Alginite-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-PAD chromatograms for guluronate oligomers taken at different timepoints during hydrolysis (95°C, pH 3.62).](image)

Oligomers with defined DP$_n$ were prepared by GFC, and DP$_n$ was verified by $^1$H-NMR (Fig. S3). Isolated triguluronate was annotated according to literature$^1$. 

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

Fig. S3 $^1$H-NMR (82 °C, 400 MHz) spectrum of isolated triguluronate, peaks were assigned according to literature $^1$. 
Conjugation with PDHA

Fig. S4 Stack of $^1$H-NMR spectra obtained after different timepoints for the reaction with $G_3$ (7 mM) and PDHA (2 equiv.) in 500 mM AcOH[$d_4$] pD 4 (27 °C, 600 MHz). Pure $G_3$ (in buffer) is included for comparison.
The equilibrium reaction mixture with G₃ and PDHA was characterized by heteronuclear NMR.

**Fig. S5** a,b) $^{13}$C 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) $^1$H-NMR spectra of G₃-PDHA after purification by GFC and dialysis in D₂O at pH 6.5 and 11.8 (27 °C, 600 MHz)
Table S1: Assignment of chemical shifts for the reaction mixture with G3 and PDHA obtained from HSQC, h2BC and HMBC (G3 (7 mM) and PDHA (14 mM) in 500 mM AcOH[d4] pD 4) (82 °C, NEO 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_1 and k_-1) for the combined yield.

Fig. S6 Conjugation with G3 (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 1H of the non-reducing ends as internal standard. A simple model assuming first order kinetics based on combined yield is included.
**Fig. S7** \(^1\)H-NMR spectra of the equilibrium reaction mixture with G\(_3\) (7mM) and PDHA (2 equiv.) (in 500 mM AcOHd4 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 AcOHd4 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 (7 mM) and b) Maltotriose (7 mM) and PDHA (2 equiv.) (500 mM AcOHd4 at pH 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 $^1$H-NMR spectra obtained at different time points for the reaction with $G_3$ (7 mM) and PDHA (2 equiv.) at pH 5 (500 mM AcOHd4, 27 °C, 600 MHz).

**Fig. S11** Conjugation with $G_3$ (7 mM) and PDHA (2 equiv.) at pH 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 literature²,³. 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** $^1$H-$^1$H COSY spectra of the oxyamine modified trimannuronate ($M_3$-PDHA) (after purification by GFC, dialysis and freeze drying) in D$_2$O 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$_2$O 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; |
|          | 4.40; | 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; |
|          | 4.98; | 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 pH 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 $^1$H-NMR spectra (27 °C, 600 MHz) obtained at different time points for the reaction with $G_2$ (7mM) and ADH (2 equiv.) at pD 4 (500 mM AcOHd4).
**Fig. S17** Plot with combined yield for conjugation with G₂ (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 $^1$H-NMR (27 °C, 600 MHz) spectra obtained at different time points for the reaction with $\text{M}_3$ (7mM) and ADH (2 equiv.) at pD 4 (500 mM AcOHd4).

Fig. S19 $^1$H-NMR (27 °C, 600 MHz) spectrum of the equilibrium reaction mixture ($t > 20$ h) for the reaction with $\text{M}_3$ (7mM) and ADH (10 equiv.) (500 mM AcOHd4 pD 4).
**Fig. S20** Stack of $^1$H-NMR (27 °C, 600 MHz) spectra obtained at different time points for the reaction with Galacturonic acid DP 3 (TriGalA$_3$) (7mM) and ADH (2 equiv.) at pH 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 G$_{10}$ and PDHA (10 equiv.) with PB (3 equiv).

Product having elution times corresponding to peak B (Fig. S21) is in agreement with G$_{10}$-PDHA. Peak A has elution volumes corresponding to a G$_{10}$-PDHA-G$_{10}$ based on comparison to a G$_n$ hydrolysate.

**Attachment of the second block: A-b-B diblock polysaccharides**
Fig. S22 $^1$H-NMR (27 °C, 600 MHz) spectrum of the reaction mixture with G$_3$ (7mM) and maltotriose DP 3 with PDHA (Glc$_3$-PDHA) (2 equiv.) (500 mM AcOHd4 pD 4). Purified Glc$_3$-PDHA (in 500 mM AcOHd4 pD 4) is included for comparison.

Fig. S23 Stack of $^1$H-NMR (27 °C, 600 MHz) spectra obtained at different time points for the reaction with G$_7$ (20.1 mM) and aminoox-PEG$_5$-N$_3$ (2 equiv.) (500 mM AcOHd4, pD 4).

Fig. S24 Reaction scheme for the reaction with G$_7$ and aminoox-PEG$_5$-N$_3$. 
Reduction of oximes/hydrazones/N-pyranosides with α-picoline borane

**Fig. S25** $^1$H-NMR (27 °C, 600 MHz) spectra of equilibrium reaction mixture with G$_3$ (7mM) and PDHA (2 equiv.) before reduction ($t_{red} = 0$). 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** $^1$H-NMR (27 °C, 600 MHz) spectra of equilibrium reaction mixture with M$_3$ (7mM) and PDHA (2 equiv.) before reduction ($t_{red} = 0$). 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** $^1$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** $^1$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 G2-PDAH (with reduction, after purification by GFC, dialysis and freeze drying) in D2O at pH 5.1 (25 °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 |
| NRT pH 5.1 | 5.08/5.17 | 3.94/3.98 | 3.92 | 4.11 | 4.53 |

**Figure a)**

- TOCSY 2.77
- 1H-NMR
Fig. S30 Assignment of key reducing end resonances from the oxyamine modified reducing end of G\textsubscript{2}-PDAH (with reduction, after purification by GFC, dialysis and freeze drying) in D\textsubscript{2}O at pH 10.2 (25 °C, 800 MHz) using 1D selective COSY and TOCSY NMR. Red arrows indicate the ppm of the pulse.

|        | 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  |

Table S4: Assignment of proton resonances of G\textsubscript{2}-PDHA (with reduction, purified by GFC, dialysis and freeze drying. Dissolved in D\textsubscript{2}O, pH 5.1) by 1D selective COSY and TOCSY. RE denotes is the oxyamine modified reducing end of G\textsubscript{2}-PDHA. N.d. denotes not determined.
**Fig. S31** Assignment of key reducing end resonances from the oxyamine modified reducing end of M₃-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 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** $^1$H-NMR (27 °C, 600 MHz) spectra of G$_2$-PDHA after purification by GFC and dialysis. The sample was dissolved in D$_2$O and pH was adjusted from 5.1 to 10.2 with NaOD.

**Fig. S33** $^1$H-NMR spectra of G$_6$ (20.1 mM) with PB (20 equiv.) in 500 mM AcOHd$_4$ pH 4 at incubated in water bath at 40°C, recorded after 24 h (82°C, 400 MHz).
Fig. S34 $^1$H-NMR (27 °C, 600 MHz) spectra of the reduction of the equilibrium rx. mixture with G7 (20.1 mM) and aminoox-PEG$_2$-N$_3$ 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 G$_{10}$ 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 G$_{10}$ was reacted with 0.5 equivalents PDHA, and the reaction mixture was analysed by SEC-MALS (red). A G$_{10}$ was included for comparison (blue). The coupling to form a diblock (G$_{10}$-PDHA-G$_{10}$) is manifested by a significant shift in elution volume (peak 1). Unreacted G$_{10}$ and G$_{10}$-PDHA comprise approximately 50 – 60 % of the sample (peak 2), and elutes close to pure G$_{10}$. Data are summarized in table S6.

Table S6: Molar mass averages of a G10-b-G10 block (prepared by reacting G$_{10}$ 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$_n$ | [n]$_w$ (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 |

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