, 1H; H-5′), 2.10 (s, 3H; $O(CO)CH_3$), 1.05 (s, 9H; CH_3-tBu) ppm; ^{13}C NMR: δ=170.6, 137.8, 137.2, 135.5, 135.4, 132.8, 132.5, 129.9, 129.8, 129.0, 128.9, 128.4, 128.2, 102.8, 101.5, 97.4, 78.1, 76.5, 75.8, 72.9, 72.8, 68.3, 67.4, 64.8, 63.0, 61.84, 61.1, 57.2, 26.7, 20.7, 19.0; ESI-HRMS: calcd for $C_{45}H_{52}N_6O_{10}Si$ [M+NH_4]: 882.3852; found: 882.3867.

Methyl 4-*O*-acetyl-2-azido-3-*O*-(2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosyl)-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-L-galactopyranoside (12): Disaccharide **11β** (858 mg; 0.99 mmol) was dissolved in MeOH/THF (30 mL, 2:1) and CSA (30 mg) was added. After 18 h, TLC analysis (ethyl acetate/light petroleum 1:5) showed full consumption of the starting material had occurred and Et_3N (200 μL) was added. Concentration of the reaction mixture followed by silica gel column chromatography (ethyl acetate/light petroleum 1:4→1:1) afforded diol **12** (471 mg, 0.61 mmol, 62%) as a white foam: $[\alpha]_D^{25}$ −62.0 (c=1, $CHCl_3$); IR (thin film): $\tilde{\nu}$=2978, 2960, 2073, 1748, 1450, 1428, 1371, 1247, 1063 cm^{-1}; ^{1}H NMR: δ=7.62 (m, 4H; H-arom.), 7.39 (m, 11H; H-arom.), 5.45 (d, J=2.8 Hz, 1H; H-4), 4.78 (s, 1H; H-1′), 4.76 (d, J=11.6 Hz, 1H; C*H*Ph), 4.63 (d, J=11.6 Hz, 1H; C*H*Ph), 4.15 (d, J=8.4 Hz, 1H; H-1), 3.92 (t, J=10.8 Hz, 1H; H-6′), 3.83 (m, 5H; H-2′, H-3, H-6′, H-4′, H-6), 3.65 (m, 2H; H-6, H-5), 3.52 (m, 5H; H-3′, OMe, H-2), 3.35 (m, 1H; H-5′), 2.80 (brs, 1H; OH), 2.61 (brs, 1H; OH), 2.02 (s, 3H; $O(CO)CH_3$), 1.06 (s, 9H; CH_3-tBu) ppm; ^{13}C NMR: δ=170.6, 137.3, 135.5, 135.5, 132.8, 129.9, 129.9, 128.7, 128.2, 128.0, 127.8, 102.6, 97.2, 80.2, 76.1, 72.8, 71.9, 66.5, 65.0, 62.4, 61.9, 61.1, 60.7, 57.2, 26.7, 20.7, 19.0 ppm; ESI-HRMS: calcd for $C_{38}H_{48}N_6O_{10}Si$ [M+NH_4]: 794.3539; found: 794.3564.

Methyl 4-*O*-acetyl-2-azido-3-*O*-(2-azido-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-mannopyranosyl)-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-L-galactopyranoside (13): TBSCl (101 mg, 0.67 mmol) and DMAP (15 mg) were added to a solution of diol **12** (471 mg, 0.61 mmol) in pyridine (10 mL). After stirring for 6 h, MeOH (500 μL) was added and the volatile compounds were evaporated. Column chromatography (ethyl acetate/light petroleum 1:10→1:5) furnished **13** (383 mg, 0.44 mmol, 72%) as a colourless oil: $[\alpha]_D^{25}$ −38.1 (c=1, $CHCl_3$); IR (thin film): $\tilde{\nu}$=2980, 2950, 2071, 1744, 1483, 1356, 1244, 1062 cm^{-1}; ^{1}H NMR: δ=7.67 (m, 4H; H-arom.), 7.44 (m, 11H; H-arom.), 5.46 (d, J=2.8 Hz, 1H; H-4), 4.80 (s, 1H; H-1′), 4.77 (d, J=14.6 Hz, 2H; C*H*Ph), 4.74 (d, J=14.6 Hz, 2H; C*H*Ph), 4.10 (d, J=8.4 Hz, 1H; H-1), 3.91 (m, 2H; 2×H-6′), 3.87 (dd, J=10.4, 2.8 Hz, 1H; H-3), 3.85 (t, J=9.6 Hz, 1H; H-4′), 3.75 (m, 2H; H-2′, H-6), 3.67 (t, J=8.0 Hz, 1H; H-6), 3.61 (m, 1H; H-5), 3.53 (m, 5H; H-2, OMe, H-3′), 3.33 (m, 1H; H-5′), 2.95 (s, 1H; OH), 2.04 (s, 3H; $O(CO)CH_3$), 1.05 (s, 9H; CH_3-tBu), 0.91 (s, 9H; CH_3-tBu), 0.12 (s, 3H; Si-CH_3), 0.11 (s, 3H; Si-CH_3) ppm; ^{13}C NMR: δ=170.3, 137.5, 135.4, 135.4, 132.7, 132.6, 129.8, 129.8, 128.4, 127.9, 127.7, 102.7, 96.5, 80.3, 77.2, 75.8, 75.5, 72.8, 72.1, 68.2, 64.7, 64.0, 61.9, 61.2, 60.8, 57.0, 26.6, 25.7, 20.6, 18.9, 18.1, −5.6, −5.6 ppm; ESI-HRMS: calcd for $C_{44}H_{62}N_6O_{10}Si_2$ [M+H]: 891.4139; found: 891.4177.

Methyl 4-*O*-acetyl-2-azido-3-*O*-{2-azido-3-*O*-benzyl-4-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-mannopyranosyl}-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-L-galactopyranoside (2): Tf_2O (47 μL, 0.28 mmol) was added to a mixture of **3** (137 mg, 0.24 mmol), BSP (55 mg, 0.26 mmol), TTBP (120 mg, 0.48 mmol) and 3-Å molecular sieves (≈200 mg) in dry DCM (5 mL) at −60 °C. After stirring at −60 °C for 15 min, **13** (180 mg, 0.20 mmol) in DCM (2 mL) was added. The mixture was allowed to warm to −20 °C over 2 h, after which Et_3N (500 μL) was added. The mixture was washed with saturated aqueous $NaHCO_3$ solution and the organic phases were dried ($MgSO_4$), filtered and evaporated under reduced pressure to afford a yellow oil which was purified by column chromatography (light petroleum→ethyl acetate/light petroleum 1:10) to give trisaccharide **2** (192 mg, 0.14 mmol, 71%) as a white foam: $[\alpha]_D^{25}$ −2.8 (c=1, $CHCl_3$); IR (thin film): $\tilde{\nu}$=2982, 2187, 2114, 1713, 1384, 1080 cm^{-1}; ^{1}H NMR: δ=7.61 (m, 4H), 7.31 (m, 21H; H-arom.), 6.93 (m, 4H; H-arom.), 5.55 (s, 1H; C*H*-benzylidene), 5.42 (d, 1H; J=8.4 Hz, H-1″), 5.36 (d, J=3.2 Hz, 1H; H-4), 4.85 (d, J=12.0 Hz, 1H; C*H*Ph), 4.79 (d, J=12.0 Hz, 1H; C*H*Ph), 4.73 (d, J=12.0 Hz, 1H; C*H*Ph), 4.64 (s, 1H; H-1′), 4.47 (d, J=12.4, 1H; C*H*Ph), 4.45 (t, J=10.4 Hz, 1H; H-3″), 4.20 (m, 2H; H-6″, H-2″), 4.01 (d, J=8.0 Hz, 1H; H-1), 3.95 (t, J=9.2 Hz, 1H; H-6′), 3.73 (m, 3H; H-3, H-4″, H-6″), 3.65 (d, J=3.1 Hz, 2H; H-2′), 3.59 (m, 6H; H-6, H-3′, H-6, H-6″, H-5, H-5″), 3.49 (s, 3H; OMe), 3.44 (dd, J=10.4, 8.4 Hz 1H; H-2), 3.34 (dd, J=11.6, 5.2 Hz1H; H-4′), 3.07 (m, 1H; H-5′), 1.98 (s, 3H; $O(CO)CH_3$), 1.03 (s, 9H; CH_3-tBu), 0.85 (s, 9H; CH_3-tBu), 0.01 (s, 3H; Si-CH_3), −0.03 (s, 3H; Si-CH_3) ppm; ^{13}C NMR: δ=170.3 (C=O), 167.6 (C=O), 138.3, 137.9, 137.3 (3×C_q-arom.), 135.54, 135.47, 133.9 (3×CH-arom.), 132.8, 132.7, 131.5 (3×C_q-arom.), 130.6, 129.9, 129.8, 128.9, 128.7, 128.4, 128.2, 128.0, 127.8, 127.7, 127.5, 127.3, 127.2, 126.2 (14×CH-arom.), 102.8 (C-1), 101.2 (*C*H-benzylidene), 98.3 (C-1″), 96.2 (C-1′), 83.0 (C-4″), 79.0 (C-3′), 76.3 (C-5′), 75.2 (C-3), 74.8 (C-3″), 74.1 (CH_2Ph), 73.2 (C-4′), 72.9 (C-5″), 72.7 (CH_2Ph), 68.7 (C-6′), 65.8 (C-5), 64.5 (C-4), 62.0 (C-2), 62.0 (C-2′), 61.6 (C-6), 61.1 (C-6′), 57.1 (OCH_3), 56.6 (C-2″), 26.7 (CH_3-tBu), 26.1 (C_q-tBu), 25.7 (CH_3-tBu), 23.9 (C_q-tBu), 20.6 (CH_3-acetyl), −5.5 (Si-CH_3), −5.5 (Si-CH_3) ppm; ESI-HRMS: calcd for $C_{72}H_{85}N_7O_{16}Si_2$ [M+Na]: 1382.5484; found: 1382.5481.

Methyl 4-*O*-acetyl-2-azido-3-*O*-{2-azido-3-*O*-benzyl-4-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-6-*O*-*tert*-butyl-

dimethylsilyl-2-deoxy-β-D-mannopyranosyl}-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-L-galactopyranoside (14): Trisaccharide **2** (90 mg, 66 μmol) was dissolved in dry *n*BuOH (4 mL) and 3-Å molecular sieves (≈100 mg) were added. After the reaction mixture had been stirred for 1 h, EDA (1 mL) was added and the mixture was stirred at 90 °C for 12 h. The volatile compounds were removed by rotary evaporation under reduced pressure, the resulting solid was dissolved in pyridine (3 mL) and Ac_2O (1 mL) was added. After 3 h, the reaction mixture was concentrated and applied to a silica gel column (ethyl acetate/light petroleum 1:7→1:4) to give **14** (74 mg, 58 μmol, 88 %) as a white solid: $[\alpha]_D^{25}$ −17.4 (c = 1, $CHCl_3$); IR (thin film): $\tilde{\nu}$ = 2980, 2130, 2114, 1718, 1712, 1380, 1080, 1002 cm^{-1}; ^1H NMR: δ = 7.37 (m, 4H; H-arom.), 7.31 (m, 21H; H-arom.), 5.51 (s, 1H; C*H*-benzylidene), 5.40 (d, J = 3.2 Hz, 1H; H-4), 5.33 (d, J = 8.4 Hz, 1H; H-1″), 4.91 (d, J = 8.4 Hz, 1H; NH,), 4.88 (d, J = 11.6 Hz, 1H; C*H*Ph), 4.75 (d, J = 12.0 Hz, 1H; C*H*Ph), 4.73 (s, 1H; H-1′), 4.70 (d, J = 12.0 Hz, 1H; C*H*Ph), 4.62 (d, J = 11.6 Hz, 1H; C*H*Ph), 4.15 (dd, J = 10.4, 4.8 Hz, 1H; H-6′), 4.08 (d, J = 8.0 Hz, 1H; H-1), 3.92 (m, 3H; H-6′, H-4′, H-3″), 3.85 (dd, J = 10.4, 3.2 Hz, 1H; H-3), 3.76 (m, 2H; H-6, H-5), 3.71 (d, J = 3.6 Hz, 1H; H-2′), 3.62 (m, 6H; H-6, H-6″, H-3′, H-6″, H-4″, H-2″), 3.53 (s, 3H; OMe), 3.49 (dd, J = 10.5, 7.8 Hz, 1H; H-2), 3.35 (m, 1H; H-5″), 3.26 (m, 1H; H-5′), 2.04 (s, 3H; $O(CO)CH_3$), 1.84 (s, 3H; $N(CO)CH_3$), 1.26 (s, 9H; CH_3-*t*Bu), 1.06 (s, 9H; CH_3-*t*Bu), 0.16 (s, 3H; Si-CH_3), 0.12 (s, 3H; Si-CH_3) ppm; ^{13}C NMR: δ = 170.4, 170.0, 138.3, 138.0, 137.3, 132.9, 132.7, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 127.8, 127.7, 127.3, 102.8, 101.1, 101.0, 96.4, 82.3, 79.2, 77.3, 76.6, 75.4, 74.1, 74.0, 73.8, 73.0, 72.5, 68.7, 65.9, 64.6, 62.2, 62.1, 61.8, 61.2, 57.1, 56.9, 26.7, 26.1, 25.9, 25.8, 23.5, 20.7, −5.3, −5.4 ppm; ESI-HRMS: calcd for $C_{66}H_{85}N_7O_{15}Si_2$ [M+H]: 1272.5715; found: 1272.5729.

Methyl 2-acetamido-4-*O*-acetyl-3-*O*-{2-acetamido-3-*O*-benzyl-4-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-β-D-mannopyranosyl}-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-L-galactopyranoside (15):*AcSH method*: AcSH (1.6 mL) was added to a solution of diazide **14** (74 mg, 58 μmol) in pyridine (500 μL) and the resulting mixture was stirred for 48 h. Concentration under reduced pressure followed by column chromatography (ethyl acetate/light petroleum 2:1→MeOH/ethyl acetate 1:10) afforded **15** (34 mg, 26 μmol, 44 %) as a glass.

Me_3P method: **14** (60 mg, 47 μmol) was dissolved in THF (2 mL), Me_3P (140 μL, 1 M in THF) and H_2O (200 μL) were added and the mixture was stirred for 3 days. Subsequently, the mixture was concentrated and coconcentrated with toluene (2×). The resulting oil was dissolved in pyridine (2 mL) and Ac_2O (500 μL) was added. After stirring for 16 h, the mixture was concentrated and the resulting product was coevaporated with toluene (2×). Column chromatography (ethyl acetate/light petroleum 2:1→ MeOH/ethyl acetate 1:10) afforded **15** (31 mg, 24 μmol, 50 %) as a slightly yellow glass.

15: $[\alpha]_D^{25}$ −15.0 (c = 0.5, MeOH); IR (thin film): $\tilde{\nu}$ = 2984, 1738, 1712, 1370 cm^{-1}; ^1H NMR (MeOD): δ = 7.64 (m, 4H; H-arom.), 7.27 (m, 21H; H-arom.), 5.59 (d, 1H; H-4, J = 3.2 Hz), 5.54 (s, 1H; C*H*-benzylidene), 4.76 (m, 3H; H-1″, 2×C*H*Ph), 4.63 (br s, 1H; H-1′), 4.58 (d, J = 12.0 Hz, 1H; C*H*Ph), 4.48 (d, J = 11.6 Hz, 1H; C*H*Ph), 4.32 (d, J = 8.4 Hz, 1H; H-1), 4.07 (dd, J = 11.6, 3.2 Hz, 1H; H-6′), 4.00 (m, 2H; H-4″, H-3), 3.86 (dd, 1H; H-2, J = 10.8, 8.8 Hz), 3.73 (m, 10H; H-3′, H-2′, H-5′, H-5, H-2″, H-3″, 2×H-6, 2×H-6″), 3.48 (m, 2H; H-6′, H-4′), 3.45 (s, 3H; OMe), 3.07 (m, 1H; H-5″), 2.14 (s, 3H; $O(CO)CH_3$), 2.06 (s, 1H; $N(CO)CH_3$), 2.04 (s, 1H; $N(CO)CH_3$), 1.99 (s, 1H; $N(CO)CH_3$), 1.04 (s, 9H; CH_3-*t*Bu), 0.97 (s, 9H; CH_3-*t*Bu), 0.18 (s, 3H; Si-CH_3), 0.16 (s, 3H; Si-CH_3) ppm; ^{13}C NMR: δ = 172.0, 171.3, 170.6, 170.2, 138.5, 137.6, 137.3, 135.6, 132.8, 129.8, 129.0, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.3, 126.0, 101.3, 101.2, 100.5, 98.5, 82.7, 79.1, 76.7, 76.4, 76.1, 74.9, 73.9, 73.5, 72.6, 72.6, 72.4, 66.1, 65.0, 60.9, 56.5, 55.9, 55.9, 45.4, 26.7, 26.1, 23.9, 23.5, 22.9, 20.6, 19.0, 18.8, −5.1, −5.4 ppm; ESI-HRMS: calcd for $C_{70}H_{93}N_3O_{17}Si_2$ [M+H]: 1304.6116; found: 1304.6151.

Methyl 2-acetamido-4-*O*-acetyl-3-*O*-{2-acetamido-3-*O*-benzyl-4-*O*-(2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-mannopyranosyl}-2-deoxy-β-L-galactopyranoside (16): HF·pyridine (70 % in pyridine, 24 μL) was added to a solution of **15** (28 mg, 21 μmol) in THF/pyr (250 μL, 4:1) in an Eppendorf vial. After stirring overnight, the reaction mixture was poured into water and the aqueous layer was extracted with ethyl acetate (3×). The combined organic phases where dried ($MgSO_4$), filtered and concentrated in vacuo. Column chromatography (ethyl acetate/light petroleum 1:1→MeOH/ethyl acetate 1:5) afforded diol **16** (15 mg, 16 μmol, 75 %) as a colourless glass: $[\alpha]_D^{25}$ −8.6 (c = 0.2, MeOH); ^1H NMR (MeOD): δ = 7.30 (m, 15H; H-arom.), 5.53 (s, 1H; C*H*-benzylidene), 5.46 (d, J = 3.2 Hz, 1H; H-4), 4.79 (m, 2H; C*H*Ph, H-1″), 4.70 (d, J = 0.8 Hz, 1H; H-1′), 4.66 (m, 1H; H-2′), 4.59 (d, J = 12.4 Hz, 1H; C*H*Ph), 4.43 (d, J = 12.4 Hz, 1H; C*H*Ph), 4.40 (d, J = 8.4 Hz, 1H; H-1), 4.07 (dd, J = 10.8, 3.6 Hz, 1H; H-3), 3.93 (dd, J = 10.0, 4.8 Hz, 1H; H-6″), 3.87 (dd, J = 9.2, 2.0 Hz, 1H; H-3″), 3.78 (dd, J = 8.4, 2.4 Hz, 1H; H-2), 3.69 (m, 8H; H-2″, H-4′, H-6′, H-3′, H-6, H-5, H-6, H-4″), 3.55 (m, 2H; H-6″, H-6′), 3.46 (s, 3H; OMe), 3.40 (m, 1H; H-5′), 3.10 (m, 1H; H-5″), 2.13 (s, 3H; $O(CO)CH_3$), 1.96 (s, 3H; $NH(CO)CH_3$), 1.94 (s, 3H; $NH(CO)CH_3$), 1.83 (s, 3H; $NH(CO)CH_3$) ppm; ^{13}C NMR (MeOD): δ = 173.7, 173.7, 173.6, 172.8 (4×C=O), 139.98, 139.97, 139.1 (3×C_q-arom.), 129.27, 129.26, 129.1, 128.9, 128.6, 128.4, 128.1, 127.3 (8× CH-arom.), 103.7 (C-1), 102.7 (C-1″), 102.4 (C*H*-benzylidene), 98.0 (C-1′), 83.2 (C-4″), 80.3 (C-3″), 80.0 (C-3′), 76.95 (C-3), 75.9 (C-5), 75.8 (C-5′), 75.3 (CH_2Ph), 74.9 (C-4′), 71.7 (CH_2Ph), 69.7 (C-6″), 67.5 (C-4), 67.3 (C-5″), 62.0 (C-6), 61.6 (C-6′), 57.6 (C-2″), 57.0 (OCH_3), 52.9 (C-2), 50.2 (C-2′), 23.2, 23.1, 22.6, 20.8 (4×CH_3-acetyl) pp,; ESI-HRMS: calcd for $C_{48}H_{61}N_3O_{17}$ [M+H]: 952.4074; found: 952.4085

Methyl 2-acetamido-4-*O*-acetyl-3-*O*-{2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-mannopyranosyluronic acid}-2-deoxy-β-L-galactopyranosyluronic acid (1): A solution of KBr (7.8 mg), Bu_4NBr (10.4 mg) and TEMPO (cat.) in saturated aqueous $NaHCO_3$ (1.4 mL) was added to a solution of diol **16** (15 mg, 16 μmol) in DCM (130 μL). A mixture of brine (0.14 mL), saturated aqueous $NaHCO_3$ (78 μL) and NaOCl (36 μL, 10 % in H_2O) was added to the resulting biphasic mixture with vigorous stirring. After 30 min, TLC analysis (MeOH/ethyl acetate 1:4) showed full transformation of the starting material into a higher-running protracted spot. After addition of another batch of NaOCl (50 μL, 10 % in H_2O) and stirring for an additional 16 h, TLC analysis (MeOH/ethyl acetate 1:4) showed full consumption of this intermediate. The phases were separated and the organic phase was washed with saturated aqueous $NaHCO_3$. The aqueous phase was extracted with DCM (2×), subsequently acidified to pH≈3 (1 M HCl) and extracted with ethyl acetate (4×). The combined organic phases were dried ($MgSO_4$), filtered and concentrated to give the crude diacid, which was used in the next step without further purification: ESI-HRMS: calcd for $C_{48}H_{57}N_3O_{19}$ [M+H]: 980.3659; found: 980.3693.

The diacid was dissolved in *t*BuOH/H_2O (200 μL, 11:4), then HCl (1 M, 30 μL) and Pd/C (25 mg) were added and argon was bubbled through the mixture for 30 min. H_2 was bubbled through for 1 h and stirring under an H_2 atmosphere was continued for 18 h, after which the reaction mixture was filtered, concentrated in vacuo and lyophilised. Gel filtration of the resulting oil afforded the desired trisaccharide **1** (4.1 mg, 5.9 μmol, 37 % over two steps) as a white foam: $[\alpha]_D^{25}$ −4.6 (c = 0.1, H_2O); ^1H NMR (D_2O): δ = 5.71 (d, J = 3.2 Hz, 1H; H-4), 4.80 (s, 1H; H-1′, obscured by D_2O residual solvent peak), 4.47 (d, J = 8.4 Hz, 1H; H-1″), 4.46 (d, J = 8.6 Hz, 1H; H-1), 4.29 (d, J = 4.0 Hz, 1H; H-2′), 4.18 (s, 1H; H-5), 4.09 (dd, J = 10.8, 3.6 Hz, 1H; H-3), 3.92 (t, J = 11.4 Hz, 1H; H-6″), 3.82 (m, 3H; H-3′, H-2, H-4′), 3.74 (dd, J = 7.2, 5.1 Hz, 1H; H-6″), 3.68 (m, 2H; H-2″, H-5′), 3.54 (m, 4H; H-3″, OMe), 3.44 (m, 2H; H-4″, H-5″), 2.10 (s, 3H; $O(CO)CH_3$), 2.07 (s, 3H; $NH(CO)CH_3$), 2.01 (s, 3H; $NH(CO)CH_3$), 2.00 (s, 3H; $NH(CO)CH_3$) ppm; ^{13}C NMR (D_2O): δ = 175.7, 172.8, 168.4, 162.2, 102.1 (C-1), 101.7 (C-1″), 97.5 (C-1′), 79.0 (C-4′), 78.0 (C-5′), 76.7 (C-5), 76.0 (C-3), 74.5 (C-3″), 74.2 (C-5′), 70.8 (C-3′), 70.4 (C-4″), 69.4 (C-4), 61.3 (C-6″), 57.6 (OMe), 56.2 (C-2″), 53.1 (C-2′), 49.7 (C-2), 23.1 ($NH(CO)CH_3$), 23.0 ($NH(CO)CH_3$), 22.7 ($NH(CO)CH_3$), 20.8 ($O(CO)CH_3$) ppm; ESI-HRMS: calcd for $C_{27}H_{41}N_3O_{19}$ [M+H]: 712.2407; found: 712.2398.

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