

Synthesis of Glycoconjugates Utilizing the Regioselectivity of a Lytic Polysaccharide Monooxygenase

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A)

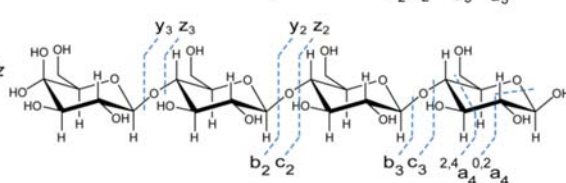
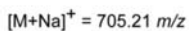
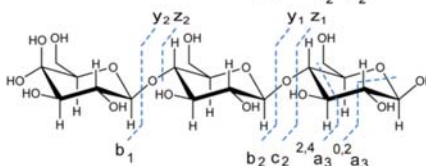
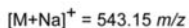
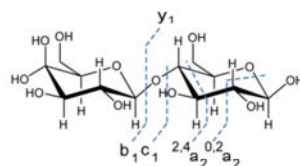
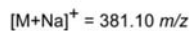
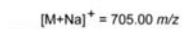
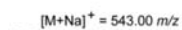
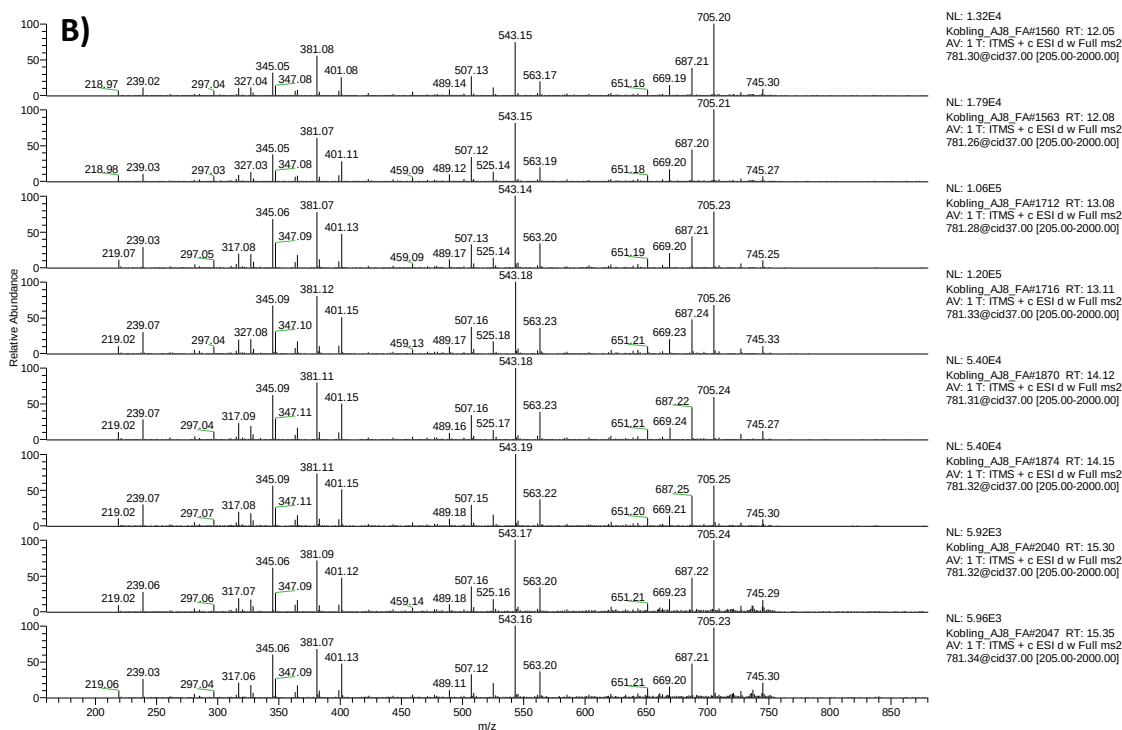
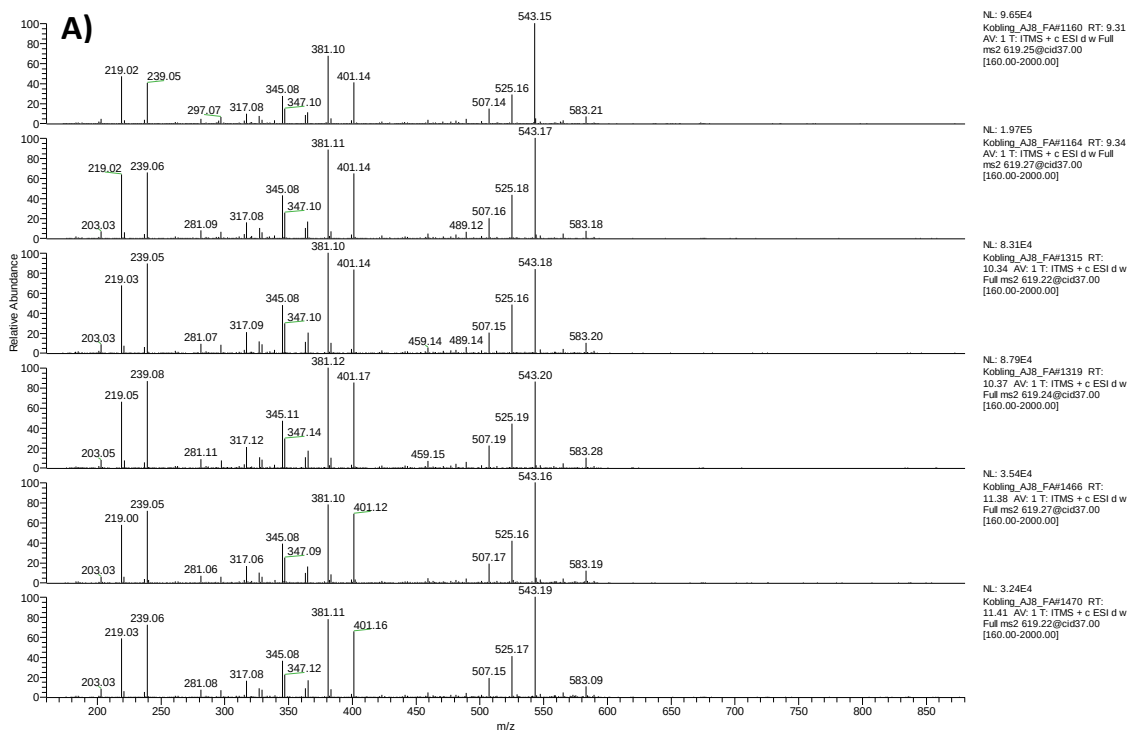


Figure S1. Direct injection MS analysis of C4-oxidized cello-oligosaccharides. Oxidized soluble products were analyzed using an LTQ Velos Pro mass spectrometer and direct injection. A) A survey scan of all ions detected in the m/z -range 150-750. Peaks annotated with an asterisk are sodium adducts of the hydrated forms of oxidized products and were selected for fragmentation. Note that the keto-sugars are in equilibrium with their gemdiols and that under the conditions used in this study, the keto-form is predominant in MALDI ToF MS analysis (Fig. 1), whereas the gemdiol is predominant in ESI-MS. Peaks corresponding to native cello-oligosaccharides are also visible (m/z 365, 527 and 689), which is due to the samples being taken at an early time point in the reaction when there is some release of non-modified sugars from chain ends, while the oxidized oligosaccharides are mostly still bound to the polymer part of the cellulose. B) MS/MS spectra of the three C4-oxidized products show a dominant cleavage of the glycosidic bonds and ring cleavage of the downstream end-sugar. Fragments including the C4-oxidation (a,b,c fragments) tend to lose water as has been described previously [1]. This characteristic double loss of water from the molecular mass is evident for all three products. The MS fragmentation pattern observed here as well as the elution pattern in HPAEC-PAD chromatography are in accordance with previous observations on NMR validated C4-oxidized cello-oligosaccharides [1]. C) The structures of the three C4-oxidized products; the observed fragmentation patterns and the theoretical masses are indicated.



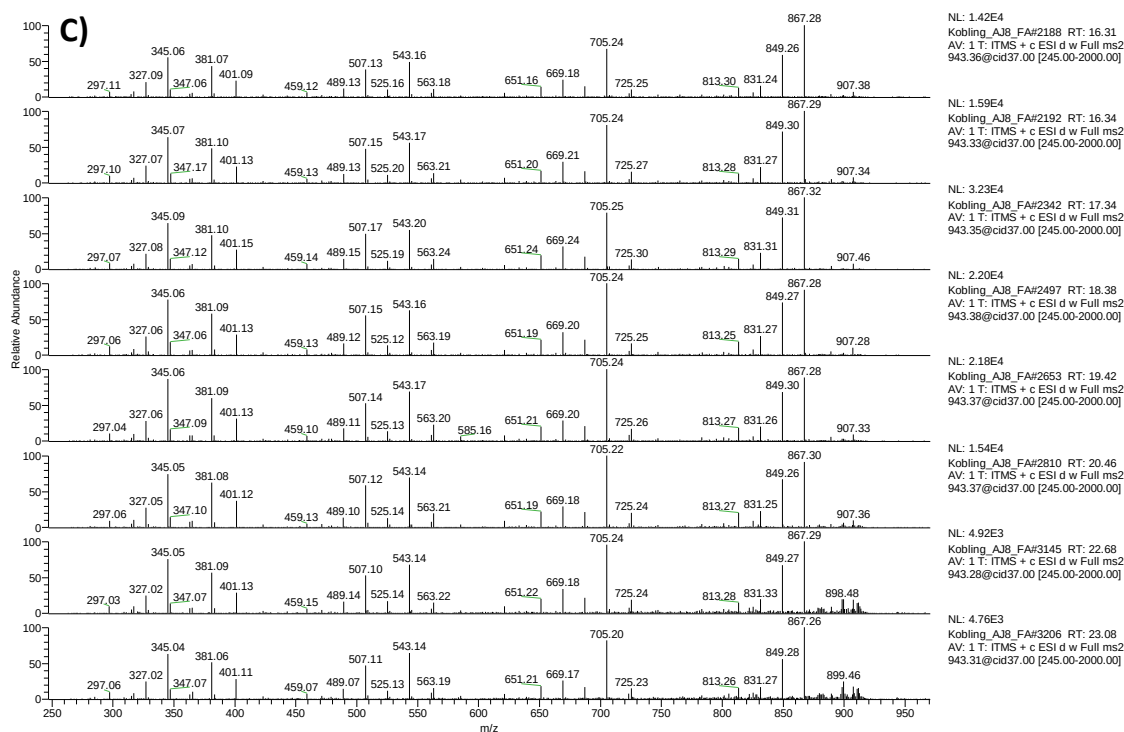


Figure S2. PGC-MS/MS analysis of the oxime bi-functionalized cello-oligosaccharides displaying fragmentation data on the three peaks in Fig. 6A. A) Fragments of 619.25 from RT 9.31 to 11.41, B) Fragments of 781.28 from RT 12.05 to 15.35, and C) Fragments of 943.35 from RT 16.31 to 23.08

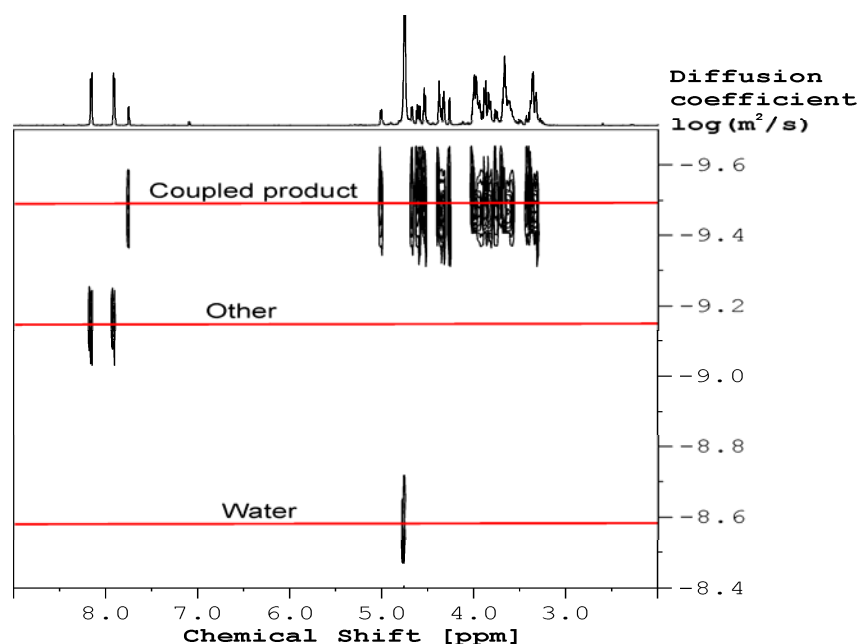


Figure S3. **Diffusion-Ordered Spectroscopy (DOSY) on the aminoxy-linked product (notion coupled product in spectrum).** DOSY spectrum of the aminoxy-linked product in 99.9 % D₂O recorded at 25°C. Red lines indicate the diffusion of the different molecules in the sample. If the aminoxy-linked products have the same diffusion coefficient it indicates that the C4-oxidized Glc and the oxime linker are covalently linked, while, if they have a different diffusion coefficient, they are not covalently bonded at all. Thus DOSY provides an indirect qualitative method to validate if a coupling reaction has been achieved and helps to identify signals belonging to the aminoxy-linked product (more specifically the azomethine (-C=N-) group which is formed upon coupling). A complete structural elucidation by NMR (Fig.6) was further performed to obtain direct proof of the structure for the coupled product. The [¹H-¹³C] HMBC spectrum provided long range bond correlations allowing to connect the monosaccharides for the aminoxy-linked product as well as identification of the carbon chemical shift for a C4-oxidized end at C4 (see figure 6A).

Table S1: The chemical shift values and correlations of the aminoxy-linked product. Chemical shifts were assigned for the main product in 99.9% D₂O at 25 °C. Numbers 1-6 represent ring carbon numbers to which the chemical shift values [¹H, ¹³C] are assigned. The water signal (4.75 ppm) was used as chemical shift reference for ¹H and ¹³C was referenced indirectly using the absolute frequency ratio [2].

Position	¹ H [ppm]	¹³ C [ppm]	COSY correlations - correlated nuclei	HMBC correlations - correlated nuclei
C4ox-1	4.67	107.0	C4ox-2	C4ox-2, C4ox-3, C4ox-5
C4ox-2	3.89	76.7	C4ox-1, C4ox-3	C4ox-1, C4ox-3, C4ox-4
C4ox-3	4.27	73.4	C4ox-2	C4ox-1, C4ox-2, C4ox-4, C4ox-5
C4ox-4	-	159.2		C4ox-2, C4ox-3, C4ox-5, C4ox-6
C4ox-5	5.01	77.1	C4ox-6	C4ox-1, C4ox-3, C4ox-4, C4ox-6
C4ox-6	3.99;3.89	63.5	C4ox-5	C4ox-5, C4ox-4
Glc3-1	4.60	104.9	Glc3-2	Glc3-2, Glc3-3, Glc3-5
Glc3-2	3.39	75.6	Glc3-1, Glc3-3	Glc3-1, Glc3-3, Glc3-4
Glc3-3	3.57	77.2	Glc3-2, Glc3-4	Glc3-1, Glc3-2, Glc3-4, Glc3-5
Glc3-4	3.63	81.0	Glc3-3, Glc3-5	C4ox-1, Glc3-2, Glc3-3, Glc3-5, Glc3-6
Glc3-5	3.64	76.7	Glc3-4, Glc3-6	Glc3-1, Glc3-4, Glc3-3, Glc3-6
Glc3-6	3.95;3.87	62.5	Glc3-5	Glc3-5, Glc3-4
Glc2-1	4.54	105.1	Glc2-2	Glc2-2, Glc2-3, Glc2-5
Glc2-2	3.37	75.7	Glc2-1, Glc2-3	Glc2-1, Glc2-3, Glc2-4
Glc2-3	3.67	76.6	Glc2-2, Glc2-4	Glc2-1, Glc2-2, Glc2-4, Glc2-5
Glc2-4	3.66	81.0	Glc2-3, Glc2-5	Glc3-1, Glc2-2, Glc2-3, Glc2-5, Glc2-6
Glc2-5	3.62	77.5	Glc2-4, Glc2-6	Glc2-1, Glc2-4, Glc2-3, Glc2-6
Glc2-6	3.98;3.84	62.4	Glc2-5	Glc2-5, Glc2-4
Glc1-1	7.76	155.4	Glc1-2	B', Glc1-2, Glc1-3, Glc1-5
Glc1-2	4.61	71.9	Glc1-1, Glc1-3	Glc1-1, Glc1-3, Glc1-4
Glc1-3	3.99	73.7	Glc1-2, Glc1-4	Glc1-1, Glc1-2, Glc1-4, Glc1-5
Glc1-4	3.95	80.7	Glc1-3, Glc1-5	Glc2-1, Glc1-2, Glc1-3, Glc1-5, Glc1-6
Glc1-5	3.96	73.6	Glc1-4, Glc1-6	Glc1-1, Glc1-3, Glc1-4, Glc1-6
Glc1-6	3.88;3.76	64.6	Glc1-5	Glc1-5
A (NH ₂ -CH ₂ -CH ₂ -O-R)	3.34	41.4	B	B
A' (NH ₂ -CH ₂ -CH ₂ -O-R')	3.29	41.2	B'	B'
B (NH ₂ -CH ₂ -CH ₂ -O-R)	4.37	72.9	A	B
B' (NH ₂ -CH ₂ -CH ₂ -O-R')	4.32;4.01	72.5	A'	Glc1-1, A'

References

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2. Zheng, D. *et al.* Letter to the Editor: ¹H, ¹³C and ¹⁵N resonance assignments for methionine sulfoxide reductase B from *Bacillus subtilis*. *J. Biomol. NMR* **27**, 183–184 (2003).