

Supporting Information

Alginate-based diblock polymers: Preparation, characterization and Ca-induced self-assembly

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Preparation and characterization of guluronate oligomers (G_n)

Pure guluronate oligomers (G_n) were prepared by acid precipitation of guluronate rich alginates, removing any oligomers containing one or more M-residue(s).

A study using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was performed, monitoring the acid hydrolysis of a guluronate with DP_n 20, enabling tailoring of the resulting DP_n .

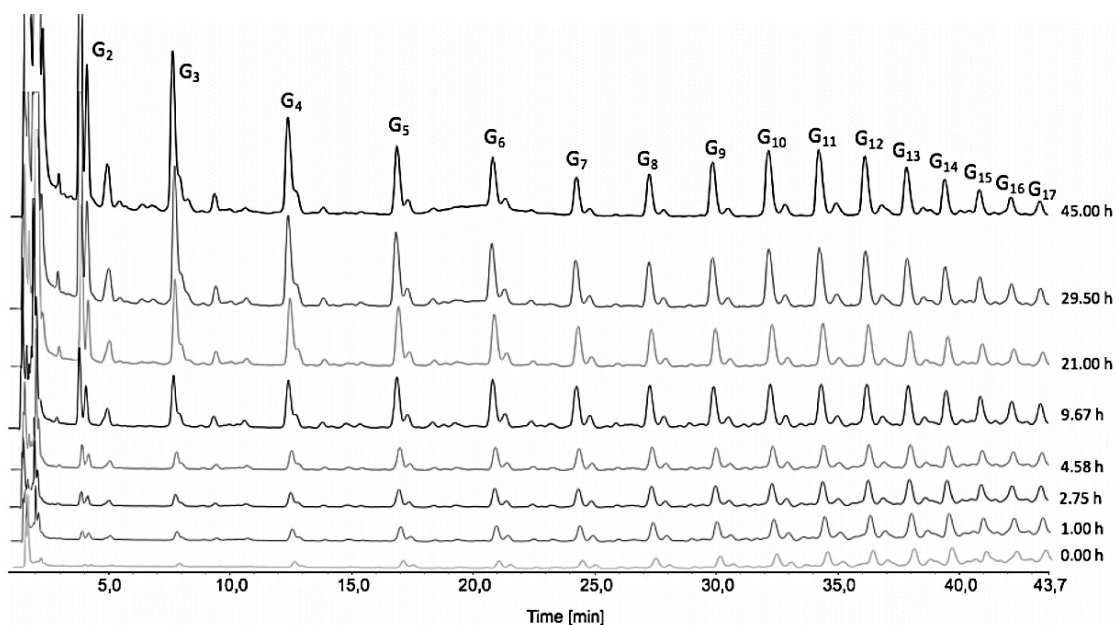


Fig. S1 HPAEC-PADH chromatograms for guluronate oligomers taken at different timepoints during hydrolysis (95°C, pH 3.62).

Oligomers with defined DP_n were prepared by GFC, and DP_n was verified by 1H -NMR (Fig. S3). Isolated triguluronate was annotated according to literature¹.

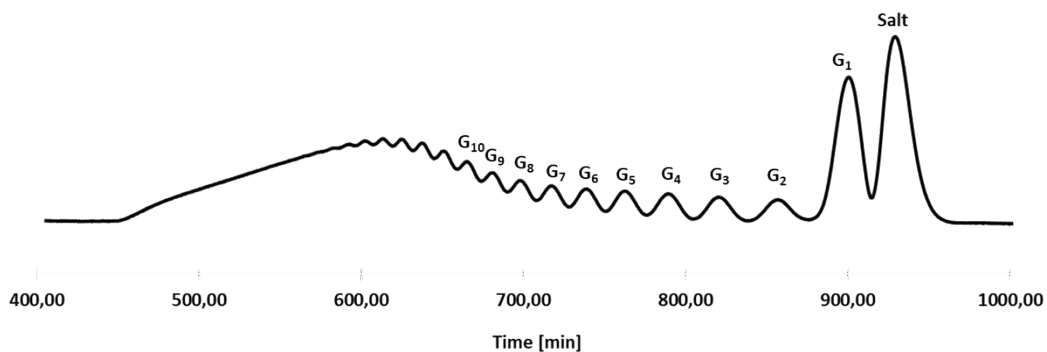


Fig. S2 GFC fractionation of guluronate oligomers (guluronate with DPn 20 hydrolyzed for 9 h at 95°, pH 3.61).

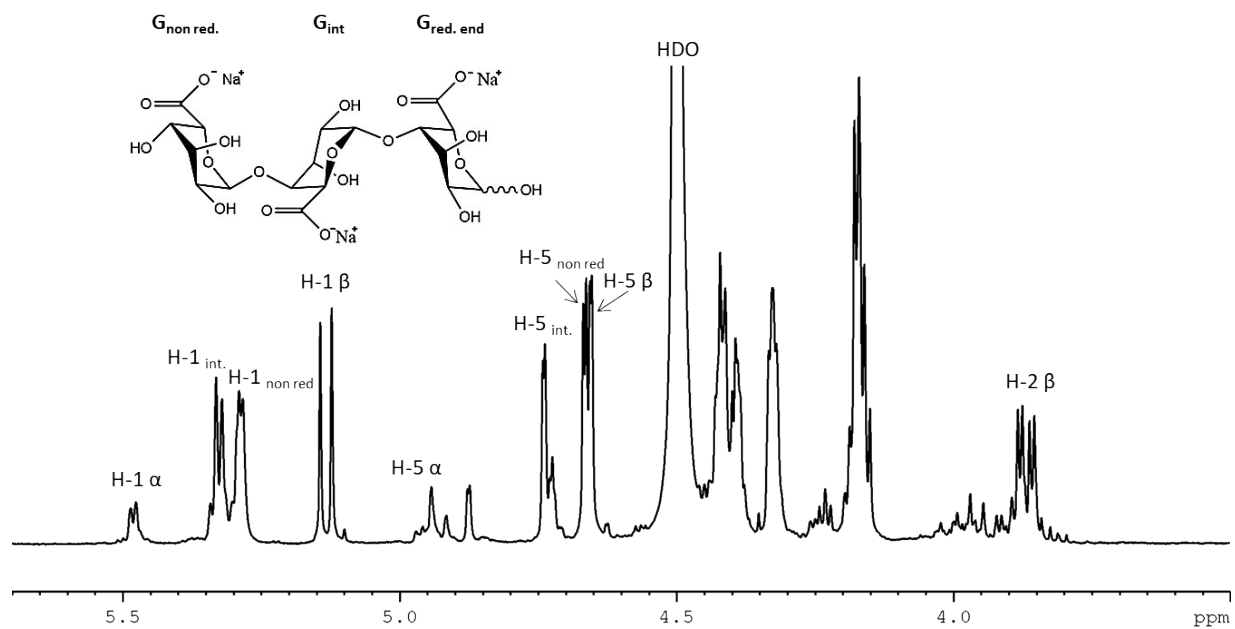


Fig. S3 $^1\text{H-NMR}$ (82 °C, 400 MHz) spectrum of isolated triguluronate, peaks were assigned according to literature ¹.

Conjugation with PDHA

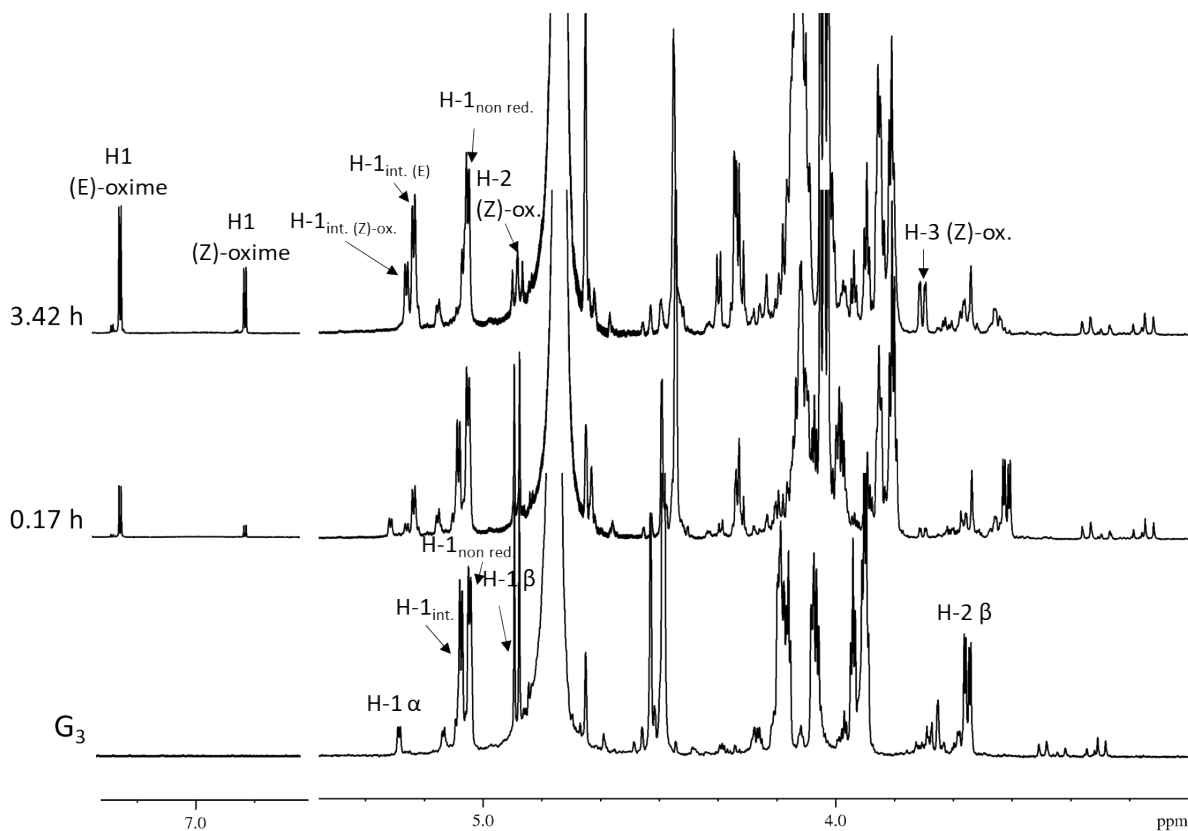


Fig. S4 Stack of ¹H-NMR spectra obtained after different timepoints for the reaction with G₃ (7mM) and PDHA (2 equiv.) in 500 mM AcOH[d₄] pD 4 (27 °C, 600 MHz). Pure G₃ (in buffer) is included for comparison.

The equilibrium reaction mixture with G₃ and PDHA was characterized by heteronuclear NMR.

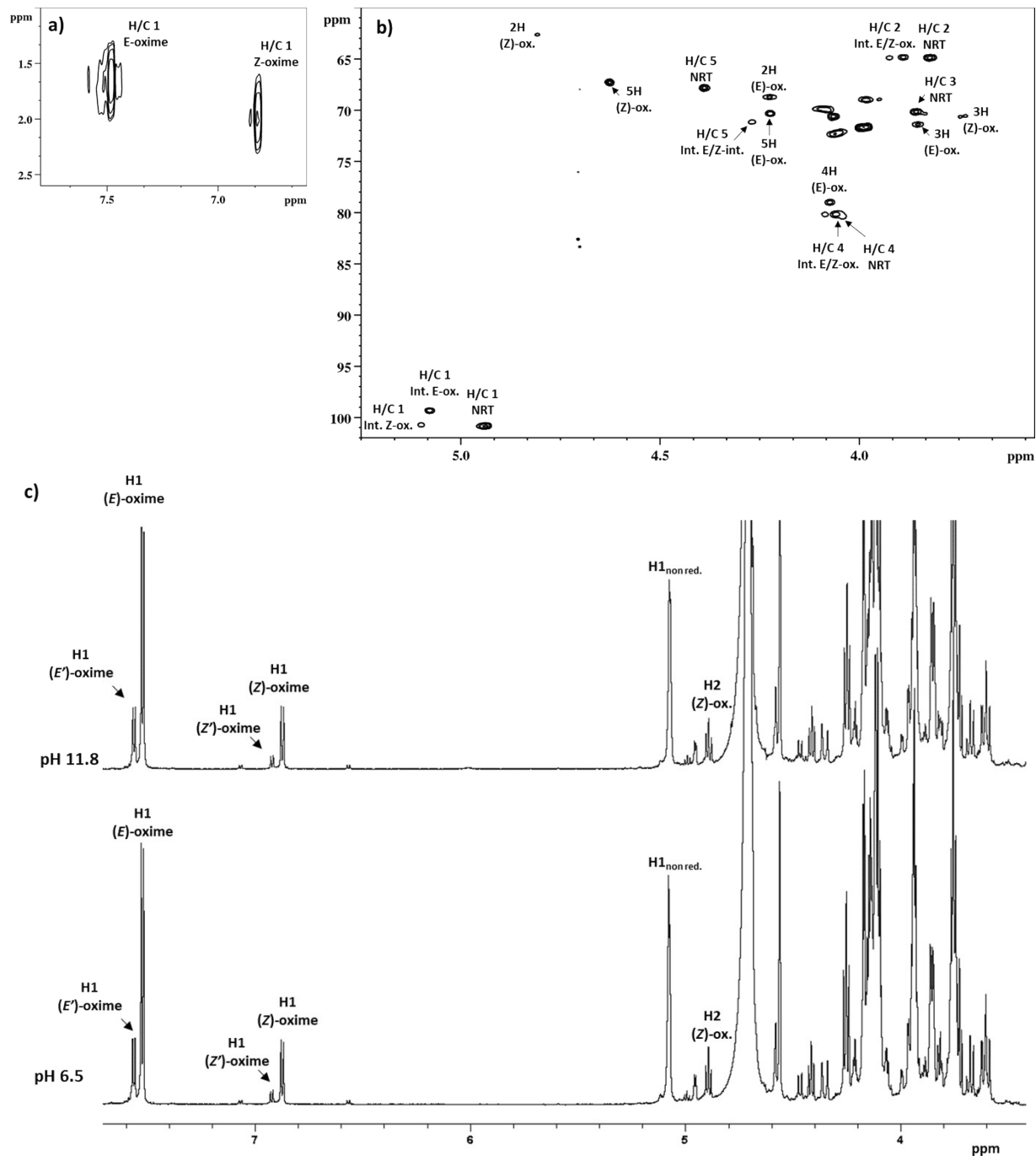


Fig. S5 a,b) ¹³C HSQC of the reaction mixture obtained for the conjugation with G₃ (7mM) and PDHA (2 equiv.) (500 mM AcOH[d₄] pD 4) recorded at t > 24 h (82 °C, NEO 600 MHz magnet). c) ¹H-NMR spectra of G₃-PDHA after purification by GFC and dialysis in D₂O at pH 6.5 and 11.8 (27 °C, 600 MHz)

	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	C6
(E)-oxime	7.48; 151.5	4.23; 68.6	3.86; 71.38	4.07; 79.0	4.22; 70.3	177.1
(Z)-oxime	6.83; 152.0	4.81; 62.63	3.74; 70.65	4.03; 80.35	4.27; 71.24	175.2
Int. (E)-oxime	5.07; 99.22	3.89; 64.86	3.97; 68.92	4.08; 79.0	4.27; 71.16	177.1
Int. (Z)-oxime	5.09; 100.5	3.92; 64.89	3.99; 68.92	4.04; 80.42	4.27; 71.16	177.1
NRT	4.94; 100.77	3.82; 70.19	3.86; 70.65	4.07; 80.2	4.39; 67.8	175.2

The reaction kinetics was determined by integrating the ¹H-NMR spectra, using H1 of the non-reducing end and TSP as internal standards. A simple model based on first order kinetics was used to determine the rate constants (k_T and k_{-T}) for the combined yield.

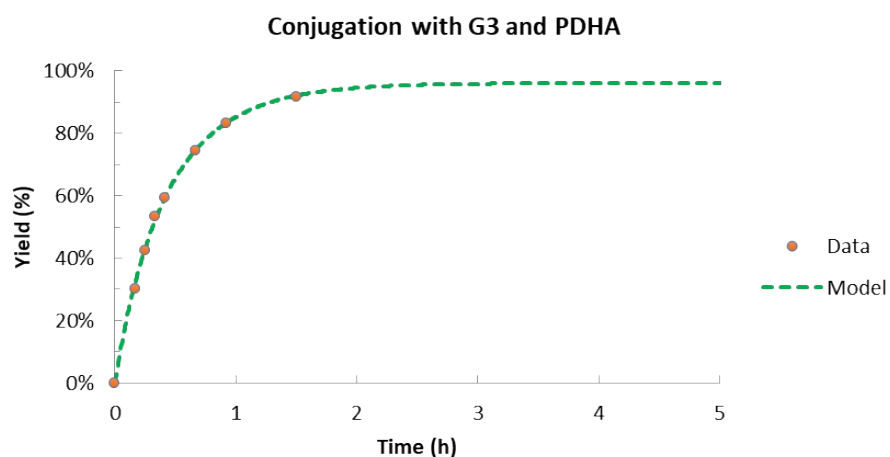


Fig. S6 Conjugation with G₃ (7mM) and PDHA (2 equiv.) (500 mM AcOHd₄ pD 4) studied by time course NMR (27 °C, 600 MHz). Data obtained by integration using ¹H of the non-reducing ends as internal standard. A simple model assuming first order kinetics based on combined yield is included.

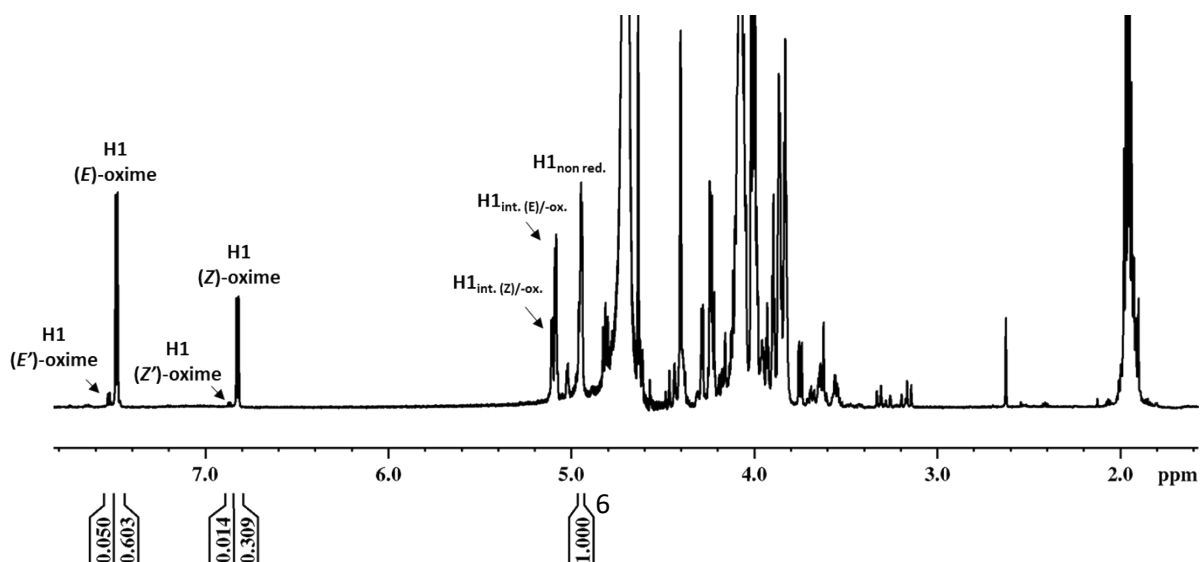


Fig. S7 $^1\text{H-NMR}$ spectra of the equilibrium reaction mixture with G_3 (7mM) and PDHA (2 equiv.) (in 500 mM AcOHd_4 pD 4 at 27 °C, 600 MHz) recorded after 4 h. Integration using H1 of the non-reducing end used to estimate yield is included.

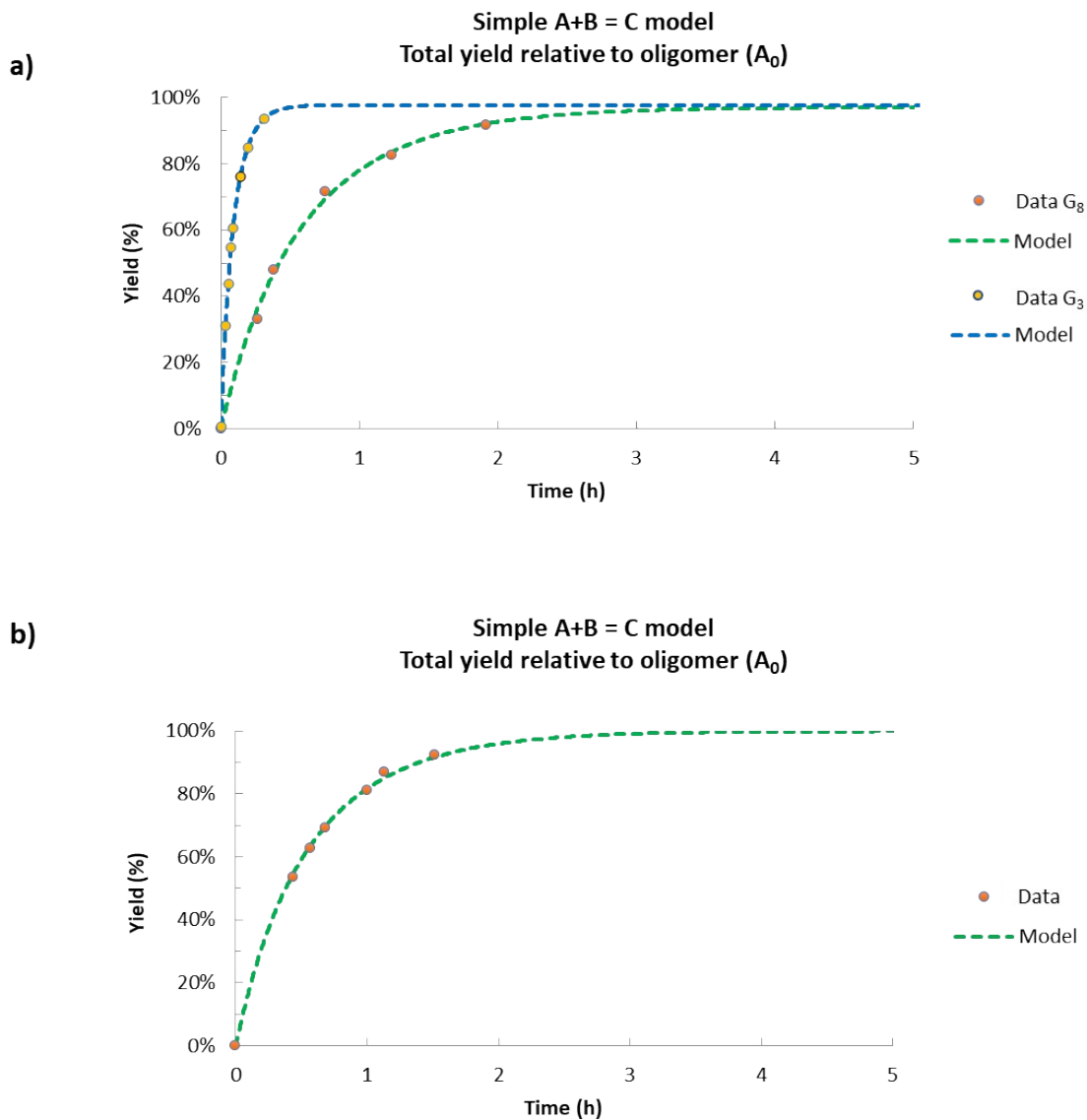


Fig. S8 Plot with combined yield for conjugation with a) G_8 (7mM) and b) G_6 (20 mM) and PDHA (2 equiv.) (500 mM AcOHd_4 pD 4) studied by time course NMR. Data obtained by integration using H1 of the non-reducing ends as internal standard. A simple model assuming first order kinetics based on combined yield is included.

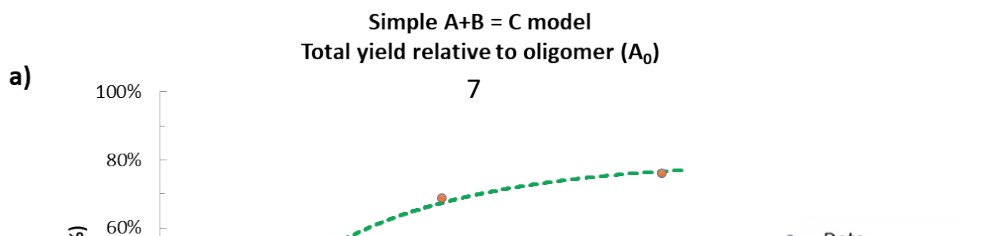


Fig. S9 Plot with combined yield for conjugation with a) Dextran DP 3 (7mM) and b) Maltotriose (7 mM) and PDHA (2 equiv.) (500 mM AcOHd4 pD 4) studied by time course NMR (27 °C, 600 MHz). Data obtained by integration using H1 of the non-reducing ends or TSP as internal standard. A simple model assuming first order kinetics based on combined yield is included.

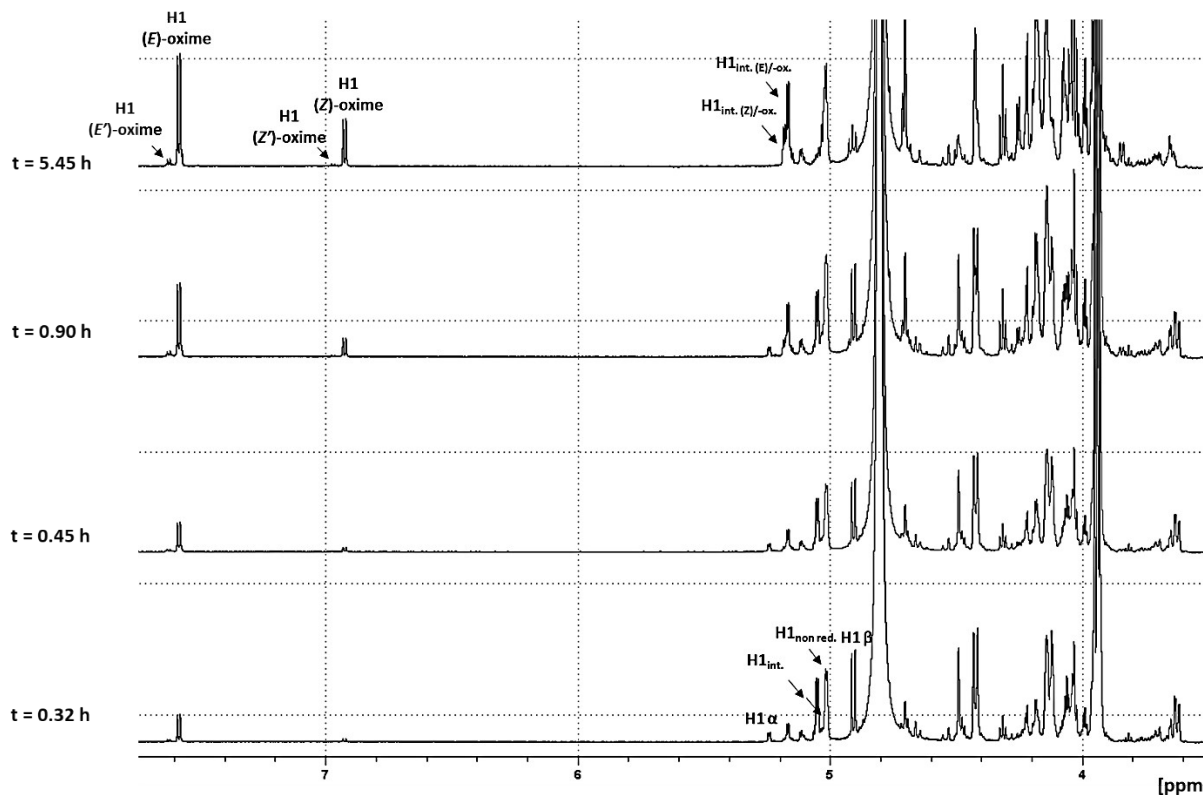


Fig. S10 Stack of ^1H -NMR spectra obtained at different time points for the reaction with G_3 (7mM) and PDHA (2 equiv.) at pD 5 (500 mM AcOHd4, 27 °C, 600 MHz).

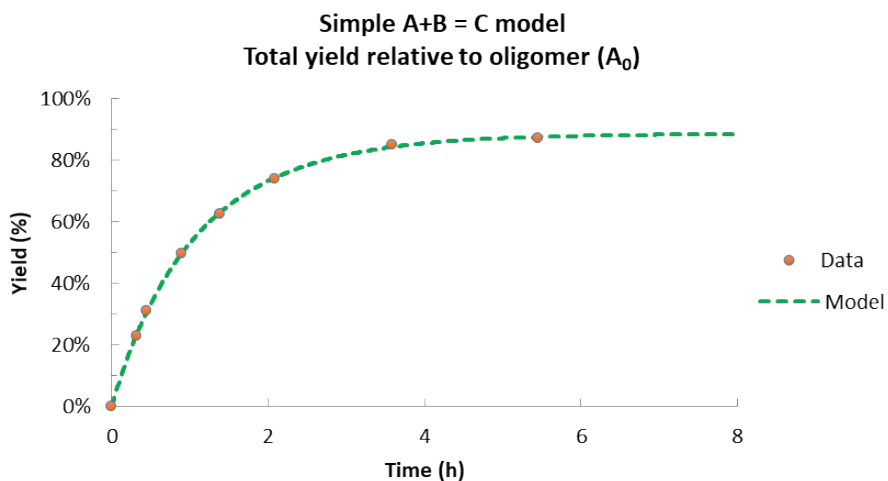


Fig. S11 Conjugation with G_3 (7mM) and PDHA (2 equiv.) at pD 5 (500 mM AcOHd4) studied by time

course NMR (obtained at 27 °C, using a 600 MHz magnet). Data obtained by integration using H1 of the non-reducing ends as internal standard. A simple model assuming first order kinetics based on combined yield is included.

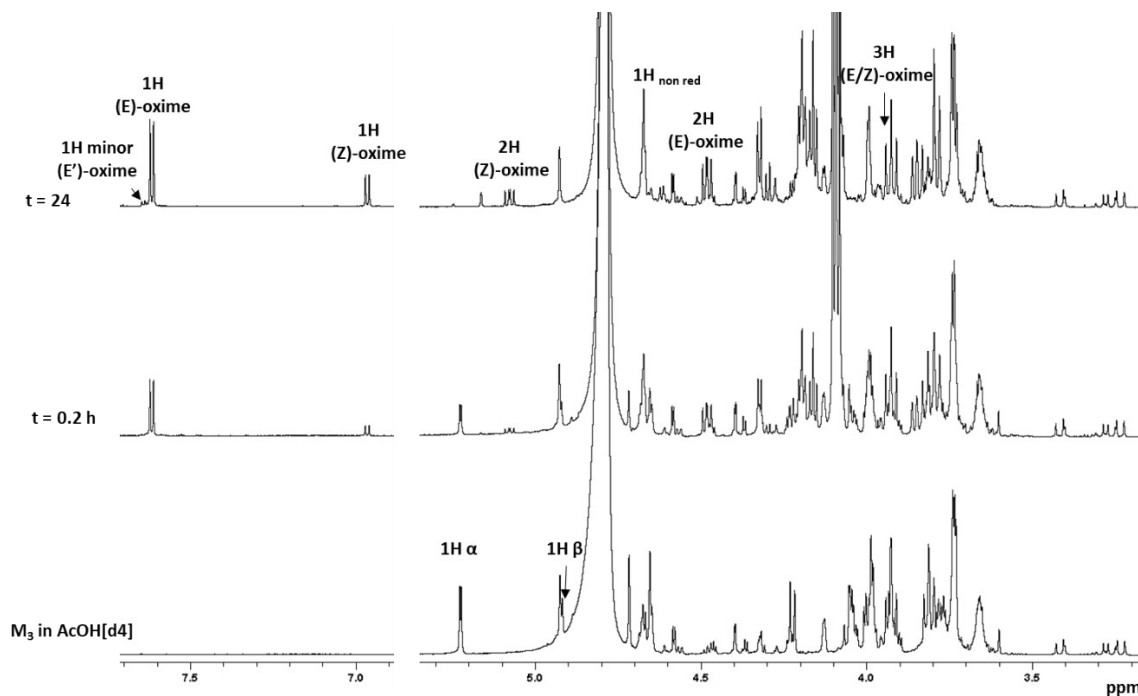


Fig. S12 Stack of 1D ¹H-NMR spectra obtained at different timepoints for the reaction with M₃ (7mM) and PDHA (2 equiv.) in 500 mM AcOH[d₄] (27 °C, 600 MHz) (M₃ is included for comparison). Key resonances indicative of the reaction are annotated.

The reaction mixture with M₃ and PDHA (24 h reaction time, 10 equiv. PDHA, 500 mM NaAc-buffer pH 4) was purified by GFC, dialysis and freeze drying.

H1 of the oxime conjugates were assigned based on literature^{2,3}. H2 and H3 of the (E)- and (Z)-oxime was assigned using 1D selective COSY and TOCY NMR (Fig. S13). Complete assignment was obtained by heteronuclear NMR, Fig. S14.

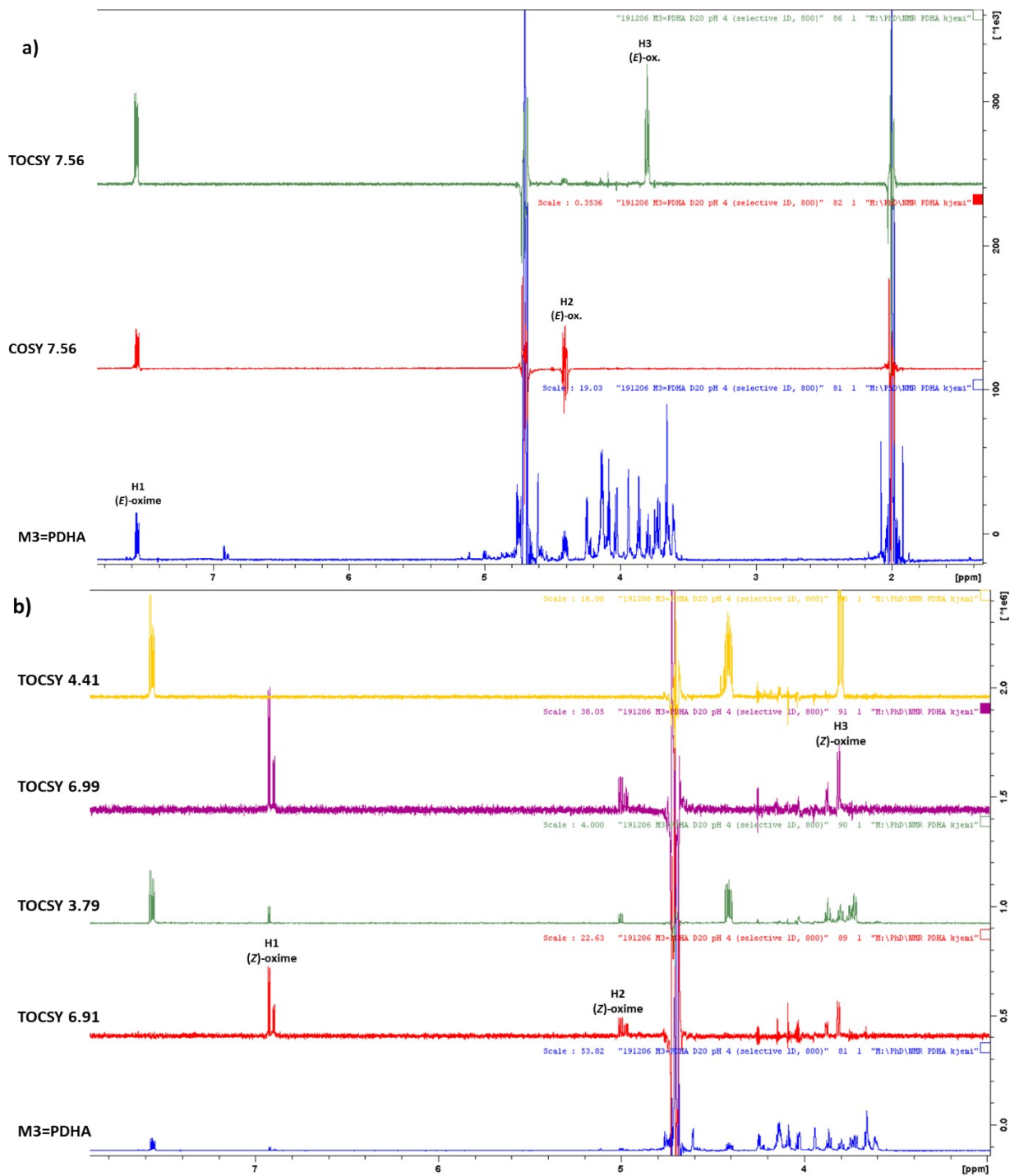


Fig. S13 1D selective COSY and TOCSY NMR spectra of M₃-PDHA (after purification) in D₂O, pH 4 (25 °C, 800 MHz). a) Assignment of H1-H3 of the (E)-oxime, and b) assignment of H1-H3 of the (Z)-oxime. The ppm of the pulse is indicated to the left.

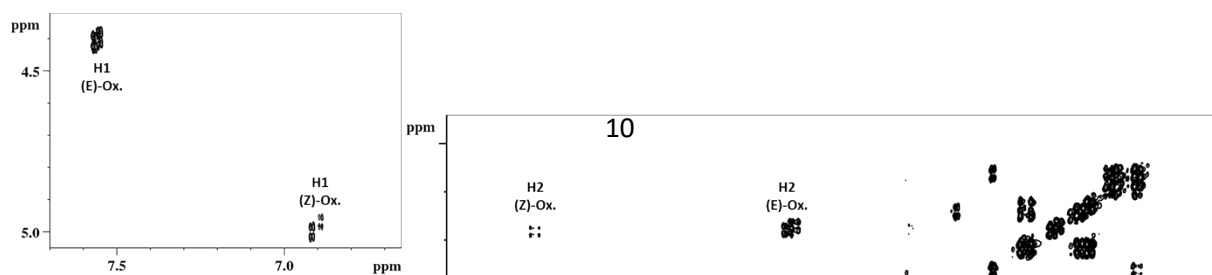


Fig. S14 ^1H - ^1H COSY spectra of the oxyamine modified trimannuronate (M_3 -PDHA) (after purification by GFC, dialysis and freeze drying) in D_2O pH 4 (25 °C, 800 MHz).

Table S2: Assignment of chemical shifts for oxyamine modified trimannuronate (M_3 -PDHA) (after purification by GFC, dialysis and freeze drying) in D_2O pH 4 obtained from 1D selective and heteronuclear NMR experiments. Prime indicates the minor form.

	H/C 1	H/C 1'	H/C 2	H/C 3	H/C 4	H/C 5	C 6
(E)-oxime	7.61; 151.7	7.65; 151.7	4.40; 68.04	3.79; 75.65	4.11; 77.6	4.25; 71.31	178.1
(Z)-oxime	6.96; 151.6	7.00; 151.3	4.98; 63.1	3.79; 75.65	4.11; 77.6	4.25; 71.31	178.1

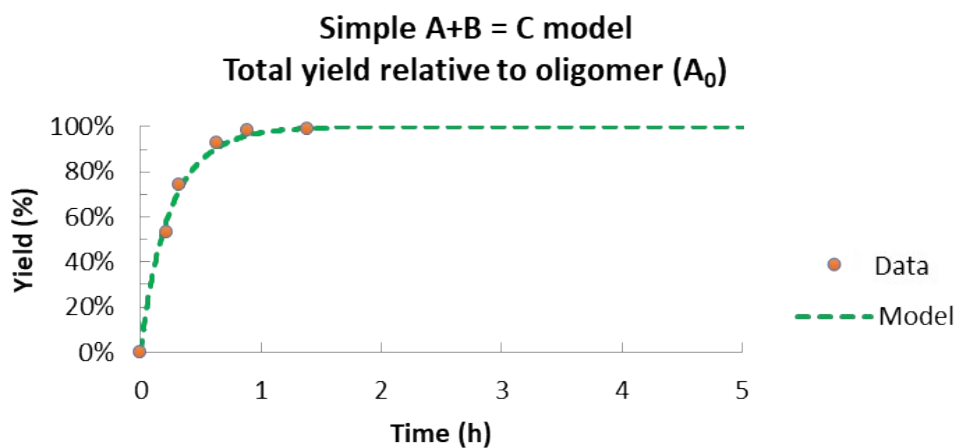


Fig. S15 Plot with combined yield for conjugation with M_3 (7 mM) and PDHA (2 equiv.) (500 mM AcOHd4 pD 4) studied by time course NMR (at 27 °C using the 600 MHz). Data obtained by integration using TSP as internal standard. A simple model assuming first order kinetics based on combined yield is included.

Conjugation with ADH

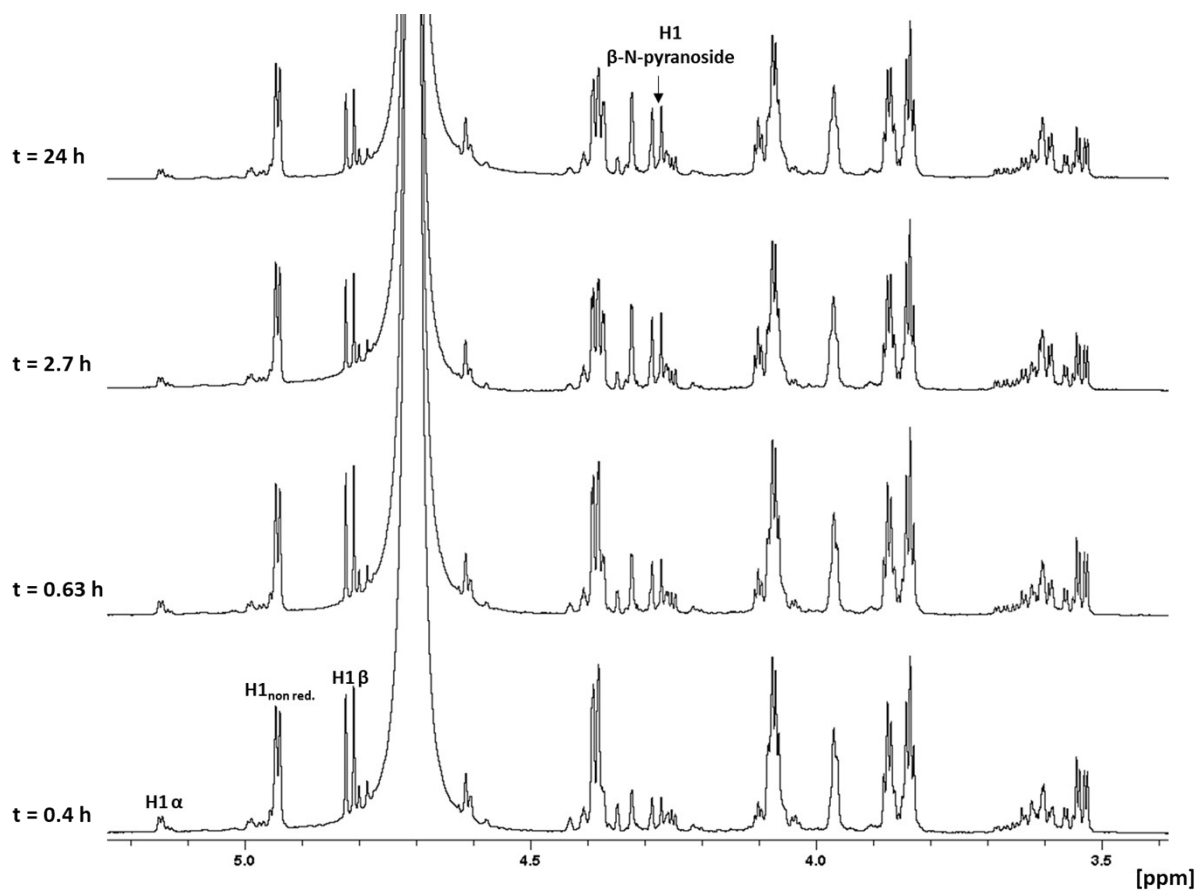


Fig. S16 Stack of ¹H-NMR spectra (27 °C, 600 MHz) obtained at different time points for the reaction with G₂ (7mM) and ADH (2 equiv.) at pD 4 (500 mM AcOHd₄).

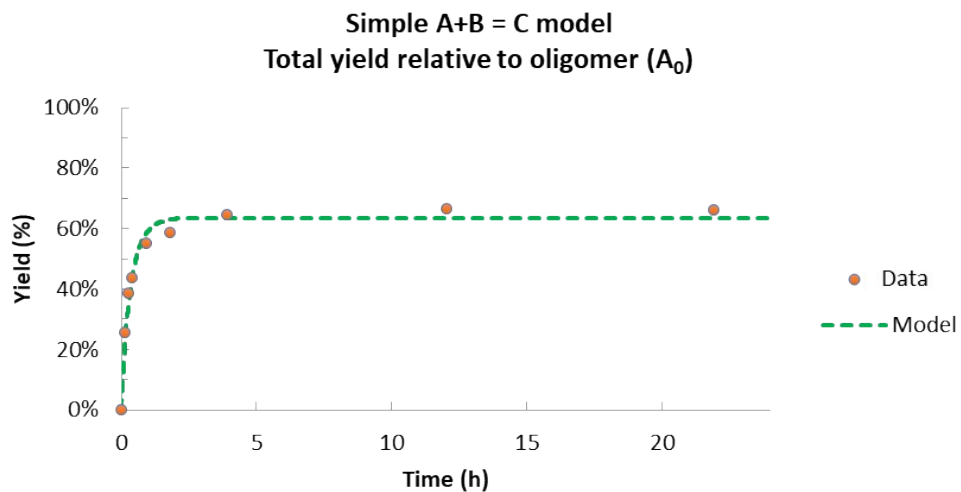


Fig. S17 Plot with combined yield for conjugation with G_2 (7 mM) and ADH (2 equiv.) (500 mM AcOHd4 pD 4) studied by time course NMR (at 27 °C using a 600 MHz magnet). Data obtained by integration using H1 of the non-reducing end as internal standard. A simple model assuming first order kinetics based on combined yield is included.

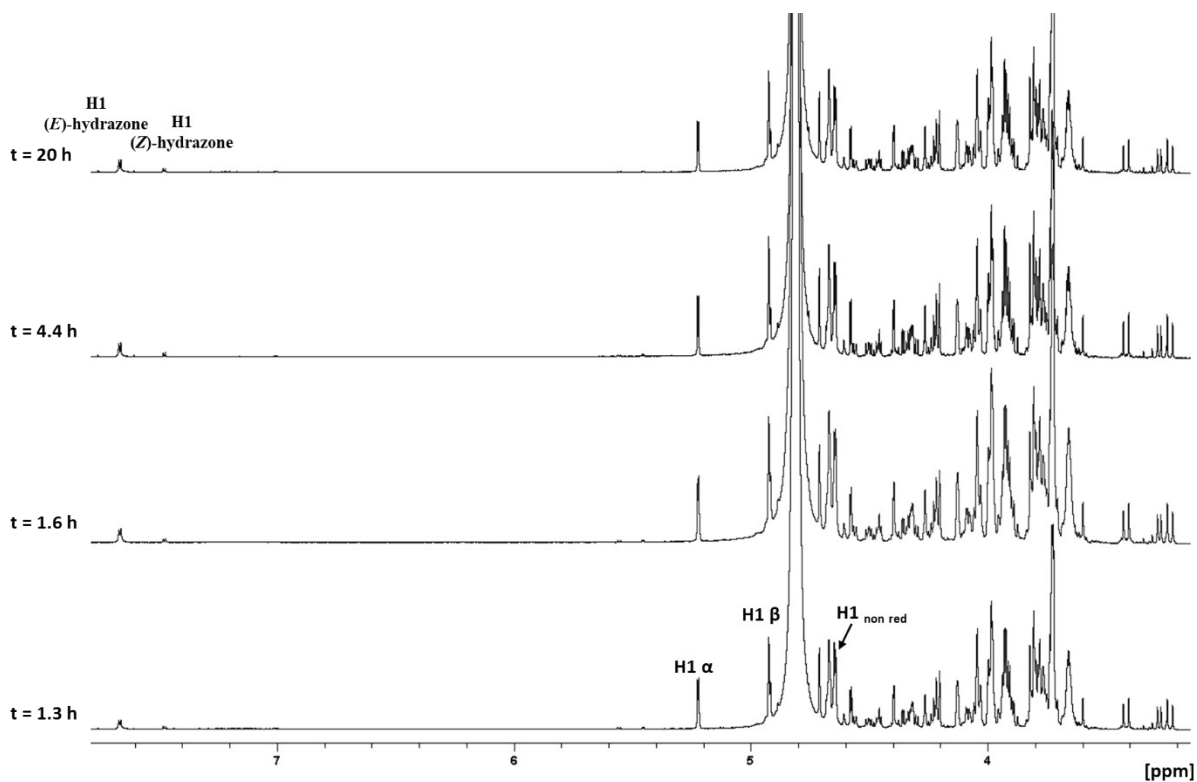


Fig. S18 Stack of $^1\text{H-NMR}$ (27 °C, 600 MHz) spectra obtained at different time points for the reaction with M_3 (7mM) and ADH (2 equiv.) at pD 4 (500 mM AcOHd_4).

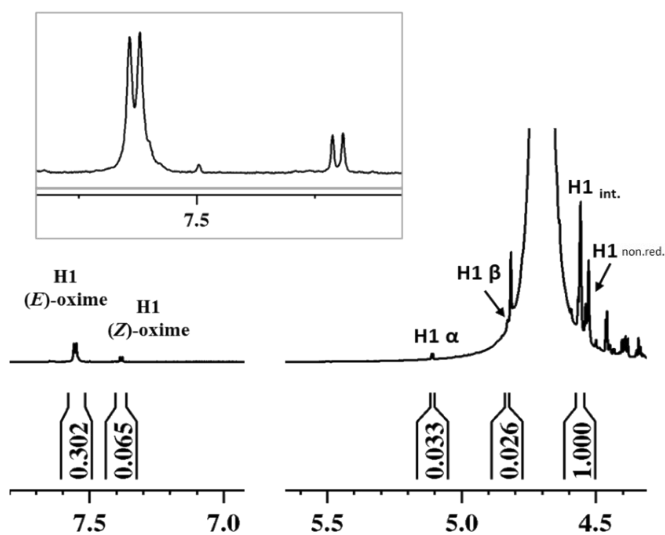


Fig. S19 $^1\text{H-NMR}$ (27 °C, 600 MHz) spectrum of the equilibrium reaction mixture ($t > 20$ h) for the reaction with M_3 (7mM) and ADH (10 equiv.) (500 mM AcOHd_4 pD 4).

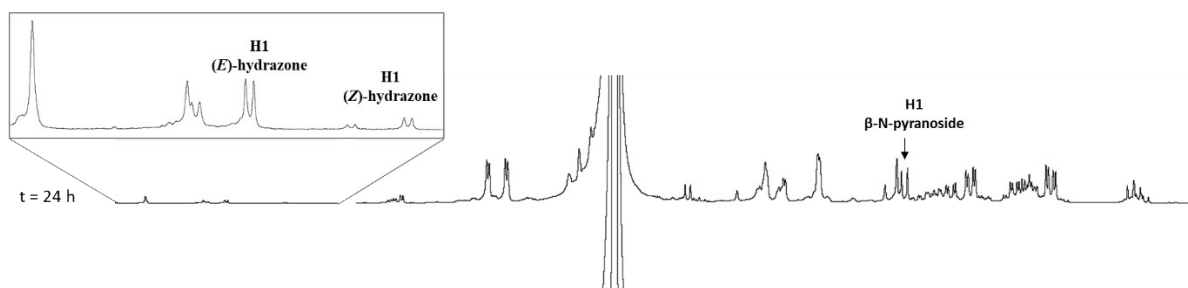


Fig. S20 Stack of $^1\text{H-NMR}$ (27 °C, 600 MHz) spectra obtained at different time points for the reaction with Galacturonic acid DP 3 (TriGalA₃) (7mM) and ADH (2 equiv.) at pD 4 (500 mM AcOHd4). A spectrum of pure TriGalA is included for comparison.

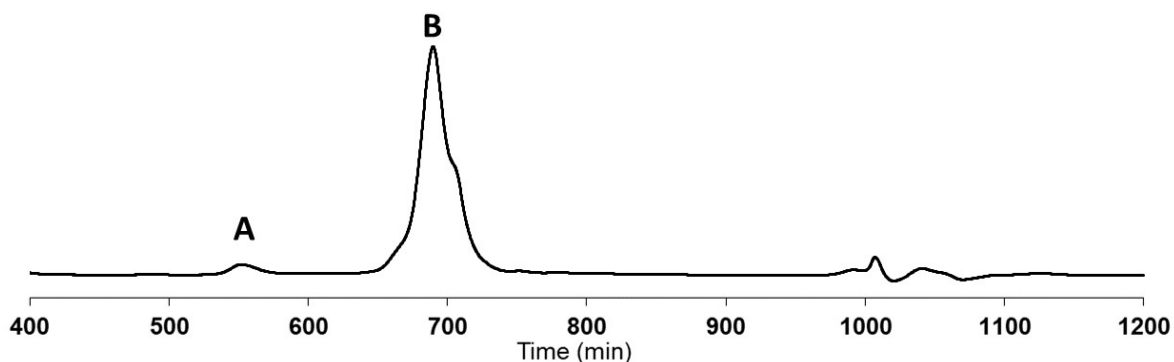


Fig. S21 GFC fractions of the products formed in the reaction with G₁₀ and PDHA (10 equiv.) with PB (3 equiv).

Product having elution times corresponding to peak B (Fig. S21) is in agreement with G₁₀-PDHA. Peak A has elution volumes corresponding to a G₁₀-PDHA-G₁₀ based on comparison to a G_n hydrolysate.

Attachment of the second block: A-b-B diblock polysaccharides

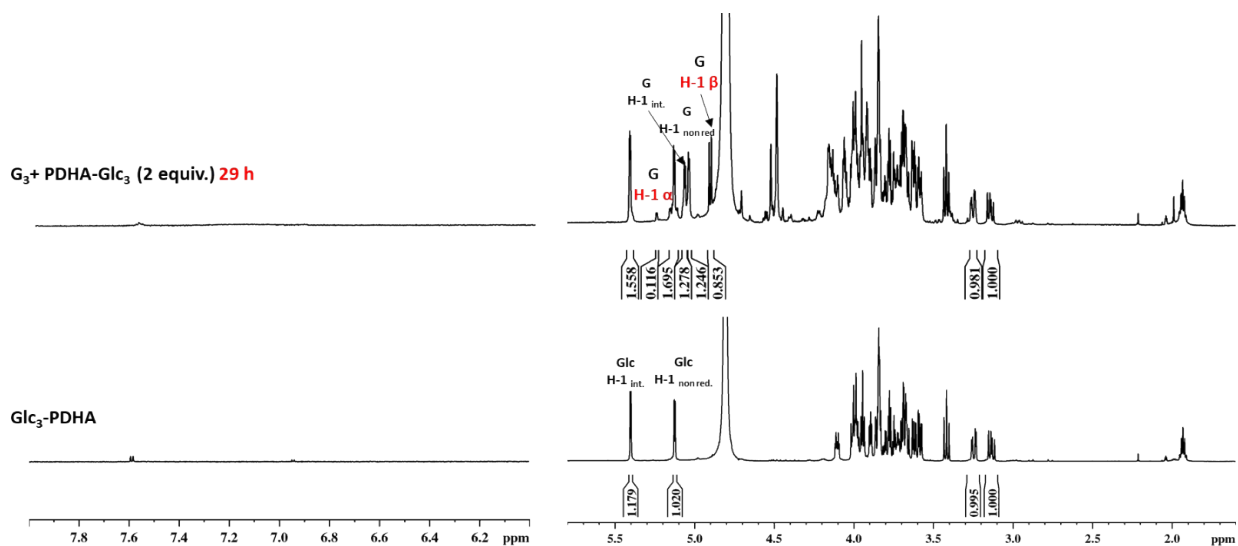


Fig. S22 $^1\text{H-NMR}$ (27 °C, 600 MHz) spectrum of the reaction mixture with G_3 (7mM) and maltotriose DP 3 with PDHA ($\text{Glc}_3\text{-PDHA}$) (2 equiv.) (500 mM AcOHd4 pD 4). Purified $\text{Glc}_3\text{-PDHA}$ (in 500 mM AcOHd4 pD 4) is included for comparison.

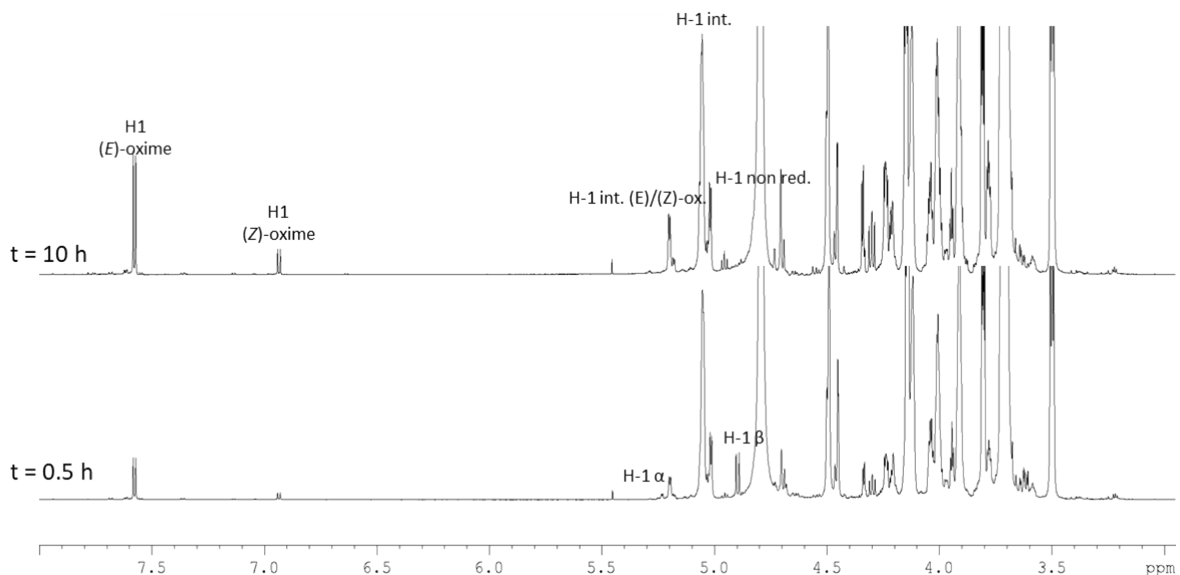


Fig. S23 Stack of $^1\text{H-NMR}$ (27 °C, 600 MHz) spectra obtained at different time points for the reaction with G_7 (20.1 mM) and aminoxy- $\text{PEG}_5\text{-N}_3$ (2 equiv.) (500 mM AcOHd4 , pD 4).

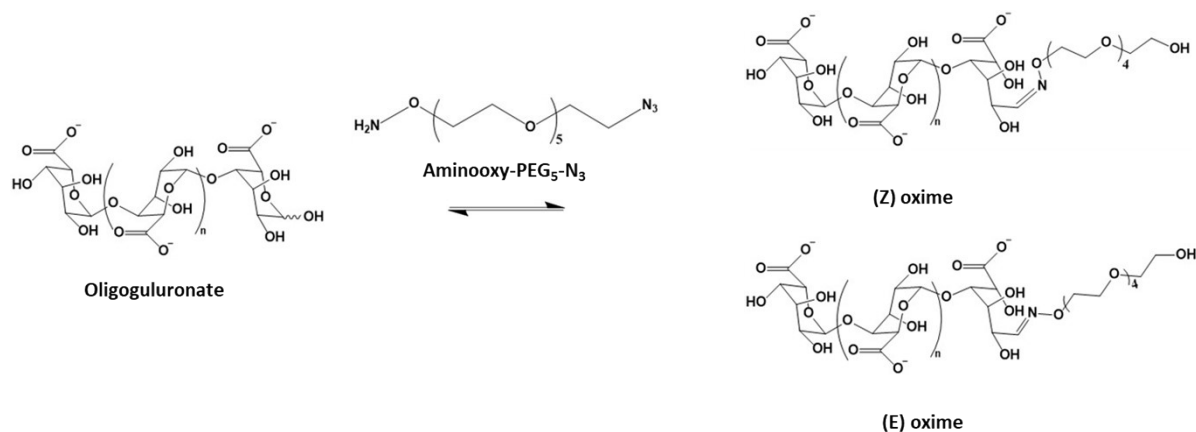


Fig. S24 Reaction scheme for the reaction with G_7 and aminoxy- $\text{PEG}_5\text{-N}_3$.

Reduction of oximes/hydrazones/N-pyranosides with α -picoline borane

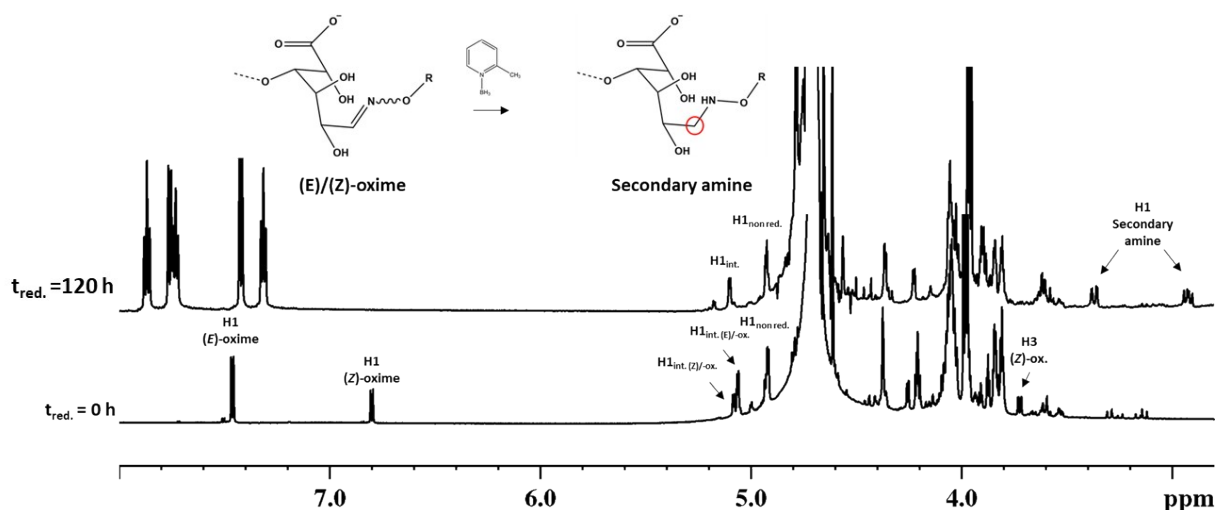


Fig. S25 $^1\text{H-NMR}$ (27 °C, 600 MHz) spectra of equilibrium reaction mixture with G_3 (7mM) and PDHA (2 equiv.) before reduction ($t_{\text{red.}} = 0$). PB (3 equiv.) was added to the reaction mixture and the spectrum obtained after 120 h is shown with annotation of the resonances from the secondary amine characteristic of the reduced conjugate.

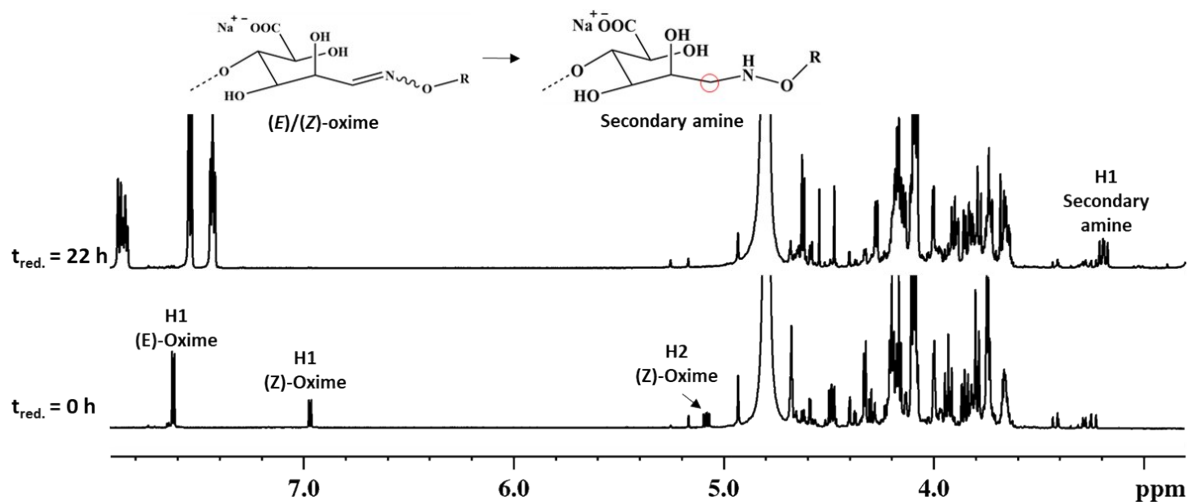


Fig. S26 $^1\text{H-NMR}$ (27 °C, 600 MHz) spectra of equilibrium reaction mixture with M_3 (7mM) and PDHA (2 equiv.) before reduction ($t_{\text{red.}} = 0$). PB (3 equiv.) was added to the reaction mixture and the spectrum obtained after 22 h is shown with annotation of key resonances.

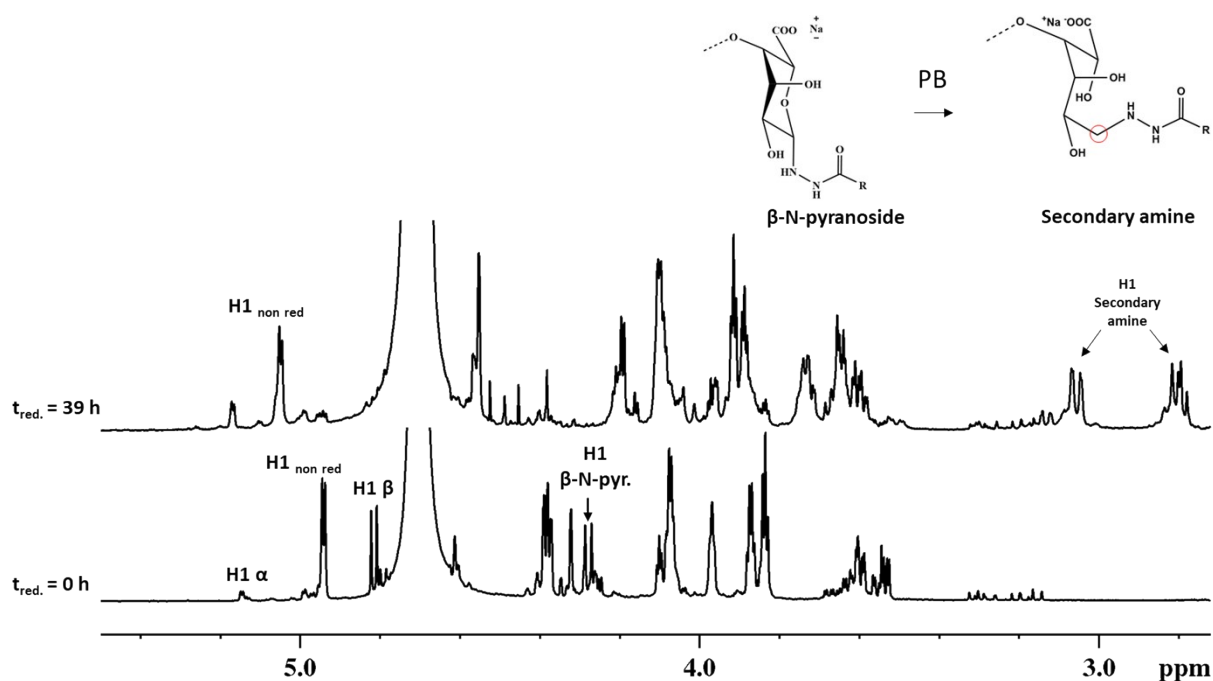


Fig. S27 $^1\text{H-NMR}$ (27 °C, 600 MHz) spectra of equilibrium reaction mixture with G_2 (7mM) and ADH (2 equiv.) before reduction ($t_{\text{red}} = 0$). PB (3 equiv.) was added to the reaction mixture and the spectrum obtained after 39 h is shown with annotation of key resonances.

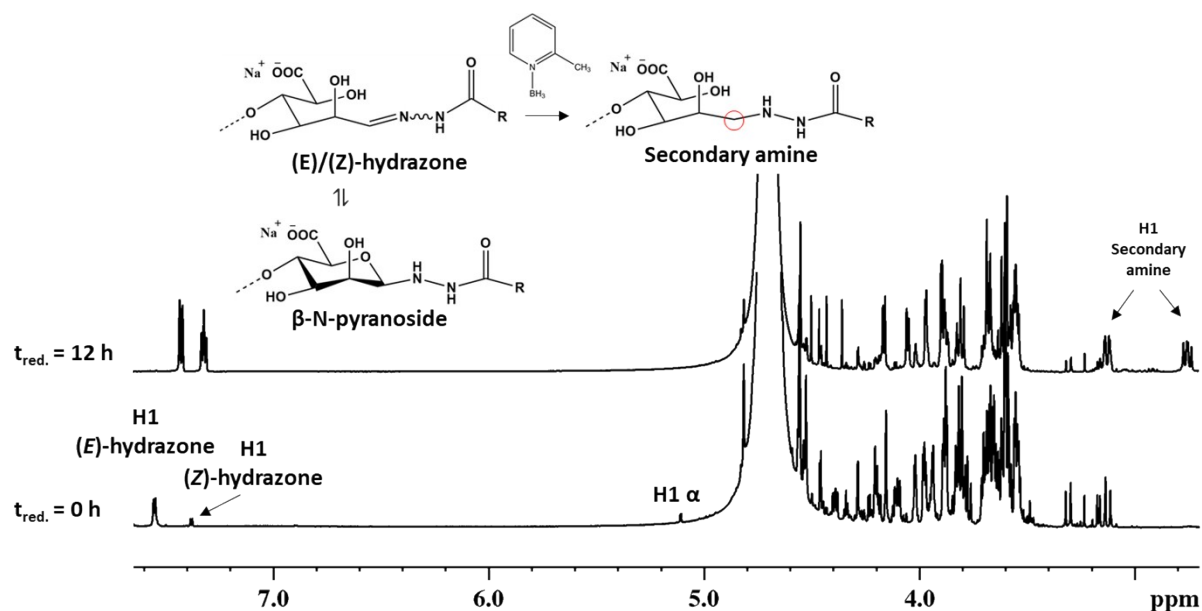


Fig. S28 $^1\text{H-NMR}$ (27 °C, 600 MHz) spectra of equilibrium reaction mixture with M_3 (7mM) and ADH (10 equiv.) before reduction ($t_{\text{red}} = 0$). PB (3 equiv.) was added to the reaction mixture and the spectrum obtained after 12 h is shown with annotation of key resonances.

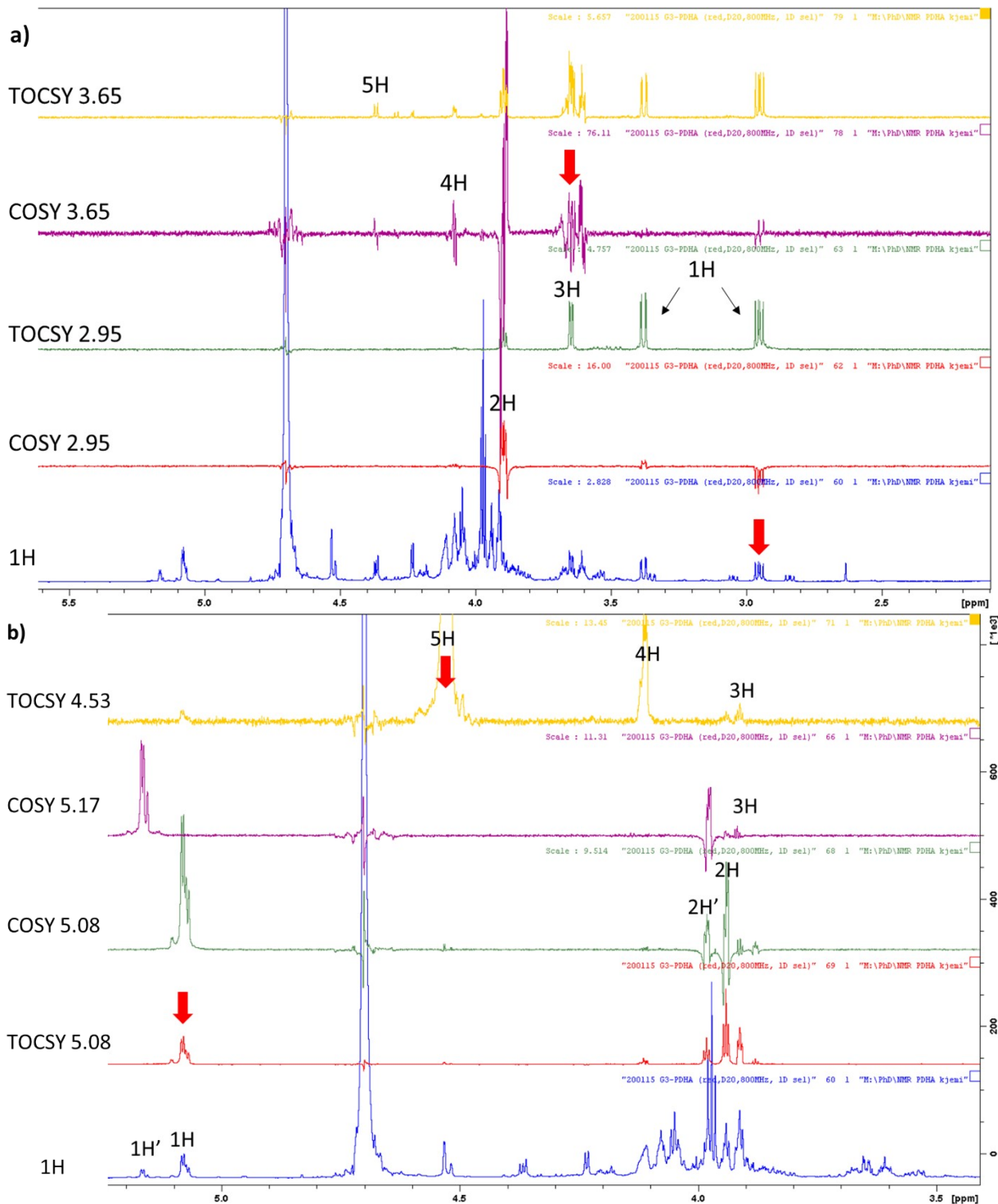
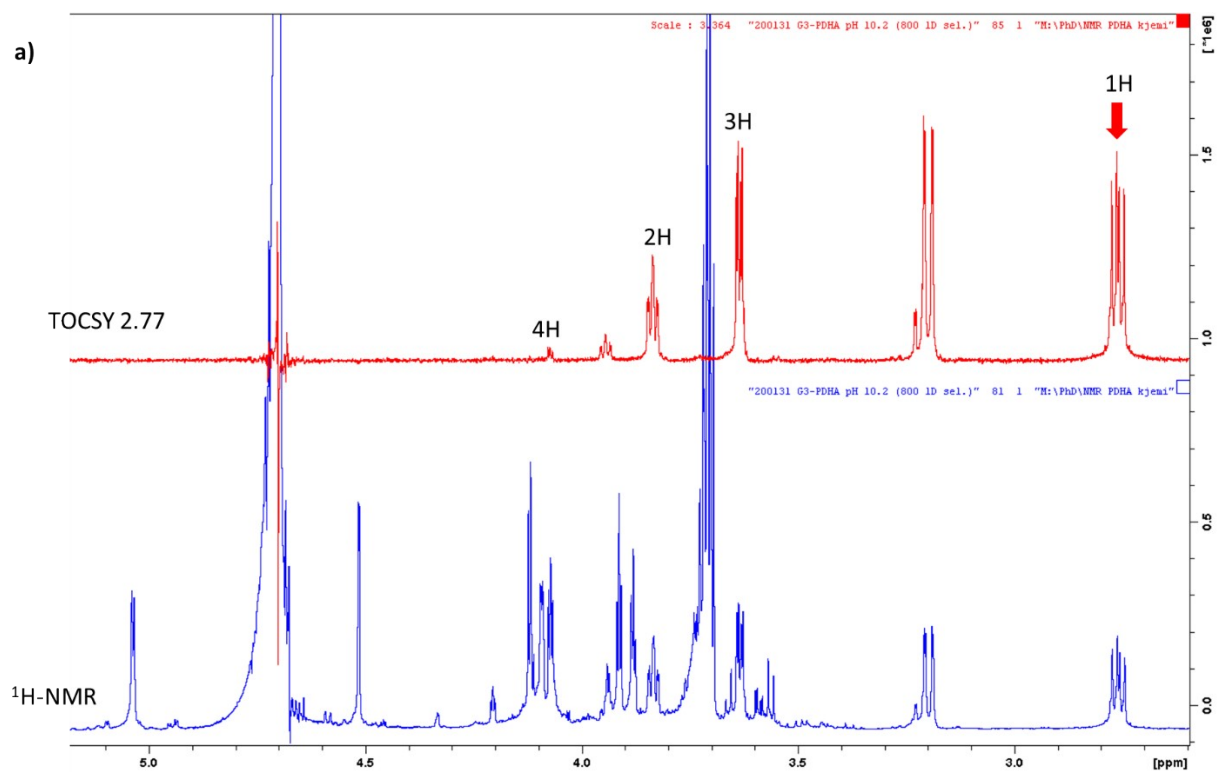


Fig. S29 Assignment of key reducing end resonances from the oxyamine modified reducing end of G_2 -PDAH (with reduction, after purification by GFC, dialysis and freeze drying) in D_2O at pH 5.1 (25 $^{\circ}C$,

800 MHz) using 1D selective COSY and TOCSY NMR. Red arrows indicate the ppm of the pulse.

Table S3: Assignment of proton resonances of G₂-PDHA (with reduction, purified by GFC, dialysis and freeze drying). Dissolved in D₂O, pH 5.1 by 1D selective COSY and TOCSY. RT is the modified reducing termini of G2-PDHA.

	H1	H2	H3	H4	H5
RT pH 5.1	2.96/3.38	3.90	3.65	4.08	4.37
	H1/H1'	H2/H2'			
NRT pH 5.1	5.08/5.17	3.94/3.98	3.92	4.11	4.53



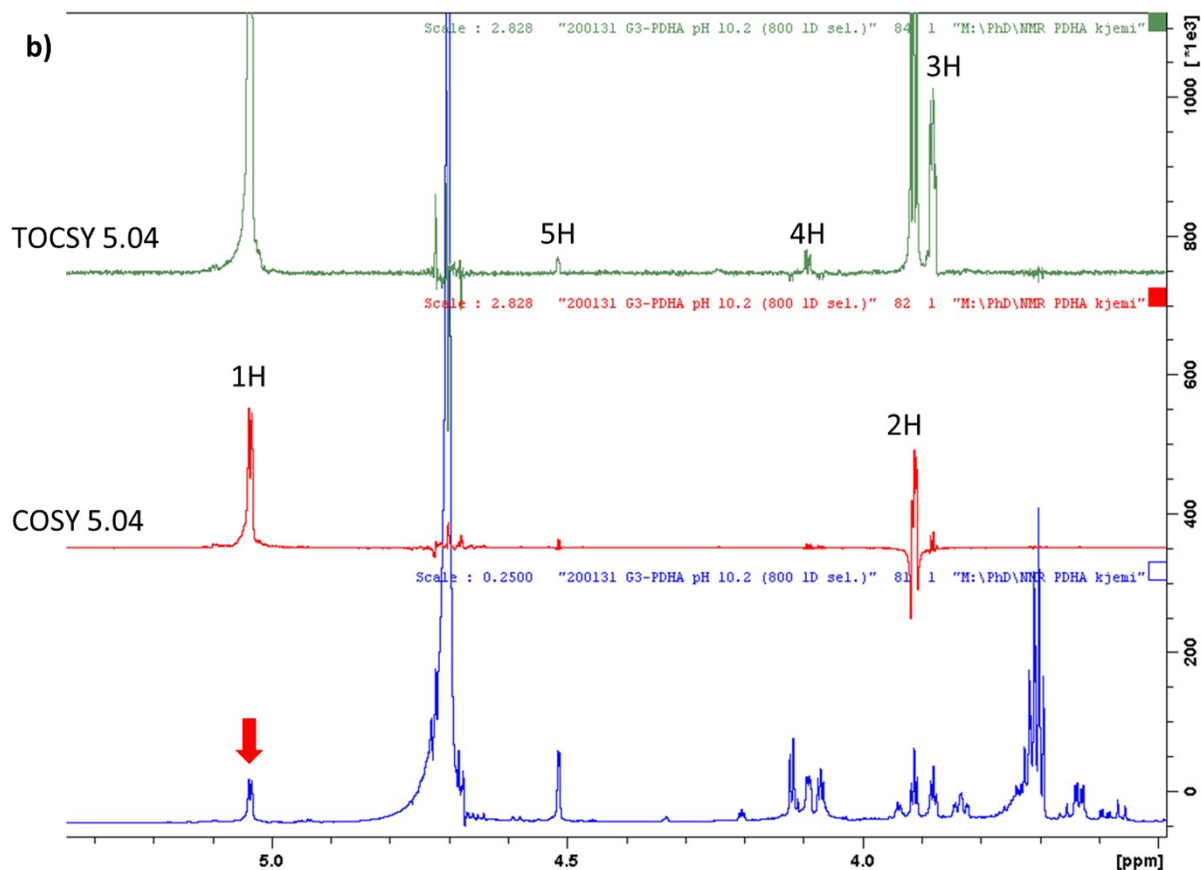


Fig. S30 Assignment of key reducing end resonances from the oxyamine modified reducing end of G₂-PDAH (with reduction, after purification by GFC, dialysis and freeze drying) in D₂O at pH 10.2 (25 °C, 800 MHz) using 1D selective COSY and TOCSY NMR. Red arrows indicate the ppm of the pulse.

Table S4: Assignment of proton resonances of G₂-PDHA (with reduction, purified by GFC, dialysis and freeze drying. Dissolved in D₂O, pH 5.1) by 1D selective COSY and TOCSY. RE denotes is the oxyamine modified reducing end of G₂-PDHA. N.d. denotes not determined.

	H1	H2	H3	H4	H5
RE pH 10.2	3.20/2.76	3.84	3.63	4.07	n.d.
NRT pH 10.2	5.04	3.91	3.88	4.09	4.52

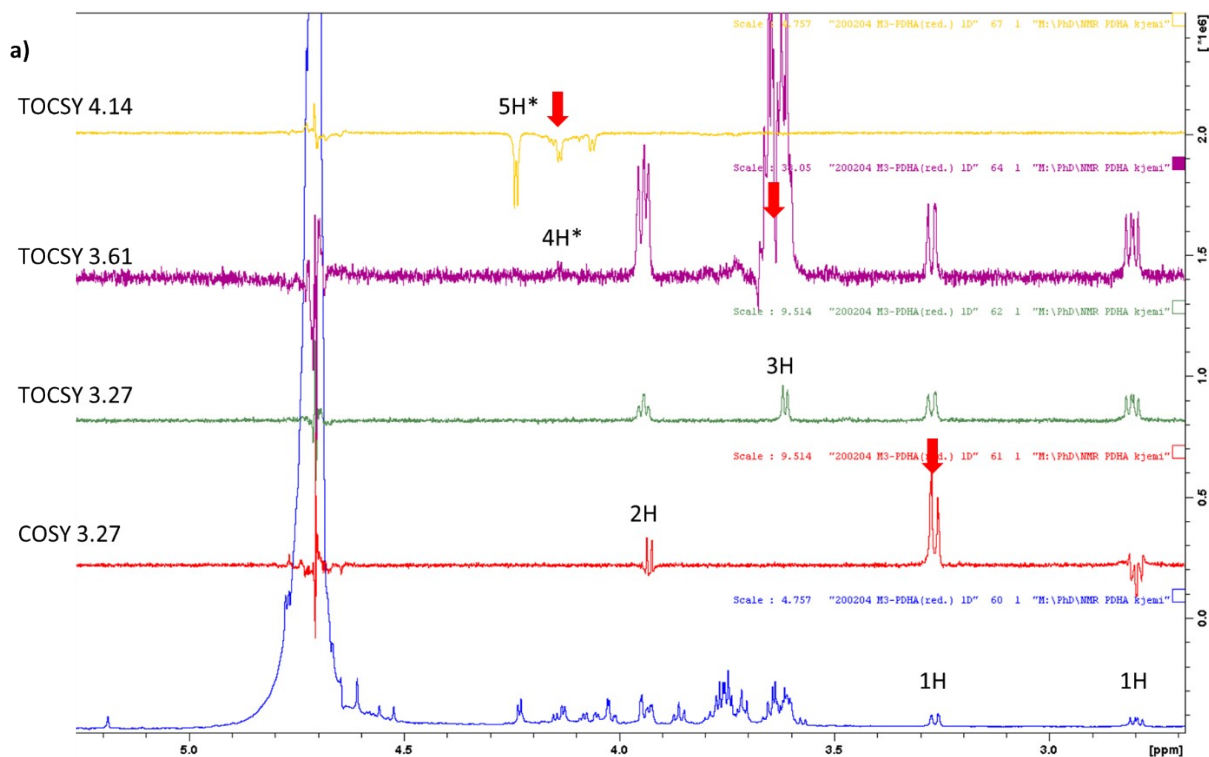


Fig. S31 Assignment of key reducing end resonances from the oxyamine modified reducing end of M₃-PDHA (with reduction, after purification by GFC, dialysis and freeze drying) in D₂O at pH 10.2 (25°C, 800 MHz) using 1D selective COSY and TOCSY NMR. Red arrows indicate the ppm of the pulse.

Table S5: Assignment of the modified reducing end (RE) of M₃-PDHA (with reduction, after purification by GFC, dialysis and freeze drying) in D₂O at pH 10.2 (800 MHz) using 1D selective COSY and TOCSY NMR. *Weak magnetization transfer

	H1	H2	H3	H4	H5
RE pH 10.7	2.80/3.27	3.93	3.61	4.14*	n.d.

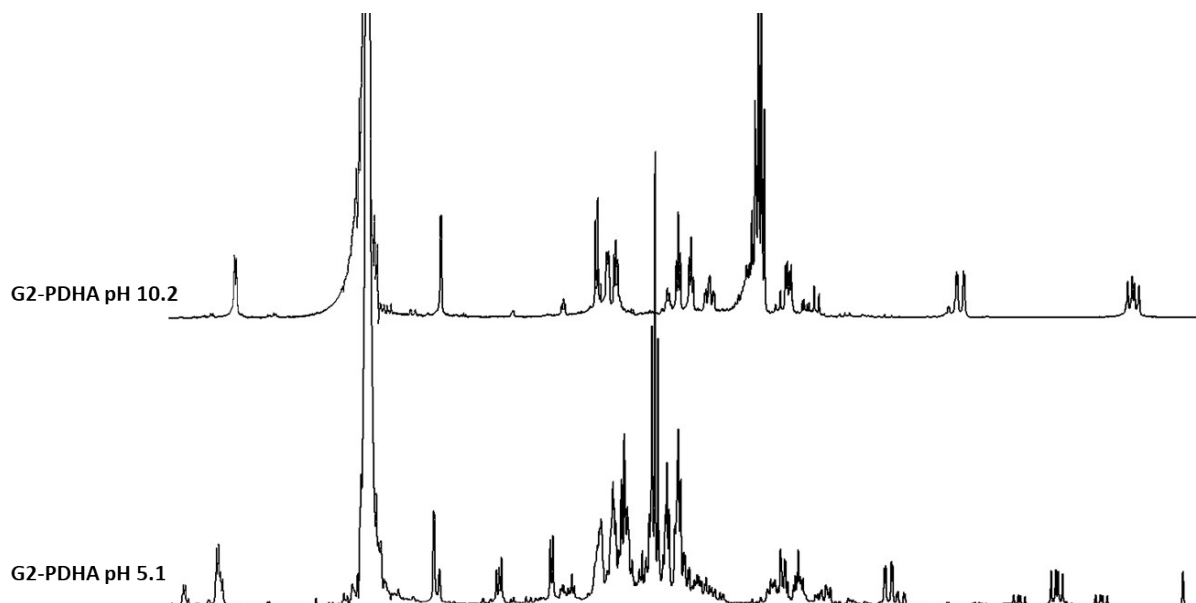


Fig. S32 $^1\text{H-NMR}$ (27 °C, 600 MHz) spectra of G_2 -PDHA after purification by GFC and dialysis. The sample was dissolved in D_2O and pH was adjusted from 5.1 to 10.2 with NaOD.

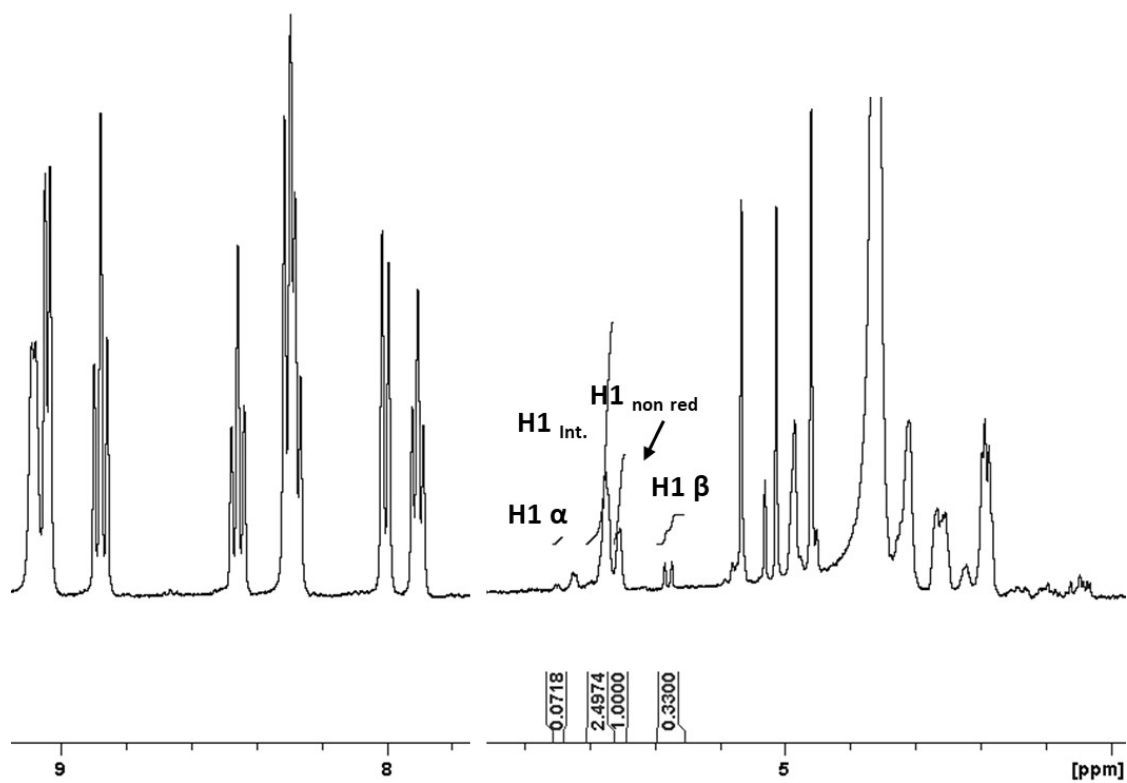


Fig. S33 $^1\text{H-NMR}$ spectra of G_6 (20.1 mM) with PB (20 equiv.) in 500 mM AcOHd4 pD 4 at incubated in water bath at 40°C, recorded after 24 h (82°C, 400 MHz).

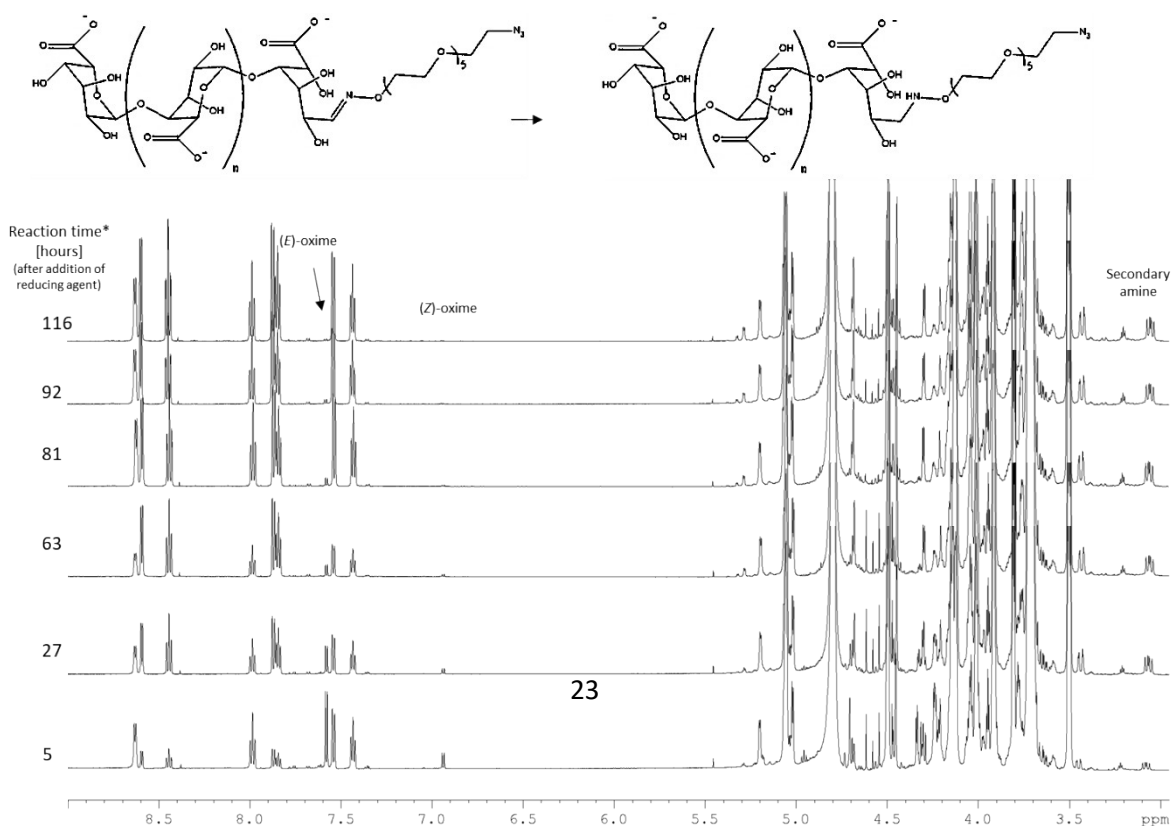


Fig. S34 $^1\text{H-NMR}$ (27 °C, 600 MHz) spectra of the reduction of the equilibrium rx. mixture with G7 (20.1 mM) and aminoox-PEG₅-N₃ after addition of PB (3 equiv. added at t₀, another 3 equiv. added after 72 h).

Alginate-based diblock polysaccharides

A symmetrical diblock was prepared with G₁₀ and PDHA (using 0.5 equiv.) and by reduction with PB. Excess PB was removed by dialysis and the sample was freeze dried. The reaction mixture was analysed by SEC-MALS (the sample was not purified by GFC) with an in-line viscosity detector.

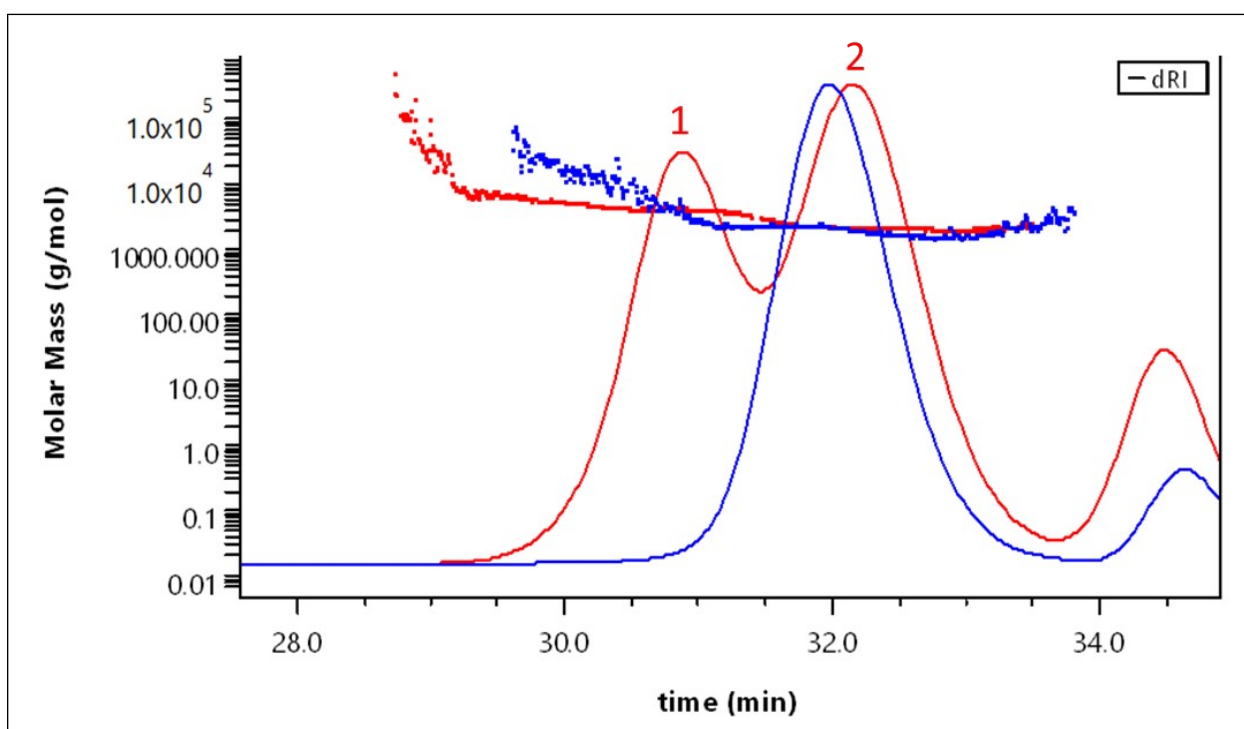


Fig. S35 A symmetrical G₁₀ was reacted with 0.5 equivalents PDHA, and the reaction mixture was analysed by SEC-MALS (red). A G₁₀ was included for comparison (blue). The coupling to form a diblock (G₁₀-PDHA-G₁₀) is manifested by a significant shift in elution volume (peak 1). Unreacted G₁₀ and G₁₀-PDHA comprise approximately 50 – 60 % of the sample (peak 2), and elutes close to pure G₁₀. Data are summarized in table S6.

Table S6: Molar mass averages of a G₁₀-b-G₁₀ block (prepared by reacting G₁₀ with 0.5 equiv. PDHA with reduction by PB, without purification by GFC) and the starting material (G₁₀). G₂₃ is included for comparison of intrinsic viscosities (see text). The data were obtained from SEC-MALS with an in-line viscosity detector, the plot of molar mass vs. time is shown in Fig. S35.

Sample	M _n (kDa)	M _w (kDa)	DP _n	[η] _w (mL/g)
G ₁₀	2.0	2.0	10	6.7

G ₁₀ -PDHA-G ₁₀ (peak 1, Fig. S35)	4.1	4.2	20	10.1
G ₁₀ (peak 2, Fig. S35)	2.1	2.2	10	6.7

References

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2. Baudendistel, O. R.; Wieland, D. E.; Schmidt, M. S.; Wittmann, V., Real-Time NMR Studies of Oxyamine Ligations of Reducing Carbohydrates under Equilibrium Conditions. *Chemistry* **2016**, *22* (48), 17359-17365.
3. Mo, I. V.; Feng, Y.; Dalheim, M. Ø.; Solberg, A.; Aachmann, F. L.; Schatz, C.; Christensen, B. E., Activation of enzymatically produced chitooligosaccharides by dioxyamines and dihydrazides. *Carbohydrate Polymers* **2020**, *232*, 115748.