Total synthesis of alginate and zwitterionic SP1 oligosaccharides

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Acceptor Reactivity in the Total Synthesis of α-L-guluronic acid and β-D-mannuronic acid containing Alginate fragments

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4.1 introduction

Alginates, naturally occurring anionic polysaccharides, are composed of 1,2-*cis* linked D-mannuronic acid (M) and L-guluronic acid (G, the C-5 epimer of M) residues that are arranged in homopolymer (polymannuronate, -MM-, or polyguluronate -GG-) or heteropolymer -MG- segments[1] (Figure 4.1).[2] They are found in marine brown algae and various bacteria, including *Pseudomonas aeruginosa*, and have found wide application in the biomaterial and food industry because of their gelling properties.[3] Notably, they have also received attention because of their putative anti-tumour, antiviral, antigenic and immunomodulatory activity.[2,4] To firmly establish structure-activity relationships for this class of compounds, well-defined single molecules of a defined length are indispensable.[5] In this framework the fully stereoselective assembly of -MM- fragments employing mannuronic acid donor glycosides for the construction of the β-D-mannosidic linkages has previously been reported.[6] Using an automated solid phase approach, a set ManA alginate fragments up to the dodecamer level was generated.[7] Furthermore, the synthesis of short L-guluronic acid oligomers has also been reported.[8,9]

The assembly of mixed alginate sequences, containing both M and G residues has never been achieved and is particularly challenging because it requires the construction of both β-D-mannuronic acid and α-L-guluronic acid linkages. While D-mannuronic acid donor glycosides can be used for the stereoselective construction of *cis*-glycosidic linkages, L-guluronic acid donors are less stereoselective in glycosylation reactions.[8,10] In addition, as described in Chapter 3, the guluronic acid C-4 hydroxyl group is a very poor nucleophile. To circumvent this low reactivity, Hung and co-workers employed 1,6-anhydro-gulose synthons to lock the C4-OH in a more accessible environment and increasing the reactivity of the alcohol.[9]

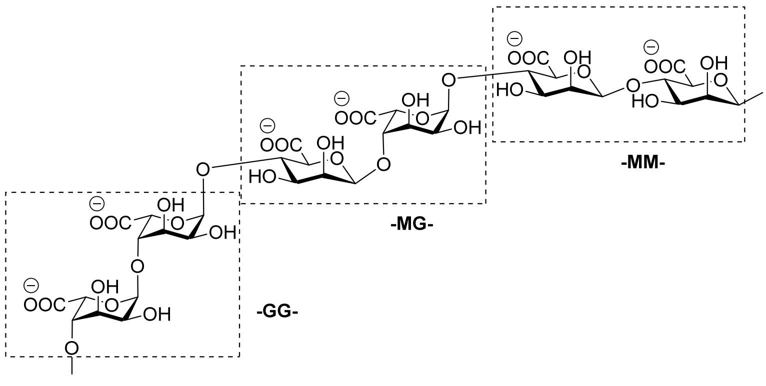


Figure 4.1 Alginates are composed of -GG-, -MM- and -MG- blocks.

Various approaches can be envisioned for the construction of mixed sequence alginate oligomers, using either monomeric or GM or MG dimer building blocks in a pre-glycosylation oxidation or post-glycosylation oxidation approach.[11] Because of the high fidelity of mannuronic acid donor synthons in the construction of β-mannosidic linkages an approach using GM building blocks, featuring a mannuronic acid donor part, is very attractive. To minimize functional group manipulation at a late stage of the syntheses the use of a guluronic acid acceptor part (as opposed to the use of a gulose acceptor) in the GM building blocks, would be most favorable.[12]

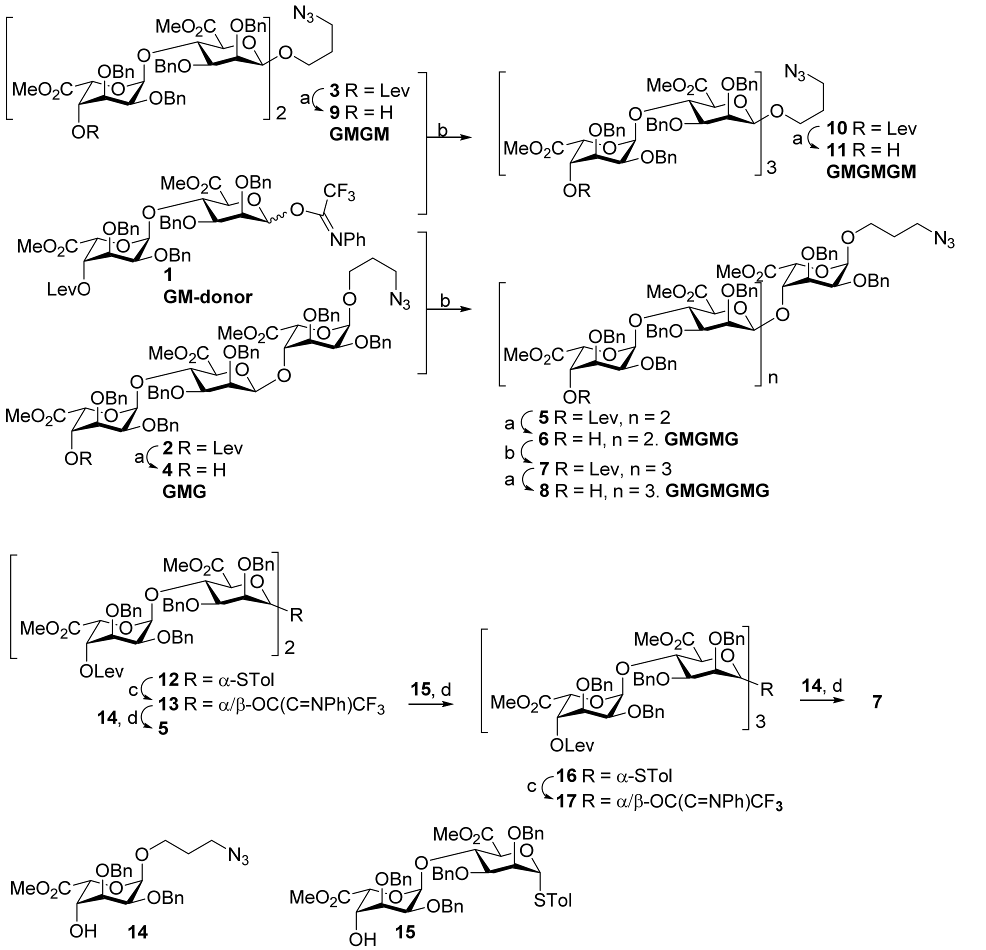
In Chapter 3 is presented a first study on the reactivity of gulose and guluronic acid acceptors in glycosylations with mannuronic acid donors. It was revealed that the nature of the substituent at the C5 position of these acceptors had relatively little influence on the yield and stereoselectivity of the glycosylations. It was shown however, that the conformational freedom of the acceptors, which in the case of GM-disaccharides is a function of the aglycon at the reducing end, was all-important. The use of disaccharide acceptors featuring a β-mannuronic acid *O*-glycoside at the reducing end provided relatively low yields in condensations with both monomer and dimer glycosyl donors. Contrary, the α-*S*-tolyl mannuronic acid counterparts could be condensed in high yield and excellent stereoselectivity with the two donor building blocks studied. This Chapter further compares the two types of dimer building blocks in the assembly of mixed sequence alginates.

4.2 Results and discussion

The synthesis of disaccharide donor **1**, trisaccharide **2** and tetrasaccharide **3** is described in Chapter 3. Although tetrasaccharide **3** was prepared in low yield, the assembly of longer oligomers was continued as shown in Scheme 4.1. Delevulinoylation of **2** and **3** gave the GMG and GMGM acceptors **4** and **9**, which were condensed with GM donor **1** to give pentamer GMGMG **5** and hexamer GMGMGM **10** with excellent stereoselectivity but again in a low yield (31% and 30% respectively). The levulinoyl group in pentamer **5** was removed ($\rightarrow$ **6**) to set the stage for another glycosylation with GM donor **1**, which led to GMGMGMG heptamer **7** in 34% yield. Delevulinoylation of **10** and **7** gave the GMGMGM and GMGMGMG oligosaccharides **11** and **8**, which were then ready for global deprotection, as described in Scheme 4.3. Clearly, the reactivity of all the *O*-linked GM oligosaccharide acceptors was poor leading to constant moderate yields in the glycosylations.

Next, an alternative approach, using thio-disaccharide acceptors, was explored, as it was found that the flexible disaccharide acceptor **15** is an apt nucleophile (see Chapter 3). Building on this finding larger GM oligosaccharides were assembled by hydrolysis of the thioacetal in GMGM tetramer **12** and transforming the resulting hemi-acetal into imidate donor **13** (See Scheme 4.1). Subsequent condensation of donor **13** with guluronic acid acceptor **14** and flexible GM dimer acceptor **15** to give the GMGMG pentamer **5** and the GMGMGM-STol hexamer **16** in 63% and 73% yield, respectively, confirming the good nucleophilicity of acceptor **15**. Elongation of the GMGMGM hexamer **16** with another guluronic acid moiety was accomplished by transformation of thioglycoside **16** into the corresponding imidate **17** and ensuing glycosylation with guluronic acid acceptor **14** to provide GMGMGMG heptamer **7** in 42% yield. The decreased yield in this glycosylation is due to partial hydrolysis of the large hexasaccharide donor.[17] It is clear that the approach using the conformational flexible acceptor **15** is overall significantly more effective.

Scheme 4.1 Synthesis of oligosaccharides by using rigid and flexible acceptors.

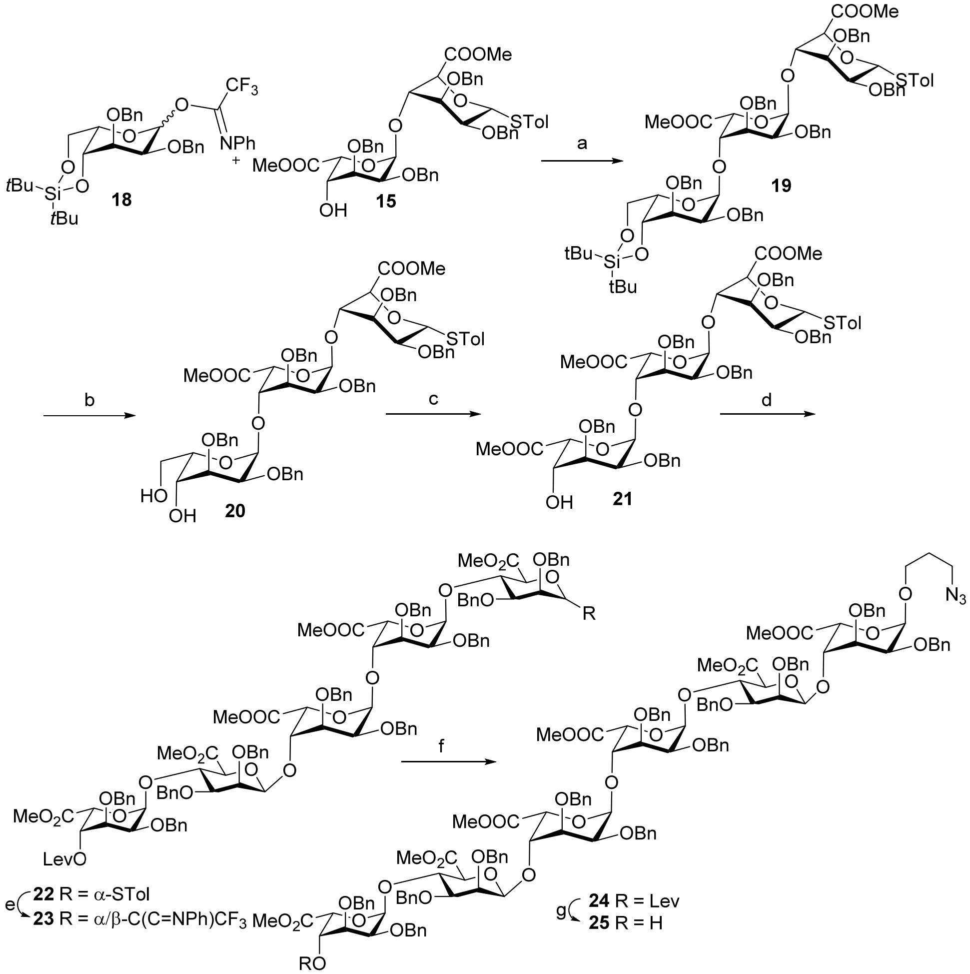


Regents and conditions: (a) N_2H_4/H_2O, acetic acid, pyridine, **4**: 89%; **6**: 98%; **8**: 83%; **9**: 78%; **11**: 86%. (b) TBSOTf (cat.), CH_2Cl_2, -78 ^oC to -45 ^oC. **5**: 31%; **7**: 34%; **10**: 30%. (c) i. NIS, TFA, CH_2Cl_2; ii. $F_3CC(=NPh)Cl$, K_2CO_3, acetone, **13**: 92%; **17**: 80%. (d) TBSOTf (cat.), CH_2Cl_2, -78 ^oC to -45 ^oC. **5**: 63%; **7**: 42%; **16**: 73%.

Then a 'random' alginate sequence was generated and GMGGMG hexasaccharide **24** was synthesized using a [2+3+1] approach as depicted in Scheme 4.2. First, trimer **21**, featuring a $^1C_4$ chair mannuronic acid residue attached to the acceptor guluronic acid moiety, was generated by condensation of gulose donor **18** with the flexible GM acceptor

15 to yield trisaccharide **19** in 87% yield and excellent stereoselectivity. Removal of the silylidene group of **19** gave diol **20**, which was oxidized and transformed into the methyl ester to yield **21** in 75% over the three steps. Then **21** was condensed with GM donor **1**.

Scheme 4.2 synthesis of a 'random' alginate sequence. GMGGMG hexasaccharide.

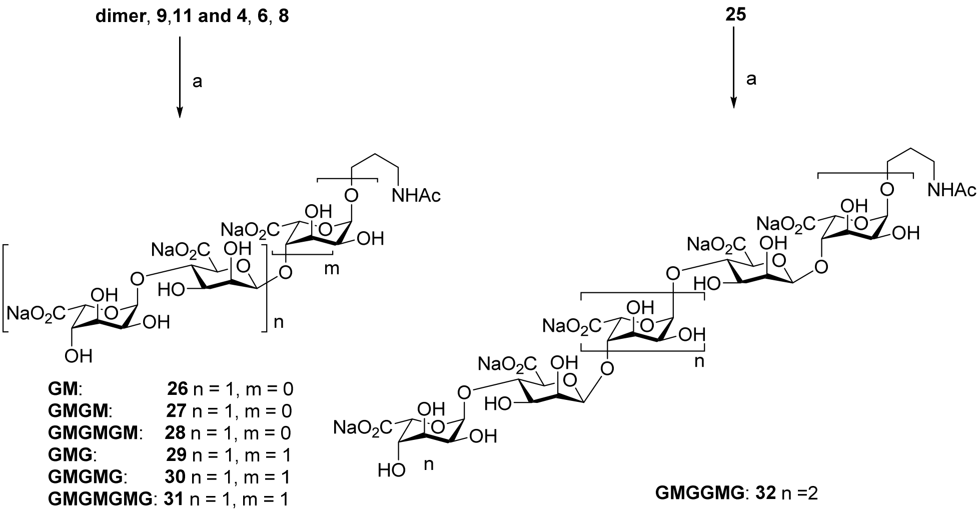


Regents and conditions: (a) TBSOTf (cat.), CH_2Cl_2, 0 °C, 92%. (b) HF/Py, pyridine, THF, 0 °C to rt, 2 h, 99%. (c) i. TEMPO, BAIB, *t*BuOH/DCM/H_2O, ii. MeI, K_2CO_3, DMF, 76% (2 steps). (d) **1**, TBSOTf (cat.), CH_2Cl_2, -78 °C to -45 °C, 87%. (e) i. NIS, TFA, CH_2Cl_2; ii. $F_3CC(=NPh)Cl$, K_2CO_3, acetone, 82%. (f) TBSOTf (cat.), CH_2Cl_2, -78 °C to -45 °C, 43%. (g) N_2H_4/H_2O, acetic acid, pyridine, 87%.

This condensation proceeded uneventfully to provide pentamer GMGGM **22** in 87% yield This oligosaccharide was transformed into the corresponding imidate donor **23** and then coupled with monosaccharide **14** to give GMGGMG hexamer **24** in 43% yield.[17]

Finally, all prepared oligomers were deprotected by i) saponification of the methyl esters, ii) high pressure debenzylation and azide reduction, and finally iii) acetylation of the formed spacer amine group. Purification of the oligomers was accomplished by HW-40 gel size exclusion chromatography, after which the alginate fragments were transformed into the sodium salts (Scheme 4.3).

Scheme 4.3 Deprotection of the oligosaccharides.



Regents and conditions: (a) i. LiOH, H_2O_2, H_2O, THF; ii. *t*BuOH, THF, H_2O, Pd/C, H_2 (4.5 bar); iii. Ac_2O, $NaHCO_3$, THF, H_2O; v. Dowex-H^+. 26: 46%; 29: 43%; 27: 50%; 30: 25%; 28: 50%; 31: 60%; 32: 60%.

4.3 Conclusion

In conclusion, the fully stereoselective assembly of a set of mixed sequence alginate oligomers has been reported for the first time, making these oligosaccharides available for biochemical studies. A set of alginate fragments, comprised of GM, GMG, GMGM, GMGMG, GMGMGM, GMGMGMG and GMGGMG sequences was assembled. During the assembly of the oligomers the conformational flexibility of the GM acceptors was revealed as an all-important factor determining the efficiency of the coupling reactions. While conformational restriction of carbohydrate building blocks has often been used to develop more efficient glycosylation strategies,[18] it is shown here that the use of inflexible building blocks can compromise the yield of a glycosylation reaction. The use of conformationally flexible building blocks can be an effective approach to overcome steric interactions in the crowded transition state of a glycosylation reaction, by allowing the acceptor to adopt a sterically most favourable shape. In future glycosylations, involving poor nucleophiles this can be an important factor to consider when optimizing the reaction.

4.4 experimental section

General experimental procedures

All reagents were of commercial grade and used as received. All moisture sensitive reactions were performed under an argon atmosphere. DCM used in the glycosylation reactions was distilled over P_2O_5 and stored on activated 5Å molecular sieves before being used. Reactions were monitored by TLC analysis with detection by UV (254 nm) and where applicable by spraying with 20% sulfuric acid in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/L) in 10% sulfuric acid (aq.) followed by charring at ~150 °C. Flash column chromatography was performed on silica gel (40-63µm). ^1H and ^{13}C spectra were recorded on a Bruker AV 400, Bruker AV 600 or Bruker AV 850 in $CDCl_3$, CD_3OD, CD_3COCD_3 or D_2O. Chemical shifts (δ) are given in ppm relative to tetramethylsilane as internal standard (^1H NMR in $CDCl_3$) or the residual signal of the deuterated solvent. Coupling constants (*J*) are given in Hz. All ^{13}C spectra are proton decoupled. NMR peak assignments were made using COSY and HSQC experiments. Where applicable NOESY, Clean TOCSY, HMBC, HMBC and GATED experiments were used to further elucidate the structure. The anomeric product ratios were analyzed through integration of proton NMR signals.

General procedure for hydrolysis of thioglycosidic bond

NIS (5.0 mmol) and TFA (462 ul, 6.0 mmol) were added to a solution of thioglycoside (5.0 mmol) in CH_2Cl_2 (40 ml) at 0 °C. After analysis by TLC showed complete consumption of the starting material, the reaction was quenched with Et_3N. Saturated $Na_2S_2O_3$ (aq) was added to the reaction mixture, which was then stirred for 30 min. The aqueous layer was extracted twice with CH_2Cl_2, and concentrated *in vacuo*. Purification by column chromatography yielded hydrolysized product as a colourless oil in good yield.

General procedure for yield *N*-phenyl-trifluoroacetimidate donor

The starting material (8 mmol) was dissolved in acetone (75 ml) and the solution was cooled to 0 °C. *N*-phenyl-trifluoroacetimidoyl chloride (12 mmol) and cesium carbonate (8 mmol) were added and the resulting suspension was stirred overnight at room temperature. Then Et_3N was added to the reaction mixture, after which it was filtered and the filtrate was concentrated *in vacuo*. Purification by column chromatography (silica gel, pentane/EtOAc/Et_3N, 20/1/trace, v/v/trace) yielded *N*-phenyl-trifluoroacetimidate donor in good yield.

General procedure for the glycosylation reactions

Imidate donor (1.5-3.0 eq) and acceptor (1.0 eq) were co-evaporated with toluene (three times). The residue was dissolved in dry DCM (0.1 M acceptor in DCM). The solution was cooled to -78 °C and followed by adding TBSOTf (0.2-0.6 eq) and the reaction was allowed to stir for 1 day at -78 °C and then slowly warmed to -45 °C and stirred for 2 days. The reaction was quenched with Et_3N, diluted with EtOAc, washed with sat. aq. NaCl and the organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography yielded the products.

General procedure for delevulinoylation

The starting material was dissolved in a mixture of acetic acid and pyridine (1/4, v/v), the mixture was cooled to 0°C and hydrazine monohydrate (5.0 eq) was added to the solution. The reaction was allowed to stir for 20 min at room temperature. Then the mixture was diluted with EtOAc, washed with 1 N aq. HCl, sat. aq. $NaHCO_3$ and sat. aq. NaCl. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography yielded the product.

General procedure for deprotection of the di-*tert*-butyl silylidene

A HF/Pyridine solution (5.0 eq) was added to a solution of starting material in a mixture of THF and pyridine at 0 °C. The reaction was allowed to stir for overnight at room temperature. Then sat. aq. $NaHCO_3$ was added to neutralize the mixture, which was subsequently, diluted with EtOAc, washed with sat. aq. NaCl. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography yielded the deprotected product.

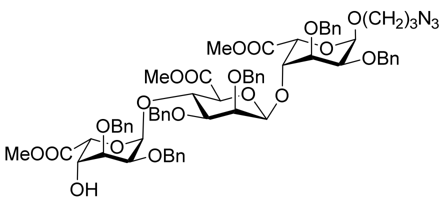
General procedure for the oxidation and methyl ester formation

The starting material was dissolved in DCM/*tert*-BuOH/H_2O (4/4/1, v/v/v). The mixture was cooled to 0 °C and TEMPO (0.2 eq) and BAIB (2.5 eq) were added. After stirring the mixture overnight at 4 °C, $Na_2S_2O_3$ was added and the heterogeneous mixture was stirred for 30 minutes, diluted with EtOAc and washed with sat. aq. NaCl. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue was dissolved in DMF, followed by the addition of K_2CO_3 (1.0 eq) and MeI (> 2.0 eq) at 0 °C. The mixture was allowed to stir overnight at 4 °C, and was then diluted with EtOAc and washed with sat. aq. NaCl. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (silica gel, pentane/EtOAc, v/v) yielded the methyl ester product.

General procedure for saponification, hydrogenation and acetylation of the oligosaccharides

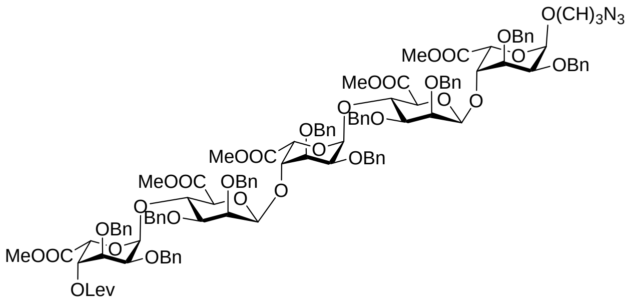
The starting material was dissolved in THF (0.4 ml), and a mixture of $LiOH{\cdot}H_2O$/ 35% H_2O_2 solution/H_2O (42 mg/520 ul/480 ul) was added to the reaction mixture. The reaction was allowed to stir for 48h at 37 °C. The reaction was cooled to 0 °C and neutralized by Amberlite IR120 (H^+) resin. After filtration, the filtrate was concentrated *in vacuo*. The residue was dissolved in THF/H_2O/*tert*-BuOH (2 ml/2 ml/0.8 ml) before a catalytic amount of Pd/C was added. The reaction mixture was stirred for 48 h under an H_2 atmosphere (4.5 bar), filtered and concentrated *in vacuo*. The ^{1}H NMR of the thus obtained crude products showed complete removal of all benzyl protecting groups. The resulting product was dissolved in H_2O (1 ml) and THF (0.5 ml), and then $NaHCO_3$ (20eq) and Ac_2O (10eq) were added to the reaction mixture, which was stirred overnight at room temperature, after which it was concentrated *in vacuo*. A white powder was obtained, which was purified by gel filtration (HW-40, 0.15M NH_4OAc in H_2O). The product containing fractions were pooled and lyophilized (4x) to yield the final products as a white solid. The products were transformed into the sodium salts by passing an aqueous solution of the compounds over a short Dowex Na^+ column, after which the compounds were lyophilized.

Methyl (3-Azidopropyl 2,3-di-*O*-benzyl-4-*O*-[methyl 2,3-di-*O*-benzyl-4-*O*-{methyl 2,3-di-*O*-benzyl-α-L-gulopyranosyl uronate}-β-D-mannopyranosyl uronate]-α-L-gulopyranosyl uronate) (4): See General procedure for delevulinoylation. Purification by column chromatography (silica gel, pentane/DCM/EtOAc, 2/1/1, v/v) yielded **4** as a colourless oil (230 mg, 96%). TLC: R_f = 0.32 (toluene/EtOAc, 2/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.52 – 6.95 (m, 30H, CH_{arom}), 5.32 (d, *J* = 3.9 Hz, 1H, H-$1_{Gul'}$), 5.13 (d, *J* = 2.0 Hz, 1H, H-$5_{Gul'}$), 5.00 – 4.20 (m, 17H, H-1_{Gul}, H-5_{Gul}, H-4_{Mann}, H-1_{Mann}, H-3_{Gul}, 6xCH_2Bn), 4.12 (m, 2H, H-4_{Gul}, H-$4_{Gul'}$), 4.00 (d, *J* = 8.4 Hz, 1H, H-5_{Mann}), 3.90-3.75 (m, 4H, H-$3_{Gul'}$, H-2_{Gul}, H-$2_{Gul'}$, -OC*H*$_2CH_2CH_2N_3$), 3.67 (s, 3H, CH_3 $COOCH_3$), 3.59 (d, *J* = 2.9 Hz, 1H, H-2_{Mann}), 3.55 – 3.40 (m, 7H, -OC*H*$_2CH_2CH_2N_3$, 2xCH_3 $COOCH_3$), 3.40 – 3.26 (m, 3H, H-3_{Mann}, -OCH_2CH_2C*H*$_2N_3$), 1.99 – 1.66 (m, 2H, -OCH_2C*H*$_2CH_2N_3$); ^{13}C–APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.5, 170.2, 168.6(-COO-), 139.0, 138.8, 138.7, 138.1, 138.0, 137.8 ($C_{q\ arom}$), 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.4, 127.3(CH_{arom}), 103.4(C-1_{Mann}), 98.1(C-1_{Gul}),

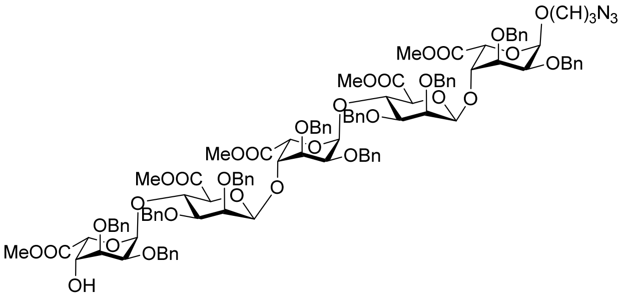


97.0(C-1$_{Gul'}$), 79.2(C-3$_{Mann}$), 78.2(C-4$_{Gul}$), 76.1(C-5$_{Mann}$), 75.1(C-3$_{Gul'}$), 74.8(C-3$_{Gul}$), 74.2(C-2$_{Mann}$), 74.1, 73.5(CH_2Bn), 72.9(CH_2Bn), 72.9, 72.8(C-2$_{Gul}$, C-2$_{Gul'}$, C-4$_{Mann}$), 71.5, 71.5, 71.3(CH_2Bn), 69.9(C-4$_{Gul'}$), 68.1(C-5$_{Gul'}$), 67.0(C-5$_{Gul}$), 65.3(-O*C*$H_2CH_2CH_2N_3$), 52.3(-COOCH_3), 52.3(-COOCH_3), 52.1(-COOCH_3), 48.3(-OCH_2CH_2*C*H_2N_3), 28.9(-OCH_2*C*$H_2CH_2N_3$); [13]C -HMBC ($CDCl_3$, 100 MHz): 103.4($J_{C1,H1}$ = 157Hz, C-1$_{Mann}$), 98.1($J_{C1,H1}$ = 168Hz, C-1$_{Gul}$), 97.0($J_{C1,H1}$ = 170Hz, C-1$_{Gul'}$). $[\alpha]^{20}_{D}$ = -78° (c = 0.8, $CHCl_3$). IR (neat): 696, 735, 908, 1028, 1038, 1065, 1103, 1115, 1177, 1206, 1238, 1304, 1362, 1454, 1749, 2095, 2878. HR-MS: [M+Na^+] Calculated for $C_{66}H_{73}O_{19}N_3$: 1234.47305; found: 1234.47434.

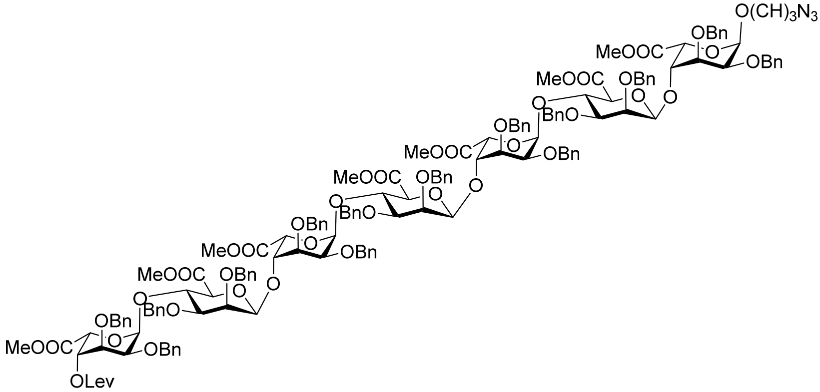
Pentasaccharide (5): See General procedure for the glycosylation reactions. Purification by size exclusion and column chromatography (silica gel, pentane/DCM/EtOAc, 5/1/1, v/v) yielded **5** as a colourless syrup (63 mg, 37%, β:α > 20:1). TLC: R_f = 0.56 (toluene/acetone, 3/1, v/v); [1]H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.49 – 6.96 (m, 50H, CH$_{arom}$), 5.31 – 5.24 (m, 2H, H-1$_{Gul''}$, H-1$_{Gul'}$), 5.23 (dd, *J* = 3.6, 1.9 Hz, 1H, H-4$_{Gul''}$), 5.19 (d, *J* = 2.0 Hz, 1H, H-5$_{Gul''}$), 4.92 – 4.79 (m, 3H, H-1$_{Gul}$, CH_2 Bn), 4.78 – 4.66 (m, 3H, H-5$_{Gul}$, CH_2 Bn), 4.63 – 4.21 (m, 22H, 2xH-1$_{Mann}$, 2xH-4$_{Mann}$, H-3$_{Gul}$, H-3$_{Gul'}$, 8x CH_2 Bn), 4.15 – 4.09 (m, 1H, H-4$_{Gul}$), 4.06 (dd, *J* = 3.7, 1.8 Hz, 1H, H-4$_{Gul'}$), 3.98 (t, *J* = 8.8 Hz, 2H, 2xH-5$_{Mann}$), 3.88 (t, *J* = 3.5 Hz, 1H, H-3$_{Gul''}$), 3.78 (dt, *J* = 7.9, 2.5 Hz, 3H, -O*C*$H_2CH_2CH_2N_3$, H-2$_{Gul}$, H-2$_{Gul'}$), 3.68 (s, 3H, CH_3 COOCH_3), 3.65 (d, *J* = 3.8 Hz, 1H, H-2$_{Gul''}$), 3.61 (d, *J* = 2.6 Hz, 1H, H-2$_{Mann}$), 3.57 (d, *J* = 2.9 Hz, 1H, H-2$_{Mann'}$), 3.55 – 3.27 (m, 17H, 2xH-3$_{Mann}$, -O*C*$H_2CH_2CH_2N_3$, 4xCH_3 COOCH_3, -OCH_2CH_2*C*H_2N_3), 2.66 (m, 2H, CH_2 Lev), 2.44 (m, 2H, CH_2 Lev), 2.15 (s, 3H, COCH_3), 1.98 – 1.73 (m, 2H, -OCH_2*C*$H_2CH_2N_3$); [13]C–APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.3(C=O Lev), 171.6, 170.3, 169.1, 168.6, 168.6(-COO-), 139.3, 139.0, 138.8, 138.8, 138.6, 138.2, 138.1, 138.0, 137.8, 137.7(C$_{q\ arom}$), 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4(CH$_{arom}$), 103.5(C-1$_{Mann'}$), 103.3(C-1$_{Mann}$), 98.2(C-1$_{Gul}$), 97.1(C-1$_{Gul'}$), 96.7(C-1$_{Gul''}$), 79.5, 79.3(2xC-3$_{Mann}$), 78.4, 78.2(C-4$_{Gul}$, C-4$_{Gul'}$), 77.5, 77.2(2xC-5$_{Mann}$), 76.8(C-3$_{Gul}$, C-3$_{Gul'}$), 76.2, 76.1(C-2$_{Mann}$, C-2$_{Mann'}$), 76.0, 75.9, 74.9, 74.4(4xCH_2Bn), 74.3, 73.6(2xC-4$_{Mann}$), 73.3(CH_2Bn), 72.8, 72.5, 72.3(C-2$_{Gul'}$, C-2$_{Gul''}$,C-3$_{Gul''}$), 71.5, 71.3, 71.1(3xCH_2Bn), 71.0(C-4$_{Gul'}$), 67.4(C-5$_{Gul'}$), 67.1(C-5$_{Gul}$), 66.3(C-5$_{Gul''}$), 65.3(-O*C*$H_2CH_2CH_2N_3$), 52.4, 52.4, 52.3, 52.2, 52.0(5x-COOCH_3), 48.4(-OCH_2CH_2*C*H_2N_3), 37.9(CH_2 Lev), 29.89(COCH_3), 29.0(CH_2 Lev), 28.1(-OCH_2*C*$H_2CH_2N_3$). $[\alpha]^{20}_{D}$ = -110° (c = 1, $CHCl_3$). IR (neat): 696, 733, 841, 908, 961, 1026, 1055, 1092, 1115, 1177, 1206, 1238, 1302, 1362, 1454, 1748, 2097, 2872, 2924. HR-MS: [M+NH_4^+] Calculated for $C_{113}H_{123}O_{33}N_3$: 2067.83770; found: 2067.84914.



Pentasaccharide (6): See General procedure for delevulinoylation. Purification by column chromatography (silica gel, pentane/DCM/EtOAc, 4/3/3, v/v) yielded **15** as a colourless oil (79 mg, 98%). TLC: R_f = 0.29 (toluene/acetone, 3/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.43 – 7.04 (m, 50H), 5.31 – 5.24 (m, 2H, H-$1_{Gul''}$, H-$1_{Gul'}$), 5.12 (d, *J* = 1.8 Hz, 1H, H-$5_{Gul''}$), 5.08 (d, *J* = 1.8 Hz, 1H, H-$5_{Gul'}$), 4.86-4.82 (m, 3H, H-1_{Gul}, CH_2 Bn), 4.76 (d, *J* = 1.8 Hz, 1H, H-5_{Gul}), 4.75 – 4.20 (m, 24H, 2xH-1_{Mann}, 2xH-4_{Mann}, H-3_{Gul}, H-$3_{Gul'}$, 9xCH_2 Bn), 4.15 – 4.04 (m, 3H, 3xH-4_{Gul}), 3.97 (dd, *J* = 13.5, 8.5 Hz, 2H, 2xH-5_{Mann}), 3.87 (t, *J* = 3.9 Hz, 1H, H-$3_{Gul''}$), 3.78-3.73(m, 4H, -$OCH_2CH_2CH_2N_3$, 3xH-2_{Gul},), 3.68 (s, 3H, CH_3 $COOCH_3$), 3.61 (d, *J* = 3.4 Hz, 1H, H-2_{Mann}), 3.57 (d, *J* = 3.6 Hz, 1H, H-2_{Mann}), 3.52 (s, 3H, CH_3 $COOCH_3$), 3.49 (s, 3H, CH_3 $COOCH_3$), 3.48-3.46(m, 1H, -$OCH_2CH_2CH_2N_3$), 3.45 (s, 3H, CH_3 $COOCH_3$), 3.39 (s, 3H, CH_3 $COOCH_3$), 3.37 – 3.29 (m, 4H, 2xH-3_{Mann}, -$OCH_2CH_2CH_2N_3$), 1.98 – 1.71 (m, 2H, -$OCH_2CH_2CH_2N_3$); ^{13}C–APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 170.3, 170.3, 168.7, 168.6(-COO-), 139.3, 139.1, 138.8, 138.3, 138.2, 138.1, 137.9($C_{q\ arom}$), 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.4, 127.3(CH_{arom}), 103.5, 103.3(2xC-1_{Mann}), 98.2(C-1_{Gul}), 97.1, 97.0(C-$1_{Gul''}$, C-$1_{Gul'}$), 79.5, 79.3(2xC-3_{Mann}), 78.4, 78.2(C-4_{Gul}, C-$4_{Gul'}$), 77.5, 77.2(2xC-5_{Mann}), 76.8, 76.2, 76.1, 75.2, 74.9(3xC-3_{Gul}, 2xC-2_{Mann}), 74.4, 74.3, 74.2, 73.6(4xCH_2Bn),74.2, 73.5(2xC-4_{Mann}), 73.3, 73.0(CH_2Bn),73.3, 73.1, 72.8(3xC-2_{Gul}), 71.6, 71.5, 71.3, 71.1 (4xCH_2Bn), 70.0(C-$4_{Gul''}$), 68.1(C-$5_{Gul''}$), 67.4(C-$5_{Gul'}$), 67.1(C-5_{Gul}), 65.4(-$OCH_2CH_2CH_2N_3$), 52.4, 52.3, 52.3, 52.2, 51.9(5x-$COOCH_3$), 48.4(-$OCH_2CH_2CH_2N_3$), 29.0(-$OCH_2CH_2CH_2N_3$); ^{13}C -HMBC ($CDCl_3$, 100 MHz): 103.5, 103.3($J_{C1,H1}$ = 157Hz, C-1_{Mann}), 98.2($J_{C1,H1}$ = 169Hz, C-1_{Gul}), 97.1, 97.0($J_{C1,H1}$ = 169Hz, C-$1_{Gul'}$, C-$1_{Gul''}$). $[\alpha]^{20}_{D}$ = -84° (c = 1, $CHCl_3$). IR (neat): 698, 737, 1028, 1038, 1065, 1115, 1207, 1238, 1456, 1749, 2096, 2924. HR-MS: [$M+NH_4^+$] Calculated for $C_{108}H_{117}O_{31}N_3$: 1969.80093; found: 1969.80604.

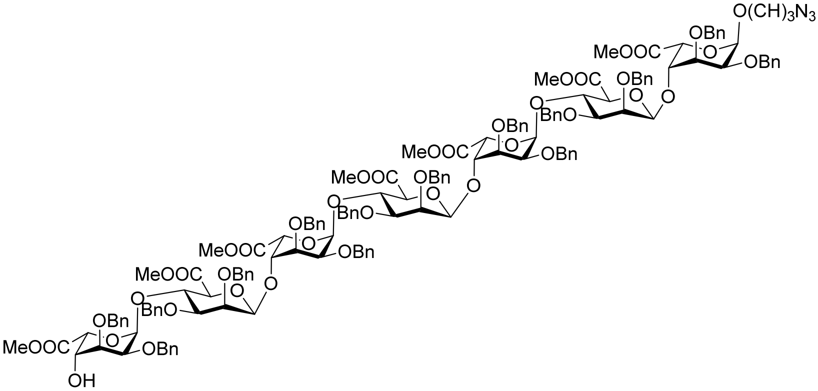


Heptasaccharide (7): See General procedure for the glycosylation reactions. Purification by column chromatography

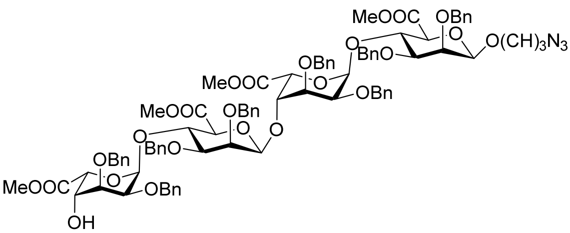


(silica gel, pentane/DCM/EtOAc, 3/1/1, v/v) yielded **7** as a colourless oil (36 mg, 34%, β:α > 20:1). TLC: R_f = 0.29 (toluene/acetone, 3/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.50 – 6.95 (m, 70H, CH_{arom}), 5.31 – 5.20 (m, 4H, H-$1_{Gul'''}$, H-$1_{Gul''}$, H-$1_{Gul'}$, H-$4_{Gul'''}$), 5.19 (d, *J* = 1.8 Hz, 1H, H-$5_{Gul'''}$), 5.07 (bs, 2H, H-$5_{Gul''}$, H-$5_{Gul'}$), 4.92 – 4.79 (m, 4H, H-1_{Gul}, CH_2 Bn), 4.75 (s, 1H, H-5_{Gul}), 4.71 (dd, *J* = 12.1, 6.0 Hz, 3H, CH_2 Bn), 4.60-4.22 (m, 31H, 3xH-4_{Mann}, 3xH-1_{Mann}, 3xH-3_{Gul}, 11x CH_2 Bn), 4.12 (dd, *J* = 5.9, 0.0 Hz, 1H, H-4_{Gul}), 4.05 (d, *J* = 3.4 Hz, 2H, 2xH-4_{Gul}), 4.02 – 3.90 (m, 3H, 3xH-5_{Mann}), 3.88 (t, *J* = 3.5 Hz, 1H, H-$3_{Gul'''}$), 3.77 (m, 3H, -$OCH_2CH_2CH_2N_3$, 2xH-2_{Gul}), 3.70 – 3.25 (m, 31H, H-2_{Gul}, 3xH-2_{Mann}, 3xH-3_{Mann}, -$OCH_2CH_2CH_2N_3$, 7xCH_3 $COOCH_3$, -$OCH_2CH_2CH_2N_3$), 2.76 – 2.55 (m, 2H, CH_2 Lev), 2.42 (ddd, *J* = 10.9, 7.7, 4.5 Hz, 2H, CH_2 Lev), 2.14 (s, 3H, $COCH_3$), 1.96 – 1.75 (m, 2H, -$OCH_2CH_2CH_2N_3$); ^{13}C–APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.2(C=O Lev), 171.6, 170.3, 169.1, 168.6, 168.6(-COO-), 139.3, 139.1, 138.9, 138.9, 138.8, 138.6, 138.3, 138.1, 138.0, 137.9, 137.9, 137.7($C_{q\ arom}$), 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.2(CH_{arom}), 103.5, 103.3(3xC-1_{Mann}), 98.2, 97.2, 97.1, 96.7(4xC-1_{Gul}), 79.5, 79.4(3xC-3_{Mann}), 78.4, 78.3(C-4_{Gul}, C-$4_{Gul'}$, C-$4_{Gul''}$), 76.3, 76.2, 76.2(3xC-5_{Mann}), 74.9, 74.5, 74.3(3xC-3_{Gul} , 3xC-2_{Mann}), 74.3, 74.2, 73.6, 73.3, 73.0 (4xCH_2Bn), 73.2, 73.1, 72.9, 72.6, 72.4(4xC-2_{Gul}, C-$3_{Gul'''}$), 71.6, 71.3, 71.2, 71.2, 71.1(5xCH_2Bn), 71.0(C-$4_{Gul'''}$), 67.4, 67.1, 66.3(4xC-5_{Gul}), 65.4(-$OCH_2CH_2CH_2N_3$), 52.3, 52.3, 52.3, 52.2, 52.2, 51.9(7x-$COOCH_3$), 48.4(-$OCH_2CH_2CH_2N_3$), 38.0(CH_2 Lev), 29.8($COCH_3$), 29.0(CH_2 Lev), 28.1(-$OCH_2CH_2CH_2N_3$); ^{13}C -HMBC ($CDCl_3$, 100 MHz): 103.5, 103.3($J_{C1,H1}$ = 158Hz, 3xC-1_{Mann}), 98.2($J_{C1,H1}$ = 168Hz, C-1_{Gul}), 97.2, 97.1, 97.0($J_{C1,H1}$ = 170Hz, C-$1_{Gul'}$, C-$1_{Gul''}$, C-$1_{Gul'''}$). $[\alpha]^{20}_{D}$ = -95° (c = 0.88, $CHCl_3$). IR (neat): 698, 737, 1028, 1038, 1059, 1096, 1117, 1207, 1240, 1362, 1456, 1749, 2098, 2924.HR-MS: [M+Na^+] Calculated for $C_{155}H_{167}O_{45}N_3$: 2813.07618; found: 2813.08957.

heptasaccharide (8): See General procedure for delevulinoylation. Purification by column chromatography (silica gel, DCM/MeOH, 100/1, v/v) yielded **8** as a colourless oil (45 mg, 83%). TLC: R_f = 0.31 (toluene/acetone, 3/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.49 – 7.01 (m, 70H, CH_{arom}), 5.30 – 5.21 (m, 3H, H-$1_{Gul'''}$, H-$1_{Gul''}$, H-$1_{Gul'}$), 5.12 (d, *J* = 1.9 Hz, 1H, H-5_{Gul}), 5.07 (d, *J* = 1.8 Hz, 2H, 2xH-5_{Gul}), 4.89 – 4.78 (m, 4H, H-1_{Gul}, CH_2 Bn), 4.78 – 4.19 (m, 29H, H-5_{Gul}, 3xH-4_{Mann}, 3xH-1_{Mann}, 3xH-3_{Gul}, 12.5x CH_2 Bn), 4.16 – 4.02 (m, 4H, 4xH-4_{Gul}), 4.02 – 3.91 (m, 3H, 3xH-5_{Mann}), 3.87 (t, *J* = 3.6 Hz, 1H, H-$3_{Gul'''}$), 3.77 (m, 5H, -O$CH_2$$CH_2$$CH_2$$N_3$, 4xH-$2_{Gul}$), 3.68 (s, 3H, CH_3 $COOCH_3$), 3.64 – 3.54 (m, 3H, 3xH-2_{Mann}), 3.56 – 3.37 (m, 19H, -O$CH_2$$CH_2$$CH_2$$N_3$, 6x$CH_3$ $COOCH_3$), 3.34 (m, 5H, 3xH-3_{Mann}, -O$CH_2$$CH_2$$CH_2$$N_3$), 1.98 – 1.73 (m, 2H, -O$CH_2$$CH_2$$CH_2$$N_3$). ^{13}C–APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.6, 170.3, 168.7, 168.6(-COO-), 139.3, 139.1, 138.9, 138.8, 138.3, 138.2, 138.1, 137.9, 137.9($C_{q\ arom}$), 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4(CH_{arom}), 103.5, 103.4(3xC-1_{Mann}), 98.2, 97.2, 97.1(4xC-1_{Gul}), 79.5, 79.5, 79.3(3xC-3_{Mann}), 78.4, 78.3(C-4_{Gul} , C-$4_{Gul'}$, C-$4_{Gul''}$), 76.3, 76.2, 76.1(3xC-5_{Mann}), 75.2, 74.9, 74.5, 74.4, 74.3(4xC-3_{Gul} , 3xC-2_{Mann}), 74.3, 74.2, 73.6, 73.3, 73.0(5xCH_2Bn), 73.5, 73.3, 73.3, 73.2, 73.1, 73.1, 72.9(4xC-2_{Gul}, 3xC-4_{Mann}), 71.7, 71.6, 71.3, 71.2, 71.1(4xCH_2Bn), 70.0(C-$4_{Gul'''}$),, 68.2, 67.4, 67.1(4xC-5_{Gul}), 65.4(-O$CH_2$$CH_2$$CH_2$$N_3$), 52.3, 52.3, 52.3, 52.3, 52.2, 52.0(7x-$COOCH_3$), 48.4(-O$CH_2$$CH_2$$CH_2$$N_3$), 29.0(-O$CH_2$$CH_2$$CH_2$$N_3$); ^{13}C -HMBC ($CDCl_3$, 100 MHz): 103.5, 103.4($J_{C1,H1}$ = 158Hz, 3xC-1_{Mann}), 98.2($J_{C1,H1}$ = 168Hz, C-1_{Gul}), 97.2, 97.1 ($J_{C1,H1}$ = 170Hz, C-$1_{Gul'}$, C-$1_{Gul''}$, C-$1_{Gul'''}$). $[\alpha]^{20}_D$ = -94° (c = 0.44, $CHCl_3$). HR-MS: [M+NH_4^+] Calculated for $C_{150}H_{161}O_{43}N_3$: 2710.08421; found: 2710.10289.

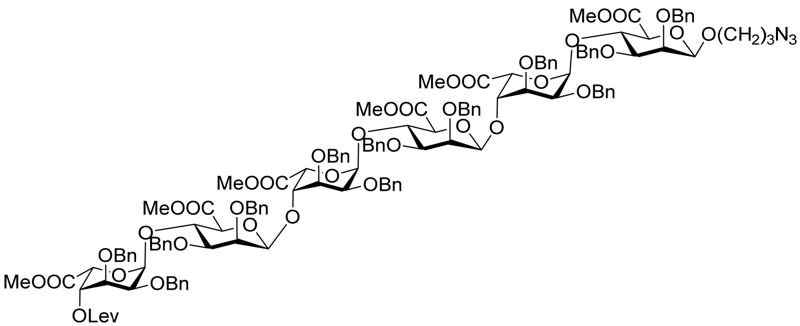


Tetrasaccharide (9): See General procedure for delevulinoylation. starting material **3** (120 mg, 0.071 mmol) was dissolved in a mixture of acetic acid and pyridine (1.25 ml, 1/4, v/v), the mixture was cooled to 0 °C and hydrazine hydrate (20 ul) was added to the solution. The reaction was allowed to stir for 20 min at room temperature. Then the reaction was diluted with EtOAc, washed with 1 N HCl, sat. aq. $NaHCO_3$ and sat. aq. NaCl, the organic phase was dried over Na_2SO_4 and



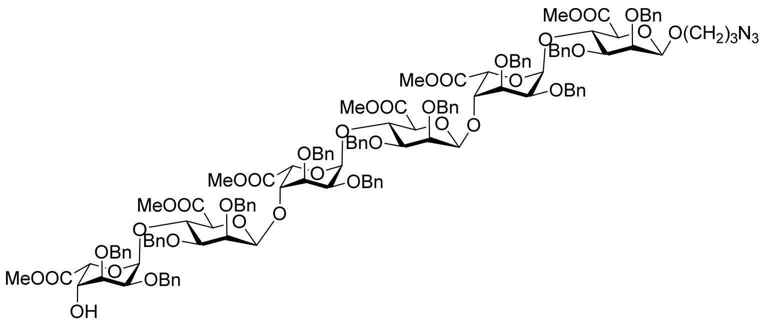
concentrated *in vacuo*. Purification by column chromatography (silica gel, pentane/DCM/EtOAc, 3/1/1, v/v/v) yielded product **9** (88 mg, 78%). TLC: R_f = 0.40 (toluene/acetone, 3/1, v/v); ^{1}H NMR ($CDCl_3$, 400 MHz, HH-COSY, HSQC): δ 7.49 – 7.03 (m, 40H, CH_{arom}), 5.29 (d, *J* = 3.9 Hz, 1H, H-$1_{Gul'}$), 5.20 (d, *J* = 4.0 Hz, 1H, H-1_{Gul}), 5.13 (d, *J* = 1.9 Hz, 1H, H-$5_{Gul'}$), 5.03 (d, *J* = 1.8 Hz, $1H_{I}$, H-5_{Gul}), 4.91 – 4.18 (m, 21H, 2xH-1_{Mann}, 2xH-4_{Mann}, H-3_{Gul}, 8x CH_2 Bn), 4.10 (dt, *J* = 4.1, 1.8 Hz, 1H, H-$4_{Gul'}$), 4.08 – 3.99 (m, 3H, -OC$H_2CH_2CH_2N_3$, H-4_{Gul}, H-5_{Mann}), 3.96 (d, *J* = 8.6 Hz, 1H, H-$5_{Mann'}$), 3.87 (t, *J* = 3.8 Hz, 1H, H-$3_{Gul'}$), 3.83 (d, *J* = 3.4 Hz, 1H, H-2_{Mann}), 3.77 (dt, *J* = 6.9, 3.7 Hz, 2H, H-$2_{Gul'}$, H-2_{Gul}), 3.57 (m, 1H, H-$2_{Mann'}$), 3.56 – 3.44 (m, 11H, H-3_{Mann}, -OC$H_2CH_2CH_2N_3$, 3xCH_3 $COOCH_3$), 3.41 (s, 3H, CH_3 $COOCH_3$), 3.39 – 3.28 (m, 3H, H-$3_{Mann'}$, -$OCH_2CH_2CH_2N_3$), 1.96 – 1.75 (m, 2H, -$OCH_2CH_2CH_2N_3$); ^{13}C–APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.5, 170.2, 168.7, 168.7(-COO-), 139.3, 138.8, 138.7, 138.2, 138.1, 138.0, 137.8($C_{q\ arom}$), 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.3, 127.3, 127.2(CH_{arom}), 103.3(C-$1_{Mann'}$), 101.5(C-1_{Mann}), 97.3(C-1_{Gul}), 97.0(C-$1_{Gul'}$), 79.5(C-3_{Mann}), 79.2(C-$3_{Mann'}$), 78.5(C-4_{Gul}), 76.1(C-$5_{Mann'}$), 75.7(C-5_{Mann}), 75.1(C-$3_{Gul'}$), 74.4, 74.3(C-3_{Gul} ,C-$2_{Mann'}$), 74.2, 73.9, 73.8, 73.2(4xCH_2Bn), 73.4, 73.4, 73.1, 72.9, 72.9(C-$2_{Mann'}$, C-4_{Mann}, C-$4_{Mann'}$, C-2_{Gul} ,C-$2_{Gul'}$), 71.6, 71.3,71.3, 71.1(4xCH_2Bn), 70.0(C-$4_{Gul'}$), 68.1(C-$5_{Gul'}$), 67.4(C-5_{Gul}), 66.8(-O$CH_2CH_2CH_2N_3$), 52.4, 52.3, 52.2, 52.0(4x-$COOCH_3$), 48.4(-$OCH_2CH_2CH_2N_3$), 29.1(-$OCH_2CH_2CH_2N_3$); ^{13}C –HMBC ($CDCl_3$, 100 MHz): 103.3($J_{C1,H1}$ = 156Hz, C-$1_{Mann'}$), 101.5($J_{C1,H1}$ = 156Hz, C-1_{Mann}), 97.3($J_{C1,H1}$ = 170Hz, C-1_{Gul}), 97.0($J_{C1,H1}$ = 170Hz, C-$1_{Gul'}$). $[\alpha]^{20}_{D}$ = -80° (c = 0.84, $CHCl_3$). IR (neat): 698, 737, 1028, 1038, 1065, 1101, 1115, 1207, 1238, 1456, 1749, 2097, 2924. HR-MS: [M+Na^+] Calculated for $C_{87}H_{95}O_{25}N_3$: 1606.61469; found: 1606.61593.

Hexasaccharide (10): As described for the general procedure for glycosylation reactions, purification by size exclusion (LH-20, MeOH/DCM 1 :1) and column chromatography yielded (silica gel, pentane/DCM/EtOAc, 4/1/1, v/v/v) **10** as a colourless syrup (17 mg, 30%, β:α > 20:1). TLC: R_f = 0.53 (toluene/acetone, 3/1, v/v); $[\alpha]^{20}_{D}$ = -95° (c = 0.48, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 850 MHz,HH-COSY, HSQC): δ 7.50 – 6.93 (m, 60H, CH_{arom}), 5.28 – 5.16 (m, 5H, H-$1_{Gul'}$, H-$1_{Gul''}$, H-1_{Gul} , H-$4_{Gul''}$, H-5_{Gul}), 5.07 (d, *J* = 1.8 Hz, 1H , H-5_{Gul}), 5.02 (d, *J* = 1.8 Hz, 1H , H-5_{Gul}), 4.85 (dd, *J* = 12.0, 9.4 Hz, 4H), 4.79 (d, *J* = 12.2 Hz, 1H), 4.74 – 4.67 (m, 4H), 4.61 – 4.18 (m, 29H, 3xH-1_{Mann}), 4.08 – 3.92 (m, 6H, 3xH-5_{Mann}, H-4_{Gul}), 3.90 – 3.84 (m, 2H), 3.82 (dd, *J* = 2.7, 1.3 Hz, 1H), 3.78 – 3.72 (m, 3H), 3.64 (t, *J* = 3.7 Hz, 1H), 3.59 (d, *J* = 3.6 Hz, 1H), 3.56 (t, *J* = 3.2 Hz, 1H), 3.53 – 3.37 (m, 20H, 6xCH_3 $COOCH_3$, -OC$H_2CH_2CH_2N_3$, H-3_{Mann}), 3.37– 3.27 (m, 4H, -$OCH_2CH_2CH_2N_3$, 2xH-3_{Mann}), 2.73 – 2.56 (m, 2H, CH_2 Lev), 2.49 – 2.37 (m, 2H, CH_2 Lev), 2.15 (s, 3H, $COCH_3$), 1.93 –

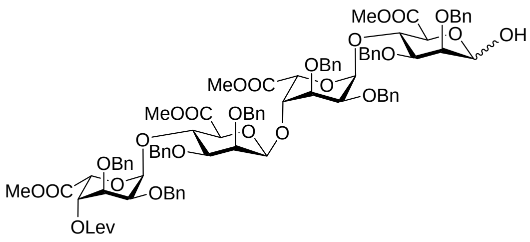


1.79 (m, 2H, -OCH$_2$CH_2CH$_2$N$_3$). ^{13}C –APT NMR (CDCl$_3$, 213 MHz, HSQC): δ 206.33 (C=O Lev), 171.6, 170.3, 170.3, 169.1, 168.8, 168.7, 168.6 (-COO-), 139.3, 139.3, 138.9, 138.8, 138.7, 138.6, 138.3, 138.2, 138.2, 138.2, 138.0, 137.8, 137.7(C$_{q\ arom}$), 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.2, 103.4, 103.3, 101.5 (3xC-1$_{Mann}$), 97.3, 97.1, 96.7(3xC-1$_{Gul}$), 79.5, 79.5, 79.4 (3xC-3$_{Mann}$), 78.4, 78.4 (2xC-4$_{Gul}$), 77.4, , 76.2, 76.1, 75.7(3xC-5$_{Mann}$), 74.5, 74.4, 74.3, 74.3, 74.3, 74.2, 73.9, 73.9, 73.8, 73.6, 73.4, 73.4, 73.3, 73.3, 73.3, 73.2, 73.2, 73.0, 73.0, 72.6, 72.5, 71.9, 71.3, 71.3, 71.2, 71.2, 71.1, 71.1, 71.1, 71.1, 71.0(C-4$_{Gul''}$), 67.5, 67.4(3xC-5$_{Gul}$), 66.8(-OCH$_2$CH$_2$CH$_2$N$_3$), 52.4, 52.4, 52.3, 52.2, 52.0, 52.0 (6x-COOCH$_3$), 48.5 (-OCH$_2$CH$_2$CH$_2$N$_3$), 38.0 (CH$_2$ Lev), 29.9 (COCH$_3$), 29. (CH$_2$ Lev)2, 28.1 (-OCH$_2$CH$_2$CH$_2$N$_3$) ; ^{13}C -HMBC (CDCl$_3$, 213 MHz): 103.4 ($J_{C1,H1}$ = 158Hz, C-1$_{Mann}$), 103.3 ($J_{C1,H1}$ = 158Hz, C-1$_{Mann}$), 101.5 ($J_{C1,H1}$ = 157Hz, C-1$_{Mann}$), 97.3 ($J_{C1,H1}$ = 172Hz, C-1$_{Gul}$), 97.1 ($J_{C1,H1}$ = 172Hz, C-1$_{Gul}$), 96.7 ($J_{C1,H1}$ = 172Hz, C-1$_{Gul'}$). HR-MS: [M+Na$^+$] Calculated for $C_{134}H_{145}O_{39}N_3$: 2442.93474; found: 2442.94918.

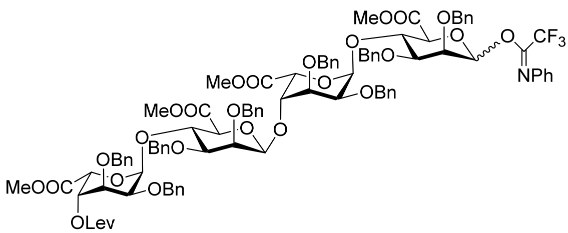
Hexasaccharide (11): See General procedure for delevulinoylation. Purification by column chromatography (silica gel, DCM/MeOH, 100/1, v/v) yielded **11** as a colourless syrup (28 mg, 86%). TLC: R$_f$ = 0.33 (toluene/acetone, 3/1, v/v); $[\alpha]^{20}_D$ = -100° (c = 0.56, CHCl$_3$). ^{1}H NMR (CDCl$_3$, 400 MHz, HH-COSY, HSQC): δ 7.48 – 6.97 (m, 60H, CH$_{arom}$), 5.28 (d, J = 3.8 Hz, 1H, H-1$_{Gul}$), 5.24 (d, J = 4.0 Hz, 1H, H-1$_{Gul}$), 5.18 (d, J = 4.0 Hz, 1H, H-1$_{Gul}$), 5.12 (d, J = 1.9 Hz, 1H, H-5$_{Gul}$), 5.07 (bs, 1H, H-5$_{Gul}$), 5.02 (bs, 1H, H-5$_{Gul}$), 4.92 – 4.15 (m, 43H), 4.13 – 3.99 (m, 6H), 3.95 (d, J = 8.5 Hz, 2H), 3.87 (t, J = 3.6 Hz, 2H), 3.82 (d, J = 3.1 Hz, 1H), 3.76 (dt, J = 12.1, 3.6 Hz, 4H), 3.62 – 3.27 (m, 31H), 1.93 – 1.77 (m, 2H). ^{13}C –APT NMR (CDCl$_3$, 100 MHz, HSQC): δ 170.5, 170.3, 170.2, 168.8, 168.7, 168.6(-COO-), 139.2, 139.2, 138.9, 138.8, 138.7, 138.2, 138.2, 138.1, 138.0, 137.8, 137.8 (C$_{q\ arom}$), 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 127.3, 127.3, 127.2, 127.2(CH $_{arom}$), 103.4, 101.5(3xC-1$_{Mann}$), 97.3, 97.0(3xC-1$_{Gul}$), 79.5, 79.2, 78.4, 76.1, 76.0, 75.2, 74.4, 74.4, 74.4, 74.3, 74.3, 74.2, 73.9, 73.9, 73.5, 73.3, 73.3, 73.2, 73.1, 73.0, 72.9, 71.6, 71.3, 71.3, 71.1, 71.1, 70.0, 68.1, 67.4, 66.8, 52.3, 52.3, 52.2, 48.5, 29.8, 29.8, 29.2. HR-MS: [M+Na$^+$] Calculated for $C_{129}H_{139}O_{37}N_3$: 2344.89796; found: 2344.91284.



Tetrasaccharide (12): The tetrasaccharide was obtained as general procedure for hydrolysis of thioglycosidic bond in 96% yield (350 mg). TLC: R_f = 0.24 (toluene/EtOAc, 4/3, v/v); $[\alpha]^{20}_{D}$ = -75° (c = 0.58, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.55 – 7.03 (m, 40H, CH_{arom}), 5.48 (d, *J* = 6.1 Hz, 1H, H-1$_{Gul'}$), 5.31 (d, *J* = 3.9 Hz, 1H, H-1$_{Gul}$), 5.25 (dd, *J* = 3.6, 1.8 Hz, 1H, H-4$_{Gul'}$), 5.20 (d, *J* = 1.9 Hz, 1H, H-5$_{Gul'}$), 5.08 (d, *J* = 3.8 Hz, 1H, H-1$_{Mann}$), 4.93 – 4.78 (m, 3H, CH_2Bn), 4.77 – 4.69 (m, 3H, H-5$_{Gul}$, CH_2Bn), 4.61 – 4.22 (m, 20H, H-1$_{Mann'}$, H-4$_{Mann'}$, H-4$_{Mann}$, CH_2Bn), 4.13 (dd, *J* = 3.8, 1.8 Hz, 1H, H-5$_{Mann}$), 4.04 (d, *J* = 8.2 Hz, 1H, H-5$_{Mann'}$), 3.89 (d, *J* = 3.5 Hz, 1H, H-3$_{Gul'}$), 3.84 (q, *J* = 3.4, 2.8 Hz, 2H, H-2$_{Mann}$, H-3$_{Mann}$), 3.67 (t, *J* = 3.7 Hz, 1H, H-2$_{Gul}$), 3.61 (d, *J* = 2.7 Hz, 1H, H-2$_{Mann'}$), 3.56 (s, 3H, CH_3OCO-), 3.53 (d, *J* = 2.8 Hz, 4H, CH_3OCO-, H-2$_{Gul'}$), 3.48 (s, 1H, CH_3OCO- β isomer), 3.46 (d, *J* = 1.1 Hz, 6H, 2xCH_3OCO-), 3.37 (dd, *J* = 9.2, 2.7 Hz, 1H, H-3$_{Mann'}$), 2.79 – 2.53 (m, 2H, CH_2 Lev), 2.52 – 2.32 (m, 2H, CH_2 Lev), 2.13 (s, 3H, CH_3CO); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.2(C=O Lev), 171.5, 169.9, 169.8, 169.0, 168.6(-$COOCH_3$), 139.1, 138.6, 138.6, 138.5, 138.0, 138.0, 137.9, 137.6($C_{q\ arom}$), 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.3, 127.2(CH_{arom}), 103.3(C-1$_{Mann'}$), 98.1(C-1$_{Mann}$), 96.6(C-1$_{Gul}$), 93.7(C-1$_{Mann}$, β isomer), 92.6(C-1$_{Gul'}$), 79.3(C-3$_{Mann'}$), 78.4(C-4$_{Mann}$), 77.5, 77.4(C-2$_{Mann}$), 77.2, 76.8, 76.7(C-2$_{Gul'}$, C-5$_{Mann'}$), 76.5, 76.1, 75.2, 74.4, 74.2, 74.1, 73.7, 73.3, 73.2, 73.1, 72.9, 72.8, 72.4, 72.3, 72.2, 71.3, 71.2, 70.9(C-4$_{Gul'}$), 67.6(C-5$_{Gul}$), 66.2(C-5$_{Gul'}$), 52.4, 52.3, 52.1, 52.0(-$COOCH_3$), 37.8(CH_2 Lev), 29.7(CH_3CO), 28.0(CH_2 Lev); ^{13}C-HMBC ($CDCl_3$, 100 MHz): 103.3($J_{C1,H1}$ = 158Hz, C-1$_{Mann'}$), 98.1($J_{C1,H1}$ = 169Hz, C-1$_{Mann}$), 96.6($J_{C1,H1}$ = 171Hz, C-1$_{Gul}$), 92.6($J_{C1,H1}$ = 171Hz, C-1$_{Gul'}$). IR (neat): 601, 698, 737, 908, 957, 1028, 1067, 1090, 1119, 1175, 1206, 1238, 1286, 1302, 1331, 1362, 1437, 1454, 1497, 1719, 1744, 2870, 2949, 3030, 3462. HR-MS: [$M+Na^+$] Calculated for $C_{89}H_{96}O_{27}$: 1619.60312; found: 1619.60402.

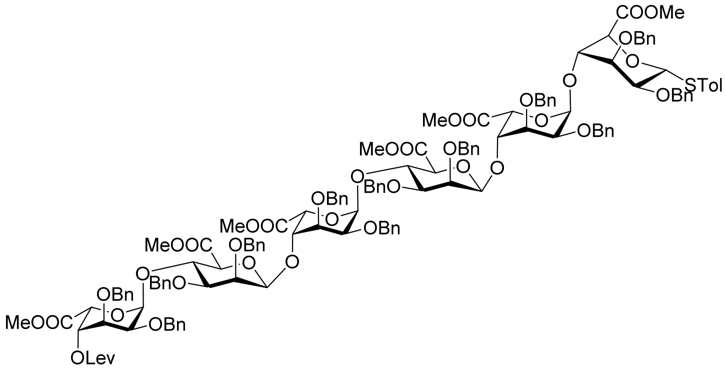


The tetrasaccharide imidate donor 13 was obtained as described for yield *N*-phenyl-trifluoroacetimidate donor (360 mg, 96%, α: β = 2.4:1). TLC: R_f = 0.40 (pentane/DCM/EtOAc, 2/1/1, v/v/v); ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.56 – 7.00 (m, 43H, CH_{arom}), 6.87 – 6.78 (m, 2H, CH_{arom}), 6.46 (bs, 0.80H, H-1$_{Mann,}$ α isomer), 6.26 (bs, 0.34H, H-1$_{Mann,}$ β isomer), 5.31 (t, *J* = 3.6 Hz, 1H, H-1$_{Gul'}$), 5.28 – 5.23 (m, 1H, H-4$_{Gul'}$), 5.21 (d, *J* = 1.9 Hz, 1H, H-5$_{Gul'}$), 5.12 (d, *J* = 4.0 Hz, 0.72H, H-1$_{Gul}$), 5.09 (d, *J* = 4.0 Hz, 0.32H, H-1$_{Gul}$), 4.98 – 4.78 (m, 3H), 4.77 – 4.63 (m, 3H), 4.63 – 4.23 (m, 13H), 4.23 – 4.12 (m, 1H), 4.06 (dd, *J* = 8.2, 4.9 Hz, 1H), 3.95 (t, *J* = 3.0 Hz, 0H), 3.88 (dt, *J* = 14.6, 3.5 Hz, 2H), 3.75 (dd, *J* = 7.7, 4.7 Hz, 2H), 3.67 (t, *J* = 3.7 Hz, 1H), 3.62 (d, *J* = 4.2 Hz,



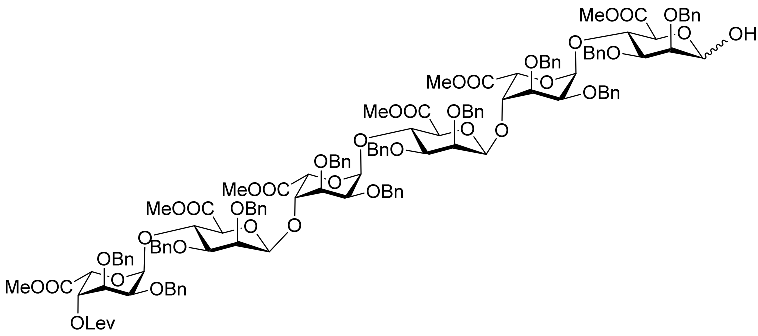
2H), 3.59 (s, 2H), 3.54 (s, 2H), 3.53 (s, 1H), 3.50 (s, 3H), 3.47 (s, 3H), 3.40 – 3.34 (m, 1H), 2.76-2.53(m, 1H), 2.48 – 2.31 (m, 2H), 2.14 (s, 3H); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.2, 171.5, 170.0, 169.9, 169.0, 168.9, 168.6, 139.1, 138.7, 138.6, 138.1, 138.0, 137.7, 137.6, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.3, 128.1, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.5, 127.5, 127.5, 127.3, 127.3, 127.2, 124.3, 123.9, 103.3(C-1$_{Mann'}$), 98.5, 98.0(C-1$_{Gul}$), 96.7(C-1$_{Gul'}$), 94.4(C-1$_{Mann}$) 92.7(C-1$_{Mann}$), 77.5, 77.2, 76.8, 74.2, 73.7, 73.5, 73.0, 72.5, 72.5, 71.5, 71.3, 71.3, 52.3, 52.3, 52.2, 52.1, 37.9, 28.0; ^{13}C-HMBC ($CDCl_3$, 100 MHz): 103.3($J_{C1,H1}$ = 157Hz, C-1$_{Mann'}$), 98.5, 98.0($J_{C1,H1}$ = 169Hz, C-1$_{Gul}$), 96.7($J_{C1,H1}$ = 169Hz, C-1$_{Gul'}$). IR (neat): 601, 638, 696, 735, 777, 908, 961, 1028, 1058, 1092, 1119, 1152, 1206, 1240, 1304, 1323, 1362, 1437, 1454, 1497, 1597, 1719, 1746, 2952. HR-MS: [M+Na$^+$] Calculated for $C_{97}H_{100}F_3NO_{27}$: 1790.63270; found: 1790.63398.

Texasaccharide (16): As described for the general procedure for the glycosylation reactions. Purification by size exclusion and column chromatography (silica gel, pentane/DCM/EtOAc, 3/1/1, v/v/v) yielded **16** as a colourless oil (116 mg, 73%, β:α > 20:1). TLC: R_f = 0.55 (toluene/EtOAc, 4/3, v/v); $[\alpha]^{20}_D$ = +42° (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.52 (d, J = 8.2 Hz, 2H), 7.48 – 6.96 (m, 62H), 5.67 (d, J = 7.8Hz, 1H, H-1$_{Mann}$), 5.29 (d, J = 4.0 Hz, 1H, H-1$_{Gul}$), 5.27 – 5.22 (m, 2H, H-1$_{Gul}$, H-4$_{Gul''}$, 2xH-5$_{Gul}$), 5.20 (d, J = 1.9 Hz, 1H, H-5$_{Gul}$), 5.09 (d, J = 1.7 Hz, 1H, H-1$_{Gul}$), 5.05 (d, J = 4.0 Hz, 1H, H-1$_{Gul}$), 4.97 – 4.81 (m, 3H), 4.81 – 4.65 (m, 4H), 4.64 – 4.21 (m, 21H), 4.15 (dd, J = 3.8, 1.8 Hz, 1H, H-4$_{Gul}$), 4.07 (dd, J = 3.7, 1.8 Hz, 1H, H-4$_{Gul}$), 4.02 (d, J = 8.2 Hz, 1H, H-5$_{Gul}$), 3.97 (d, J = 8.3 Hz, 1H, H-5$_{Gul}$), 3.89 (t, J = 3.7 Hz, 1H), 3.84 (t, J = 3.5 Hz, 1H), 3.81 – 3.69 (m, 2H), 3.68 – 3.61 (m, 2H), 3.58 (d, J = 2.4 Hz, 3H), 3.52 (s, 3H), 3.49 (d, J = 2.7 Hz, 0H), 3.47 (s, 2H), 3.45 (s, 6H), 3.40 (s, 3H), 3.39 – 3.35 (m, 1H, H-3$_{Mann}$), 3.34 – 3.29 (m, 1H, H-3$_{Mann}$), 2.79 – 2.51 (m, 2H), 2.44 (dd, J = 6.4, 4.3 Hz, 2H), 2.27 (s, 3H), 2.14 (s, 3H); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.2, 171.5, 170.2, 169.9, 169.5, 169.0, 168.6, 139.2, 139.1, 138.8, 138.8, 138.5, 138.2, 138.2, 138.1, 138.0, 137.9, 137.8, 137.6, 131.8, 130.4, 129.6, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 127.4, 127.2, 127.2, 103.3, 103.3(C-1$_{Mann''}$, C-1$_{Mann'}$), 97.9, 97.1, 96.6(3xC-1$_{Gul}$), 79.5, 79.3(C-3$_{Mann''}$, C-3$_{Mann'}$), 78.7, 78.3(C-4$_{Gul}$, C-4$_{Gul'}$), 77.5, 77.4($HCCl_3$), 77.2, 76.8($DCCl_3$), 76.3, 76.1(C-5$_{Mann''}$, C-5$_{Mann'}$), 74.9, 74.4, 74.3, 74.3, 74.3, 74.2, 74.2, 74.1, 73.5, 73.3, 73.2, 73.2, 73.0, 72.9, 72.5, 72.5, 72.4, 72.3, 71.4, 71.2, 71.2, 71.1, 71.0, 67.8, 67.4, 66.3, 52.3, 52.2, 52.1, 52.1, 52.0, 51.9, 37.9, 29.8, 28.0, 21.1. IR (neat): 698, 737, 908, 1028, 1057, 1094, 1119, 1207, 1240, 1362, 1437, 1454, 1497, 1747,

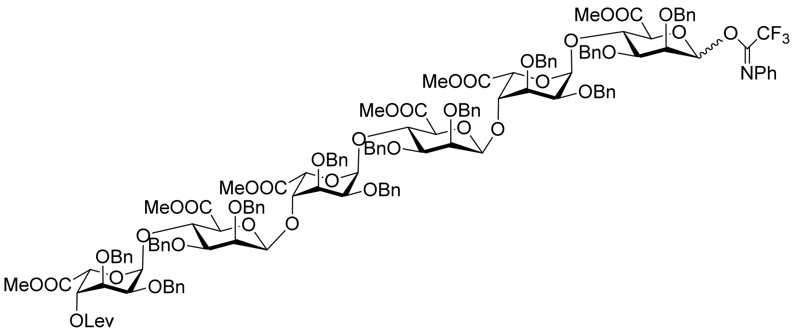


2951. HR-MS: [M+Na$^+$] Calculated for $C_{138}H_{146}SO_{38}$: 2465.91050; found: 2465.89507.

The hexasaccharide was obtained as general procedure for hydrolysis of thioglycosidic bond (74 mg, 81%). TLC: R_f = 0.38 (toluene/EtOAc, 4/3, v/v); $[\alpha]^{20}_{D}$ = -77° (c = 1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.54 – 6.93 (m, 60H), 5.47 (d, *J* = 6.7 Hz, 1H, H-1$_{Mann}$), 5.28 (d, *J* = 4.0 Hz, 1H, H-1$_{Gul}$), 5.27 – 5.21 (m, 2H, H-1$_{Gul}$, H-4$_{Gul''}$), 5.19 (d, *J* = 2.0 Hz, 1H), 5.08 (d, *J* = 1.8 Hz, 1H, H-5$_{Gul}$), 5.05 (d, *J* = 3.8 Hz, 1H, H-1$_{Gul}$), 4.90 – 4.78 (m, 4H), 4.72 (d, *J* = 2.0 Hz, 2H), 4.69 (s, 2H), 4.63 – 4.20 (m, 26H), 4.14 (dd, *J* = 3.8, 1.8 Hz, 1H, H-4$_{Gul}$), 4.07 (dd, *J* = 3.9, 1.5 Hz, 1H, H-4$_{Gul}$), 4.03 (d, *J* = 8.2 Hz, 1H, H-5$_{Mann}$), 3.97 (d, *J* = 8.5 Hz, 1H, H-5$_{Mann}$), 3.88 (t, *J* = 3.6 Hz, 1H), 3.83 (dt, *J* = 7.7, 3.1 Hz, 1H), 3.77 (t, *J* = 3.6 Hz, 1H), 3.68 – 3.61 (m, 3H), 3.59 (s, 2H), 3.57 (d, *J* = 2.5 Hz, 1H), 3.52 (d, *J* = 9.7 Hz, 4H), 3.48 (s, 1H), 3.47 (s, 3H), 3.45 (s, 4H), 3.44 (s, 2H), 3.40 (s, 3H), 3.39 – 3.36 (m, 1H), 3.32 (dd, *J* = 9.2, 2.8 Hz, 1H, H-3$_{Mann}$), 2.81 – 2.53 (m, 3H), 2.44 (m, 2H), 2.14 (s, 3H); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.2, 171.6, 170.2, 169.9, 169.8, 169.0, 168.6, 139.2, 139.1, 138.8, 138.8, 138.6, 138.5, 138.2, 138.1, 138.0, 138.0, 137.9, 137.7, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.3, 127.2, 103.4, 103.3(C-1$_{Mann''}$, C-1$_{Mann'}$), 98.3, 97.1, 96.7(3xC-1$_{Gul}$), 92.7(C-1$_{Mann}$), 79.5, 79.3, 78.3, 77.5, 77.4, 77.2, 76.8, 76.7, 76.3, 76.1, 75.4, 74.5, 74.3, 74.2, 74.2, 73.9, 73.4, 73.4, 73.3, 73.2, 73.1, 73.0, 72.9, 72.9, 72.5, 72.4, 72.4, 71.5, 71.3, 71.2, 71.1, 71.0(C-4$_{Gul''}$), 67.7, 67.4, 66.3(3xC-5$_{Gul}$), 52.3, 52.3, 52.2, 52.1, 52.1, 51.9, 37.9, 29.8, 28.1; ^{13}C-HMBC ($CDCl_3$, 100 MHz): 103.4, 103.3($J_{C1,H1}$ = 158Hz, C-1$_{Mann''}$, C-1$_{Mann'}$), 98.3, 97.1, 96.7($J_{C1,H1}$ = 168Hz, $J_{C1,H1}$ = 171Hz, $J_{C1,H1}$ = 171Hz, 3xC-1$_{Gul}$), 92.7($J_{C1,H1}$ = 171Hz, C-1$_{Mann}$). IR (neat): 698, 737, 910, 1028, 1061, 1098, 1117, 1206, 1238, 1304, 1362, 1454, 1748, 2870, 2949, 3030. HR-MS: [M+Na$^+$] Calculated for $C_{131}H_{140}O_{39}$: 2359.88639; found: 2359.88290.

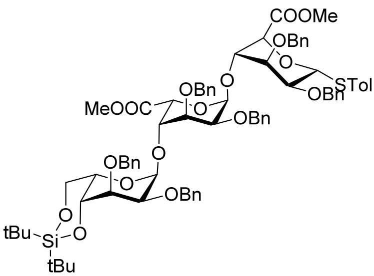


The hexasaccharide imidate donor 17: was obtained as general procedure for yield *N*-phenyl-trifluoroacetimidate



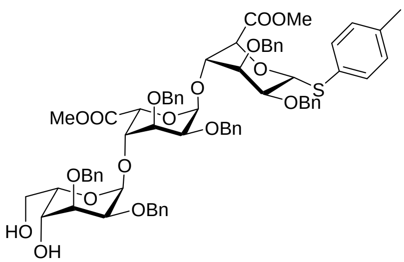
donor (74 mg, 99%, α: β = 3.2:1). TLC: R_f = 0.65 (toluene/EtOAc, 4/3, v/v); $[\alpha]^{20}_D$ = -80° (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.56 – 6.96 (m, 63H), 6.82 (dd, *J* = 8.2, 1.9 Hz, 2H), 6.45 (s, 0.76H, H-1_{Mann} α isomer), 6.25 (s, 0.24H, H-1_{Mann} β isomer), 5.28 (d, *J* = 3.9 Hz, 1H, H-1_{Gul}), 5.27 – 5.21 (m, 2H, H-1_{Gul}, H-$4_{Gul''}$), 5.19 (d, *J* = 1.9 Hz, 1H, H-5_{Gul}), 5.12 – 5.05 (m, 2H, H-1_{Gul}, H-5_{Gul}), 4.94 – 4.77 (m, 3H), 4.78 – 4.63 (m, 4H), 4.64 – 4.19 (m, 18H), 4.15 (dd, *J* = 3.8, 1.9 Hz, 1H), 4.07 (dd, *J* = 3.8, 1.8 Hz, 1H), 4.02 (d, *J* = 8.3 Hz, 1H), 3.97 (d, *J* = 8.3 Hz, 1H), 3.88 (t, *J* = 3.6 Hz, 1H), 3.84 (t, *J* = 3.6 Hz, 1H), 3.80 – 3.69 (m, 2H), 3.64 (t, *J* = 3.6 Hz, 2H), 3.58 (d, *J* = 6.3 Hz, 3H), 3.51 (s, 3H), 3.49 (s, 3H), 3.45 (s, 6H), 3.40 (s, 3H), 3.37 (m, 1H), 3.35 – 3.29 (m, 1H), 2.80 – 2.50 (m, 2H), 2.53 – 2.22 (m, 2H), 2.15 (d, *J* = 9.5 Hz, 3H); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.2, 171.6, 170.3, 170.0, 169.1, 168.6, 139.2, 139.1, 138.8, 138.8, 138.6, 138.2, 138.1, 138.0, 137.8, 137.7, 128.7, 128.7, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.5, 127.2, 127.2, 127.1, 124.3, 123.9, 119.7, 119.6, 103.4, 103.3(C-$1_{Mann'}$, C-$1_{Mann''}$), 98.5, 98.1, 97.1, 96.7(3xC-1_{Gul}), 94.4(C-1_{Mann}), 79.5, 79.3, 78.6, 78.4, 76.8, 76.6, 76.3, 76.1, 75.7, 74.8, 74.4, 74.3, 74.2, 74.2, 73.7, 73.3, 73.3, 73.0, 73.0, 72.5, 72.5, 72.3, 71.3, 71.3, 71.2, 71.2, 71.1, 71.0, 67.7, 67.4, 66.3, 53.9, 53.0, 52.3, 52.3, 52.3, 52.2, 51.9, 46.2, 37.9, 29.8, 29.4, 28.1; ^{13}C-HMBC ($CDCl_3$, 100 MHz): 103.4, 103.3($J_{C1,H1}$ = 158Hz, C-$1_{Mann''}$, C-$1_{Mann'}$), 98.5, 97.1, 96.7($J_{C1,H1}$ = 169Hz, $J_{C1,H1}$ = 169Hz, $J_{C1,H1}$ = 171Hz, 3xC-1_{Gul}). IR (neat): 696, 735, 908, 959, 1028, 1055, 1094, 1117, 1206, 1238, 1304, 1329, 1360, 1454, 1748, 2872. HR-MS: [M+Na$^+$] Calculated for $C_{139}H_{144}F_3NO_{39}$: 2530.91598; found: 2530.91172.

Trisaccharide 19: As described for the general procedure for the glycosylation reactions. **19** was obtained (1.86 g,



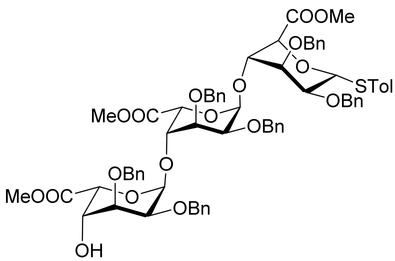
92%). TLC: R_f = 0.10 (Pentane/EtOAc, 10/1, v/v). $[\alpha]^{20}_D$ = -47° (c = 0.98, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.62 – 6.92 (m, 34H, CH_{arom}), 5.68 (d, *J* = 7.9 Hz, 1H, H-1_{Mann}), 5.24 – 5.11 (m, 1H, H-1_{Gul}), 5.11 – 4.94 (m, 2H, H-$1_{Gul'}$, C*H*H Bn), 4.83 (d, *J* = 11.9 Hz, 1H, CH*H* Bn), 4.78 – 4.42 (m, 8H), 4.41 – 3.99 (m, 4H), 4.00 – 3.26 (m, 17H), 2.26 (s, 3H, CH_3 STol), 1.03 (s, 9H, 3xCH_3 *tert*-Bu), 0.91 (s, 9H, 3xCH_3 *tert*-Bu); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.0, 169.7(-COO-), 139.6, 138.9, 138.3, 138.2, 138.1, 137.9($C_{q\ arom}$), 131.8(CH_{arom}), 130.4($C_{q\ arom}$), 129.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.0, 127.9, 127.8, 127.6, 127.6, 127.5(CH_{arom}), 99.9(C-1_{Gul}), 97.2(C-1_{Gul}), 77.6, 77.3, 75.8, 75.3, 75.2, 74.9, 74.1, 73.7, 73.2, 72.5, 72.5, 71.6, 71.1, 68.2(C-$5_{Gul'}$), 66.8(C-$6_{Gul'}$), 64.8(C-5_{Gul}), 52.1(-$COOCH_3$), 27.7(CH_3 *tert*-Bu), 27.3(CH_3 *tert*-Bu), 23.3(C_q *tert*-Bu), 21.2(CH_3 STol), 20.4(C_q *tert*-Bu). IR (neat): 650, 698, 739, 799, 827, 868, 1028, 1088, 1117, 1140, 1207, 1306, 1364, 1454, 1472, 1494, 1734, 1753, 2859, 2932. HR-MS: [M+Na^+] Calculated for $C_{77}H_{90}O_{17}SSi$: 1369.55602; found: 1369.55666.

Trisaccharide 20: was obtained as described by the general procedure for removal of the di-*tert*-butyl silylidene



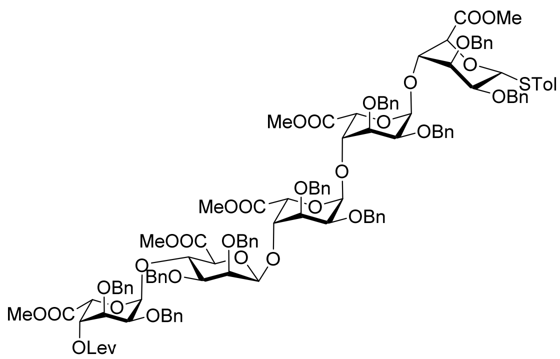
group (1.61 mg, 99%). TLC: R_f = 0.40 (pentane/EtOAc, 1/1, v/v). $[\alpha]^{20}_D$ = -44° (c = 0.92, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.70 – 6.72 (m, 34H, CH_{arom}), 5.68 (d, *J* = 7.9 Hz, 1H), 5.26 – 4.97 (m, 2H), 4.91 (d, *J* = 11.3 Hz, 1H), 4.87 – 4.29 (m, 11H), 4.29 – 3.94 (m, 2H), 3.91 – 3.28 (m, 15H), 2.25 (s, 3H, CH_3 STol)); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.0, 169.7(-COO-), 139.4, 138.9, 138.3, 138.2, 138.2, 137.9($C_{q\ arom}$), 131.9(CH_{arom}), 130.2($C_{q\ arom}$), 129.6, 128.3, 128.3, 127.9, 127.8, 127.6, 127.5, 127.3(CH_{arom}), 100.0(C-1_{Gul}), 97.2(C-1_{Gul}), 77.4, 75.0, 74.8, 74.6, 74.3, 74.1, 73.9, 73.0, 72.8, 72.5, 72.4, 72.1, 71.7, 71.4, 68.1, 66.4, 64.0, 52.1(-$COOCH_3$), 21.1(CH_3 STol). IR (neat): 612, 698, 735, 779, 810, 914, 949, 1028, 1074, 1088, 1103, 1209, 1242, 1282, 1306, 1323, 1362, 1393, 1437, 1454, 1495, 1734, 1749, 2870, 2922. HR-MS: [M+Na^+] Calculated for $C_{69}H_{74}O_{17}S$: 1229.45389; found: 1229.45418.

The trisaccharide acceptor 21 was obtained as described by the general procedure for oxidation and methylation



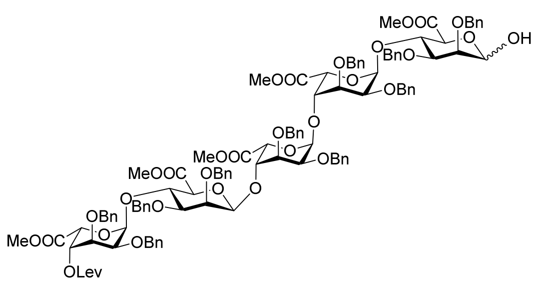
(85 mg, 76%). TLC: R_f = 0.38 (pentane/DCM/EtOAc, 2/1/1, v/v). $[\alpha]^{20}_D$ = -42° (c = 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.52 (d, *J* = 8.3 Hz, 2H, CH_{arom}), 7.47 – 7.08 (m, 30H, CH_{arom}), 7.03 (d, *J* = 8.4 Hz, 2H, CH_{arom}), 5.68 (d, *J* = 7.9 Hz, 1H), 5.16 (dd, *J* = 17.4, 3.7 Hz, 2H), 4.85 (dd, *J* = 11.6, 5.1 Hz, 2H), 4.70 (bs, 1H), 4.68 – 4.09 (m, 15H), 4.00 (s, 1H), 3.96 – 3.60 (m, 7H), 3.47 (d, *J* = 27.9 Hz, 6H), 2.26 (s, 3H, CH_3 STol); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.3, 169.7, 169.6(-COO-), 139.0, 138.9, 138.2, 138.1, 138.0, 137.9, 137.0($C_{q\ arom}$), 131.8(CH_{arom}), 130.4($C_{q\ arom}$), 129.6, 128.4, 128.2, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.5(CH_{arom}), 100.1(C-1_{Gul}), 97.5(C-1_{Gul}), 78.1, 77.4, 75.0, 75.0, 74.8, 74.8, 74.1, 73.9, 73.8, 73.4, 72.9, 72.5, 71.9, 71.7, 69.9, 68.7, 68.0, 52.5, 52.1, 52.0(-$COOCH_3$), 21.1(CH_3 STol). IR (neat): 698, 737, 1028, 1076, 1092, 1119, 1209, 1240, 1306, 1358, 1454, 1495, 1736, 1751, 2870, 3030, 3497. HR-MS: [M+Na^+] Calculated for $C_{70}H_{74}O_{18}S$: 1257.44881; found: 1257.44898.

Pentasaccharide 22 was obtained as described for the general procedure for the glycosylation reactions.



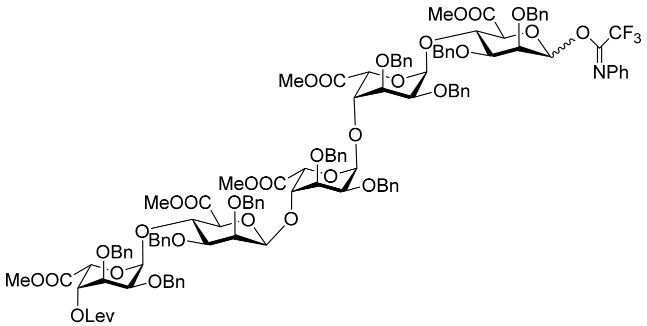
Purification by size exclusion (LH-20, DCM/MeOH, 1:1) and column chromatography (silica gel, DCM/acetone, 30/1, v/v) yielded **29** as a colourless syrup (112 mg, 87%, β:α > 20:1). TLC: R_f = 0.58 (toluene/EtOAc, 4/3, v/v); $[\alpha]^{20}_{D}$ = -66° (c = 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.69 – 6.72 (m, 54H, CH_{arom}), 5.69 (d, *J* = 8.2 Hz, 1H, H-1_{Mann}), 5.37 – 4.96 (m, 5H, H-1_{Gul}, H-$1_{Gul'}$, H-$1_{Gul''}$, H-$4_{Gul''}$, H-$5_{Gul''}$), 4.93 – 4.78 (m, 3H), 4.73 (s, 1H), 4.70 (s, 1H), 4.65 (s, 1H), 4.62 – 4.49 (m, 7H), 4.45 (s, 3H), 4.41 – 4.33 (m, 2H), 4.33 – 4.23 (m, 3H), 4.16 (dd, *J* = 4.1, 1.8 Hz, 1H), 4.12 (d, *J* = 2.0 Hz, 1H), 4.05 (d, *J* = 8.2 Hz, 1H), 3.90 (dd, *J* = 8.1, 4.6 Hz, 2H), 3.83 (dd, *J* = 4.0, 2.8 Hz, 1H), 3.78 – 3.74 (m, 1H), 3.73 (t, *J* = 3.3 Hz, 1H), 3.70 – 3.52 (m, 6H), 3.50 (d, *J* = 0.9 Hz, 6H), 3.46-3.40 (m, 4H), 3.37 (s, 3H), 2.89 – 2.49 (m, 2H), 2.43 (m, 2H), 2.26 (s, 3H), 2.14 (s, 3H); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.2(C=O Lev), 171.5, 169.7, 169.6, 169.5, 169.0, 168.6(-$COOCH_3$), 139.2, 139.0, 138.7, 138.5, 138.2, 138.2, 138.1, 137.9, 137.9, 137.7($C_{q\ arom}$), 131.7(CH_{arom}), 130.4($C_{q\ arom}$), 129.5, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 127.2(CH_{arom}), 103.3(C-$1_{Mann'}$), 100.1, 97.4, 96.6(3xC-1_{Gul}), 79.4, 78.7, 78.0, 77.5, 77.4, 77.2, 76.8, 76.2, 75.2, 74.8, 74.2, 74.2, 73.7, 73.4, 73.3, 73.3, 73.1, 73.0, 72.5, 72.5, 72.4, 72.4, 71.7, 71.3, 71.3, 71.0, 71.0(C-$4_{Gul''}$), 68.1, 67.8, 66.3(3xC-5_{Gul}), 52.3, 52.3, 52.1, 52.0, 51.9(-$COOCH_3$), 37.9(CH_2 Lev), 29.8(CH_3CO), 28.0(CH_2 Lev), 21.1(CH_3 STol); ^{13}C-HMBC ($CDCl_3$, 100 MHz): 103.3($J_{C1,H1}$ = 158Hz, C-$1_{Mann'}$), 100.1, 97.4, 96.6 ($J_{C1,H1}$ = 170Hz, 169Hz, 172Hz). IR (neat): 696, 735, 810, 910, 953, 1026, 1063, 1090, 1117, 1177, 1207, 1238, 1306, 1360, 1454, 1744, 2870, 2922. HR-MS: [M+H^+] Calculated for $C_{117}H_{124}O_{32}S$: 2073.78692; found: 2073.78474.

This pentasaccharide was obtained as general procedure for hydrolysis of thioglycosidic bond (67 mg, 98%). TLC: R_f = 0.50 (DCM/acetone, 10/1, v/v); $[\alpha]^{20}_{D}$ = -75° (c = 0.58, $CHCl_3$).



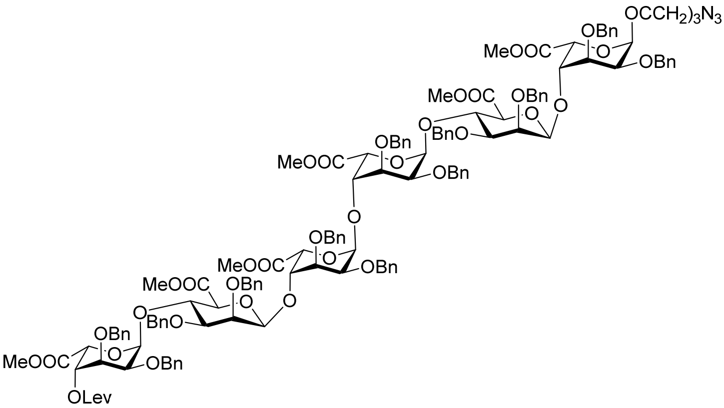
^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.52 – 6.95 (m, 50H), 5.47 (d, *J* = 7.0 Hz, 1H, H-1_{Mann}), 5.27 (d, *J* = 4.0 Hz, 1H, H-1_{Gul}), 5.24 (dd, *J* = 3.9, 2.0 Hz, 1H, H-$4_{Gul''}$), 5.19 (d, *J* = 2.0 Hz, 1H, H-5_{Gul}), 5.17 (d, *J* = 3.9 Hz, 1H, H-1_{Gul}), 5.13 (d, *J* = 3.9 Hz, 1H, H-1_{Gul}), 4.86 (dt, *J* = 11.8, 4.7 Hz, 3H), 4.73 (d, *J* = 2.3 Hz, 1H), 4.70 (d, *J* = 2.5 Hz, 1H), 4.63 (d, *J* = 1.9 Hz, 1H), 4.60 – 4.13 (m, 19H), 4.11 (d, *J* = 12.0 Hz, 1H), 4.05 (d, *J* = 8.3 Hz, 1H), 3.89 (d, J = 3.4 Hz, 2H), 3.87 – 3.77 (m, 2H), 3.72 (dd, J = 5.3, 3.2 Hz, 1H), 3.69 – 3.58 (m, 5H), 3.50 (d, J = 4.0 Hz, 5H), 3.38 (d, J = 4.6 Hz, 3H), 2.68 – 2.56 (m, 2H), 2.53 – 2.30 (m, 2H), 2.15 (s, 3H); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.3, 171.6, 170.0, 169.8, 169.5, 169.1, 168.7, 139.3, 139.1, 138.7, 138.6, 138.6, 138.1, 138.0, 138.0, 137.7, 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.3, 103.4(C-$1_{Mann'}$), 100.2, 98.0, 96.7(3xC-1_{Gul}), 92.7(C-1_{Mann}), 79.4, 78.7, 77.8, 77.5, 77.2, 76.9, 76.8, 76.2, 75.6, 75.4, 74.3, 74.1, 73.7, 73.3, 73.2, 73.2, 73.0, 72.8, 72.6, 72.5, 72.4, 71.7, 71.4, 71.3, 71.1, 71.0, 68.0, 67.8, 66.3, 52.4, 52.2, 52.0, 52.0, 37.9, 29.8, 29.7, 28.1; ^{13}C-HMBC ($CDCl_3$, 100 MHz): 103.4 ($J_{C1,H1}$ = 158Hz, C-$1_{Mann'}$), 100.2, 98.0, 96.7 ($J_{C1,H1}$ = 171Hz, $J_{C1,H1}$ = 167Hz, $J_{C1,H1}$ = 175Hz, 3xC-1_{Gul}), 92.7 ($J_{C1,H1}$ = 170Hz, C-1_{Mann}). IR (neat): 698, 739, 908, 1028, 1094, 1119, 1209, 1238, 1306, 1360, 1437, 1454, 1497, 1719, 1748, 2872, 2926, 3030. HR-MS: [M+Na$^+$] Calculated for $C_{110}H_{118}O_{33}$: 1389.74476; found: 1389.74550.

The pentasaccharide imidate donor 23 was obtained as general procedure for yield *N*-phenyl-trifluoroacetimidate donor (65 mg, 84%, α: β = 2.4:1).

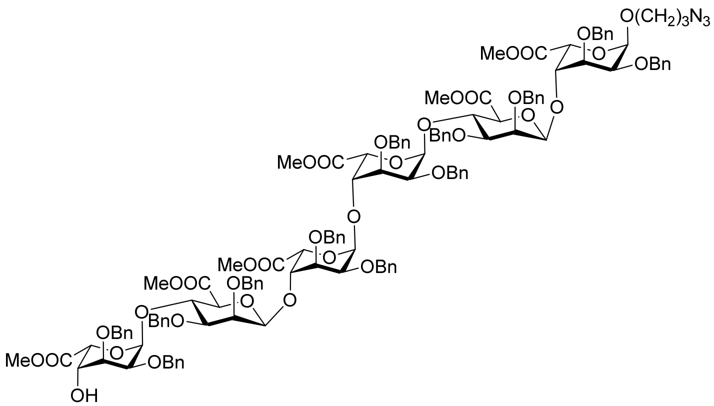


TLC: R_f = 0.33 (pentane/DCM/EtOAc, 2/1/1, v/v/v); ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.54 – 7.00 (m, 53H), 6.88 – 6.78 (m, 2H), 6.46 (s, 0.55H, H-1_{Mann} α isomer), 6.26 (s, 0.23H, H-1_{Mann} β isomer), 5.28 (d, J = 3.5 Hz, 1H, H-1_{Gul}), 5.24 (dd, J = 3.7, 1.8 Hz, 1H, H-$4_{Gul''}$), 5.20 (d, J = 1.9 Hz, 1H, H-5_{Gul}), 5.17 (d, J = 3.9 Hz, 2H, 2xH-1_{Gul}), 4.87 (dd, J = 11.7, 6.0 Hz, 2H), 4.77 – 4.64 (m, 3H), 4.61 – 4.19 (m, 15H), 4.18 – 4.11 (m, 1H), 4.11 – 4.02 (m, 2H), 3.90 (dt, J = 7.4, 3.5 Hz, 2H), 3.86 – 3.80 (m, 1H), 3.76 – 3.69 (m, 2H), 3.69 – 3.57 (m, 4H), 3.55 – 3.48 (m, 5H), 3.46 (s, 2H), 3.39 (d, J = 9.0 Hz, 3H), 2.79 – 2.57 (m, 2H), 2.51 – 2.36 (m, 2H), 2.15 (s, 3H); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.2, 171.6, 169.8, 169.6, 169.1, 169.1, 168.7, 139.3, 139.0, 138.7, 138.6, 138.2, 138.1, 138.1, 138.0, 138.0, 137.7, 137.7, 128.7, 128.7, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.5, 127.4, 127.3, 127.2, 127.1, 119.6, 103.4(C-$1_{Mann'}$), 100.4, 100.2, 98.3, 97.6, 96.7(C-1_{Gul}), 94.3(C-1_{Mann}), 79.4, 78.8, 78.0, 77.5, 77.2, 76.8, 76.6, 76.2, 75.5, 74.9, 74.4, 74.2, 74.2, 73.7, 73.5, 73.4, 73.3, 73.2, 73.0, 73.0, 72.7, 72.5, 72.4, 71.6, 71.3, 71.3, 71.1, 68.0, 67.8, 66.3, 52.4, 52.3, 46.2, 37.9, 30.4, 29.8, 28.1; ^{13}C-HMBC ($CDCl_3$, 100 MHz): 103.4 ($J_{C1,H1}$ = 158Hz, C-$1_{Mann'}$), 100.2, 98.3, 97.6 ($J_{C1,H1}$ = 170Hz, $J_{C1,H1}$ = 168Hz, $J_{C1,H1}$ = 171Hz, 3xC-1_{Gul}). IR (neat): 696, 737, 910, 1028, 1063, 1072, 1092, 1117, 1206, 1240, 1306, 1360, 1437, 1454, 1497, 1720, 1744, 2855, 2924. HR-MS: [M+Na$^+$] Calculated for $C_{118}H_{122}F_3NO_{33}$: 2160.77434; found: 2160.77214.

Hexasaccharide 24 was obtained as described general procedure for the glycosylation reactions. Purification by column chromatography (silica gel, pentane/DCM/EtOAc, 3/1/1, v/v/v) yielded **24** (mg, 43%, β:α > 20:1). $[\alpha]^{20}_{D}$ = -95° (c = 0.44, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.70 – 6.89 (m, 60H), 5.33 (d, *J* = 4.0 Hz, 1H, H-1_{Gul}), 5.27 (d, *J* = 3.8 Hz, 1H, H-1_{Gul}), 5.23 (dd, *J* = 3.8, 1.9 Hz, 1H, H-$4_{Gul'''}$), 5.19 (d, *J* = 2.0 Hz, 1H, H-5_{Gul}), 5.06 (d, *J* = 4.1 Hz, 1H, H-1_{Gul}), 5.03 (d, *J* = 1.9 Hz, 1H, H-5_{Gul}), 4.90 – 4.80 (m, 6H, H-1_{Gul}, CH_2 Bn), 4.76 (d, *J* = 1.6 Hz, 1H, H-5_{Gul}), 4.74 – 4.65 (m, 5H, CH_2 Bn), 4.64 – 4.21 (m, 25H, 2xH-1_{Mann}, 2xH-4_{Mann}, 2xH-3_{Gul}, CH_2 Bn), 4.18 (t, *J* = 3.6 Hz, 1H, H-4_{Gul}), 4.11 (m, 2H, 2xH-4_{Gul}), 4.06 – 3.94 (m, 3H, 2xH-5_{Mann}, CH_2 Bn), 3.92 – 3.75 (m, 5H, 2xH-3_{Gul}, 2xH-2_{Gul}, -O$CH_2$$CH_2CH_2N_3$), 3.70 – 3.63 (m, 5H, 2xH-2_{Gul}, CH_3OCO), 3.60 (d, *J* = 3.4 Hz, 5H, 2xH-2_{Mann}, CH_3OCO), 3.50 (s, 3H, CH_3OCO), 3.48 (s, 3H, CH_3OCO), 3.45 (d, *J* = 1.6 Hz, 4H, CH_3OCO, -O$CH_2$$CH_2CH_2N_3$), 3.41 (d, *J* = 1.0 Hz, 1H), 3.40 – 3.27 (m, 4H, 2xH-3_{Mann}, -$OCH_2CH_2$$CH_2$$N_3$), 3.21 (s, 3H, CH_3OCO), 2.65 (dd, *J* = 15.2, 6.4 Hz, 2H, CH_2 Lev), 2.59 – 2.26 (m, 2H, CH_2 Lev), 2.15 (s, 3H, CH_3CO), 2.00 – 1.46 (m, 2H, -$OCH_2$$CH_2$$CH_2N_3$).^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 206.3(C=O Lev), 171.6, 170.3, 169.9, 169.8, 169.1, 168.8, 168.7(-$COOCH_3$), 139.3, 139.3, 139.1, 138.8, 138.7, 138.6, 138.3, 138.1, 138.0, 137.9, 137.8($C_{q\ arom}$), 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 127.4, 127.3, 127.3(CH_{arom}), 103.5, 103.4(2xC-1_{Mann}), 100.2, 98.2, 96.8, 96.7(4xC-1_{Gul}), 79.7, 79.5(2xC-3_{Mann}), 78.8, 78.2(3xC-4_{Gul}), 76.2(2xC-5_{Mann}), 75.5, 74.9(2xC-3_{Gul}), 74.3(2xC-2_{Mann}), 74.2(CH_2Bn), 73.9,73.8(C-2_{Gul}, 2xC-4_{Mann}), 73.6(CH_2Bn), 73.5(C-3_{Gul}), 73.0(CH_2Bn), 72.8, 72.5(3xC-2_{Gul}), 72.4(C-3_{Gul}), 71.6, 71.5, 71.3, 71.1(CH_2Bn), 71.0(C-$4_{Gul'''}$), 67.7, 67.1, 66.3(3xC-5_{Gul}), 65.3(-O$CH_2$$CH_2CH_2N_3$), 52.4, 52.3, 51.8(6x-$COOCH_3$), 48.4(-$OCH_2CH_2$$CH_2$$N_3$), 38.0($CH_2$ Lev), 29.8(CH_3CO), 28.7(-$OCH_2$$CH_2$$CH_2N_3$), 28.0($CH_2$ Lev); ^{13}C-HMBC ($CDCl_3$, 100 MHz): 103.4 ($J_{C1,H1}$ = 158Hz, C-$1_{Mann'}$), 100.2, 98.3, 97.6 ($J_{C1,H1}$ = 170Hz, $J_{C1,H1}$ = 168Hz, $J_{C1,H1}$ = 171Hz, 3xC-1_{Gul}). IR (neat): 698, 737, 912, 1028, 1063, 1094, 1117, 1207, 1238, 1304, 1360, 1437, 1454, 1497, 2095, 2855, 2924, 3030. HR-MS: [M+Na^+] Calculated for $C_{134}H_{145}N_3O_{39}$: 2442.93494; found: 2442.92607.



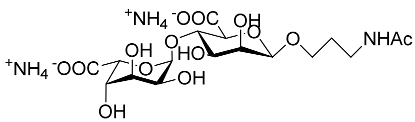
25 was obtained as described by the general procedure for delevulinoylation, saponification, high pressure hydrogenation and acetylation of oligosaccharides as a white solid. **25**, (25 mg, 87%).



$[\alpha]^{20}_{D}$ = -76° (c = 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz,HH-COSY, HSQC): δ 7.58 – 6.94 (m, 60H), 5.33 (d, *J* = 4.0 Hz, 1H, H-1_{Gul}), 5.28 (d, *J* = 4.0 Hz, 1H, H-1_{Gul}), 5.17 – 4.97 (m, 3H, H-1_{Gul}, 2xH-5_{Gul}), 4.91 – 4.81 (m, 6H, H-1_{Gul}, CH_2 Bn), 4.76 (d, *J* = 1.8 Hz, 1H, H-5_{Gul}), 4.73 (d, *J* = 3.1 Hz, 1H, CH_2 Bn), 4.70 (d, *J* = 3.1 Hz, 1H, CH_2 Bn), 4.68 – 4.22 (m, 27H, 2xH-1_{Mann}, 2xH-4_{Mann}, 2xH-3_{Gul}, CH_2 Bn), 4.21 – 4.06 (m, 4H, 4xH-4_{Gul}), 4.03 (d, *J* = 8.3 Hz, 1H, H-5_{Mann}), 3.99 – 3.93 (m, 1H, H-5_{Mann}), 3.86 (dt, *J* = 13.1, 3.8 Hz, 2H, 2xH-3_{Gul}), 3.79 (dt, *J* = 12.5, 4.1 Hz, 4H, -O$CH_2CH_2CH_2N_3$, 3xH-2_{Gul}), 3.72 – 3.56 (m, 9H, H-2_{Gul}, 2xH-2_{Mann}, 2xCH_3OCO), 3.53 (s, 3H, CH_3OCO), 3.48 (d, *J* = 1.3 Hz, 7H, 2xCH_3OCO, -O$\mathit{CH_2}CH_2CH_2N_3$), 3.43 – 3.27 (m, 4H, 2xH-3_{Mann}, -O$CH_2CH_2\mathit{CH_2}N_3$), 3.21 (s, 3H, CH_3OCO), 2.01 – 1.71 (m, 1H, -O$CH_2\mathit{CH_2}CH_2N_3$); ^{13}C –APT NMR ($CDCl_3$, 100 MHz, HSQC): δ 170.5, 170.3, 169.9, 169.8, 168.8, 168.7(-$COOCH_3$), 139.3, 139.3, 139.1, 138.9, 138.8, 138.7, 138.3, 138.1, 138.1, 137.9, 137.9($C_{q\ arom}$), 128.5, 128.5, 128.4, 128.4, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4, 127.3, 127.3(CH_{arom}), 103.5, 103.4(2xC-1_{Mann}), 100.2, 98.2, 97.0, 96.8(4xC-1_{Gul}), 79.7, 79.3(2xC-3_{Mann}), 78.8, 78.2(3xC-4_{Gul}), 77.5, 77.2, 76.8, 76.3, 76.2(2xC-5_{Mann}), 75.5, 75.2, 74.9(3xC-3_{Gul}), 74.3, 74.2(2xC-2_{Mann}), 74.2(CH_2Bn), 73.9, 73.9(2xC-2_{Gul}), 73.6, 73.5, 73.3, 73.0(CH_2Bn , 2xC-2_{Mann}), 72.9(CH_2Bn), 72.8(2xC-2_{Gul}), 71.7, 71.6, 71.5, 71.4, 71.1(CH_2Bn), 70.0(C-$4_{Gul'''}$), 68.1, 67.7, 67.1(4xC-5_{Gul}), 65.3(-O$\mathit{C}H_2CH_2CH_2N_3$), 52.4, 52.4, 52.3, 51.8(6x-$COOCH_3$), 48.4(-O$CH_2CH_2\mathit{C}H_2N_3$), 28.7(-O$CH_2\mathit{C}H_2CH_2N_3$). IR (neat): 698, 737, 910, 1028, 1065, 1115, 1207, 1238, 1306, 1360, 1437, 1454, 1497, 1752, 2095, 2855, 2922, 3030. HR-MS: [M+Na^+] Calculated for $C_{129}H_{139}N_3O_{37}$: 2344.89796; found: 2344.89193.

Disaccharide 26

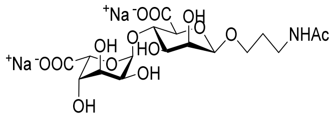
26-NH_4^+: was obtained as described the general procedure for saponification, high-pressure hydrogenation and acetylation of oligosaccharides as a white solid (10.9 mg, three steps yield: 62%).



^{1}H NMR (400 MHz, D_2O, HH-COSY, HSQC): δ 5.06 – 4.95 (bs, 1H, H-1_{Gul}), 4.71 (d, *J* = 1.9 Hz, 1H, H-4_{Gul}), 4.66 (bs, 1H, H-1_{Mann}), 4.15 (bs, 1H, H-3_{Gul}), 3.98 – 3.84 (m, 4H, H-2_{Gul}, H-2_{Mann}, H-5_{Gul}, -O$\mathit{CH_2}CH_2CH_2$NHAc), 3.81 – 3.59 (m, 4H, H-5_{Mann}, H-4_{Mann} , H-3_{Mann}, -O$\mathit{CH_2}CH_2CH_2$NHAc), 3.37 –

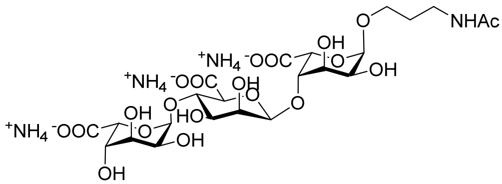
3.11 (m, 2H, $-OCH_2CH_2CH_2NHAc$), 1.95 (s, 3H, CH_3CO), 1.78 (t, *J* = 6.4 Hz, 2H, $-OCH_2CH_2CH_2NHAc$); ^{13}C–APT NMR (D_2O, 100 MHz, HSQC): δ 176.1, 175.7, 174.0(-COO-), 99.7(C-1_{Mann}), 99.5(C-1_{Gul}), 77.8(C-4_{Mann}), 75.8(C-5_{Mann}), 71.9(C-3_{Mann}), 70.8(C-3_{Gul}), 70.6(C-2_{Mann}), 70.3(C-5_{Gul}), 68.0(C-4_{Gul}), 67.2($-OCH_2CH_2CH_2NHAc$), 64.7(C-2_{Gul}), 36.2($-OCH_2CH_2CH_2NHAc$), 28.1($-OCH_2CH_2CH_2NHAc$), 21.8(CH_3CO); ^{13}C-HMBC ($CDCl_3$, 100 MHz): 99.7($J_{C1,H1}$ = 160Hz, C-1_{Mann}), 99.5($J_{C1,H1}$ = 170, C-1_{Gul}). HR-MS: [M+H$^+$] Calculated for $C_{17}H_{27}O_{14}N$: 470.15043; found: 470.15015.

26-Na$^+$: 82 mg, yield: 74%.^{1}H NMR (600 MHz, D_2O) δ 5.16 – 5.13 (m, 1H), 4.83 (d, *J* = 1.9 Hz, 1H), 4.81 (d, *J* = 1.1 Hz, 1H), 4.30 (t, *J* = 1.7 Hz, 1H), 4.11 (dd, *J* = 3.1, 1.0 Hz, 1H), 4.09 – 4.06 (m, 2H), 4.06 – 4.01 (m, 1H), 3.94 – 3.91 (m, 2H), 3.91 – 3.87 (m, 1H), 3.84 – 3.79 (m, 1H), 3.47 – 3.41 (m, 1H), 3.38-3.33 (m, 1H), 2.10 (s, 3H), 1.93 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 177.3, 176.8, 175.1, 100.72, 100.67, 79.0, 76.9, 73.0, 71.8, 71.6, 71.3, 69.1, 68.2, 65.8, 37.2, 29.2, 22.9.

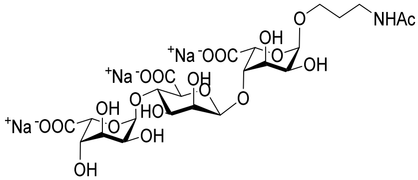


Trisaccharide 29

29-NH$_4^+$: was obtained as described the general procedure for saponification, high pressure hydrogenation and acetylation of oligosaccharides as a white solid (9.6 mg, three steps yield: 43%). ^{1}H NMR (400 MHz, D_2O, HH-COSY, HSQC): δ 4.96 (bs, 1H, H-$1_{Gul'}$), 4.90 (d, *J* = 4.3 Hz, 1H, H-1_{Gul}), 4.68 (d, *J* = 4.5 Hz, 2H, H-1_{Mann}, H-$4_{Gul'}$,), 4.42 (d, *J* = 1.6 Hz, 1H, H-5_{Gul}), 4.25 – 4.08 (m, 3H, H-3_{Gul}, H-4_{Gul}, H-$3_{Gul'}$), 3.99 (t, *J* = 3.9 Hz, 1H, H-2_{Gul}), 3.89 (d, *J* = 3.4 Hz, 3H, H-2_{Mann}, H-$5_{Gul'}$, H-$2_{Gul'}$), 3.81 – 3.66 (m, 4H, H-5_{Mann}, H-4_{Mann}, H-3_{Mann}, $-OCH_2CH_2CH_2NHAc$), 3.52 (m, 1H, $-OCH_2CH_2CH_2NHAc$), 3.34 – 3.08 (m, 2H, $-OCH_2CH_2CH_2NHAc$), 1.93 (s, 3H, CH_3CO), 1.79 (dt, *J* = 7.8, 3.6 Hz, 2H, $-OCH_2CH_2CH_2NHAc$); ^{13}C –APT NMR (D_2O, 100 MHz, HSQC): δ 176.2, 175.9, 175.7, 174.0(-CO-), 101.2(C-1_{Mann}), 99.5(C-$1_{Gul'}$), 98.7(C-1_{Gul}), 80.4(C-4_{Gul}), 77.5(C-4_{Mann}), 75.8(C-5_{Mann}), 71.5(C-3_{Mann}), 70.8(C-$3_{Gul'}$), 70.7, 70.4(C-$5_{Gul'}$, C-2_{Mann}), 69.50(C-3_{Gul}), 68.0(C-$4_{Gul'}$), 66.9($-OCH_2CH_2CH_2NHAc$), 66.70(C-5_{Gul}), 64.7(C-$2_{Gul'}$), 64.4(C-2_{Gul}), 36.9($-OCH_2CH_2CH_2NHAc$), 27.9($-OCH_2CH_2CH_2NHAc$), 21.8(CH_3CO); ^{13}C-HMBC ($CDCl_3$, 100 MHz): 101.2($J_{C1,H1}$ = 161Hz, C-1_{Mann}), 99.5($J_{C1,H1}$ = 171Hz, C-$1_{Gul'}$), 98.7($J_{C1,H1}$ = 170Hz, C-1_{Gul}). HR-MS: [M+H$^+$] Calculated for $C_{23}H_{35}O_{20}N$: 646.18252; found: 646.18250.



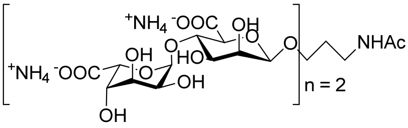
29-Na$^+$: 10.9 mg, yield: quantitative.^{1}H NMR (600 MHz, D_2O) δ 5.16 – 5.13 (m, 1H), 5.07 (d, *J* = 3.9 Hz, 1H), 4.84 (dd, *J* = 6.1, 1.4 Hz, 1H), 4.58 (d, *J* = 1.7 Hz, 1H), 4.38-4.36(m, 1H), 4.33 (m, 1H), 4.32 – 4.27 (m, 1H), 4.17 (t, *J* = 3.9 Hz, 1H), 4.09 – 4.04 (m, 3H), 3.97 – 3.85 (m, 4H), 3.69 (m, 1H), 3.48 – 3.33 (m, 2H), 2.10 (s, 3H), 1.96 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 177.3, 177.0, 176.8, 175.0, 102.3, 100.6, 99.7, 81.5,



78.6, 76.9, 72.5, 71.8, 71.7, 71.4, 70.6, 69.1, 68.0, 67.7, 65.8, 65.4, 38.0, 29.0, 22.8.

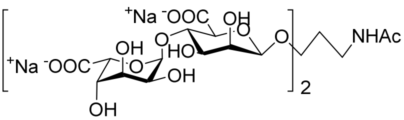
Tetrasaccharide 27

27-NH_4^+ was obtained as described by the general procedure for saponification, high pressure hydrogenation and



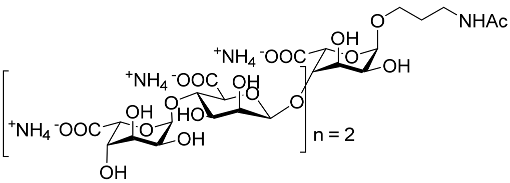
acetylation of oligosaccharides as a white solid (10.6 mg, three steps yield: 51%). ^{1}H NMR (400 MHz, D_2O, HH-COSY, HSQC): δ 5.03 (m, 2H, H-1_{Gul}, H-$1_{Gul'}$), 4.84(bs, 1H, H-$4_{Gul'}$), 4.82 (bs, 1H, H-5_{Gul}), 4.71 (bs, 1H, H-1_{Mann}), 4.68 (bs, 1H, H-$1_{Mann'}$), 4.27 – 4.20 (m, 1H, H-4_{Gul}), 4.18 (m, 2H, H-3_{Gul}, H-$3_{Gul'}$), 4.02 – 3.72 (m, 12H, H-$5_{Gul'}$, H-2_{Gul}, H-$2_{Gul'}$, H-2_{Mann}, H-$2_{Mann'}$, H-3_{Mann}, H-$3_{Mann'}$, H-4_{Mann}, H-$4_{Mann'}$, H-5_{Mann}, H-$5_{Mann'}$, -O$CH_2$$CH_2$$CH_2$NHAc), 3.70 – 3.59 (m, 1H, -O$CH_2$$CH_2$$CH_2$NHAc), 3.34 – 3.12 (m, 2H, -O$CH_2CH_2$$CH_2$NHAc), 1.96 (s, 3H, CH_3CO), 1.79 (q, J = 6.5 Hz, 2H, -O$CH_2$$CH_2$$CH_2$NHAc); ^{13}C –APT NMR ($D_2O$, 100 MHz, HSQC): δ 175.2, 174.8, 174.0(-CO-), 101.2, 99.8(C-$1_{Mann,}$ C-$1_{Mann'}$), 99.4(C-1_{Gul}, C-$1_{Gul'}$), 79.9(C-4_{Gul}), 77.4, 77.3 (C-$4_{Mann,}$ C-$4_{Mann'}$), 75.4, 75.3(C-$5_{Mann,}$ C-$5_{Mann'}$), 71.7, 71.3(C-$3_{Mann,}$ C-$3_{Mann'}$), 70.7, 70.6, 70.2, 69.1 (C-3_{Gul}, C-$2_{Mann,}$ C-$2_{Mann'}$, C-$3_{Gul'}$), 67.6, 67.1(C-5_{Gul}, C-$4_{Gul'}$), 67.2(-O$CH_2CH_2CH_2$NHAc), 64.6(C-2_{Gul}, C-$2_{Gul'}$), 36.2(-O$CH_2CH_2$$CH_2$NHAc), 28.1(-O$CH_2$$CH_2$$CH_2$NHAc), 21.8($CH_3$CO); ^{13}C-HMBC ($CDCl_3$, 100 MHz): 101.2, 99.8($J_{C1,H1}$ = 161Hz, $J_{C1,H1}$ = 160Hz, C-$1_{Mann,}$ C-$1_{Mann'}$), 99.4($J_{C1,H1}$ = 171Hz, $J_{C1,H1}$ = 170Hz, 2xC-1_{Gul}). HR-MS: [M+H^+] Calculated for $C_{29}H_{43}O_{26}N$: 822.21461; found: 822.21521.

27-Na^+: 10.6 mg, yield: 98%.^{1}H NMR (600 MHz, D_2O) δ 5.16 (d, J = 3.8 Hz, 1H), 5.15 – 5.13 (m, 1H), 4.84 – 4.79 (m, 2H), 4.33 (m, 2H), 4.31 – 4.28 (m, 1H), 4.12 (t, J = 3.9 Hz, 1H), 4.09 (dd, J = 3.2, 1.0 Hz, 1H), 4.08 – 4.01 (m, 5H), 3.94 – 3.84 (m, 7H), 3.81 (m, 1H), 3.39 (m 3H), 2.10 (s, 3H), 1.93 (p, J = 6.5 Hz, 2H); ^{13}C NMR (151 MHz, D_2O) δ 177.3, 176.9, 175.1, 102.2, 100.7, 100.6, 81.0, 78.7, 77.0, 76.9, 72.9, 72.6, 71.8, 71.7, 71.4, 70.2, 69.1, 68.5, 68.2, 65.8, 65.7, 37.2, 29.2, 22.9.



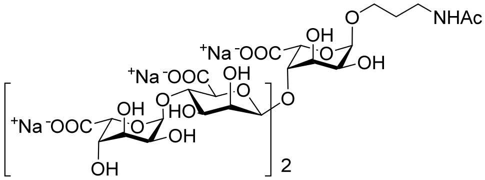
Pentasaccharide 30

30-NH_4^+ was obtained as described by the general procedure for saponification, high pressure hydrogenation and



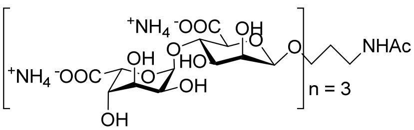
acetylation of oligosaccharides as a white solid (8.4 mg, three steps yield: 77%).^{1}H NMR (400 MHz, D_2O, HH-COSY, HSQC): δ 5.02-5.00 (m, 2H, H-$1_{Gul'}$, H-$1_{Gul''}$), 4.93 (d, J = 3.9 Hz, 1H, H-1_{Gul}), 4.75 (d, J = 1.9 Hz, 1H, H-$5_{Gul'}$), 4.71(bs, 1H, H-1_{Mann}), 4.69 (bs, 1H, H-1_{Mann}), 4.46(bs, 1H, H-$5_{Gul'}$), 4.26 – 4.12 (m, 5H, H-$4_{Gul,}$ H-$4_{Gul'}$, H-$3_{Gul,}$ H-$3_{Gul'}$, H-$3_{Gul''}$,), 4.02 (t, J = 3.9 Hz, 1H, H-2_{Gul}), 3.97 (t, J = 3.9 Hz, 1H, H-$2_{Gul'}$), 3.94-3.90 (m, 4H, H-$2_{Gul''}$, H-$5_{Gul''}$, H-2_{Mann}, H-$2_{Mann'}$), 3.84 – 3.69 (m, 7H, H-3_{Mann}, H-$3_{Mann'}$, H-4_{Mann}, H-$4_{Mann'}$, H-5_{Mann}, H-$5_{Mann'}$, -O$CH_2$$CH_2$$CH_2$NHAc), 3.55 (dt, J = 10.0, 5.8 Hz, 1H, -O$CH_2$$CH_2$$CH_2$NHAc), 3.32 – 3.15 (m, 2H, -$OCH_2CH_2CH_2$NHAc), 1.96 (s, 3H, CH_3CONH-), 1.82 (td, *J* = 6.4, 2.4 Hz, 2H, -$OCH_2CH_2CH_2$NHAc); ^{13}C –APT NMR (D_2O, 100 MHz, HSQC): δ 175.8, 175.6, 175.6,175.6, 174.0(-CO-), 101.3, 101.2(C-$1_{Mann,}$ C-$1_{Mann'}$), 99.5, 99.4, 98.7(C-1_{Gul}, C-$1_{Gul'}$, C-$1_{Gul''}$), 80.4, 80.0(C-4_{Gul}, C-$4_{Gul'}$), 77.5, 77.1 (C-$4_{Mann,}$ C-$4_{Mann'}$), 75.8, 75.7(C-$5_{Mann,}$ C-$5_{Mann'}$), 71.5, 71.4(C-$3_{Mann,}$ C-$3_{Mann'}$), 70.8, 70.7, 70.7, 70.4, 69.5, 69.3(C-$5_{Gul''}$, C-3_{Gul}, C-$3_{Gul'}$, C-$2_{Mann,}$ C-$2_{Mann'}$, C-$3_{Gul''}$), 67.9(C-$4_{Gul''}$), 67.3(C-$5_{Gul'}$), 67.0(-$OCH_2CH_2CH_2$NHAc), 66.7(C-5_{Gul}), 64.7, 64.7, 64.4(C-2_{Gul}, C-$2_{Gul'}$, C-$2_{Gul''}$), 37.0(-$OCH_2CH_2CH_2$NHAc), 28.0(-$OCH_2CH_2CH_2$NHAc), 21.8(CH_3CO) ; ^{13}C-HMBC ($CDCl_3$, 100 MHz): 101.3, 101.2($J_{C1,H1}$ = 160Hz, $J_{C1,H1}$ = 160Hz, C-$1_{Mann,}$ C-$1_{Mann'}$), 99.5, 99.4, 98.7 ($J_{C1,H1}$ = 170Hz, $J_{C1,H1}$ = 170Hz, $J_{C1,H1}$ = 170Hz, 3xC-1_{Gul}). HR-MS: [M+H^+] Calculated for $C_{35}H_{51}O_{32}N$: 998.24669; found: 998.24784.

30-Na^+: 8.4 mg, yield: 98%.^{1}H NMR (600 MHz, D_2O) δ 5.17 – 5.12 (m, 2H), 5.07 (d, *J* = 3.9 Hz, 1H), 4.86 – 4.81 (m, 2H), 4.58 (d, *J* = 1.7 Hz, 1H), 4.37 (td, *J* = 3.9, 1.2 Hz, 1H), 4.33 (ddd, *J* = 10.1, 4.8, 2.8 Hz, 3H), 4.30 – 4.27 (m, 1H), 4.16 (t, *J* = 3.9 Hz, 1H), 4.11 (t, *J* = 3.9 Hz, 1H), 4.09 – 4.03 (m, 4H), 3.96 – 3.84 (m, 7H), 3.69 (m, 1H), 3.40 (m, 2H), 2.10 (s, 3H), 1.96 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 177.3, 176.9, 176.9, 176.9, 176.8, 175.0, 102.3, 102.2, 100.6, 100.5, 99.7, 81.5, 81.0, 78.7, 78.3, 77.0, 76.9, 72.6, 72.5, 71.8, 71.7, 71.4, 70.6, 70.3, 69.1, 68.4, 68.0, 67.8, 65.8, 65.7, 65.4, 38.0, 28.9, 22.8.

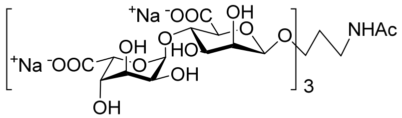


Hexasaccharide 28

28-NH_4^+ was obtained as described by the general procedure for saponification, high pressure hydrogenation and acetylation of oligosaccharides as a white solid (8.6 mg, three steps yield: 54%). ^{1}H NMR (850 MHz, D_2O, HH-COSY, HSQC): δ 5.11 – 4.95 (m, 3H, 3xH-1_{Gul}), 4.91 – 4.82 (m, 3H, H-$4_{Gul''}$, H-5_{Gul}, H-$5_{Gul'}$), 4.72(bs, 1H, H-1_{Mann}), 4.71(bs, 1H, H-1_{Mann}), 4.67(bs, 1H, H-1_{Mann}), 4.31 – 4.12 (m, 5H, H-4_{Gul}, H-$4_{Gul'}$, 3xH-3_{Gul}), 3.98 – 3.84 (m, 10H, 3xH-2_{Gul}, 3xH-2_{Mann}, 3xH1174.27878; found: 1174.280-5_{Mann}, -$OCH_2CH_2CH_2$NHAc), 3.84 – 3.72(m, 6H, 3xH-4_{Mann}, 3xH-3_{Mann}), 3.66 (m, 1H, -$OCH_2CH_2CH_2$NHAc), 3.28 (dt, *J* = 13.4, 6.7 Hz, 1H, -$OCH_2CH_2CH_2$NHAc), 3.24 – 3.13 (m, 1H, -$OCH_2CH_2CH_2$NHAc), 1.95(s, 3H, CH_3CONH-)1.77 (t, *J* = 6.5 Hz, 2H, -$OCH_2CH_2CH_2$NHAc); ^{13}C NMR (214 MHz, D_2O) δ 175.8, 175.5, 175.4, 175.0(-COO-), 102.3, 102.3, 100.9(3xC-1_{Mann}), 100.4, 100.4(3xC-1_{Gul}), 80.8, 80.8(C-4_{Gul} , C-$4_{Gul'}$), 78.3, 78.2, 77.9(3xC-4_{Mann}), 76.1, 76.1, 76.0(3xC-5_{Mann}), 72.6, 72.2, 72.2(3xC-3_{Mann}), 71.6, 71.6, 71.5, 71.1(3xC-2_{Mann}, C-3_{Gul}),, 70.1, 70.0(2xC-3_{Gul}), 68.4(C-$1_{Gul''}$), 68.2(-$OCH_2CH_2CH_2$NHAc), 68.0, 68.0(C-$5_{Gul'}$, C-5_{Gul}), 65.5, 65.5(3xC-2_{Gul}), 37.2(-$OCH_2CH_2CH_2$NHAc), 29.1(-$OCH_2CH_2CH_2$NHAc), 22.8(CH_3CO); ^{13}C-HMBC ($CDCl_3$, 214 MHz): 102.3, 102.3, 100.9 ($J_{C1,H1}$ = 160Hz, 3xC-1_{Mann}), 100.5, 100.4, 100.4 ($J_{C1,H1}$ = 170Hz, 3xC-1_{Gul}). HR-MS: [M+H^+] Calculated for $C_{41}H_{59}O_{38}N$: 1174.27878; found: 1174.28070.



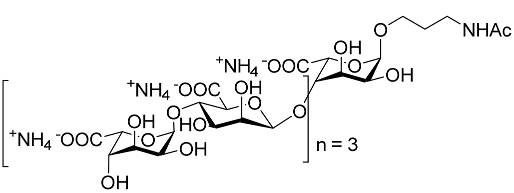
28-Na$^+$: 8.2 mg, yield: 93%. ^{1}H NMR (600 MHz, D_2O) δ 5.01 – 4.95 (m, 3H), 4.72 (dd, *J* = 4.2, 1.6 Hz, 2H), 4.69 – 4.63



(m, 4H), 4.19 – 4.11 (m, 5H), 3.98 – 3.85 (m, 9H), 3.78 – 3.68 (m, 7H), 3.68 – 3.62 (m, 1H), 3.28 (m, 1H), 3.19 (m, 1H), 1.94 (s, 3H), 1.76 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 177.3, 176.9, 176.9, 176.9, 176.8, 175.0, 102.2, 102.1, 100.7, 100.5, 100.5, 100.4, 81.0, 80.9, 78.6, 78.6, 78.3, 76.9, 76.9, 76.8, 72.9, 72.5, 72.4, 71.8, 71.7, 71.6, 71.4, 70.2, 70.1, 69.0, 68.4, 68.1, 65.7, 65.6, 37.1, 29.1, 22.8.

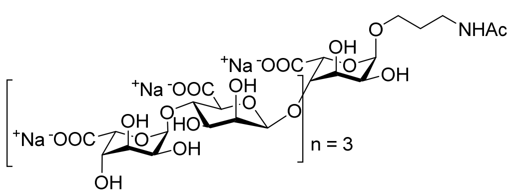
Heptasaccharide 31

31-NH$_4^+$ was obtained as described by the general procedure for saponification, high pressure hydrogenation and



acetylation of oligosaccharides as a white solid (6.0 mg, three steps yield: 63%). ^{1}H NMR (850 MHz, D_2O, HH-COSY, HSQC): δ 5.03 – 4.94 (m, 3H, H-1$_{Gul'}$, H-1$_{Gul''}$, H-1$_{Gul'''}$), 4.90 (d, *J* = 4.0 Hz, 1H, H-1$_{Gul}$), 4.82(bs, 3H, H-4$_{Gul'''}$, H-5$_{Gul'}$, H-5$_{Gul''}$) 4.73 – 4.65 (m, 3H, H-1$_{Mann}$, H-1$_{Mann'}$, H-1$_{Mann''}$), 4.49 (s, 1H, H-5$_{Gul}$), 4.26 – 4.11 (m, 7H, H-4$_{Gul}$, H-4$_{Gul'}$, H-4$_{Gul''}$, H-3$_{Gul}$, H-3$_{Gul'}$, H-3$_{Gul''}$, H-3$_{Gul'''}$), 4.02 – 3.68 (m, 18H, 4 x H-2$_{Gul}$, H-5$_{Gul''}$, 3 x H-2$_{Mann}$, 3 x H-3$_{Mann}$, 3 x H-4$_{Mann}$, 3 x H-5$_{Mann}$, (-O*C*$H_2CH_2CH_2$NHAc), 3.52 (m, 1H, (-O*C*$H_2CH_2CH_2$NHAc), 3.47 – 3.40 (m, 1H), 3.27-3.19 (m, 2H, $OCH_2CH_2CH_2$NHAc), 1.92 (s, 3H, CH_3CO), 1.86 – 1.70 (m, 2H, -$OCH_2CH_2CH_2$NHAc). ^{13}C –APT NMR (D_2O, 214 MHz, HSQC): δ 176.1, 175.0(8x-COO-), 102.2(3xC-1$_{Mann}$), 100.4, 99.7(4xC-1$_{Gul}$), 81.3, 80.8(C-4$_{Gul}$, C-4$_{Gul'}$, C-4$_{Gul''}$), 78.0(3xC-4$_{Mann}$), 76.3(3xC-5$_{Mann}$), 72.2(3xC-3$_{Mann}$), 71.6, 71.2, 70.4, 70.1(3xC-2$_{Mann}$, 4xC-3$_{Gul}$, C-5$_{Gul'''}$), 68.6(C-5$_{Gul'}$, C-5$_{Gul''}$), 68.1(C-4$_{Gul'''}$), 68.0(-O*C*$H_2CH_2CH_2$NHAc), 67.5(C-5$_{Gul}$), 65.5, 65.3(4xC-2$_{Gul}$), 37.9(-$OCH_2CH_2CH_2$NHAc), 28.8(-$OCH_2CH_2CH_2$NHAc), 22.7(CH_3CO); ^{13}C-HMBC ($CDCl_3$, 214 MHz): 102.4, 102.3, 102.2 ($J_{C1,H1}$ = 161Hz, 3xC-1$_{Mann}$), 100.4, 99.7($J_{C1,H1}$ = 170Hz, $J_{C1,H1}$ = 171Hz, 4xC-1$_{Gul}$). HR-MS: [M+H$^+$] Calculated for $C_{47}H_{67}O_{44}N$: 1350.31087; found: 1350.31198.

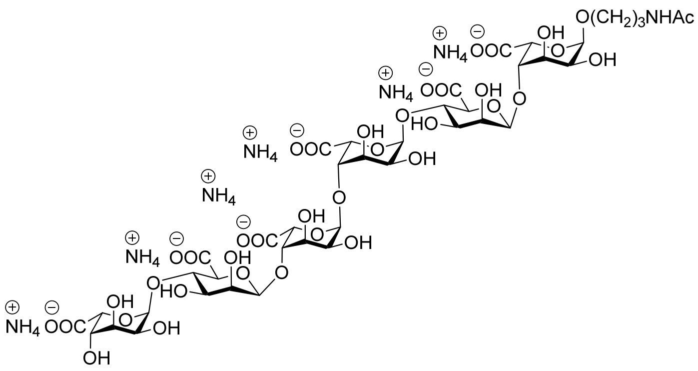
31-Na$^+$: 5.9 mg, yield: 96%. ^{1}H NMR (600 MHz, D_2O) δ 5.17 – 5.12 (m, 3H), 5.07 (d, *J* = 3.5 Hz, 1H), 4.95-4.90(m, 3H),



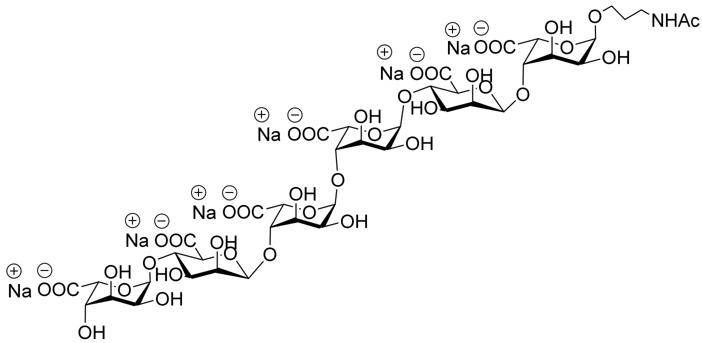
4.85 – 4.80 (m, 3H), 4.58 (d, *J* = 1.7 Hz, 1H), 4.39 – 3.83(m, 25H), 4.39 – 4.27 (m, 6-7H), 4.16 (t, *J* = 3.9 Hz, 1H), 4.11 (t, *J* = 4.0 Hz, 2H), 4.08 – 4.02 (m, 7-8H), 3.95 – 3.83 (m, 9H), 3.69 (m, 1H), 3.43 (m, 1H), 3.37 (m, 1H), 2.10 (s, 3H), 1.96 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 177.3, 176.9, 176.9, 176.8, 175.0, 102.3, 102.2, 102.2, 100.6, 100.5, 100.5, 99.7, 81.5, 81.0, 81.0, 78.7, 78.4, 78.3, 77.0, 76.9, 72.6, 72.5, 72.5, 71.8, 71.8, 71.7, 71.4, 70.6, 70.3, 70.3, 69.1, 68.4, 68.0, 67.8, 65.8, 65.7, 65.4, 38.0, 28.9, 22.8.

Hexasaccharide 32

32-NH$_4^+$: (7.9 mg, three steps yield: 61%). HR-MS: [M+Na$^+$] Calculated for $C_{41}H_{59}NO_{38}$: 1196.22073; found: 1196.26155. ^{1}H NMR (850 MHz, Deuterium Oxide) δ 5.00 (dd, *J* = 9.3, 4.5 Hz, 2H, H-1$_{Gul'}$, H-1$_{Gul''}$), 4.98 – 4.94 (m, 1H, H-1$_{Gul'''}$), 4.91 (d, *J* = 4.0 Hz, 1H, H-1$_{Gul}$), 4.76 (d, *J* = 1.7 Hz, 1H, H-5$_{Gul'}$), 4.70 – 4.63 (m, 4H, 2xH-1$_{Mann}$, H-4$_{Gul}$, H-5$_{Gul'''}$), 4.41 (dd, *J* = 7.5, 1.6 Hz, 2H, H-5$_{Gul}$, H-5$_{Gul''}$), 4.26 – 4.06 (m, 6H, H-3$_{Gul}$, H-3$_{Gul''}$, H-3$_{Gul'''}$, H-4$_{Gul}$, H-4$_{Gul'}$, H-4$_{Gul''}$), 3.99 (t, *J* = 3.9 Hz, 1H, H-2$_{Gul}$), 3.95 (t, *J* = 3.8 Hz, 1H, H-3$_{Gul'}$), 3.93 (d, *J* = 3.4 Hz, 1H, H-2$_{Gul''}$), 3.91 – 3.89 (m, 4H, H-2$_{Gul'''}$, H-5$_{Gul'''}$, 2xH-2$_{Mann}$), 3.88 (d, *J* = 4.0 Hz, 1H, H-2$_{Gul'}$), 3.82 – 3.68 (m, 7H, 2xH-3$_{Mann}$, 2xH-4$_{Mann}$, 2xH-5$_{Mann}$,-OC*H*$_2$CH$_2$CH$_2$NHAc), 3.52 (dt, *J* = 10.3, 6.0 Hz, 1H,-OC*H*$_2$CH$_2$CH$_2$NHAc), 3.30 – 3.24 (m, 1H,-OCH$_2$CH$_2$C*H*$_2$NHAc), 3.25 – 3.15 (m, 1H,-OCH$_2$CH$_2$C*H*$_2$NHAc), 1.94 (s, 3H, CH$_3$CO), 1.80 (q, *J* = 7.2 Hz, 2H,-OCH$_2$C*H*$_2$CH$_2$NHAc); ^{13}C NMR (213 MHz, D$_2$O) δ 181.9, 177.3, 176.8, 176.8, 176.5, 175.0, 102.2, 102.1(2xC-1$_{Mann}$), 101.7(C-1$_{Gul''}$), 100.4(C-1$_{Gul'''}$), 100.3(C-1$_{Gul'}$), 99.7(C-1$_{Gul}$), 81.5, 81.1(3xC-4$_{Gul}$), 78.3, 78.1(2xC-4$_{Mann}$), 76.9, 76.8(2xC-5$_{Mann}$), 72.4, 72.3(2xC-3$_{Mann}$), 71.8, 71.7, 71.7, 71.4(C-5$_{Gul'''}$, C-3$_{Gul}$, 2xC-2$_{Mann}$), 70.5, 70.2, 70.1(3xC-5$_{Gul}$), 69.0(C-4$_{Gul'''}$), 68.3, 68.2(2xC-5$_{Gul}$), 67.9(-O*C*H$_2$CH$_2$CH$_2$NHAc), 67.7(C-5$_{Gul}$), 65.9, 65.9, 65.7, 65.4(4xC-2$_{Gul}$), 37.9(-OCH$_2$CH$_2$*C*H$_2$NHAc), 28.9(CH$_3$CO), 22.8(-OCH$_2$*C*H$_2$CH$_2$NHAc); ^{13}C-HMBC (CDCl$_3$, 213 MHz): 102.2, 102.1($J_{C1,H1}$ = 161Hz, 2xC-1$_{Mann}$), 101.7, 100.4, 100.3, 99.7 ($J_{C1,H1}$ = 170Hz, 4xC-1$_{Gul}$).



32-Na$^+$: 7.9 mg, 98%. ^{1}H NMR (600 MHz, D$_2$O) δ 5.02 – 4.96 (m, 3H), 4.92 – 4.89 (m, 1H), 4.76 (d, *J* = 1.6 Hz, 1H), 4.69 – 4.65 (m, 3H), 4.42 (d, *J* = 1.7 Hz, 2H), 4.40 (d, *J* = 1.7 Hz, 1H), 4.21 – 4.14 (m, 3H), 4.13 (dd, *J* = 3.4, 2.0 Hz, 1H), 4.08 (dd, *J* = 3.8, 1.7 Hz, 1H), 4.00 (t, *J* = 3.9 Hz, 1H), 3.98 – 3.93 (m, 1H), 3.92 – 3.87 (m, 7H), 3.80 – 3.69 (m, 6H), 3.53 (m, 1H), 3.24 (m, 2H), 1.94 (s, 3H), 1.80 (m, 2H); ^{13}C NMR (150 MHz, D$_2$O) δ 177.3, 176.9, 176.9, 176.8, 176.5, 175.0, 102.2, 102.1, 101.8, 100.5, 100.4, 99.6, 81.4, 81.1, 81.0, 78.6, 78.2, 76.9, 76.8, 72.5, 72.3, 71.8, 71.8, 71.6, 71.4, 70.5, 70.0, 70.0,



69.0, 68.3, 68.1, 67.9, 67.7, 65.9, 65.7, 65.6, 65.4, 38.0, 28.9, 22.8; ^{13}C-HMBC ($CDCl_3$, 150 MHz): 102.2, 102.1($J_{C1,H1}$ = 160Hz, 2xC-1_{Mann}), 101.8($J_{C1,H1}$ = 171Hz, C-1_{Gul}), 100.5, 100.4, 99.6($J_{C1,H1}$ = 170Hz, 3xC-1_{Gul}).

4.5 references

[1] Because of its polymeric nature, mixed sequence alginate can be described, arbitrarily, as being built up from -GM- or -MG- blocks.

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