Electronic Supplementary Information (ESI)

Structurally-Analogous Trehalose and Sucrose Glycopolymers – Comparative Characterizations and Evaluations of Their Effects on Insulin Fibrillation

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EXPERIMENTAL SECTION

1. Materials and general methods

Glycomonomers 6-*O*-acryloyl-trehalose (TreA) and 6'-*O*-acryloyl-sucrose (SucA) were synthesized following our previously described methods.[1,2] The chain transfer agent (CTA), benzyl 2-hydroxyethyl carbonotrithioate, was synthesized according to the procedure reported by Skey and O'Reilly.[3] The syntheses details and relevant NMR spectra are provided below in sections 2. and 3.

Acetic acid (POCH S.A.), acetic anhydride (POCH S.A.), acryloyl chloride (Merck), benzyl bromide (Acros Organics), calcium chloride ($CaCl_2$; POCH S.A.), carbon disulphide (CS_2; Sigma Aldrich), Concanavalin A (ConA) from *Canavalia ensiformis* (Jack bean) type-IV (Sigma Aldrich), disodium hydrogen phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$; Sigma Aldrich), glycine (Acros Organics), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; Acros Organics), invertase concentrate (Fisher Chemical), lithium bromide (LiBr; Sigma Aldrich), magnesium sulphate ($MgSO_4$; Sigma Aldrich), manganese(II) chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$; Sigma Aldrich), 2-mercaptoethanol (Sigma Aldrich), orthophosphoric acid (H_3PO_4, ≥ 85%, Merck), potassium carbonate (K_2CO_3; POCH S.A.), pyridine (anhydrous, Acros Organics), sodium chloride (NaCl; POCH S.A.), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$; Sigma Aldrich), sodium hydroxide (NaOH; Sigma Aldrich), sucrose (Sigma Aldrich), trehalase (prokaryote) (Megazyme Ltd.), trehalose (anhydrous; Acros Organics), triethylamine (TEA; Acros Organics), trimethylsilyl chloride (Sigma Aldrich) were used directly without any purification.

The initiator, 2,2´-azobisisobutyronitrile (AIBN; Sigma Aldrich), was purified via recrystallization from methanol and stored at –20 °C. Thioflavin T (ThT; T3516, dye content ≥ 65%) was supplied by Sigma Aldrich, and a ~6 mM stock solution was prepared in 20 mM phosphate buffer (pH 7.4) and stored frozen. To prepare the stock solution, 10.0 mg of ThT was dissolved in 3.1 mL of buffer and passed through a 0.20 µm syringe filter (CHROMAFIL® H-PTFE Xtra), and the accurate ThT concentration was determined by spectrophotometry at 412 nm (ε = 36 000 $M^{-1} \cdot cm^{-1}$) after dilution.

Amberlyst 15 was supplied by Fluka and washed with several portions of methanol until the solvent became colourless.

Deionized water (DI H_2O) was produced using a reverse osmosis system (conductivity < 2 µS/cm).

Toluene and methanol were supplied by Sigma Aldrich. Anhydrous solvents were purchased from Acros Organics and stored over molecular sieves under inert atmosphere. Dimethyl sulfoxide (DMSO; ACS reagent) and *N,N*-dimethylformamide (DMF; HPLC grade) were supplied by Sigma Aldrich. Deutered solvents were purchased from Deutero GmbH. Solvents for column chromatography and extraction were purchased from Avantor Performance Materials Poland S.A and distilled prior use.

Dialysis tubes were purchased from Spectrum Laboratories, Inc.

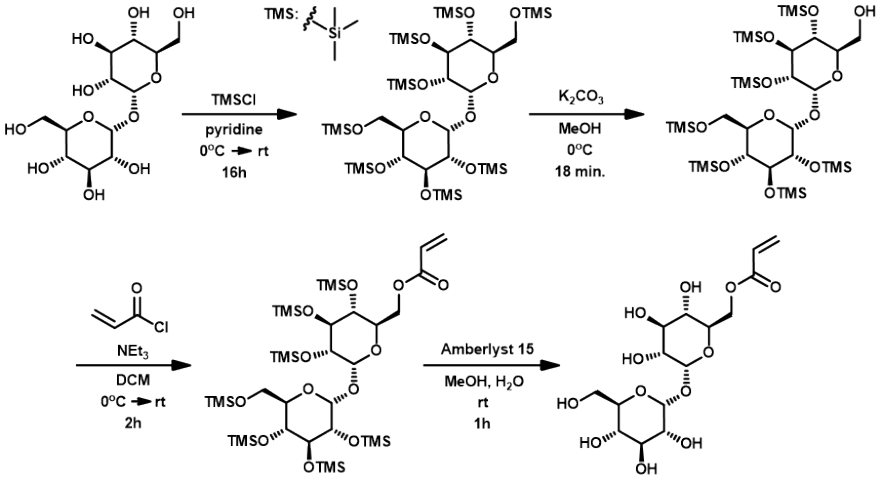
NMR spectra were recorded in deuterated solvents by using Varian NMR instrument operating at 600 MHz or Bruker NMR instrument operating at 400 MHz. Chemical shifts are reported in ppm (δ) relative to solvent residual signal or tetramethylsilane ($CDCl_3$, $DMSO_{d6}$) or 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (D_2O) as an internal reference. Freeze-drying was carried out under 0.035 mbar at -50 °C (ALPHA 1-2 LDplus, CHRIST).

2. Synthesis of trehalose and sucrose glycomonomers

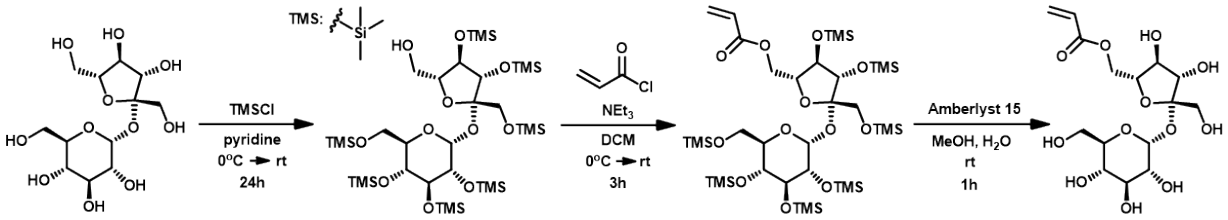
2.1. Supplementary discussion on synthesis of trehalose and sucrose glycomonomers

With numerous reactive hydroxyl groups and no chemical function being readily selected, regioselective derivatisation of carbohydrates is a prominent challenge. Thus, it usually involves multistep regioselective protection and deprotection methodologies, employment of specific catalysts/reagents and certain reaction conditions or utilization of enzymatic approaches. Synthesis of trehalose primary monoesters is especially challenging, because trehalose is composed of two glucose subunits with identical reactivity. Trehalose monomer – trehalose 6-*O*-acrylate (TreA) – was synthetized in 4 step procedure employing trimethylsilyl (TMS) protecting group strategy (**Scheme S1**), which is based on differences in the susceptibility of primary and secondary trimethylsilyl-protected hydroxyl groups for methanolysis. Anhydrous trehalose was first converted into per-*O*-trimethylsilyl trehalose, which was subsequently subjected to regioselective deprotection in methanolic solution under mild basic conditions in the presence of catalytic amount of K_2CO_3. Under such conditions primary trimethylsilyl ethers can be selectively removed in the presence of secondary trimethylsilyl ethers. Moreover, precise control of temperature and reaction time enables to obtain 2,3,4,2',3',4',6'-hepta-*O*-trimethylsilyl-trehalose deprotected at just one primary hydroxyl group as a major product and then isolate it by using column chromatography with satisfactory yield (~50%). Column chromatography affords also in recovery of ~25% of unreacted starting material (per-*O*-trimethylsilyl trehalose) as a faster moving fraction. Isolated hepta-TMS-protected trehalose was further regioselectively monoestrified with acryloyl chloride affording TMS-protected trehalose monomer bearing acrylate functionalization at *O*-6. Final deprotection from remaining TMS groups was easily accomplished upon treatment with Amberlyst 15 (Brønsted acidic resin catalyst), that cleanly furnished requisite trehalose 6-*O*-acrylate with almost quantitive yield.

A similar approach, based on temporary trimethylsilyl ethers protection of hydroxyl groups has also been developed for the regioselective derivatization of sucrose to sucrose 6'-O-acrylate (**Scheme S2**). Otherwise to trehalose, sucrose is composed from glucose and fructose subunits and it contains three primary hydroxyl groups (OH-6, OH-1' and OH-6'). Fortunately, under certain conditions those primary hydroxyls exhibit relatively well defined difference in reactivity [4] as it turned out to be the case herein. Similarly as in the synthesis of trehalose monomer, sucrose was first treated with trimethylsilyl chloride to obtain per-*O*-trimethylsilyl sucrose. However, some of TMS protecting groups in this derivative were found to be susceptible to hydrolysis during the purification of the crude by extraction with water, with one group being prominently more prone to hydrolysis than others, as judged from the TLC analysis. Sufficient differences in polarity of obtained mixture enabled to separate this major product of hydrolysis with decent yield (31%) by using column chromatography. Relative integration of signals from TMS protons (0.0 – 0.3 ppm) in ^{1}H NMR spectrum (**Fig. S8**) confirms that TMS group was cleaved from just one hydroxyl group. Furthermore, the presence of one clean signal corresponding to anomeric proton from glucopyranosyl ring (~5.1 ppm **Fig. S8**) and single signals in ^{13}C NMR spectrum (**Fig. S8**) prove the presence of one pure regioisomer in isolated fraction. The overlap of signals from the majority of sugar protons (3.2 – 3.9 ppm) makes it impossible to identify the position of deprotected hydroxyl group at this step, even analysing 2D NMR spectra (not attached). The regioisomer was however identified by analysing ^{1}H and ^{13}C NMR spectra and series of 2D NMR spectra (COSY, HSQC, HMBC) (**Fig. S9-S16**) of the products of further steps – acetylation with acryloyl chloride and deprotection from remaining TMS groups to get final monomer. The presence of the correlation signal between carbonyl carbon from acrylate functionalization and one of the protons at C-6' on HMBC spectra (**Fig. S12 and S16**) of both those products clearly indicates that the isolated regioisomer was deprotected and further functionalized at *O*-6' on fructose ring.



Scheme S1. Synthetic pathway to trehalose 6-*O*-acrylate (**TreA**)



Scheme S2. Synthetic pathway to sucrose 6'-*O*-acrylate (**SucA**)

2.2. Synthesis of 6-O-acryloyl-trehalose (TreA)

2,3,4,6,2',3',4',6'-octa-O-trimethylsilyl-trehalose

Anhydrous trehalose (21.91 mmol, 7.50 g) was dissolved in anhydrous pyridine (80 mL) and the solution was cooled on ice-water bath before trimethylsilyl chloride (362.62 mmol, 39.38 g, 46.00 mL) was added dropwise, while stirring. The reaction was allowed to warm up to room temperature and left overnight. The resulting suspension was diluted with ethyl acetate (100 mL) and extracted with water (80 mL). The organic phase was washed two more times with water (80 mL), dried over $MgSO_4$ and concentrated under vacuum. The residue was evaporated twice with toluene to afford 2,3,4,6,2',3',4',6'-okta-*O*-trimethylsilyl-trehalose as white crystals (19.70 g, 98%).

^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm]: 0.10, 0.12, 0.14, 0.14 (4x s, 72H, –Si(CH_3)$_3$); 3.38 (dd, 2H, J = 9.3, 3.2 Hz, H-2, H-2'); 3.44 (~t, 2H, J = 9.1 Hz, H-4, H-4'); 3.64-3.71 (m, 4H, H-6a, H-6'a, H-6b, H-6'b); 3.76-3.81 (ddd, 2H, J = 9.5, 4.1, 2.3 Hz, H-5, H-5'); 3.89 (~t, 2H, J = 9.0 Hz, H-3, H-3'); 4.91 (d, 2H, J = 3.2 Hz, H-1, H-1'). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ [ppm]: -0.11, 0.32, 1.10, 1.24 (8x –Si(CH_3)$_3$); 62.31 (C-6, C-6'); 71.92, 73.04, 73.41, 73.77 (C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5'); 94.53 (C-1, C-1').

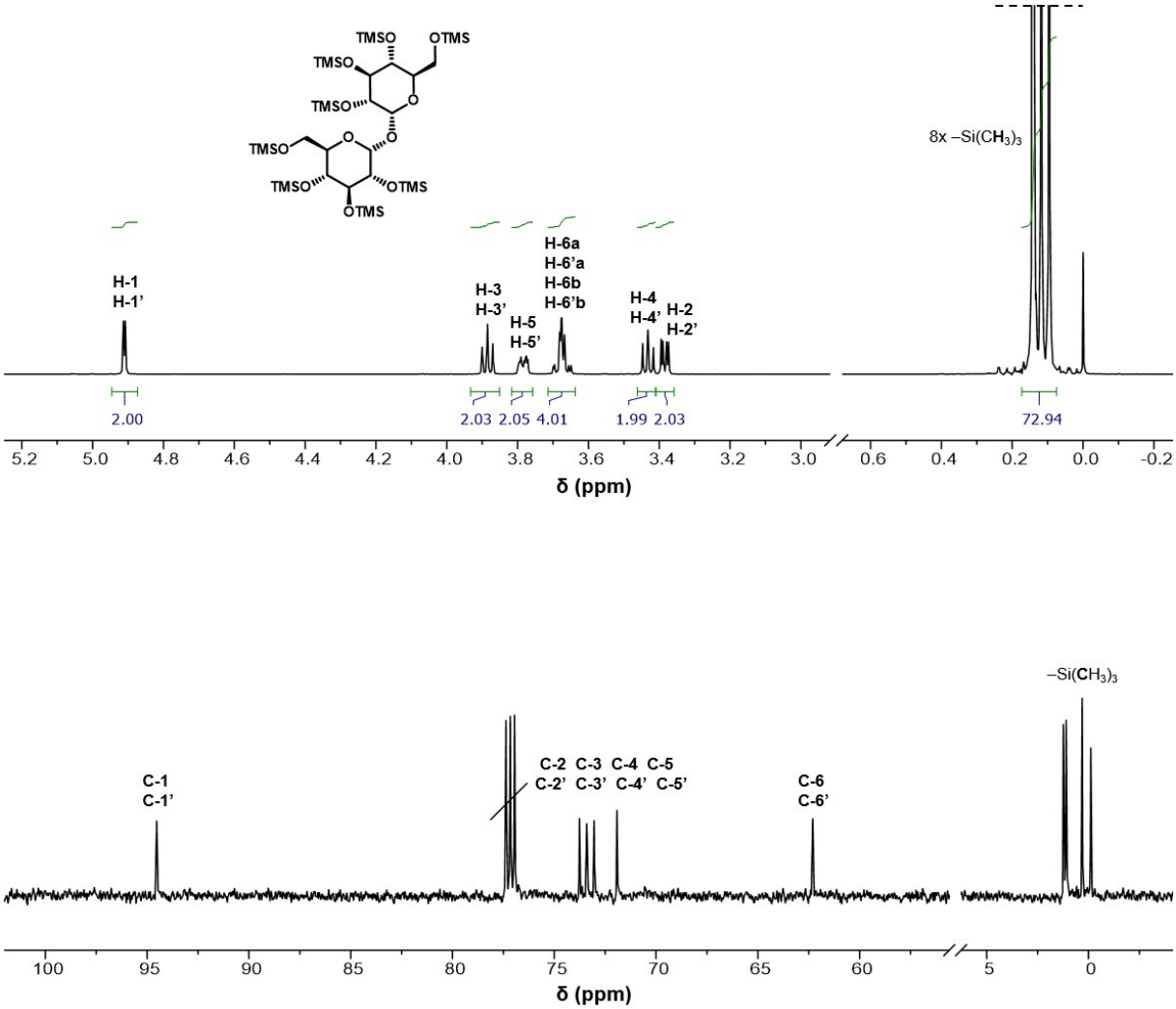


Fig. S1. ^{1}H NMR and ^{13}C NMR spectra of 2,3,4,6,2',3',4',6'-octa-*O*-trimethylsilyl-trehalose ($CDCl_3$)

2,3,4,2',3',4',6'-hepta-O-trimethylsilyl-trehalose

2,3,4,6,2',3',4',6'-okta-*O*-trimethylsilyl-trehalose (4.36 mmol, 4.00 g) was dissolved in methanol (270 mL) and cooled on ice-water bath. Then, solid K_2CO_3 (3.98 mmol, 0.55 g) was added, while intensively stirring. The reaction was quenched after 18 min by transferring into separating funnel containing 200 mL of hexane and extracting twice with water (100 mL). The organic phase was dried over $MgSO_4$ and concentrated under vacuum. Crude product containing starting material, 2,3,4,2',3',4',6'-hepta-*O*-trimethylsilyl-trehalose and 2,3,4,2',3',4'-hexa-*O*-trimethylsilyl-trehalose was separated by silica gel flash chromatography* (hexane:ethyl acetate 98:2) to afford 2,3,4,2',3',4',6'-hepta-*O*-trimethylsilyl-trehalose as a colourless oil (1.71 g, 47%). 1.02 g (25%) of starting material was recovered as a first fraction.

*column chromatography has to be run as fast as possible to avoid loss of TMS protecting groups

^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm]: 0.10, 0.11, 0.12, 0.14, 0.14, 0.14, 0.16 (7x s, 63H, $-Si(CH_3)_3$); 1.76 (br s, 1H, -OH); 3.38-3.44 (m, 3H, H-2, H-2', H-4'); 3.46 (~t, 2H, J = 9.1 Hz, H-4); 3.63-3.74 (m, 4H, H-6a, H-6b, H-6'a, H-6'b); 3.79 (ddd, 1H, J = 9.5, 4.7 Hz, 2.0 Hz, H-5'); 3.84 (~dt, 1H J = 9.5, 3.5 Hz, H-5); 3.89, 3.92 (2 t, 2H, J = 9.0 Hz, H-3, H-3'); 4.89 (d, 1H J = 3.1 Hz, H-1); 4.94 (d, 1H J = 3.1 Hz, H-1'). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ [ppm]: -0.15, 0.25, 0.27, 1.01, 1.09, 1.17, 1.20 (7x $-Si(CH_3)_3$); 61.88, 62.29 (C-6, C-6'); 71.62, 71.89, 72.87, 72.90, 72.94, 73.54, 73.55, 73.63 (C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5'); 94.40, 94.57 (C-1, C-1').

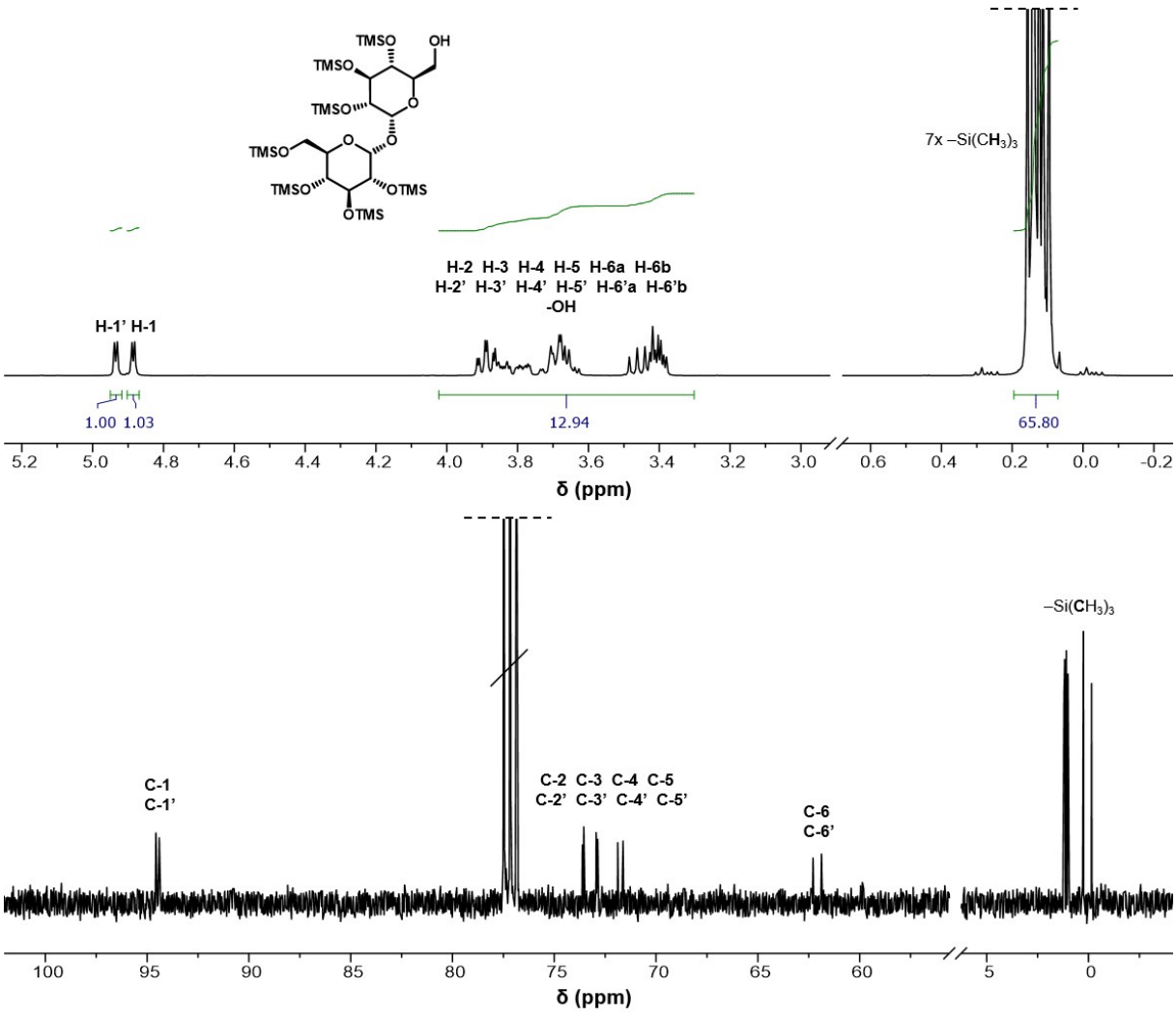


Fig. S2. ^{1}H NMR and ^{13}C NMR spectra of 2,3,4,2',3',4',6'-hepta-*O*-trimethylsilyl-trehalose ($CDCl_3$)

6-O-acryloyl-2,3,4,2',3',4',6'-hepta-O-trimethylsilyl-trehalose

2,3,4,2',3',4',6'-hepta-*O*-trimethylsilyl-trehalose (7.89 mmol, 6.68 g) and TEA (17.98 mmol, 1.82 g, 2.50 mL) were dissolved in anhydrous DCM (30 mL) and cooled on ice water bath. While stirring, second solution of acryloyl chloride (11.93 mmol, 1.08 g) in anhydrous DCM (20 mL) was added dropwise. The mixture was allowed to warm up to room temperature and left for 2 h. The resulting suspension was diluted with DCM (160 mL) and extracted twice with brine (220 mL). The organic phase was dried over $MgSO_4$ and concentrated under vacuum. Crude product was purified by silica gel flash chromatography* (hexane:ethyl acetate 98:2) to afford 6-*O*-acryloyl-2,3,4,2',3',4',6'-hepta-*O*-trimethylsilyl-trehalose as colourless oil (5.26 g, 74%).

*column chromatography has to be run as fast as possible to avoid loss of TMS protecting groups

^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm]: 0.08, 0.12, 0.13, 0.14, 0.15 (5x s, 63H, –Si$(CH_3)_3$); 3.40 (dd, 1H, J = 9.3, 3.1 Hz, H-2'); 3.42-3.47 (m, 2H, H-2, H-4'); 3.51 (dd, 1H, J = 9.5, 8.6 Hz, H-4); 3.64-3.70 (m, 2H, H-6'a, H-6'b); 3.77 (m, 2H,H-5'); 3.86-3.94 (m, 2H, H-3, H-3'); 4.05 (ddd, 1H J = 9.6, 4.4, 2.4 Hz, H-5); 4.16 (dd, 1H, J = 12.0, 4.4 Hz, H-6a); 4.37 (dd, 1H, J = 12.0, 2.4 Hz, H-6b); 4.90 (d,1H, J = 3.1 Hz, H-1'); 4.96 (d, 1H, J = 3.1 Hz, H-1); 5.84 (dd, 1H, J = 10.4, 1.4 Hz, –CH=C**H**$_2$ *cis*); 6.18 (dd, 1H, J = 17.3, 10.4 Hz, –C**H**=CH_2); 6.44 (dd, 1H, J = 17.3, 1.4 Hz, –CH=C**H**$_2$ *trans*). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ [ppm]: -0.12, 0.30, 0.35, 1.03, 1.07, 1.21, 1.23 (7x –Si$(CH_3)_3$); 62.09, 63.83 (C-6, C-6'); 70.74, 71.72, 72.14, 72.84, 72.97, 73.55, 73.64, 73.73 (C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5'); 94.39, 94.70 (C-1, C-1'); 128.31 (–**C**H=CH_2); 131.28 (–CH=**C**H_2); 166.31 (–OC(O)–).

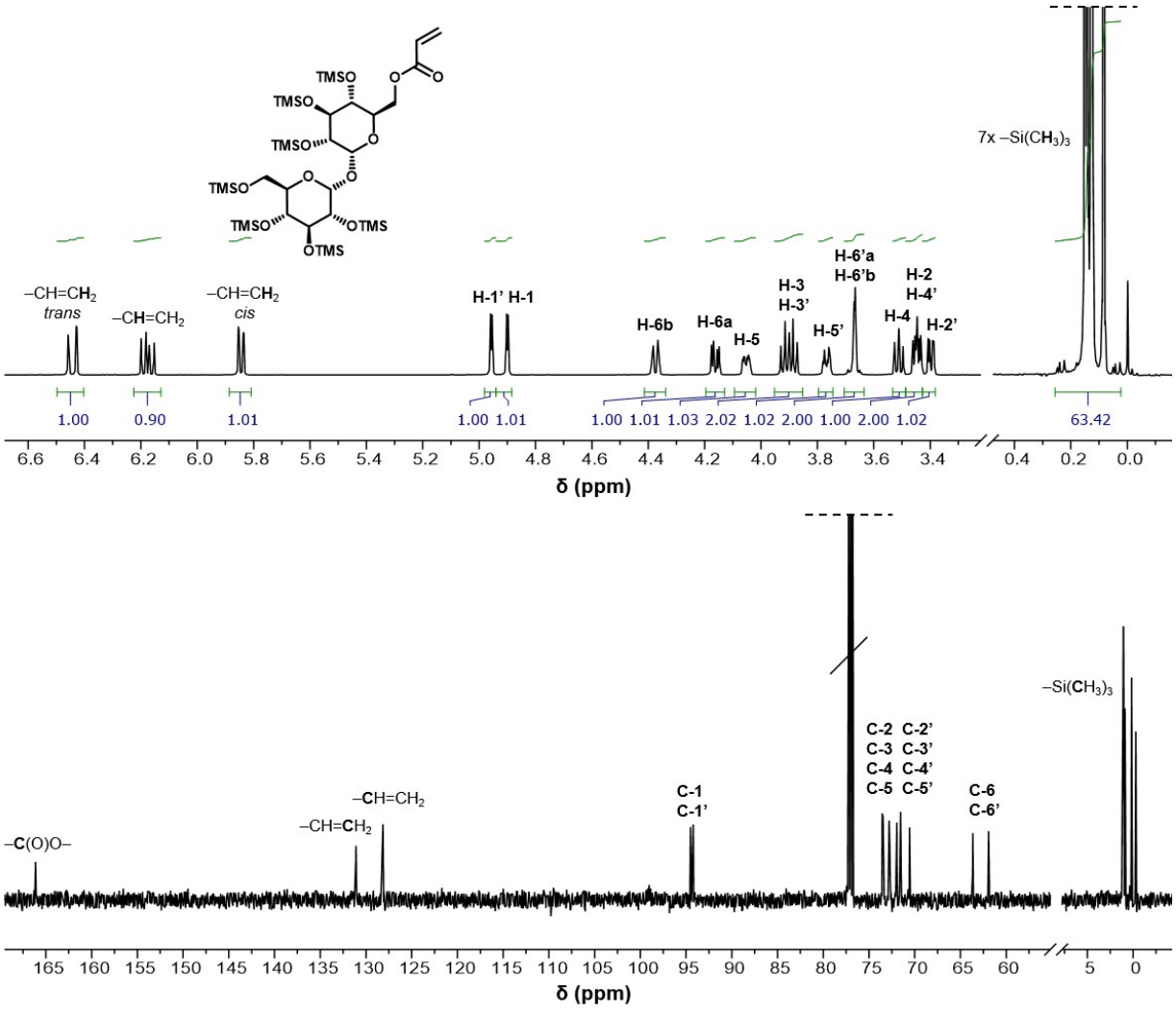


Fig. S3. ^{1}H NMR and ^{13}C NMR spectra of 6-*O*-acryloyl-2,3,4,2',3',4',6'-hepta-*O*-trimethylsilyl-trehalose ($CDCl_3$)

6-O-acryloyl-trehalose

Amberlyst 15* (8.8 g) was added to the solution of 6-*O*-acryloyl-2,3,4,2',3',4',6'-hepta-*O*-trimethylsilyl-trehalose (2.5 mmol, 2.25 g) in the mixture of methanol and milli-Q water (330 and 30 mL, respectively) and the reaction was left at room temperature under stirring for 1 h. Amberlyst 15 was filtered off and the solution was then extracted with hexane (110 mL). Aqueous phase was separated and concentrated under vacuum at 30 °C to ~15 mL. The residual solution was filtered through the syringe filter (0.45 µm) and frieze dried to afford 6-*O*-acryloyl-trehalose as a white solid (0.97 g, 98%).

*washed several times with methanol before use

^{1}H NMR (DMSO-d_6, 400 MHz) δ [ppm]: 3.21 – 3.08 (m, 2H, H-4, H-4'); 3.30 – 3.21 (m, 2H, H-2, H-2'); 3.47 (ddd, 1H, J = 11.4, 6.2, 5.0 Hz, H-6'a); 3.60 – 3.50 (m, 3H, H-3, H-3', H-6'b); 3.64 (ddd, 1H, J = 9.9, 4.9, 2.3 Hz, H-5'); 3.96 (ddd, 1H, J = 10.1, 5.6, 2.1 Hz, H-5); 4.16 (dd, 1H, J = 11.8, 5.7 Hz, H-6a); 4.29 (dd, 1H, J = 11.8, 2.1 Hz, H-6b); 4.34 (~t, 1H, J = 5.9 Hz, C-6-OH); 4.67 (d, 1H, J = 6.3 Hz, -OH); 4.69 (d, 1H, J = 6.0 Hz, -OH); 4.74 (d, 1H, J = 4.9 Hz, -OH); 4.76 (d, 1H, J = 5.2 Hz, -OH); 4.84 (d, 1H, J = 3.6 Hz, H-1); 4.85-4.89 (m, 2H, H-1', -OH); 5.09 (d, 1H, J = 5.3 Hz, -OH); 5.94 (dd, 1H J = 10.3, 1.7 Hz, 1H, –CH=C**H**$_2$ *cis*); 6.17 (dd, 1H, J = 17.3, 10.3 Hz, 1H, –C**H**=CH$_2$); 6.32 (dd, 1H, J = 17.3, 1.7 Hz, 1H, –CH=C**H**$_2$ *trans*). **^{13}C NMR** (DMSO-d_6, 100 MHz) δ [ppm]: 60.73 (C-6); 63.56 (C-6'); 69.55 (C-5); 70.06, 70.10 (C-4, C-4'); 71.45, 71.55 (C-2, C-2'); 72.59, 72.81 (C-3, C-3', C-5'); 93.25, 93.36 (C-1, C-1'); 128.37 (–**C**H=CH$_2$); 131.48 (–CH=**C**H$_2$, –C'H'=**C**'H'$_2$); 165.42 (–OC(O)–).

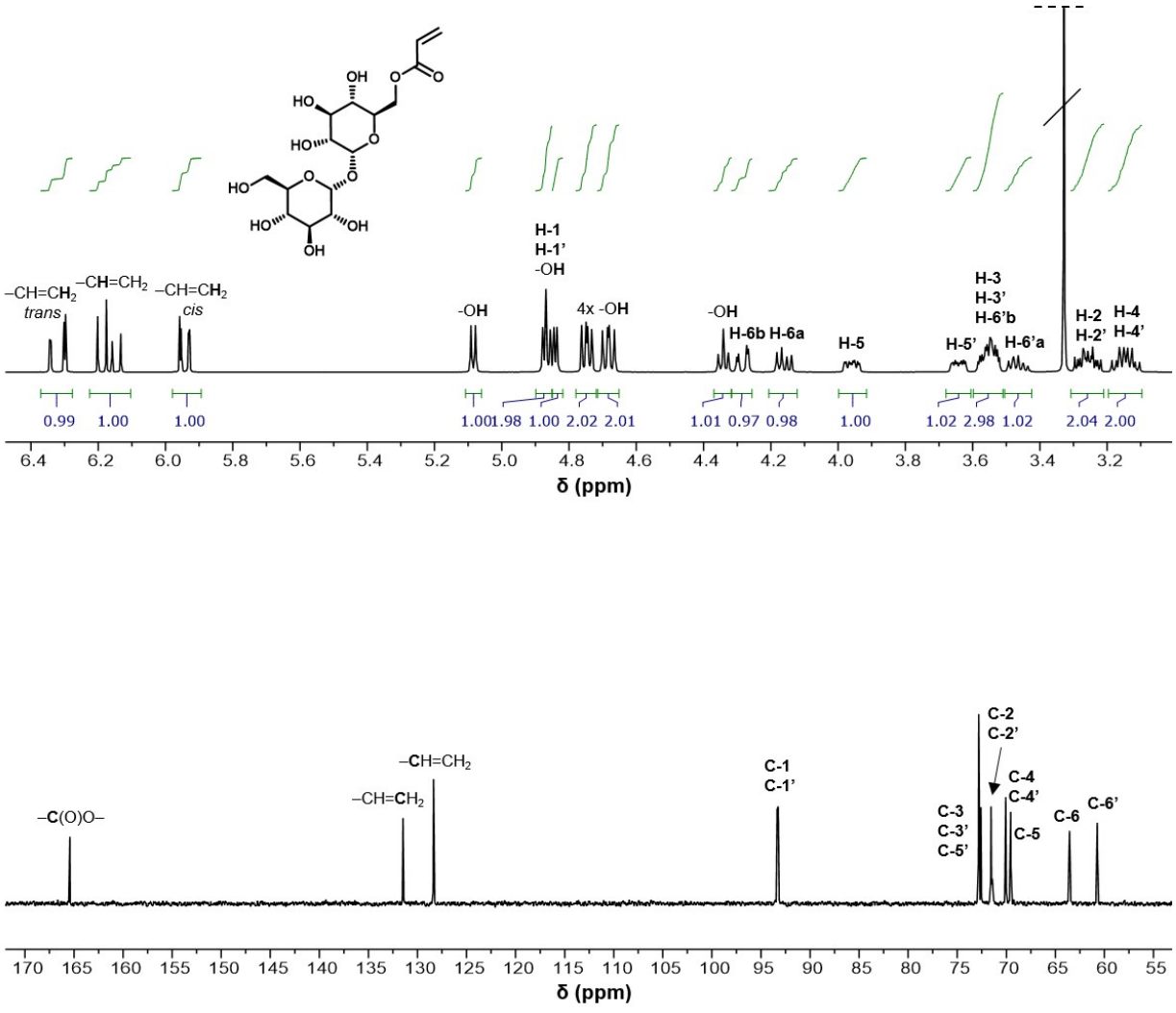


Fig. S4. ^{1}H NMR and ^{13}C NMR spectra of 6-*O*-acryloyl-trehalose ($DMSO_{d6}$)

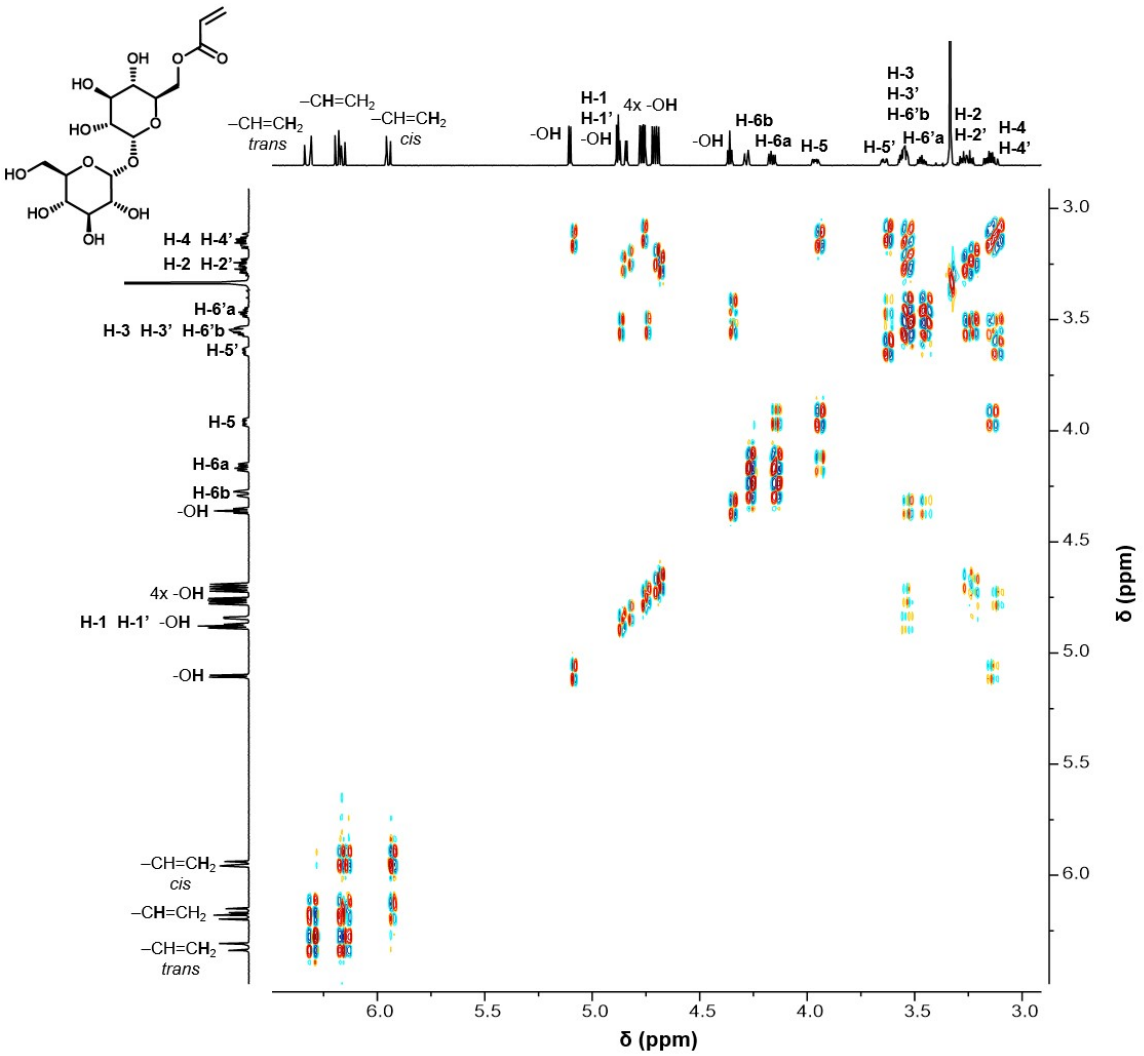


Fig. S5. COSY 2D NMR spectrum of 6'-*O*-acryloyl-sucrose ($DMSO_{d6}$)

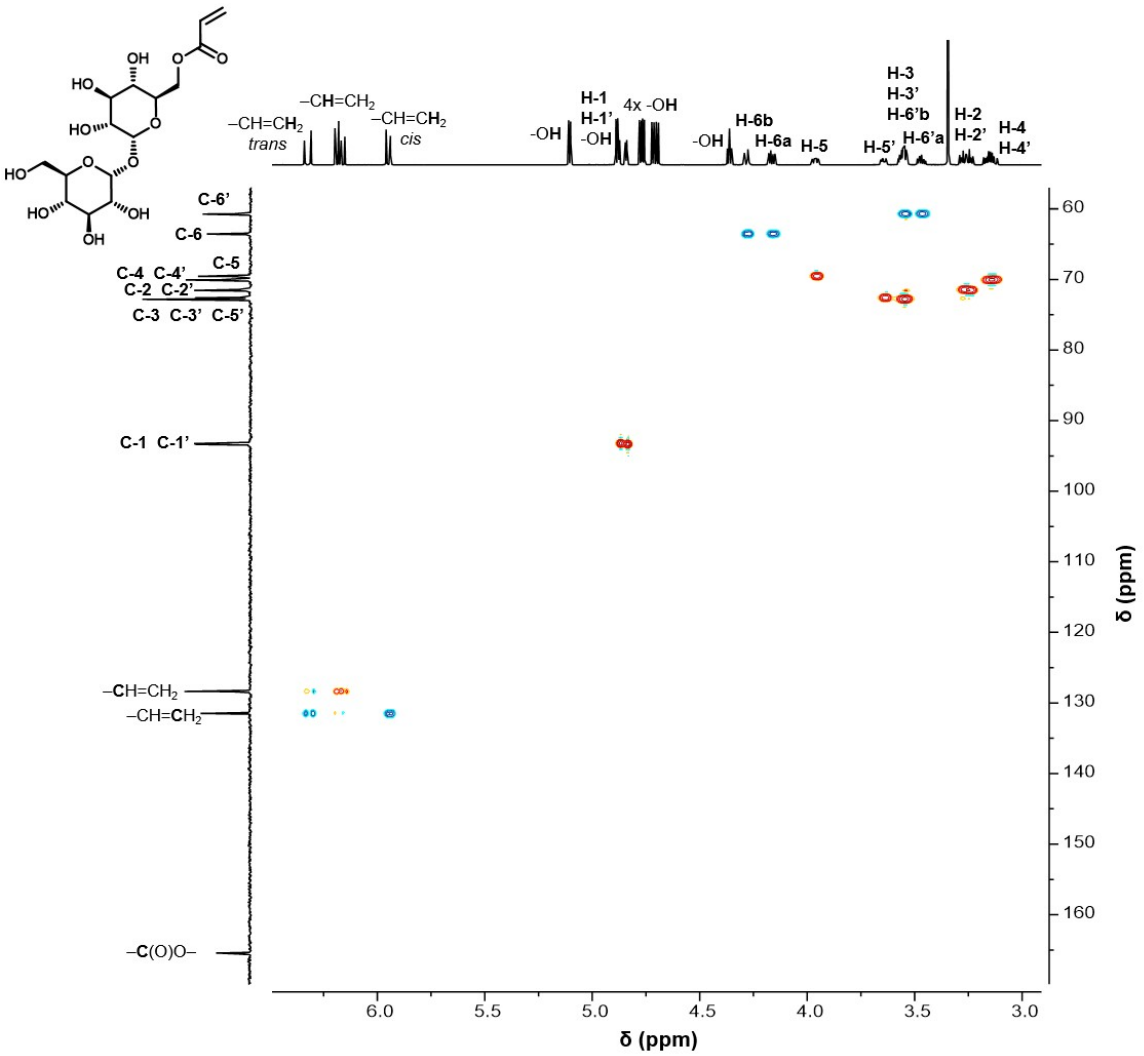


Fig. S6. HSQC 2D NMR spectrum of 6'-*O*-acryloyl-trehalose ($DMSO_{d6}$)

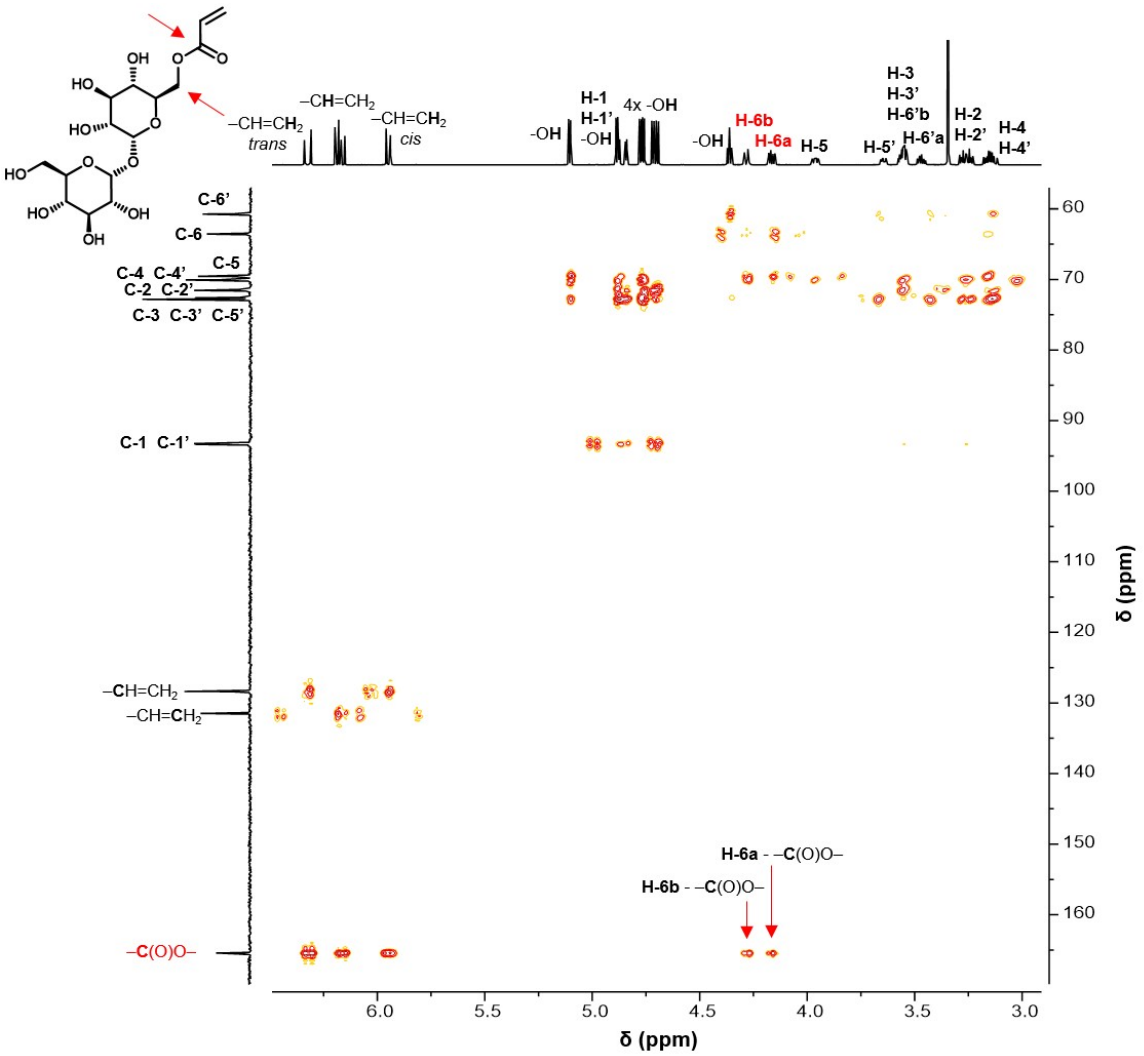


Fig. S7. CIGAR 2D NMR spectrum of 6-*O*-acryloyl-trehalose ($DMSO_{d6}$)

2.3. Synthesis of 6'-O-acryloyl-sucrose (SucA)

2,3,4,6,1',3',4'-hepta-O-trimethylsilyl-sucrose

Sucrose (5.84 mmol, 2.00 g) was suspended in anhydrous pyridine (40 mL) and the suspension was cooled on ice-water bath followed by dropwise addition of trimethylsilyl chloride (94.62 mmol, 10.28 g, 12.00 mL), while stirring. The reaction was allowed to warm up to room temperature and left overnight. The resulting suspension was diluted with ethyl acetate (50 mL) and extracted intensively with water (40 mL) until most of 2,3,4,6,1',3',4',6'-okta-*O*-trimethylsilyl-sucrose was partially deprotected to 2,3,4,6,1',3',4'-hepta-*O*-trimethylsilyl-sucrose. The progress of the deprotection was followed by TLC, as shown below. The organic phase was washed once more with water (40 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was rotevaporated twice with toluene to remove residual pyridine. The crude product was purified by silica gel flash column chromatography* (~100 g of silica gel, hexane : toluene 1:4) to afford 2,3,4,6,1',3',4'-hepta-*O*-trimethylsilyl-sucrose as white crystals (1.54 g, 31%).

*column chromatography has to be run as fast as possible to avoid loss of TMS protecting groups

^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm]: 0.11, 0.11, 0.13, 0.15, 0.16, 0.17, 0.18 (7x s, 63H, –Si(CH_3)$_3$); 3.16 – 3.90 (m, 12H, H-2; H-3; H-4; H-5; H-6a; H-6b; H-1'a; H-1'b; H-5'; H-6'a; H-6'b, –OH); 4.27 (~t, *J* = 8.4 Hz, 1H, H-4'); 4.35 (d, *J* = 8.4 Hz, 1H, H-3'); 5.08 (d, *J* = 3.2 Hz, 1H, H-1). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ [ppm]: -0.02, 0.41, 0.67, 0.71, 1.04, 1.30 (7x –Si(CH_3)$_3$); 59.96 (C-6); 60.62 (C-1'); 62.76 (C-6'); 71.28, 72.70, 73.02, 73.08, 74.08, 76.08 (C-2, C-3, C-4, C-5, C-3', C-4'); 82.06 (C-5'); 93.22 (C-1) 103.43 (C-2').

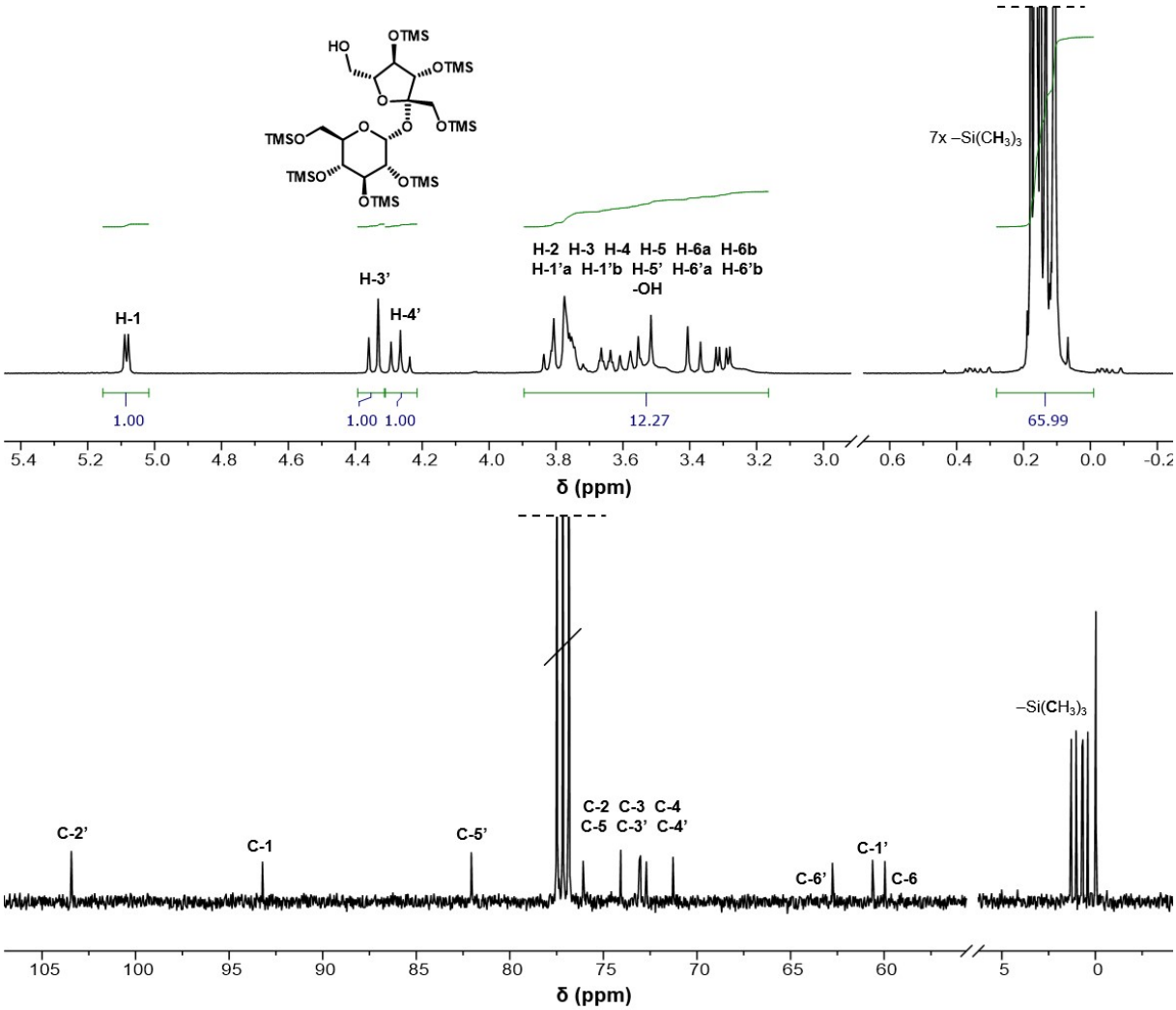


Fig. S8. ^{1}H NMR and ^{13}C NMR spectra of 2,3,4,6,1',3',4'-hepta-*O*-trimethylsilyl-sucrose ($CDCl_3$)

6'-O-acryloyl-2,3,4,6,1',3',4'-hepta-O-trimethylsilyl-sucrose

2,3,4,6,1',3',4'-hepta-*O*-trimethylsilyl-sucrose (1.76 mmol, 1.49 g) and TEA (5.34 mmol, 0.54 g, 0.74 mL) were dissolved in anhydrous DCM (7 mL) and the solution was cooled on ice water bath. While stirring, second solution of acryloyl chloride (3.52 mmol, 0.32 g) in anhydrous DCM (6 mL) was added dropwise. The reaction was allowed to warm up to room temperature and left for 3 h. The resulting suspension was diluted with DCM (40 mL) and extracted twice with brine (50 mL). The organic phase was dried over $MgSO_4$ and concentrated under vacuum. Crude product was purified by silica gel flash chromatography* (~40 g of silica gel, hexane : ethyl acetate 99:1) to afford 6'-*O*-acryloyl-2,3,4,6,1',3',4'-hepta-*O*-trimethylsilyl-sucrose as white crystals (1.05 g, 66%).

*column chromatography has to be run as fast as possible to avoid loss of TMS protecting groups

^{1}H NMR ($CDCl_3$, 400 MHz) δ [ppm]: 0.12, 0.13, 0.14, 0.15, 0.18, 0.18, 0.19 (7x s, 63H, –Si$(CH_3)_3$); 3.33 (dd, *J* = 9.3, 3.4 Hz, 1H, H-2); 3.43 (d, *J* = 11.6 Hz, 1H, H-1'a; 3.53 (~t, *J* = 9.1 Hz, 1H, H-4); 3.56 (d, *J* = 11.6 Hz, 1H); 3.70 (dd, *J* = 11.3, 1.8 Hz, 1H, H-6a); 3.81 – 3.74 (m, 2H, H-3, H-6b); 3.91 – 3.82 (m, 2H, H-5, H-5'); 4.13 (~t, *J* = 8.2 Hz, 1H); 4.29 (dd, *J* = 11.9, 7.2 Hz, 1H, H-6'a); 4.34 (d, *J* = 8.1 Hz, 1H, H-3'); 4.44 (dd, *J* = 11.9, 2.9 Hz, 1H, H-6'b); 5.25 (d, *J* = 3.4 Hz, 1H, H-1); 5.83 (dd, *J* = 10.5, 1.4 Hz, 1H, –CH=C**H**$_2$ *cis*); 6.17 (dd, *J* = 17.3, 10.5 Hz, 1H, –C**H**=CH_2); 6.46 (dd, *J* = 17.4, 1.4 Hz, 1H, –CH=C**H**$_2$ *trans*). **^{13}C NMR** ($CDCl_3$, 100 MHz) δ [ppm]: -0.06, 0.46, 0.66, 0.82, 1.12, 1.33 (7x –Si$(CH_3)_3$); 61.94 (C-6); 63.18 (C-1'); 65.23 (C-6'); 71.76, 73.05, 73.28, 73.95, 76.15, 76.40 (C-2, C-3, C-4, C-5, C-3', C-4'); 78.89 (C-5'); 91.87 (C-1); 103.81 (C-2'); 128.56 (–**C**H=CH_2); 131.04 (–CH=**C**H_2); 166.31 (–OC(O)–).

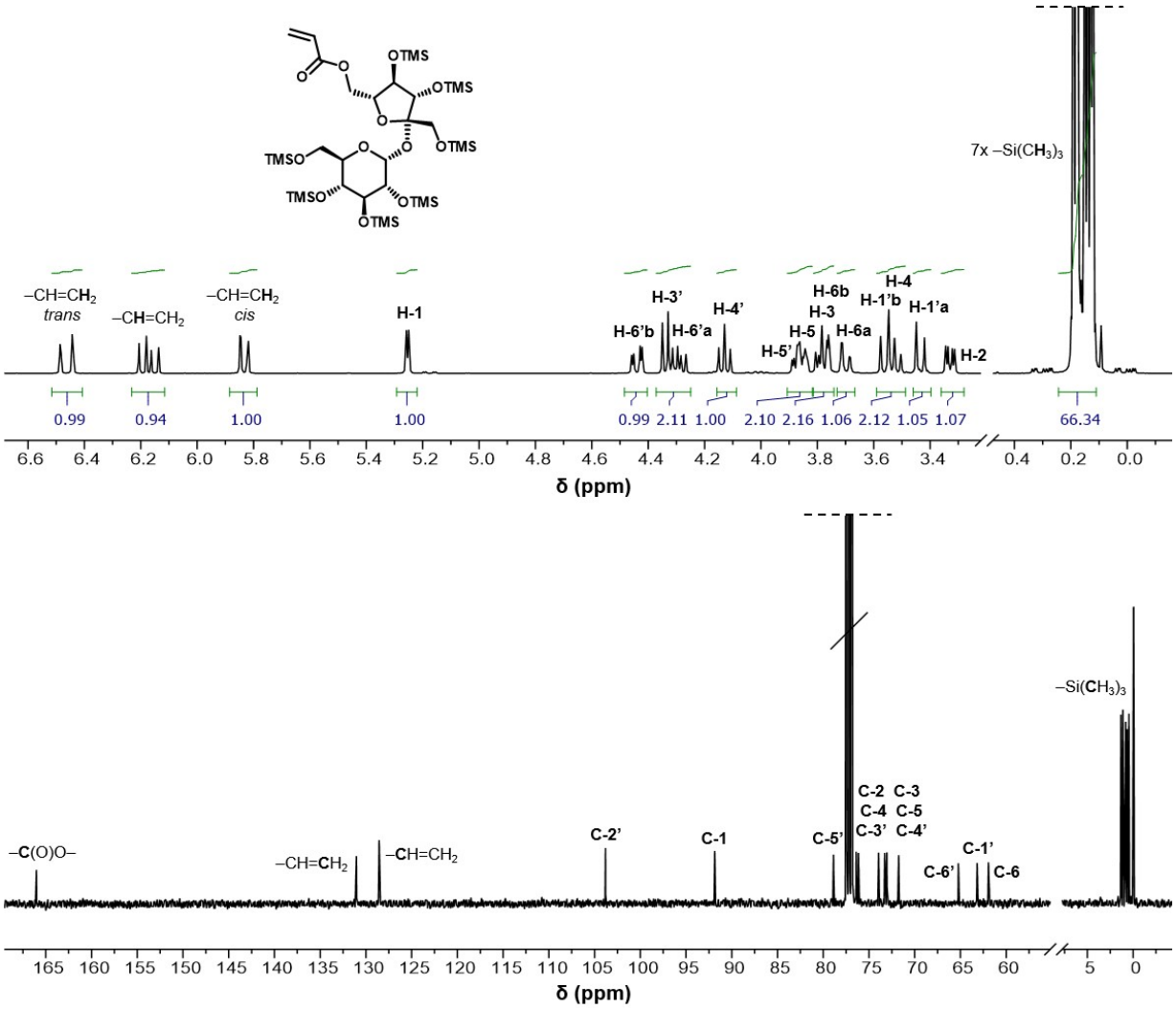


Fig. S9. ^{1}H NMR and ^{13}C NMR spectra of 6'-*O*-acryloyl-2,3,4,6,1',3',4'-hepta-*O*-trimethylsilyl-sucrose ($CDCl_3$)

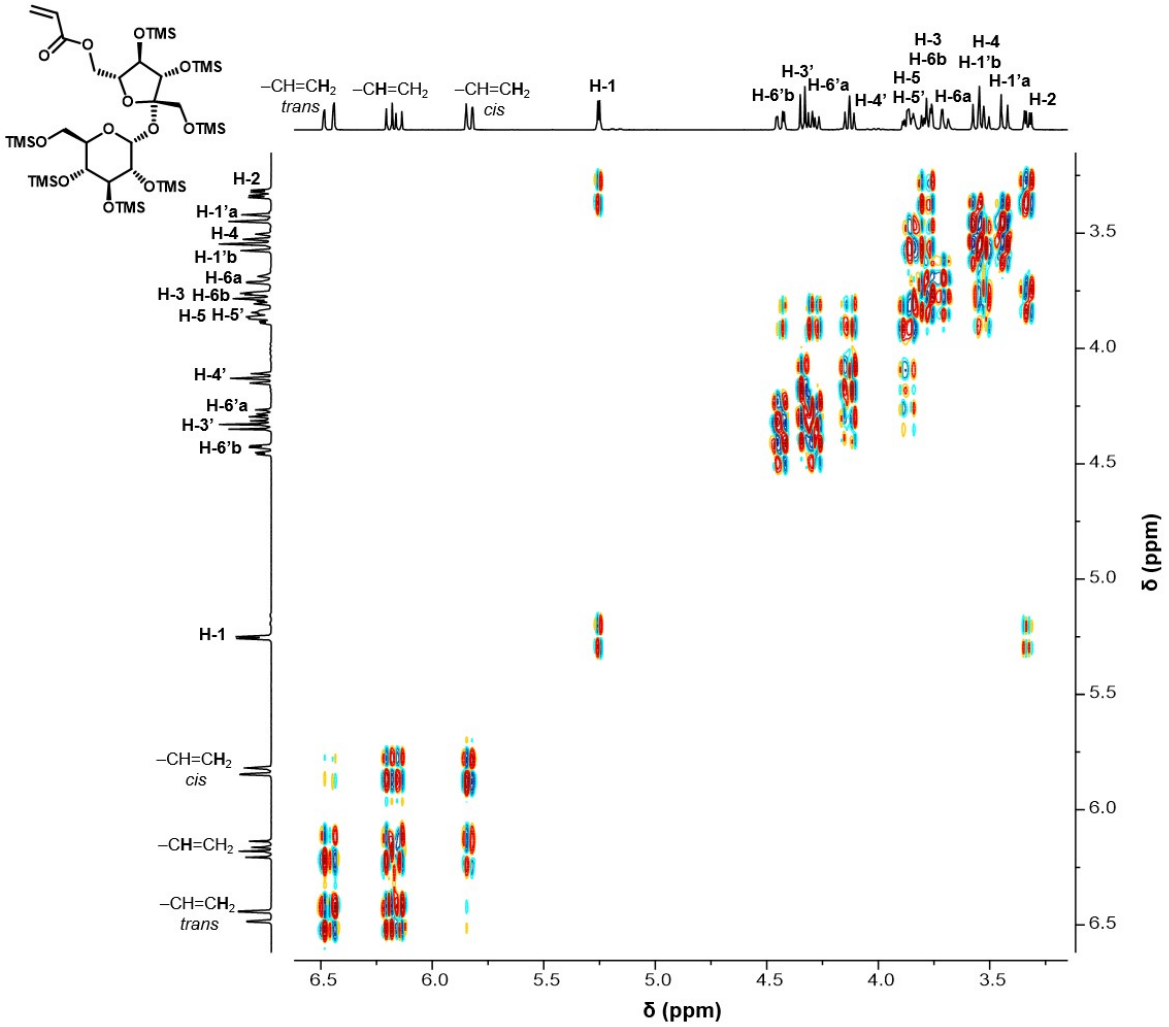


Fig. S10. COSY 2D NMR spectrum of 6'-*O*-acryloyl-2,3,4,6,1',3',4'-hepta-*O*-trimethylsilyl-sucrose ($CDCl_3$)

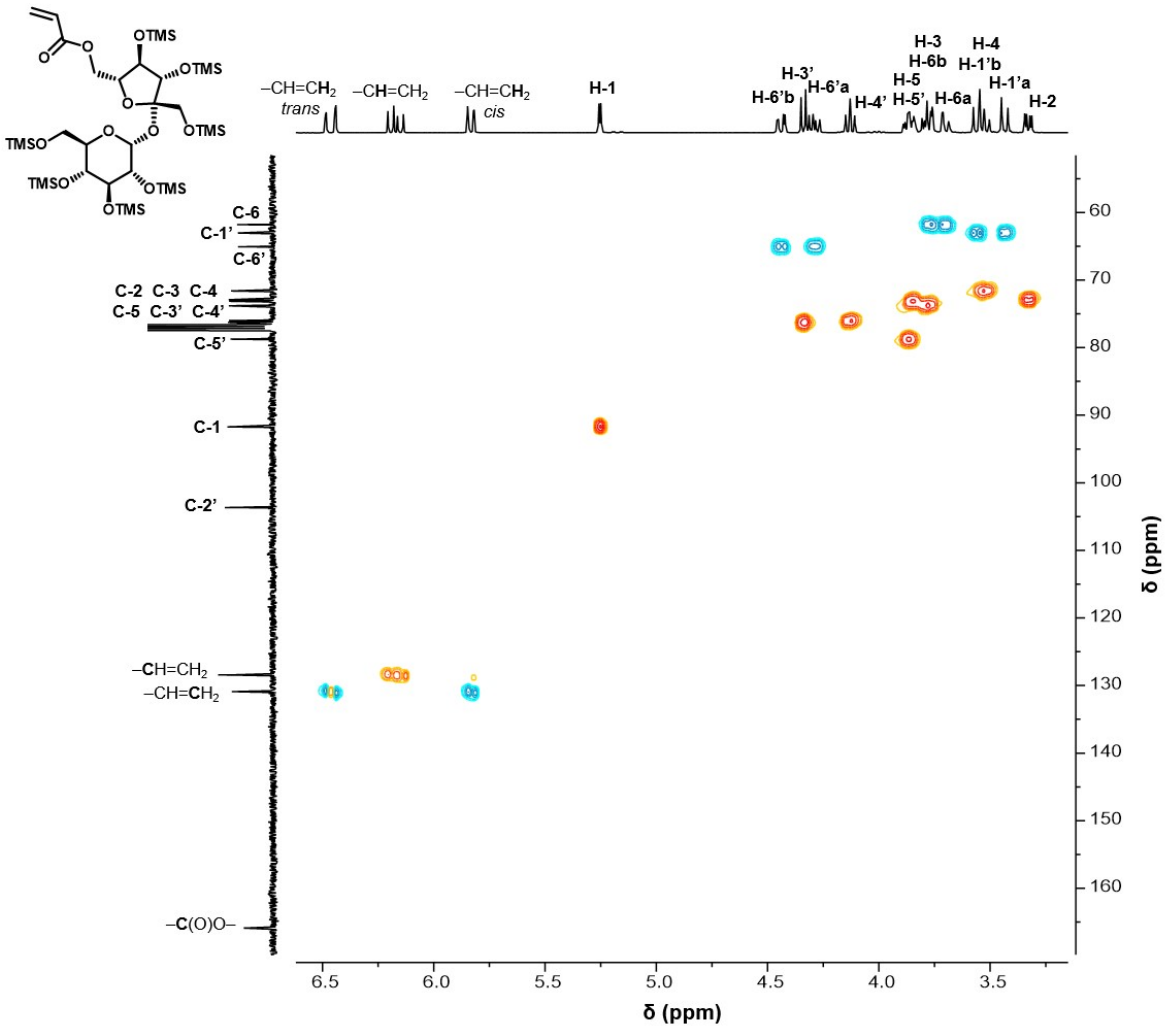


Fig. S11. HSQC 2D NMR spectrum of 6'-*O*-acryloyl-2,3,4,6,1',3',4'-hepta-*O*-trimethylsilyl-sucrose ($CDCl_3$)

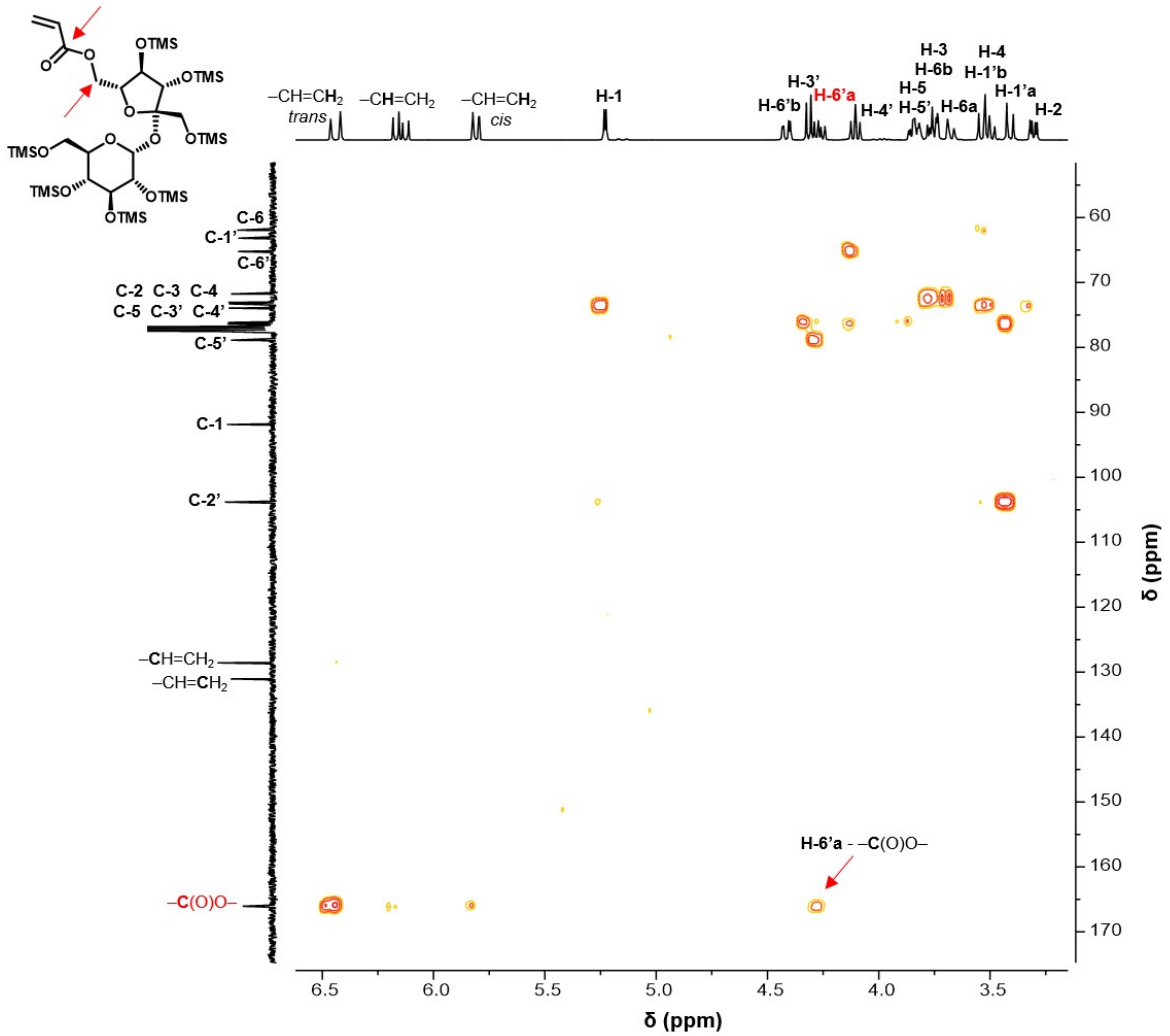


Fig. S12. HMBC 2D NMR spectrum of 6'-*O*-acryloyl-2,3,4,6,1',3',4'-hepta-*O*-trimethylsilyl-sucrose. Correlation between H-6' and –C(O)O– enables to assign the position of acryloyl functionalisation ($CDCl_3$)

6'-O-acryloyl-sucrose

Amberlyst 15* (~3.8 g) was added to the solution of 6'-*O*-acryloyl-2,3,4,6,1',3',4'-hepta-*O*-trimethylsilyl-sucrose (1.09 mmol, 0.98 g) in the mixture of methanol and milli-Q water (150 and 15 mL, respectively) and the reaction was left at room temperature under stirring for 1 h. Amberlyst 15 was filtered off and the filtrate was extracted with hexane (50 mL). Aqueous phase was separated and concentrated under vacuum at 35°C to ~10 mL. The residual solution was filtered through the syringe filter (0.45 µm) and frieze dried to afford 6'-*O*-acryloyl-sucrose as a white solid (0.42 g, 98%).

*washed several times with methanol before use

^{1}H NMR ($DMSO_{d6}$, 400 MHz) δ [ppm]: 3.10 (ddd, *J* = 9.8, 8.8, 5.5, Hz, 1H, H-4); 3.18 (ddd, *J* = 9.5, 6.1, 3.8 Hz, 1H, H-2); 3.44 – 3.38 (m, 2H, H-1'a, H-1'b); 3.53 – 3.43 (m, 2H, H-3, H-6a); 3.61 (ddd, *J* = 11.6, 4.9, 2.2 Hz, 1H, H-6b); 3.66 (ddd, *J* = 9.8, 5.1, 2.2 Hz, 1H, H-5); 3.76 (~td, *J* = 8.0, 2.7 Hz, 1H, H-5'); 3.84 (~td, *J* = 8.1, 5.6 Hz, 1H, H-4'); 3.92 (~t, *J* = 8.0 Hz, 1H, H-3'); 4.27 (~dd, *J* = 11.8, 8.1 Hz, 1H, H-6'a); 4.42 – 4.37 (m, 2H, H-6'b, –OH); 4.67 (d, *J* = 7.9 Hz, 1H, –OH); 4.77 (d, *J* = 3.4 Hz, 2H, 2x –OH); 4.85 (~t, *J* = 6.4 Hz, 1H, –OH); 5.02 (d, *J* = 6.2 Hz, 1H, –OH); 5.14 (d, *J* = 3.8 Hz, 1H, H-1); 5.40 (d, *J* = 5.6 Hz, 1H, –OH); 5.96 (dd, *J* = 10.3, 1.6 Hz, 1H, –CH=C**H**$_2$ *cis*); 6.20 (dd, *J* = 17.3, 10.3 Hz, 1H, –C**H**=CH_2); 6.35 (dd, *J* = 17.3, 1.6 Hz, 1H, –CH=C**H**$_2$ *trans*). **^{13}C NMR** ($DMSO_{d6}$, 100 MHz) δ [ppm]: 61.21 (C-6); 62.02 (C-1'); 66.46 (C-6'); 70.50 (C-4); 72.09 (C-2); 73.29, 73.31 (C-3, C-5); 75.26 (C-4'); 76.81 (C-3'); 79.55 (C-5'); 92.26 (C-1); 104.79 (C-2'); 128.67 (–**C**H=CH_2); 132.28 (–CH=**C**H_2); 165.90 (–OC(O)–).

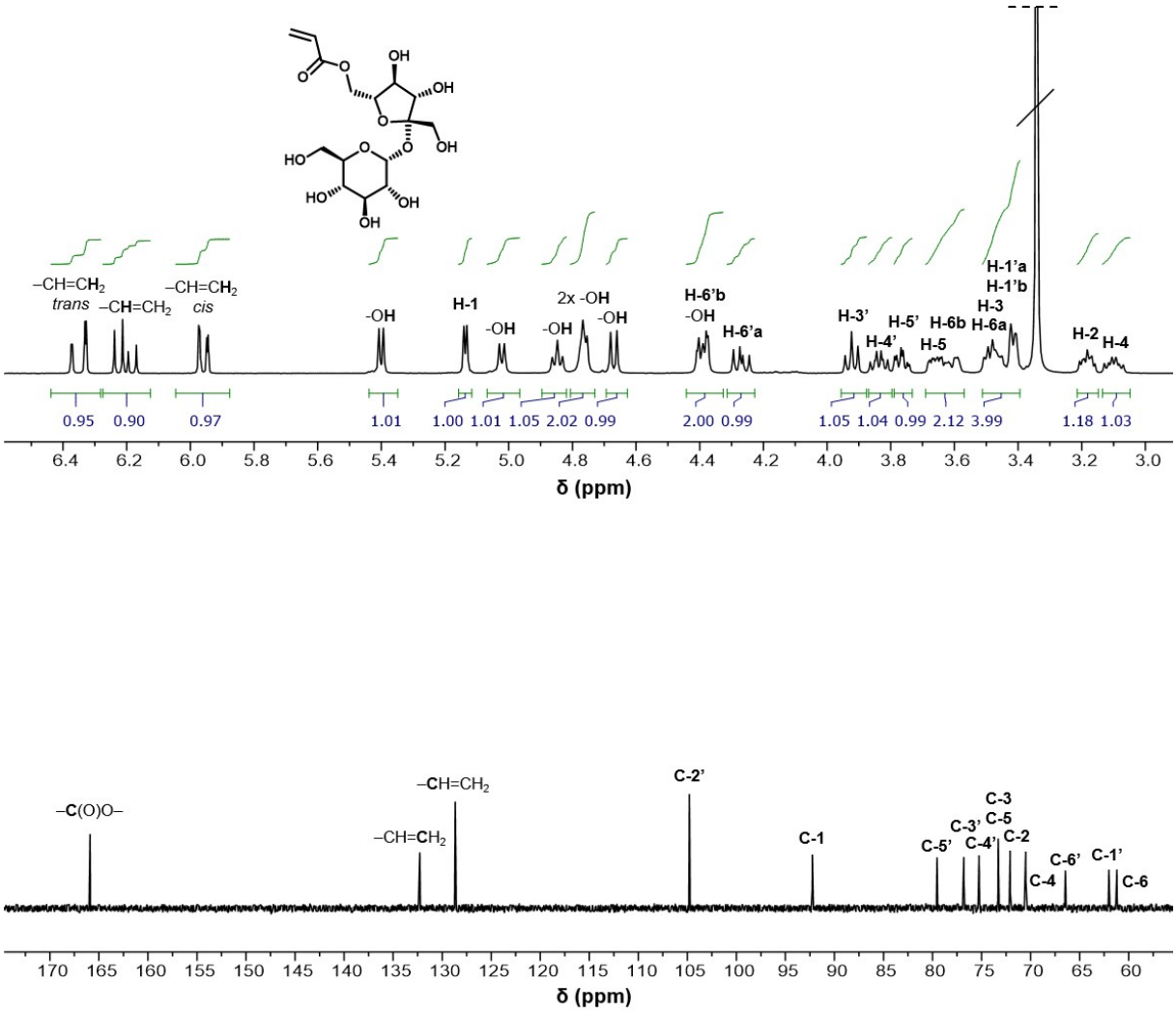


Fig. S13. ^{1}H NMR and ^{13}C NMR spectra of 6'-*O*-acryloyl-sucrose ($DMSO_{d6}$)

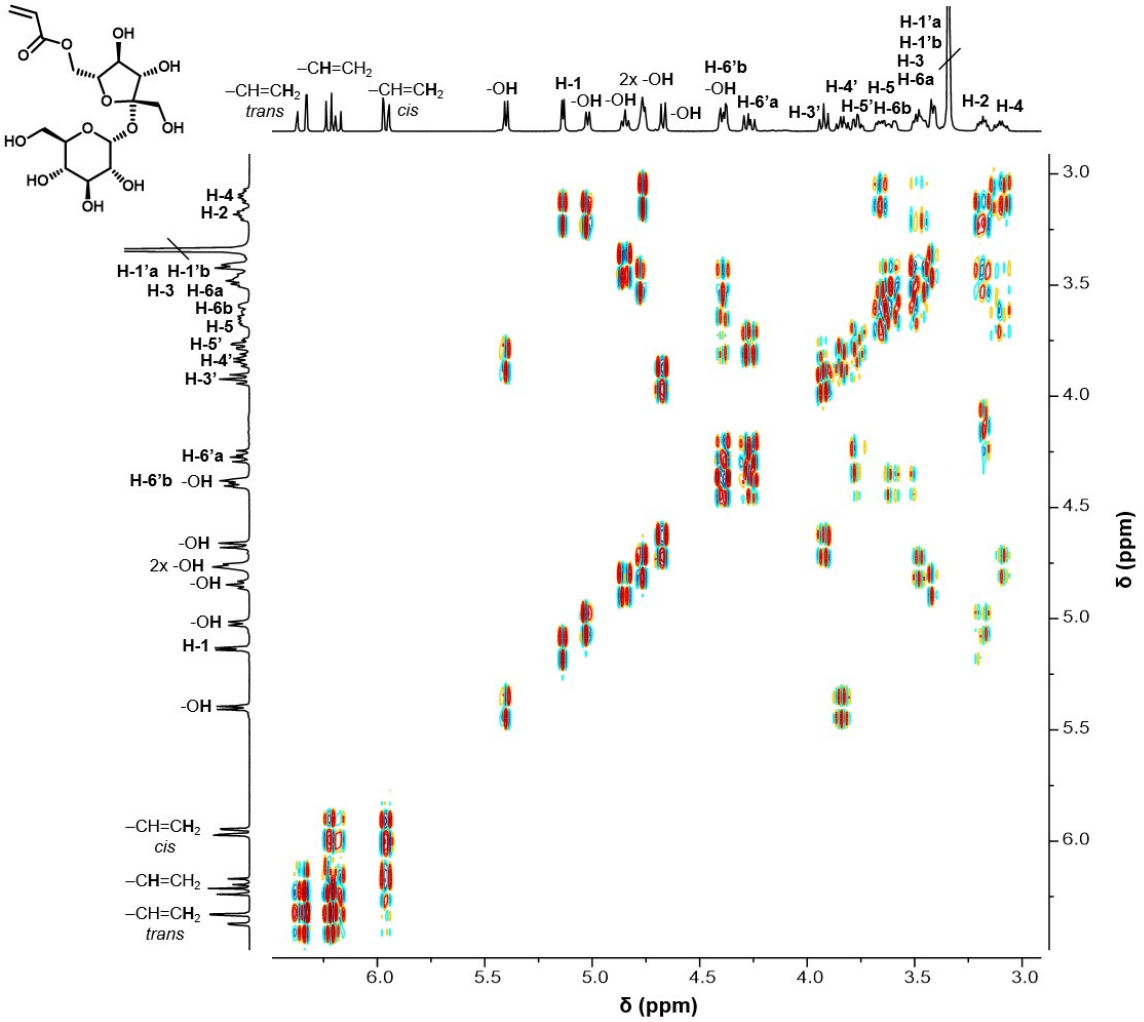


Fig. S14. COSY 2D NMR spectrum of 6'-*O*-acryloyl-sucrose ($DMSO_{d6}$)

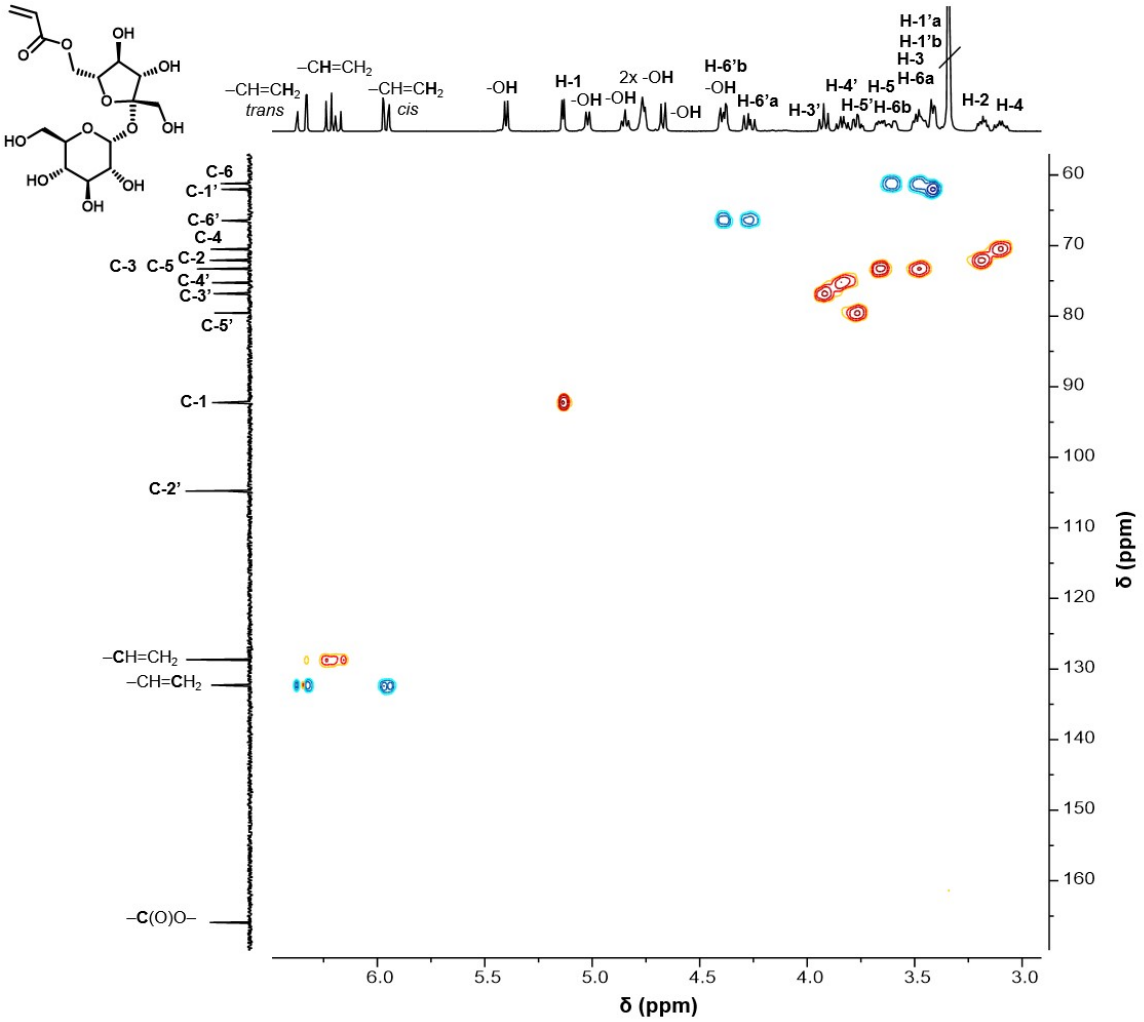


Fig. S15. HSQC 2D NMR spectrum of 6'-*O*-acryloyl-sucrose ($DMSO_{d6}$)

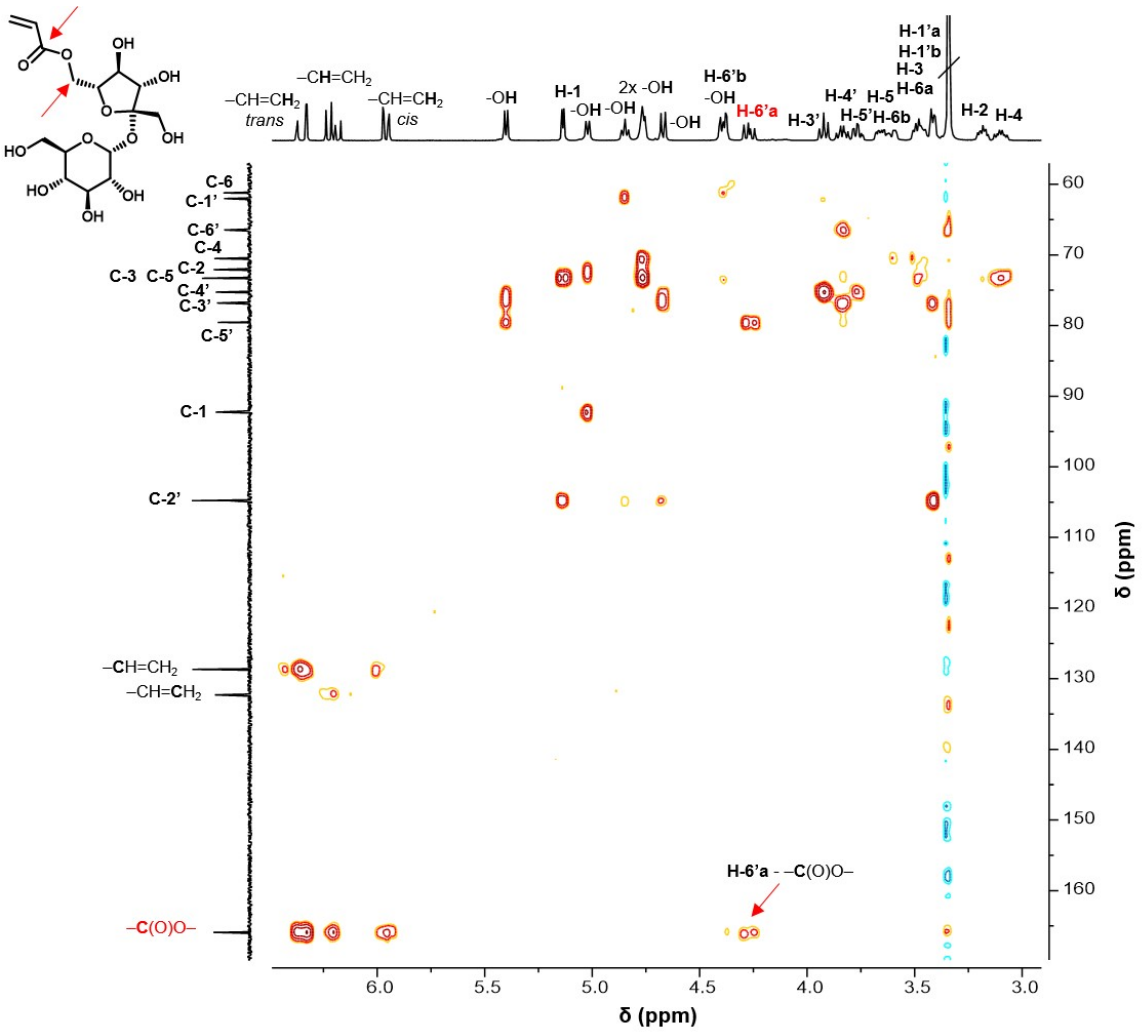


Fig. S16. HMBC 2D NMR spectrum of 6'-*O*-acryloyl-sucrose ($DMSO_{d6}$)

3. Synthesis of RAFT CTA benzyl 2-hydroxyethyl carbonotrithioate

RAFT CTA agent benzyl 2-hydroxyethyl carbonotrithioate was synthesized according to the published procedure by Skey and O'Reilly.[3] Briefly, 2-mercaptoethanol (1.00 g, 12.8 mmol) was added to the suspension of K_3PO_4 (2.72g, 12.8 mmol) in acetone (20 mL), while stirring, and was left for 15 min before CS_2 (2.92 g, 38.4 mmol) was introduced. After stirring for another 15 minutes benzyl bromide (2.19 g, 12.8 mmol) was added and the precipitate of KBr formed. The reaction was left for 1 h under stirring at room temperature and then precipitate was filtered off. The filtrate was collected, and concentrated under reduce pressure to give a yellow oily crude. The crude was purified by silica gel column chromatography using hexane:ethyl acetate gradient to yield the product as a yellow viscous oil which crystallised upon storage at -20 °C (2.51 g, 80%).

^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm]: 1.79 (bs, 1H, –O**H**); 3.62 (t, *J* = 6.1 Hz, 2H, –C**H$_2$**SC(S)S–); 3.90 (t, *J* = 6.1 Hz, 2H, –C**H$_2$**OH); 4.62 (s, 1H, –C**H$_2$**C$_6$H$_5$); 7.37 – 7.24 (m, 5H, –C$_6$**H$_5$**). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ [ppm]: 39.32 (–**C**H$_2$SC(S)S–); 41.87 (–**C**H$_2$C$_6$H$_5$); 60.80 (–**C**H$_2$OH); 127.99, 128.88, 129.41, 134.94 (–**C$_6$**H$_5$); 223.65 (–S**C**(S)S–)

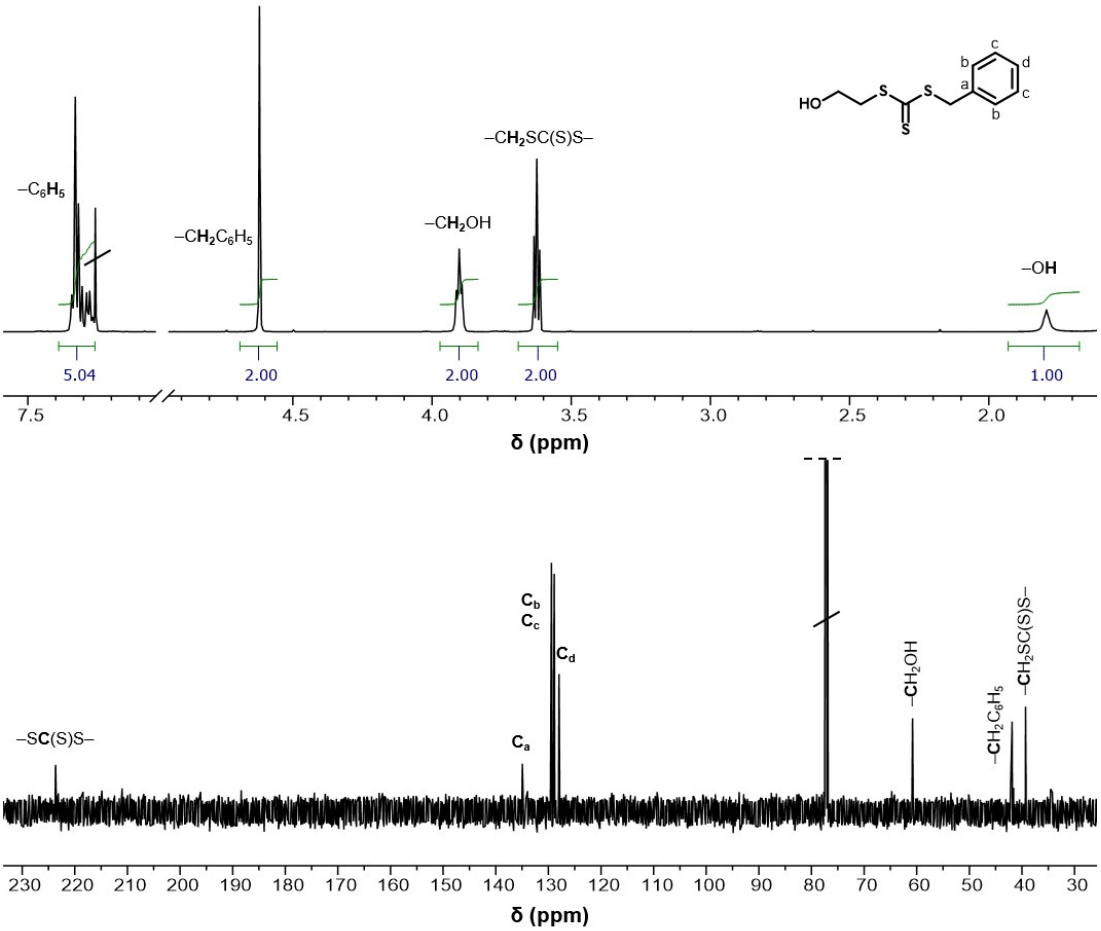


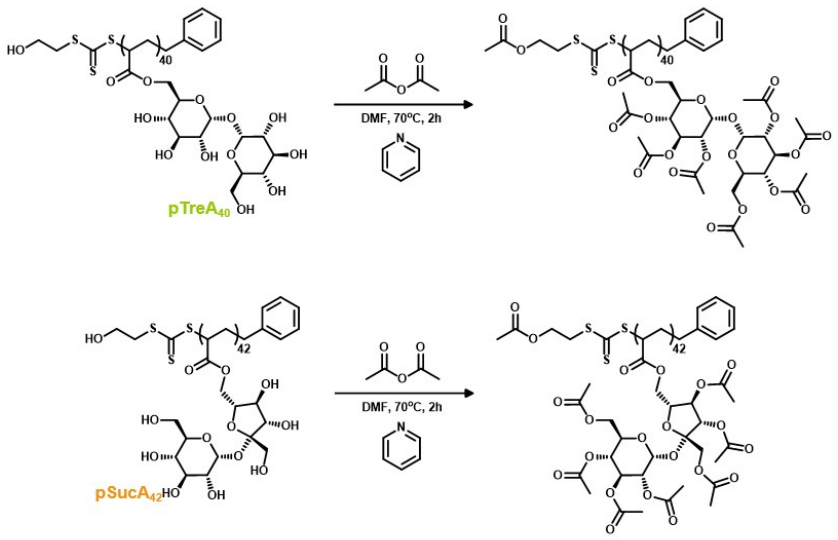
Fig. S17 ^{1}H and ^{13}C NMR spectra of benzyl 2-hydroxyethyl carbonotrithioate (600 MHz, $CDCl_3$).

4. Synthesis of glycopolymers

Trehalose and sucrose glycopolymers were synthesized from TreA and SucA, respectively, in DMSO via RAFT polymerization using 2-hydroxyethyl carbonotrithioate as the CTA and AIBN as the thermal initiator. Specifically, TreA and SucA glycomonomers (8.0 x 10^{-4} mol) were each weighed in 4-mL glass vials, to which 200 µL of DMSO, 2.0 x 10^{-5} mol of CTA (400 µL of a 50-mM stock solution in DMSO), and 4.0 x 10^{-6} mol of AIBN (200 µL of a 20-mM stock solution in DMSO) were added, such that [glycomonomer]:[CTA]:[AIBN] = 40:1:0.2. The vials were each sealed with a silicone septum, and the solutions were thoroughly deoxygenated with argon for 1 h. Each vial was then transferred to an oil bath preheated to 70 °C and stirred (400 rpm) under these conditions for 16 h. The polymerization was stopped by cooling the vials in an ice-bath and exposing the polymerization solutions to air. The monomer conversion was calculated based on the ^{1}H-NMR spectrum of the crude products in D_2O. The polymer was purified by dialysis (Spectra/Por® 6, MWCO: 1 kDa, 18 mm) against deionized water (~2.5 L, changed three times) for 24 h and then freeze-dried to afford $pTreA_{40}$ and $pSucA_{42}$ glycopolymers as pale yellow powders.

5. Size exclusion chromatography

The molecular weights and molecular weight distributions of the synthesized glycopolymers were determined by size exclusion chromatography (SEC) using a Thermo Scientific DIONEX UltiMate 3000 UHPLC+ system comprising a degasser, a pump (BX P4), an autosampler (ACC-3000(T)), a column oven (TCC-3000 SDIRS), and a refractive index detector (RefractoMax 520). A 50 × 7.5 mm guard column (5 µm particle size) and two linear columns, 300 × 7.5 mm (5 µm particle size) and 300 x 8 mm (10 µm particle size), were used for separation. DMF (HPLC grade, containing 0.03% w/v LiBr) was used as the eluent at a flow rate of 0.6 mL/min at 50 °C with an injection volume of 100 µL. The system was calibrated with polystyrene standards (1.28-1390 kDa, Agilent Technologies). To provide the solubility of trehalose and sucrose glycopolymers in DMF, the hydroxyl groups were acetylated prior the SEC analysis using acetic anhydride (**Scheme S3**) as follows: 15 µL of an acetic anhydride/pyridine mixture (v/v: 1/1) and 90 µL of DMF (containing 0.03% w/v LiBr) were added to ~2 mg of the polymer sample and heated at 70 °C for 2 h; the resulting clear solution was diluted with 900 µL of DMF (0.03% w/v LiBr), filtered through a syringe filter (0.45 µm), and analyzed.



Scheme S3 Glycopolymers acetylation prior to SEC analysis

6. Dynamic light scattering

The Z-average mean hydrodynamic diameter and polydispersity index (PDI) of glycopolymer self-assemblies were determined by dynamic light scattering (DLS) using a Zetasizer Nano S90 (Malvern) instrument equipped with a 4 mV He-Ne ion laser (λ = 633 nm) as the light source and detecting light scattered at 90°. These measurements were performed for glycopolymer solutions in selected buffers using low-volume quartz batch cuvette ZEN2112. Before each measurement, the samples were equilibrated for 20 min at the measurement temperature using a built-in Peltier temperature controller.

7. Cryogenic transmission electron microscopy

Cryogenic transmission electron microscopy (cryo-TEM) images were obtained using a Tecnai F20 X TWIN microscope (FEI Company, Hillsboro, Oregon, USA) equipped with field emission gun, operating at an acceleration voltage of 200 kV. Images were recorded with a Gatan Rio 16 CMOS 4k camera (Gatan Inc., Pleasanton, California, USA) and processed with Gatan Microscopy Suite (GMS) software (Gatan Inc., Pleasanton, California, USA). Specimens were prepared via the vitrification of aqueous solutions on grids with holey carbon film (Quantifoil R 2/2; Quantifoil Micro Tools GmbH, Großlöbichau, Germany). Prior to use, the grids were activated for 15 s in oxygen plasma using a Femto plasma cleaner (Diener Electronic, Ebhausen, Germany). Cryo-TEM samples were prepared by applying a droplet (3 µL) of the suspension to the grid, blotting with filter paper and immediately freezing in liquid ethane using a fully automated blotting device (Vitrobot Mark IV, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Once prepared, the vitrified specimens were kept under liquid nitrogen until they were inserted into a cryo-TEM holder (Gatan 626, Gatan Inc., Pleasanton, USA) and analyzed in the TEM at –178 °C.

8. Hydrolytic stability of glycopolymers

The hydrolytic stabilities of the glycopolymers at various pH values were analyzed using ^{1}H-NMR spectroscopy. The samples were prepared by dissolving 4.0 mg of glycopolymer in 200 µL of an appropriate 20-mM buffer solution in D_2O, followed by incubation at 50 °C in a dry block thermostat (Biosan, TDB-120) for 24 h. After incubation, samples were diluted to 500 µL with D_2O, and their ^{1}H-NMR spectra were recorded. Phosphate (pK_1), acetate, phosphate (pK_2), glycine, or phosphate (pK_3) buffer was used to maintain the pH of the solutions at 2.0, 5.0, 7.4, 9.0, or 12.0, respectively. The buffers were prepared at 25 °C taking into account their temperature-dependent coefficients, $d(pK_a)/dT$, so that the desired pH was reached at 50 °C.

9. Affinity of glycopolymers and corresponding disaccharides towards ConA

The affinities of the prepared glycopolymers and the corresponding disaccharides toward ConA were investigated via isothermal titration calorimetry (ITC), which was performed using a Nano ITC-SV (TA instruments) instrument with ConA as the titrand and the glycopolymer/disaccharide (ligand) as the titrant, dissolved in 100 mM acetate buffer (pH 5.2) containing 50 mM NaCl, 1 mM $MnCl_2$, and 1 mM $CaCl_2$. The concentration of ConA used in these titration experiments was [ConA] ≈ 40 µM (in experiments with trehalose or $pTreA_{40}$) or [ConA] ≈ 200 µM (in experiments with sucrose or $pSucA_{42}$). The accurate ConA concentration was determined by UV-Vis spectroscopy at 280 nm based on $A_{1\%,\ 1\ cm}$ = 12.4 at pH 5.2[5] and expressed in terms of the monomeric unit (MW = 26 500 Da). Ligands were used in the following concentrations: [trehalose] = 4.5 mM; [$pTreA_{40}$] = 112.5 µM; [sucrose] = 18.0 mM; [$pSucA_{42}$] = 428.6 µM. The glycopolymer concentrations were based on the average molecular weight of the glycopolymers calculated from the average degree of polymerization. All of the solutions were passed through 0.20-µm syringe filters (CHROMAFIL® H-PTFE Xtra) and degassed at 25 °C for 20 min prior to the ITC experiments, which were performed at 25 °C with constant stirring at 200 rpm. Injections (1 × 2 µL followed by 31 × 8 µL) of ligand solution into the ConA solution were added using a 250-µL syringe at 240-s intervals. The data point corresponding to the first 2-µL injection is usually inaccurate, and thus, it was discarded before further

analysis. The data for the ligand-ConA titration experiments were corrected by subtracting the heat of ligand dilution, which was obtained from control experiments performed by injecting ligand solution into the corresponding buffer solution. The raw ITC data were processed using NanoAnalyze software provided (TA instruments) by applying an independent one-site binding model. ITC experiments were performed under low-*c* conditions; therefore, only K_a was extracted from the obtained data as a reliable value.

10. Quantitative precipitation of ConA by glycopolymers

The ConA solutions were prepared by dissolving the lectin in 100 mM HEPES buffer (pH 7.2) containing 50 mM NaCl, 1 mM $MnCl_2$, and 1 mM $CaCl_2$ and passing it through a 0.20-µm syringe filter (CHROMAFIL® H-PTFE Xtra). The solutions were prepared at 2.0 mg·mL^{-1} to examine the glycopolymer concentration dependence and at 0.5, 1.0, 2.0, 3.0, and 4.0 mg·mL^{-1} to study ConA concentration dependence. Glycopolymer solutions were prepared in the corresponding buffer at four concentrations (500, 200, 100, or 50 µM) considering the disaccharide concentration. Then, 500 µL of the glycopolymer solution was added to 500 µL of the ConA solution, gently mixed with a pipette, and incubated at 25 °C for 10 h. A control solution was prepared in the same way by replacing the glycopolymer solution with the corresponding buffer solution. After incubation, the white suspensions were centrifuged (12 400 × *g*, 5 min, 25 °C), and 900 µL of the supernatant were collected and analyzed spectrophotometrically at 280 nm to determine the ConA concentration. The accurate ConA concentration was calculated based on $A_{1\%,\,1\,cm}$ = 13.7 at pH 7.2.[5] The percentage of precipitated ConA was calculated from the difference between the ConA concentration in the control and in the sample. Each sample was analyzed in triplicate, and the reported values represent the mean value ± the standard deviation (SD).

11. Susceptibility of glycopolymers and glycomonomers to enzymatic hydrolysis by glycosyl hydrolases

The susceptibilities of the glycopolymers and glycomonomers to enzymatic hydrolysis by the corresponding glycosyl hydrolases (trehalase for TreA and $pTreA_{40}$ or invertase for SucA and $pSucA_{42}$) were evaluated using thin layer chromatography (TLC). Hydrolysis of the corresponding free disaccharides was performed simultaneously and used as a control. The trehalase stock solution was prepared via 10× dilution of the supplied trehalase solution in 100 mM sodium maleate buffer (pH 5.5), and the invertase stock solution was prepared by 200× dilution of the supplied invertase concentrate in 100 mM acetate buffer (pH 4.7). Trehalose, TreA, and $pTreA_{40}$ solutions were prepared in 100 mM sodium maleate buffer (pH 5.5) at 25 mM regarding the trehalose concentration, and the solutions of sucrose, SucA, and $pSucA_{42}$ were prepared in 100 mM acetate buffer (pH 4.7) at 50 mM regarding the sucrose concentration. Then, 200 µL of each of these solutions was transferred to a 96-microwell plate and 20 µL of the corresponding enzyme stock solution was added from a multi-channel pipette, and the plates were incubated at 25 °C. At designated time intervals, 2 µL of the solutions were transferred onto TLC plates (developing distance = 7 cm) and dried well with compressed air. The plates were then developed in an acetonitrile:H_2O solvent mixture (v/v: 3:1) and visualized by immersing in 10% sulfuric acid solution in methanol and heating.

12. Recombinant human insulin fibrillation

The stock solution of recombinant human insulin was prepared by dissolving the protein in 20 mM phosphate buffer (pH 7.4) at 2.0 mg·mL^{-1} (recombinant human insulin is not readily soluble at this pH, so the protein/buffer mixture was left for ~2 h with occasional shaking, which allowed it to transform from a white suspension to a clear solution) and passing through a 0.20-µm syringe filter (CHROMAFIL® H-PTFE Xtra). Then, 350 µL of the insulin stock solution was added to 350 µL of the glycopolymer solution (100, 50, 20, or 10 mM, regarding the disaccharide concentration) or disaccharide solution (100 mM) in 20 mM phosphate buffer (pH 7.4) containing 200 mM NaCl and 40 µM ThT, and the mixture was gently mixed with a pipette. Similarly, the insulin stock solution was diluted by half with 20 mM phosphate buffer (pH 7.4) containing 200 mM NaCl and

40 µM ThT to study fibrillation in the absence of additives. Control solutions were prepared in the same way by replacing the insulin stock solution with 20 mM phosphate buffer (pH 7.4). Aliquots of each sample solution (6 × 100 µL) and control (3 × 100 µL) were then transferred to the black 96-microwell plate with a clear bottom (SARSTEDT, lummox®) and covered with PET sealing film to prevent sample evaporation. The plate was placed in microplate thermo-shaker (Biosan, PST-100HL) and incubated at 50 °C under vigorous shaking (900 rpm) for 30 h. The fluorescence intensity of ThT (λ_{ex} = 450 nm, 9 nm bandwidth; λ_{em} = 482 nm, 15 nm bandwidth) was recorded at 15-min intervals using a Multi-Mode Microplate Detection Platform (SpectraMax® i3, Molecular Devices®). The fluorescence was corrected by subtracting the fluorescence intensity of the corresponding control solutions without insulin. The parameters characterizing insulin fibrillation were derived by fitting the sigmoidal equation shown in Eq. (1),[6] to the experimental fluorescence intensity (*F*) versus time (*t*) data using non-linear regression by using GraphPad Prism 8.0.1.

$$F(t) = F_I + \frac{F_{MAX} - F_I}{1 + e^{-k_{app}(t_{1/2} - t)}} \qquad (1)$$

In this equation, F_I represents the initial fluorescence intensity, F_{MAX} is final fluorescence intensity in the stationary phase, $t_{1/2}$ represents the time at which the fluorescence reaches half of F_{MAX}, and k_{app} is the apparent rate constant for the growth of fibrils. The lag time (t_{lag}) can be derived and expressed as shown in Eq. (2):

$$t_{lag} = t_{1/2} - \frac{2}{k_{app}} \qquad (2)$$

The fluorescence intensities of all samples were normalized to the average F_{MAX} of insulin fibrils formed without any additives.

After completing the ThT assay, the average insulin recovery (%) was determined. All six replicates of each sample were combined and centrifuged (12 400 × g, 5 min, 25 °C). The supernatants were collected, passed through a 0.20 µm syringe filter (CHROMAFIL® H-PTFE Xtra) and the concentration of non-fibrillated insulin remaining in the solution was determined by Bradford protein assay, based on the established standard curve (**Fig. S18**). Briefly, 50 µL of the sample was added to 1000 µL of Bradford reagent, mixed, and left for 15 min in the dark. Aliquots of each sample (3 × 300 µL) were then transferred to the 96-microwell plate and the absorption intensity at 595 nm was measured. Average insulin recovery (%) was calculated from the difference between the insulin concentration in the solution before fibrillation and in the supernatant.

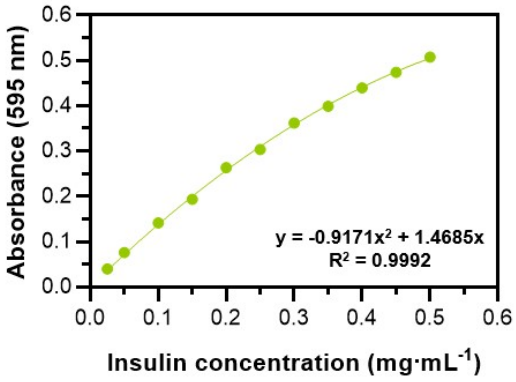


Fig. S18 Bradford protein assay standard curve for human recombinant insulin. The standard curve was established in 96-microwell plate format using 300 uL of the solution.

SUPPLEMENTARY DATA

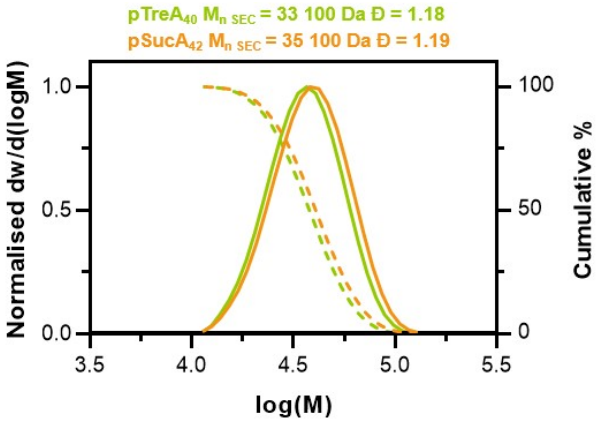


Fig. S19 Differential (solid line) and cumulative (dashed line) molecular weight distribution curves of peracetylated $pTreA_{40}$ and $pSucA_{42}$ from SEC analysis (DMF + 0.03% w/v LiBr).

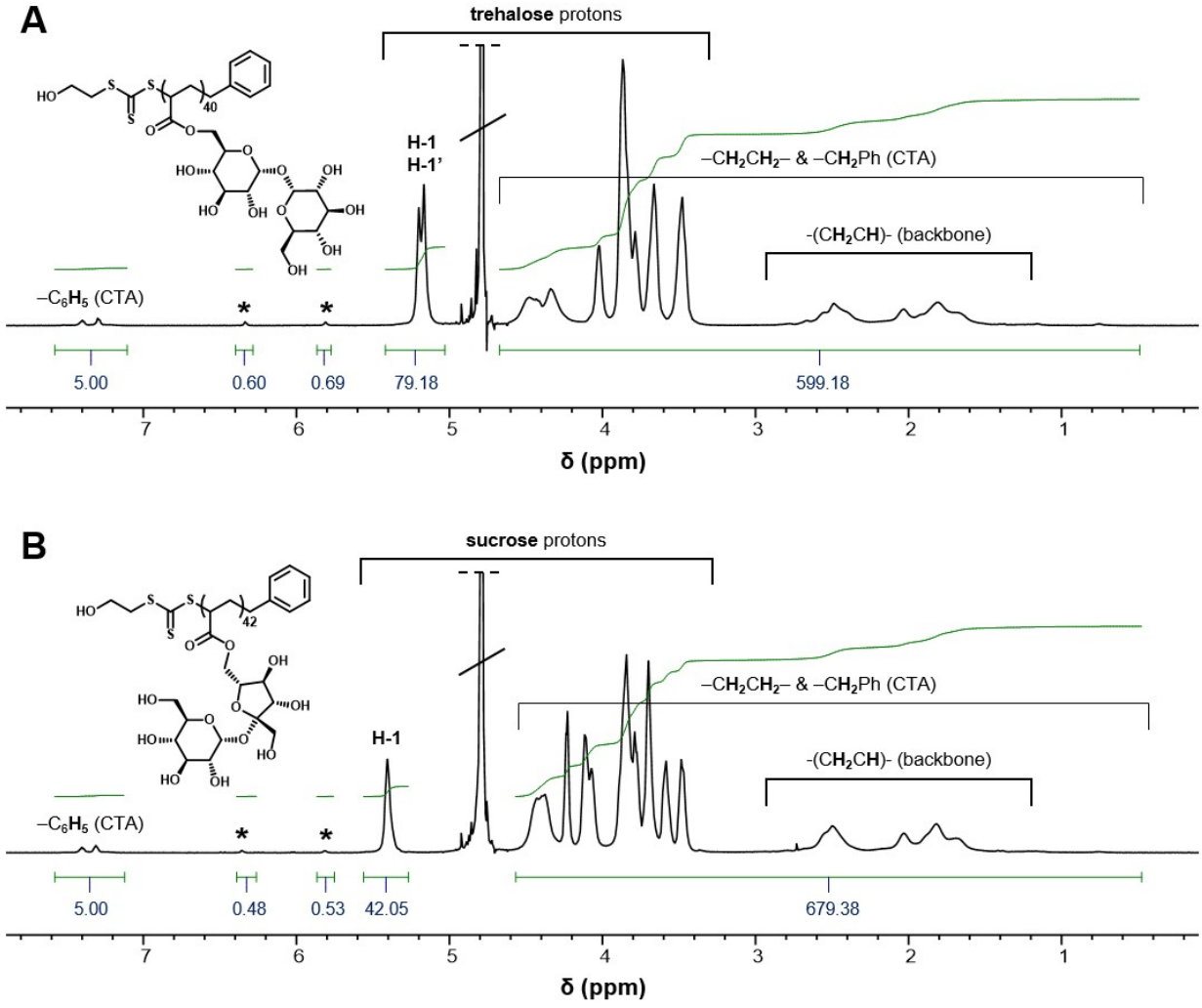


Fig. S20 ^{1}H NMR spectra of $pTreA_{40}$ (A) and $pSucA_{42}$ (B) (600 MHz, D_2O). Asterisks (*) indicate the signals originating from double bond from "dead" polymer chains, which formed after termination by disproportionation.

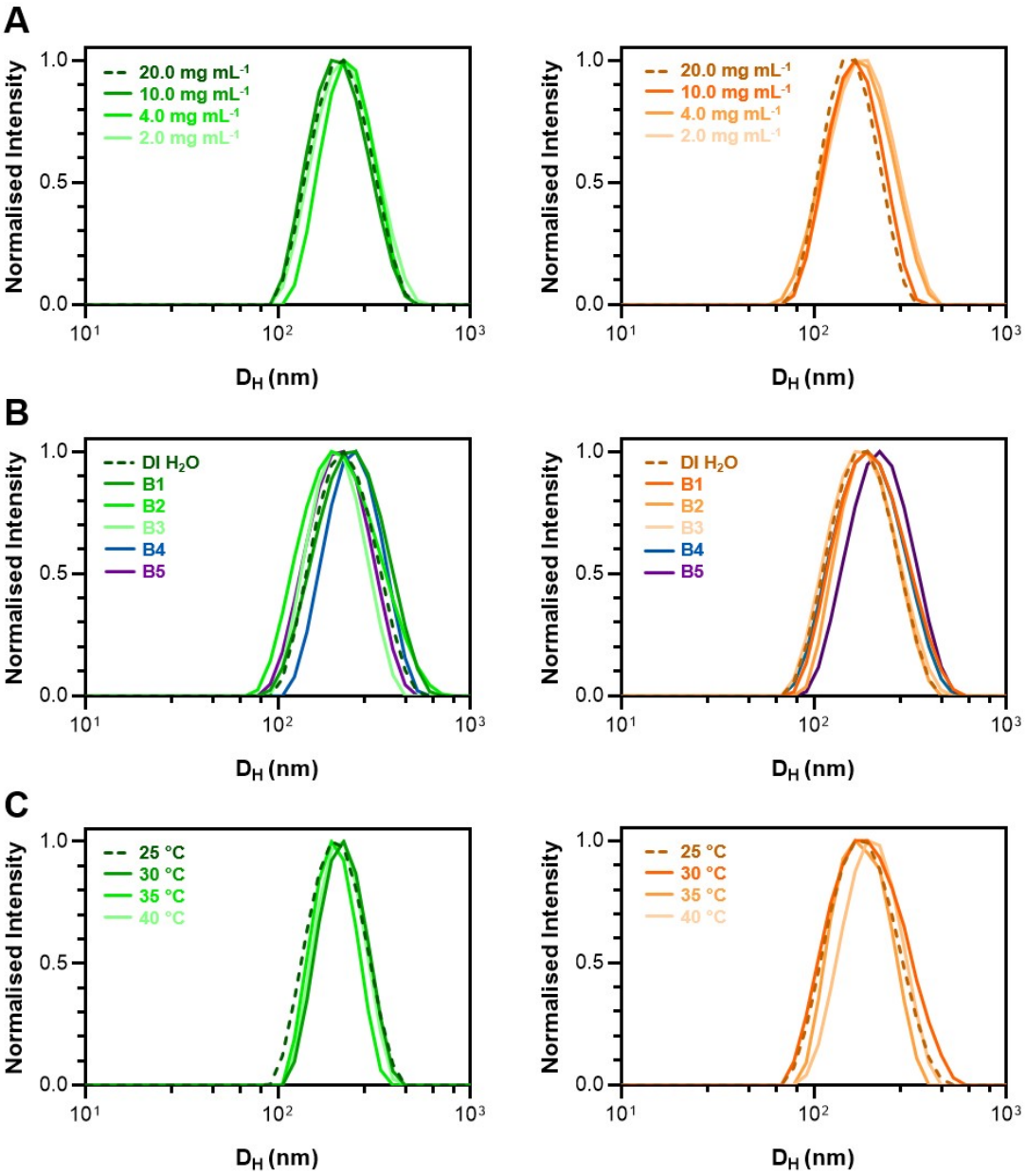


Fig. S21 DLS data for **pTreA$_{40}$** (left) and **pSucA$_{42}$** (right) self-assemblies under various conditions; (A) in deionized water at 25 °C upon dilution from 20.0 to 2.0 mg·mL^{-1}; (B) in deionized water and in various buffers at 25 °C (2.0 mg·mL^{-1}); (C) in 20 mM phosphate buffer (pH 7.4) containing 100 mM NaCl upon temperature increase from 25 to 40 °C (2.0 mg·mL^{-1}); at 45 an 50 °C no self-assemblies were detected be DLS. **B1 –** 100 mM acetate buffer (pH 5.2) containing 50 mM NaCl, 1 mM $MnCl_2$, and 1 mM $CaCl_2$; **B2 –** 100 mM HEPES buffer (pH 7.2) containing 50 mM NaCl, 1 mM $MnCl_2$, and 1 mM $CaCl_2$; **B3 –** 20 mM phosphate buffer (pH 7.4) containing 100 mM NaCl; **B4 –** 100 mM sodium maleate buffer (pH 5.5); **B5 –** 100 mM acetate buffer (pH 4.7).

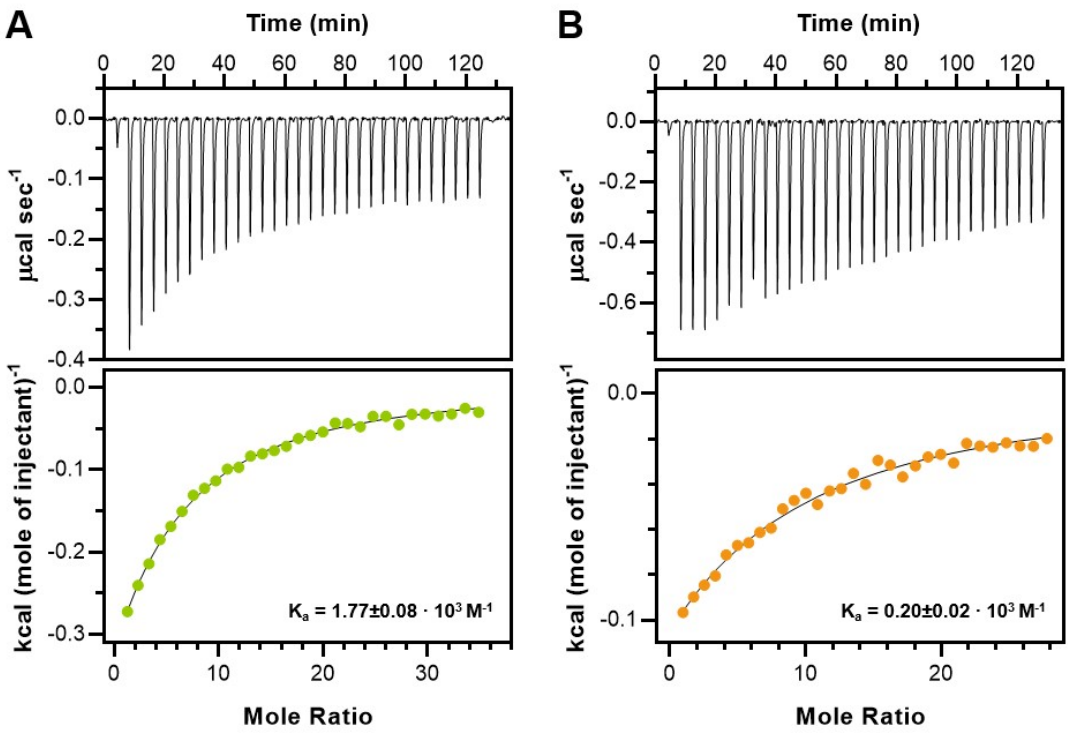


Fig. S22 Calorimetric titration of ConA with (A) trehalose and (B) sucrose. Top: raw experimental data. Bottom: Integrated experimental data (•) and the best fit of independent model (–). ITC experiments were performed in 100 mM acetate buffer (pH 5.2) containing 50 mM NaCl, 1 mM $MnCl_2$, and 1 mM $CaCl_2$ at 25 °C.

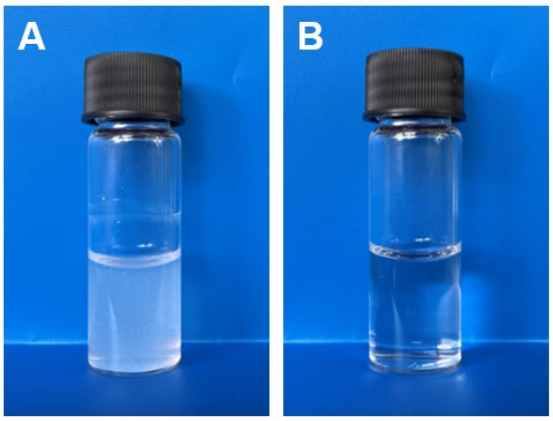


Fig. S23 Photography of (A) ConA - $pTreA_{40}$ precipitate in in 100 mM HEPES buffer (pH 7.2) containing 50 mM NaCl, 1 mM $MnCl_2$, and 1 mM $CaCl_2$ (2.0 mL, ConA: 0.5 mg·mL^{-1}, $pTreA_{40}$: 250 µM regarding the concentration of trehalose); (B) the previous solution after addition of 100 µL of 100 mg·mL^{-1} methyl-α-mannoside solution.

Table S1 Parameters of insulin fibrillation[1]

ADDITIVE	Concentration[2] (mM)	$t_{1/2}$ (h)	t_{lag} (h)	F_{MAX}	k_{app} (h^{-1})	R^2*
–	–	1.78±0.12	1.60±0.12	1.00±0.03	11.25±1.69	0.9972-0.9993
$pTreA_{40}$	5	3.40±0.17	3.14±0.17	1.75±0.12	7.89±0.31	0.9990-0.9996
	10	5.56±0.23	5.20±0.23	1.17±0.03	5.56±0.09	0.9993-0.9997
	25	12.54±0.42	12.14±0.42	0.85±0.02	5.00±0.12	0.9982-0.9998
	50	22.16±0.84	21.69±0.84	0.54±0.01	4.34±0.46	0.9980-0.9994
$pSucA_{42}$	5	3.48±0.23	3.19±0.23	1.73±0.1	7.03±0.81	0.9992-0.9995
	10	6.10±0.28	5.75±0.28	1.17±0.2	5.58±0.09	0.9992-0.9998
	25	14.15±0.48	13.75±0.48	0.84±0.1	5.03±0.15	0.9992-0.9998
	50	25.16±0.66	24.68±0.66	0.53±0.00	4.17±0.22	0.9979-0.9995
Trehalose	50	1.77±0.07	1.57±0.07	1.01±0.01	10.02±0.23	0.9972-0.9993
Sucrose	50	1.83±0.12	1.64±0.12	1.01±0.01	10.22±0.56	0.9968-0.9997

[1] Reported values are the mean ± SD of six replicates

[2] Glycopolymer concentration with regard to the concentration of the corresponding disaccharide

* R^2 range of sigmoidal model fit

$t_{1/2}$ – time at which fluorescence reaches half of F_{MAX}; t_{lag} – lag time; F_{MAX} – normalized fluorescence intensity in the stationary phase; k_{app} – apparent rate constant for the growth of fibrils

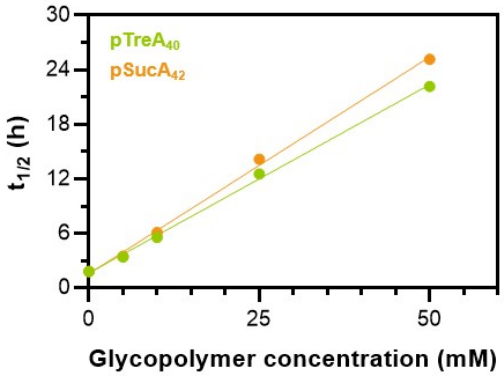


Fig. S24 Correlation between glycopolymers concentration and lag time of insulin fibrillation. Glycopolymers concentration regards the concentration of corresponding disaccharide.

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