## Electronic Supplementary Information

# Enzymatic synthesis and polymerisation of β-mannosyl acrylates produced from renewable hemicellulosic glycans

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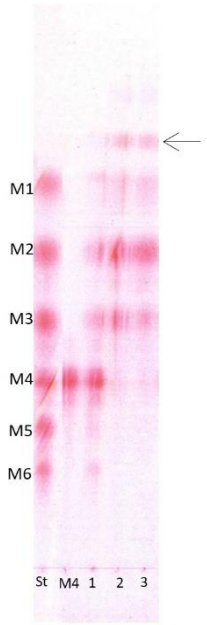


**Fig. S1**. TLC analysis of reactions with *Tr*Man5A (2 µM), $M_4$ (5 mM) and HEMA (25 vol%). Total reaction volume was 50 µl. On the TLC, 1 µl of standards and 2 µl of samples were loaded. St: Manno-oligosaccharide standards from DP1-DP6 ($M_1$-$M_6$), M4: $M_4$ control, 1: 5 min reaction, 2: 1 h reaction, 3: 5 h reaction. The arrow marks the indication that conjugates with HEMA were generated in the enzymatic reactions using $M_4$ as donor substrate.

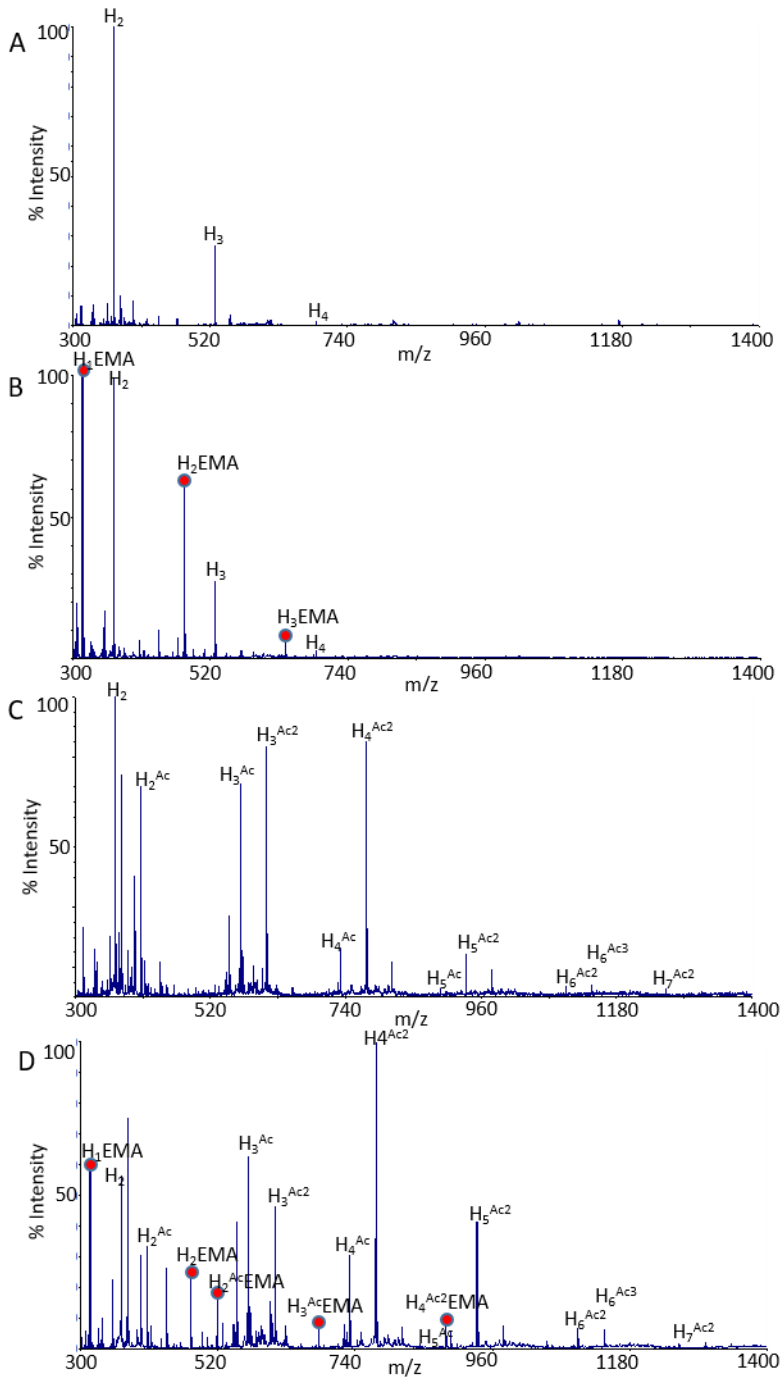


**Fig. S2**. MALDI-ToF MS analysis of 24 h reactions with *Tr*Man5A and polymeric mannans as donor substrates, with or without HEMA included. Peak assignment is presented in Table S1. Red dots mark peaks that correspond to masses of products where HEMA has been conjugated with saccharides. $H_n$= hexose with DP n, Ac(n)= number of acetylations, $H_n^{Ac(n)}EMA$ = hexose with DP n (with or without acetylation) conjugated to HEMA. **A)** MS spectra of *Tr*Man5A reaction with LBG without HEMA. **B)** MS spectra of *Tr*Man5A reaction with LBG with HEMA included. **C)** MS spectra of *Tr*Man5A reaction with AcGGM without HEMA. **D)** MS spectra of *Tr*Man5A reaction with AcGGM with HEMA included.

**Table S1**. Peak assignment for peaks shown in the Fig. S2 MALDI-ToF MS spectra for A, B, C and D. Masses of peaks corresponding to products where HEMA has been conjugated with saccharides are marked red.

| Peak assignment | Theoretical $M+Na^+$ | Found $M+Na^+$ A *Tr*Man5A + LBG | Found $M+Na^+$ B *Tr*Man5A + LBG + HEMA | Found $M+Na^+$ C *Tr*Man5A + AcGGM | Found $M+Na^+$ D *Tr*Man5A + AcGGM + HEMA |
|---|---|---|---|---|---|
| $M_1$EMA | 315.1056 | | 315.0458 | | 315.0887 |
| $H_2$ | 365.1060 | 365.0394 | 365.0424 | 365.0818 | 365.0883 |
| $H_2^{Ac}$ | 407.1165 | | | 407.0868 | 407.0986 |
| $H_2$EMA | 477.1584 | | 477.0855 | | 477.1381 |
| $H_2^{AC}$EMA | 519.1690 | | | | 519.1506 |
| $H_3$ | 527.1588 | 527.0773 | 527.0784 | | |
| $H_3^{Ac}$ | 569.1694 | | | 569.1388 | 569.1499 |
| $H_3^{Ac2}$ | 611.1799 | | | 611.1467 | 611.1586 |
| $H_3$EMA | 639.2112 | | 639.1271 | | |
| $H_3^{Ac}$EMA | 681.2218 | | | | 681.2001 |
| $H_4$ | 689.2116 | 689.1177 | 689.1110 | | |
| $H_4^{Ac}$ | 731.2222 | | | 731.1855 | 731.1995 |
| $H_4^{Ac2}$ | 773.2328 | | | 773.1956 | 773.2085 |
| $H_4^{Ac2}$EMA | 885.2852 | | | | 885.2575 |
| $H_5^{Ac}$ | 893.275 | | | 893.2343 | 893.2457 |
| $H_5^{Ac2}$ | 935.2856 | | | 935.2383 | 935.2560 |
| $H_6^{Ac2}$ | 1097.338 | | | 1097.2903 | 1097.2999 |
| $H_6^{Ac3}$ | 1139.349 | | | 1139.2938 | 1139.3103 |
| $H_7^{Ac2}$ | 1259.391 | | | 1259.3314 | 1259.3439 |

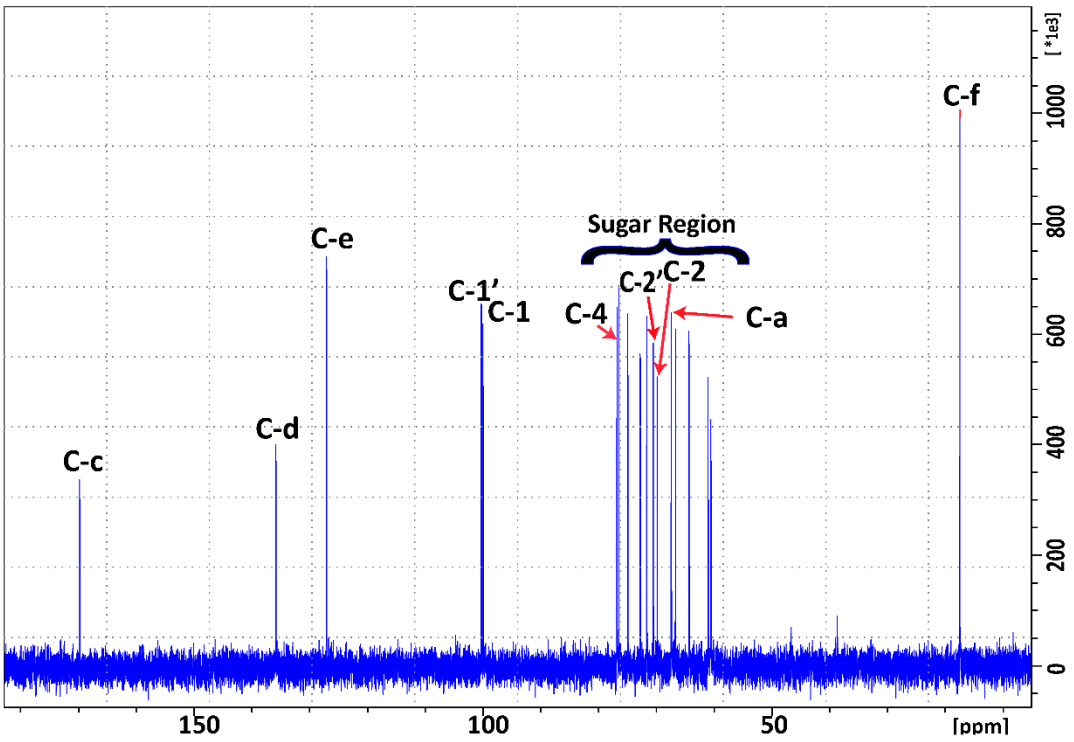


**Fig. S3**. $^{13}$ C NMR spectrum of the synthesised $M_2EMA$ collected at 10 °C.

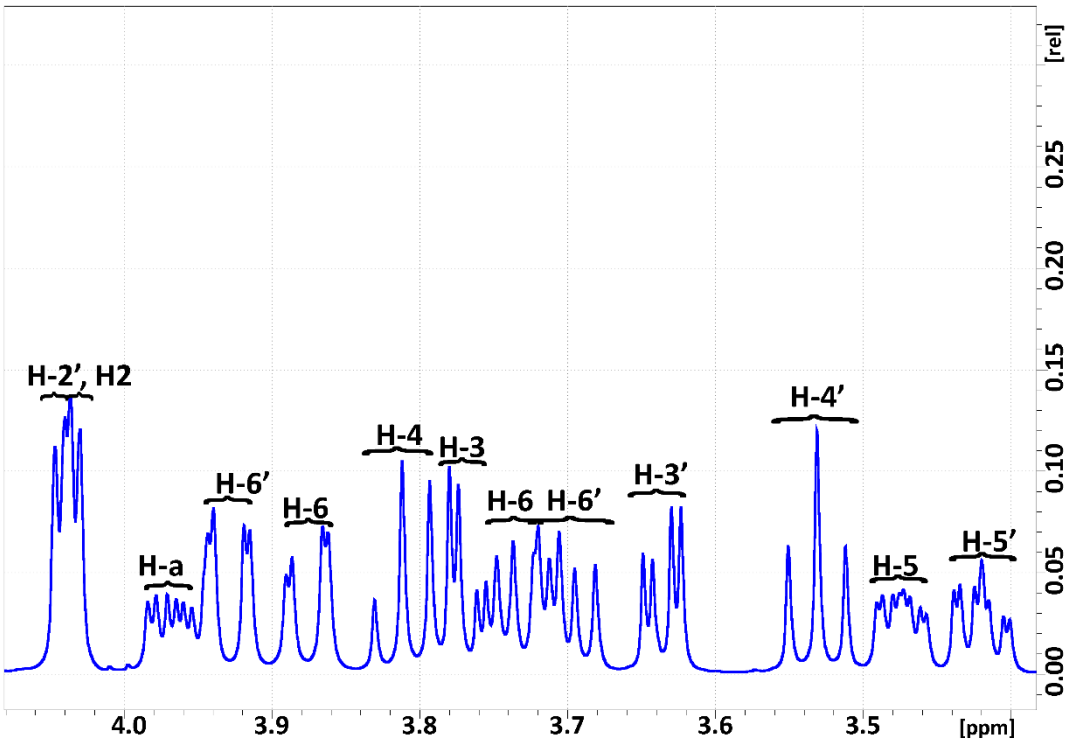


**Fig. S4**. Detailed resolved assignation of chemical shifts ($\delta_H$) corresponding to protons in the mannose units of the synthesised $M_2$EMA.

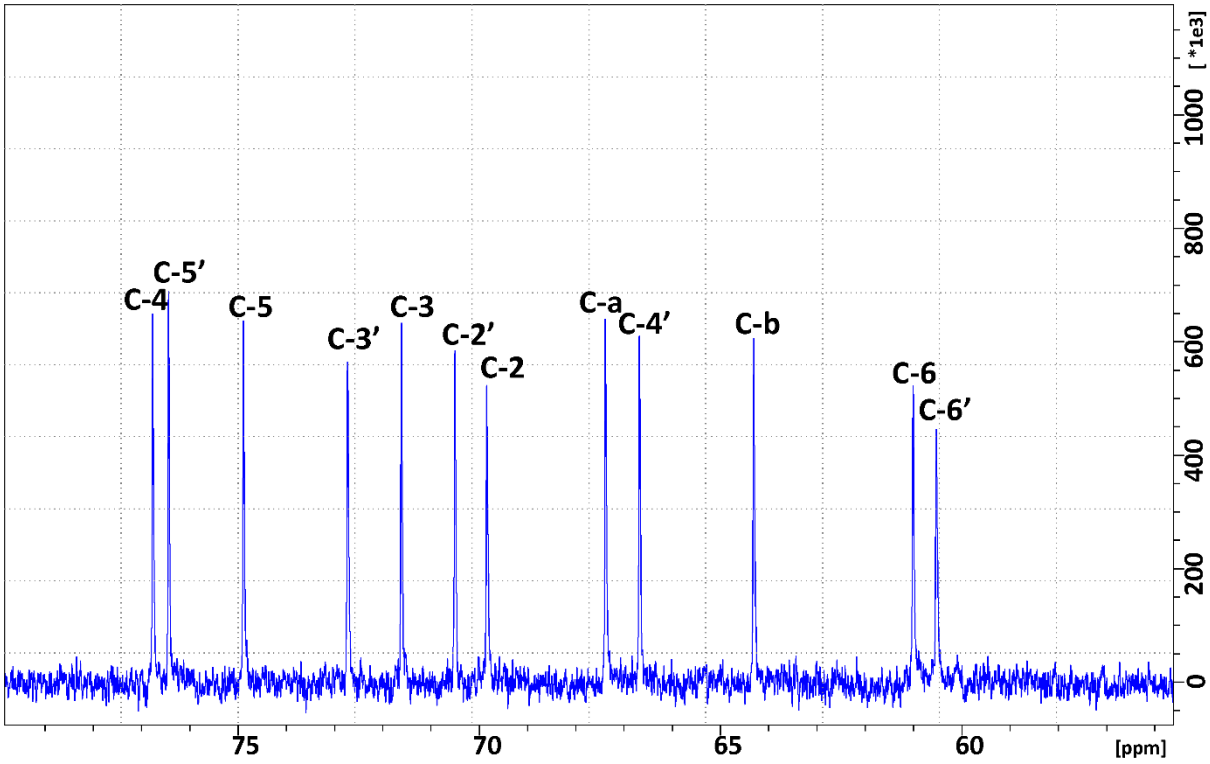


**Fig. S5.** Assignation of chemical shifts ($\delta_C$) corresponding to carbons in the mannose units of the synthesised $M_2$EMA. C-a, C-b correspond to chemical shifts of carbons in the acrylate.

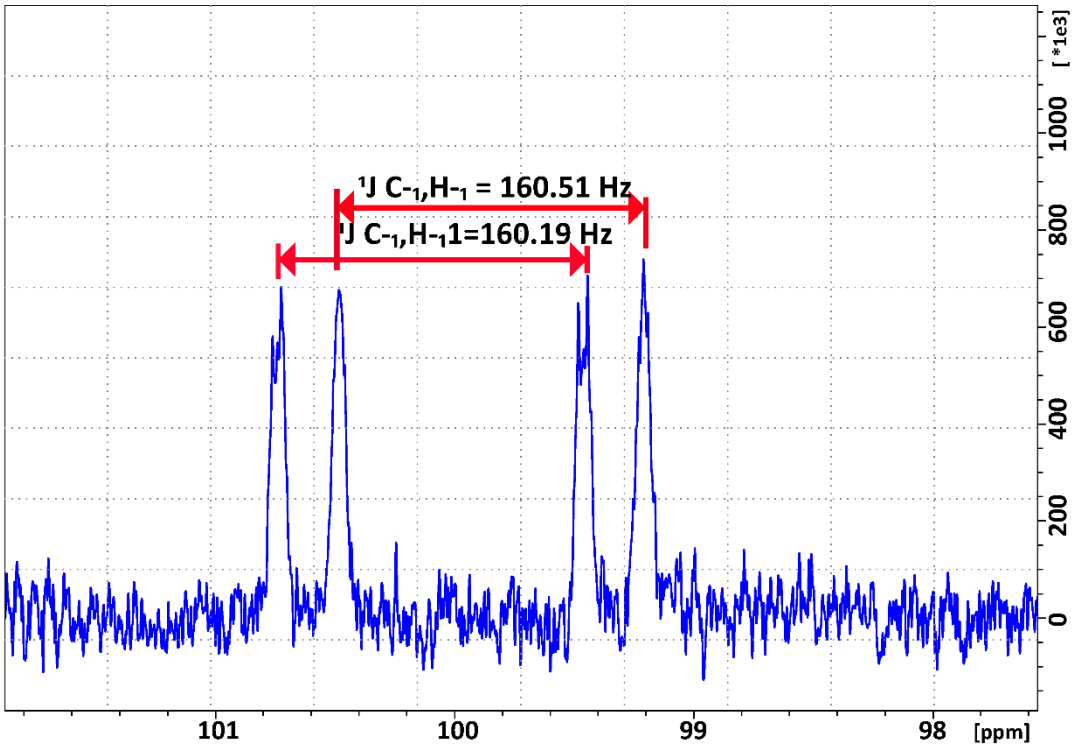


**Fig. S6**. First order coupling constants ($^{1}J_{C\text{-}1,H\text{-}1}$) of $M_2$EMA showing values of 160 Hz which correspond to conformation of a β-linkage in the anomeric position.

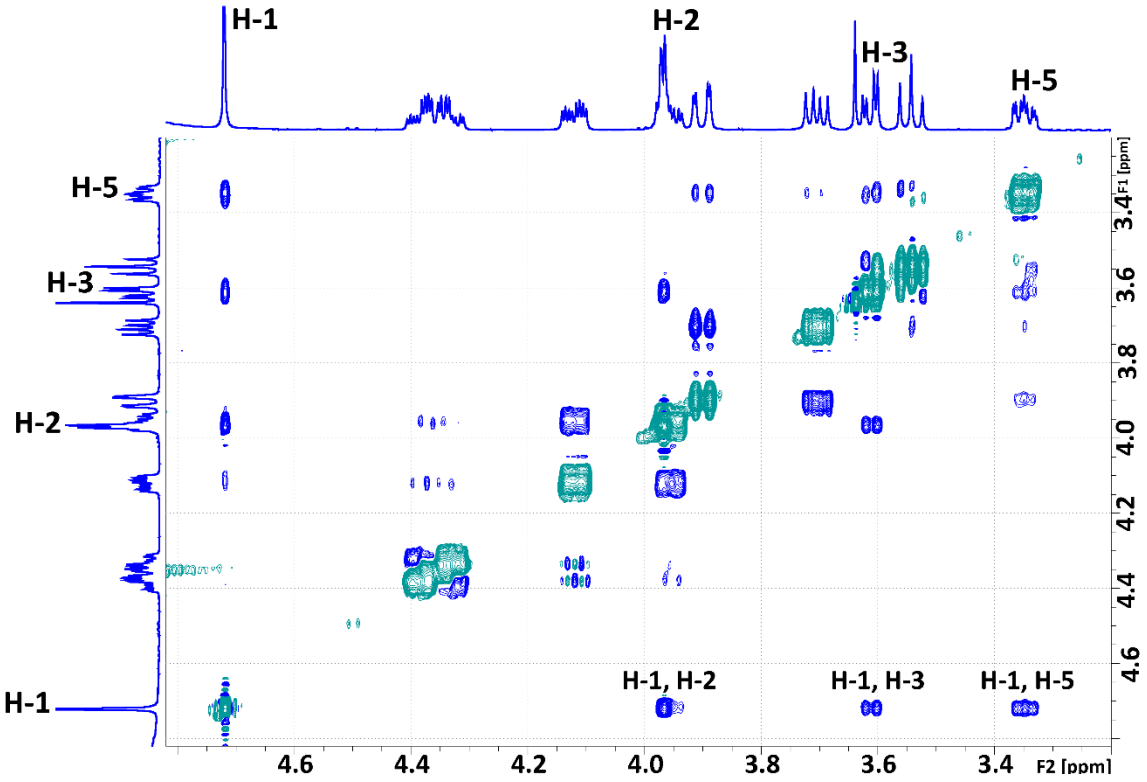


**Fig. S7**. NOESY of $M_1$EMA showing strong crosspeaks between H-1 to H-2, H-3 and H-5, which correspond to β-anomer of the mannose unit. Insert shows the possible intra-residue NOE connectivities for the β-D-mannose unit.

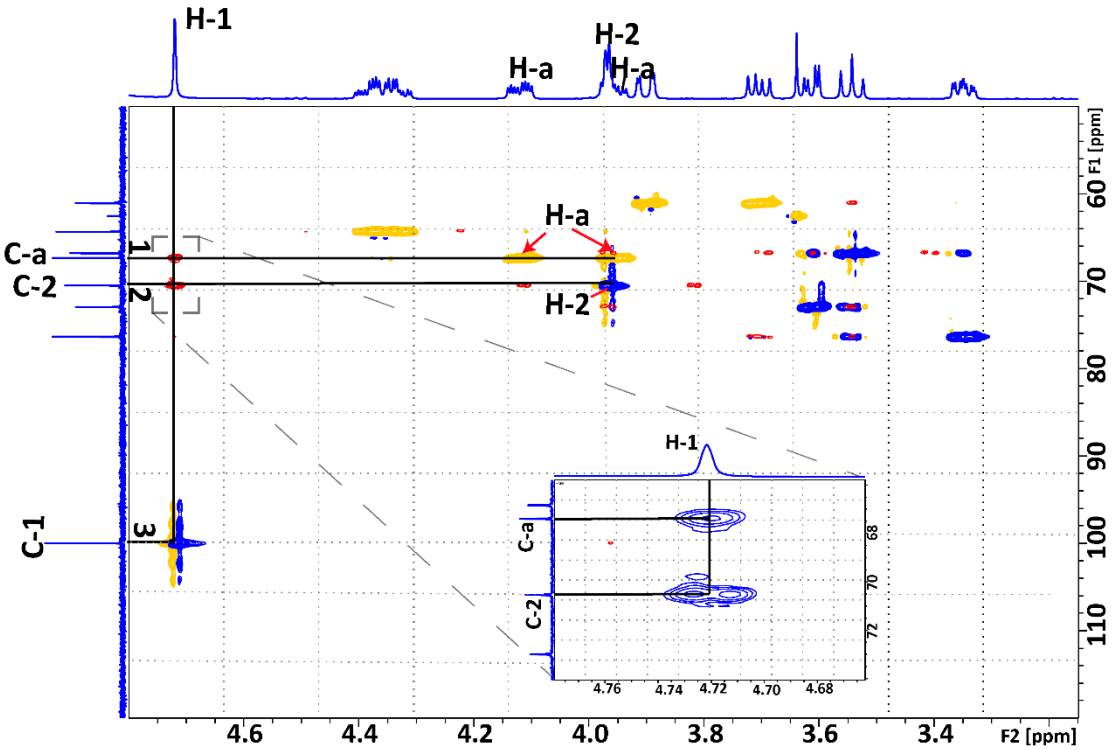


**Fig. S8**. Overlay of $^{1}H$ – $^{13}C$ HMBC (red, positive signals) and HSQC (blue, positive signals; yellow, negative signals) NMR spectra of the synthesised $M_1$EMA. Chemical shifts in ppm (δ). The horizontal lines are drawn at (from top to bottom): **(1)** $\delta_C$ 67.31 of C-a carbon, along that line direct bond J-coupling to H-a protons shown by the cross peaks in HSQC at $\delta_H$ 4.12 and at $\delta_H$ 3.94; **(2)** $\delta_C$ 70.45 of C-2 carbon, along that line direct bond J-coupling to $\delta_H$ 3.97 (H-2) shown by the cross peak in HSQC; **(3)** $\delta_C$ 99.92 of C-1 anomeric carbon with cross peak at $\delta_H$ 4.72 (H-1) in HSQC indicating a direct bond J-coupling between C-1 carbon and H-1 proton. The vertical line is drawn at $\delta_H$ 4.72 (H-1), in the **insert**, along this vertical line two long-range J-couplings to $\delta_C$ 70.45 (C-2) and to $\delta_C$ 67.31 (C-a) indicating the actual formation of the glycosidic linkage between the acrylate and the mannose unit at the anomeric carbon. All spectra were collected at 10 °C in $D_2O$.

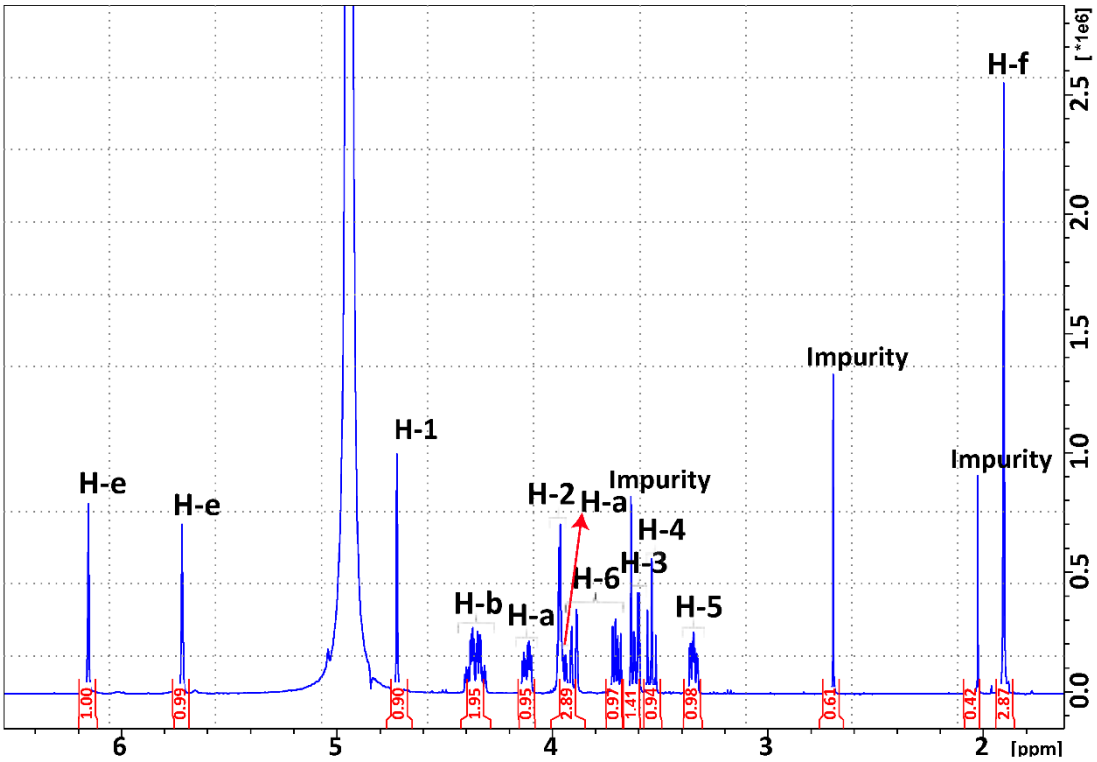


**Fig. S9**. $^{1}$H NMR spectrum of the synthesised $M_1$EMA collected at 10 °C. $^{1}$H NMR ($D_20$) δ in ppm: 6.14 (H-e), 5.71 (H-e), 4.72 (H-1, s), 4.33- 4.38 (H-b, m), 4.12 (H-a, m), 3.97 (H-2, dd), 3.94 (H-a, m), 3.90 (H-6, dd), 3.70 (H-6, dd), Impurity (3.64, s), 3.61 (H-3, dd), 3.54 (H-4, t), 3.35 (H-5, m), 2.70 (Impurity, s), 2.03 (Impurity, s), 1.92 (H-f, s). Brackets are used to group regions. Integral values for different peaks relative to the H-e of the acrylate peak are shown above the x-axis. There is only one H-1 shift as indicated in the spectra as expected due to the synthesised compound having one mannose unit.

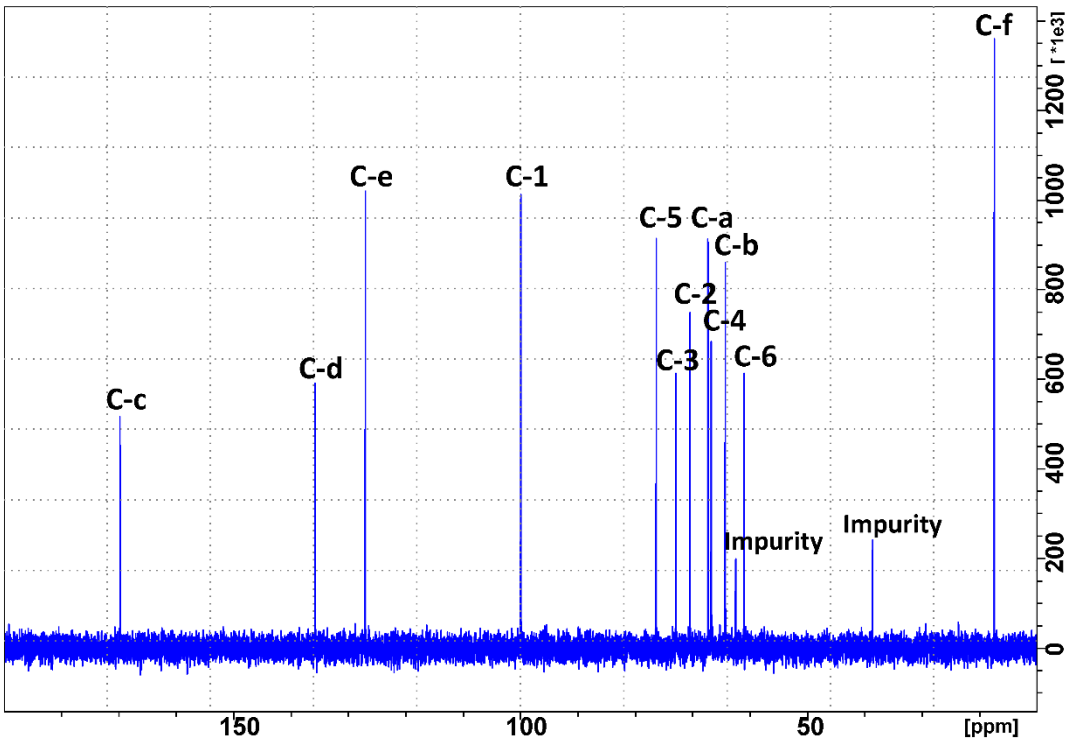


**Fig. S10**. $^{13}C$ NMR spectrum of the synthesised $M_1EMA$ collected at 10 °C. $^{13}C$ NMR ($D_20$) δ in ppm: 169.73 (C-c), 135.76 (C-d), 126.99 (C-e), 99.92 (C-1), 76.31 (C-5), 72.91 (C-3), 70.45 (C-2), 67.31 (C-a), 66.75 (C-4), 64.29 (C-b), 62.49 (Impurity), 61.02 (C-6), 38.65 (Impurity), 17.43 (C-f). One peak in the sugar region at 99.92 ppm indicates the presence of one single mannose unit.