Supporting Material

Hydrogels with novel hydrolytically-labile trehalose-based crosslinks: small changes – big differences in degradation behavior

Małgorzata Burek[a], Sylwia Waśkiewicz[b], Anna Lalik[c], Ilona Wandzik*[a]

[a] *Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Faculty of Chemistry, Silesian University of Technology, B. Krzywoustego 4, 44 100 Gliwice, Poland*

[b] *Department of Physical Chemistry and Technology of Polymers, Faculty of Chemistry, Silesian University of Technology, M. Strzody 9, 44 100 Gliwice, Poland*

[c] *Systems Engineering Group, Institute of Automatic Control, Silesian University of Technology, B. Krzywoustego 8, 44 100 Gliwice, Poland*

e-mail: ilona.wandzik@polsl.pl

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1. Materials and general methods

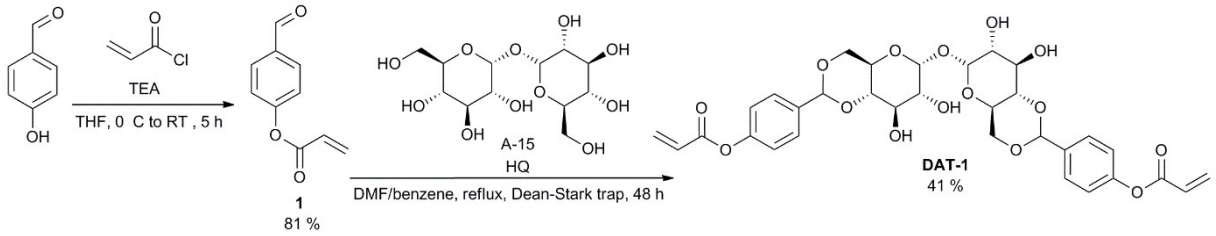
Acrylamide (AM), acryloyl chloride, amberlyst-15 (A-15), ammonium persulphate (APS), Celite 501, citric acid, Giemsa's azur-eosin-methylene blue, glycine, hydrochloric acid, hydroquinone, *p*-hydroxybenzaldehyde, lithium tetrafluoroborate, phosphate buffered saline (PBS) tablets, sodium hydroxide *N,N,N',N'*-tetramethylethylenediamine (TMEDA), *p*-toluenesulfonic acid, anhydrous trehalose, trifluoroacetic acid (TFA), triethylamine (TEA), and trimethyl orthoformate were purchased from Sigma Aldrich, Acros Organics or Fluka and used directly without any purification. *N*-(hydroxyethyl)acrylamide (HEAM) and *N,N*-dimethylacrylamide (Sigma Aldrich) were purified by passing through a column filled with basic aluminum oxide to remove the inhibitor. Anhydrous *N,N*-dimethylformamide (DMF) were purchased from Acros Organics and stored over molecular sieves under an inert atmosphere. All other solvents and inorganic salts were purchased from Avantor Performance Materials Poland S.A.

p-(2-Hydroxyethoxy)benzaldehyde[1] and 6,6'-di-*O*-acryl-α,α-D-trehalose (**DET-1**)[2] were synthesized according to the published procedures.

Reactions were monitored by TLC on precoated plates of silica gel 60 F_{254} (Merck) and visualized by charring with 10% sulphuric acid in ethanol. Purification by flash column chromatography was performed on silica gel 60 (40-63 µm, Merck) using automated system (Isolera, Biotage). Optical rotation was measured using a polarimeter (P-2000, JASCO) equipped with a sodium lamp (λ = 589 nm) at 25 °C. Frieze-drying was carried out under 0.035 mbar at -50 °C (ALPHA 1-2 LD_{plus}, CHRIST). Mass spectra were recorded using Electrospray Ionisation Mass Spectrometry (QTRAP 4000, AB Sciex). NMR spectra were recorded in deutered solvents (Deutero GmbH) with internal standards using NMR spectrometer operating at 600 MHz (Varian).

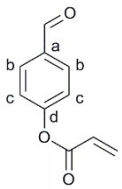
2. Synthesis and study on trehalose-based crosslinkers DAT-1 and DAT-2

2.1. Synthetic procedure for DAT-1



Scheme S1 Synthetic pathway of crosslinker **DAT-1**.

p-acryloyloxybenzaldehyde (1)

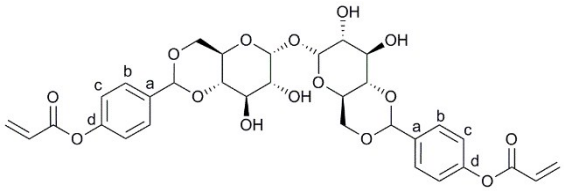


To a solution of *p*-hydroxybenzaldehyde (0.123 mol) and TEA (0.160 mol) in THF (200 mL) cooled on ice water bath, acryloyl chloride (0.160 mol) in THF (20 mL) was added dropwise. The mixture was allowed to warm up to room temperature and left overnight. Precipitated triethylamine hydrochloride was separated on a pad of Celite 501, and filtrate was concentrated under vacuum. The crude product

was purified through vacuum distillation in the presence of Giemsa's azur-eosin-methylene blue as a polymerization inhibitor to afford **1** as a dense, colorless liquid, that crystalized in refrigerator (17.52 g, 81%). **bp** 118 – 119 °C (2-3 mbar); **mp** 28 °C.

^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm]: 6.07 (dd, 1H, J = 10.5, 1.1 Hz, -CH=C**H**$_2$ *cis*); 6.34 (dd, 1H, J = 17.3, 10.5 Hz, -C**H**=CH$_2$); 6.65 (dd, 1H, J = 17.3, 1.1 Hz, -CH=C**H**$_2$ *trans*); 7.32-7.35 (m, 2H, H$_c$); 7.92-7.96 (m, 2H, H$_b$); 10.0 (s, 1H, -CHO); **^{13}C NMR** ($CDCl_3$, 150 MHz) δ [ppm]: 122.43 (C$_c$); 127.53 (–**C**H=CH$_2$); 131.31 (C$_b$); 133.60 (–CH=**C**H$_2$); 134.15 (C$_a$); 155.38 (C$_d$); 163.85 (–C(O)O–); 190.99 (–CHO). **LR ESI-MS:** m/z calcd for $C_{10}H_8O_3Na$ [M+Na]$^+$ 199.0, found 200.2

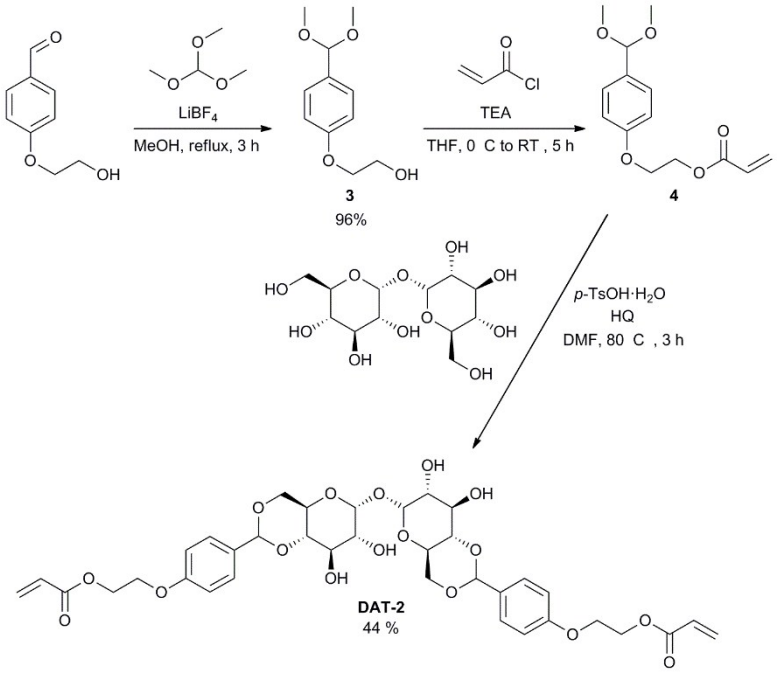
4,6:4',6'-di-*O*-(*p*-acryloyloxbenzylidene)-α,α'-D-trehalose (DAT-1)



To a suspension of trehalose (0.008 mol) in anhydrous DMF (15 mL) and benzene (30 mL), **1** (0.021 mol), hydroquinone (0.160 mmol) and A-15 (1.10 g) were added. The suspension was stirred for 48 h under reflux with azeotropic removal of water by using Dean-Stark trap. After cooling, A-15 was filtered off and benzene was evaporated under vacuum. Oily residue was poured into large excess of 5% aqueous $NaHCO_3$ and stirred for 1 h, then decanted and treated with second portion of 5% aqueous $NaHCO_3$. White precipitate were collected and dried under vacuum. The crude product was purified from unreacted *p*-acryloyloxybenzaldehyde through several dissolution in ethyl acetate and precipitation in hexane to afford **DAT-1** as a white solid after vacuum drying (2.14 g, 41%).

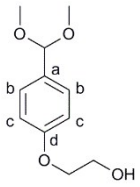
^{1}H NMR (DMSO-d$_6$, 600 MHz) δ [ppm]: 3.41 (~t, 2H, J = 9.5 Hz, H-4, H-4'); 3.44 (ddd, 2H, J = 9.6, 5.9, 3.8 Hz, H-2, H-2'); 3.67 (~t, 2H, J = 10.0 Hz, H-6a, H-6a'); 3.78 (~td, 2H, J = 9.3, 4.4 Hz, H-3, H-3'); 4.03-4.09 (m, 2H, H-5, H-5'); 4.11 (dd, 2H, J = 9.7, 4.9 Hz, H-6b, H-6b'); 4.95 (d, 2H, J = 3.8 Hz, H-1, H-1'); 5.25 (d, 2H, J = 4.9 Hz, C-3-OH, C-3'-OH'); 5.28 (d, 2H, J = 5.9 Hz, C-2-OH, C-2'-OH'); 5.61 (s, 2H, –OCHO–, –OC'H'O–); 6.66 (dd, 2H, J = 10.4, 1.3 Hz, –CH=C**H**$_2$, –C'H'=C'**H**'$_2$ *cis*); 6.41 (dd, 2H, J = 17.3, 10.4 Hz, –C**H**=CH$_2$, –C'**H'**=C'H'$_2$); 6.54 (dd, 2H, J = 17.3, 1.3 Hz, –CH=C**H**$_2$, –C'H'=C'**H'**$_2$ *trans*); 7.17-7.21 (m, 4H, H$_c$, H$_c$'); 7.50-7.54 (m, 4H, H$_b$, H$_b$'). **^{13}C NMR** (DMSO-d$_6$, 150 MHz) δ [ppm]: 62.51, 68.22, 69.40, 71.94, 81.43 (C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6, C-6'); 94.96 (C-1, C-1'); 100.21 (–OCHO–, –OC'H'O–); 121.36 (C$_c$, C$_c$'); 127.56 (–**C**H=CH$_2$, –**C'**H'=C'H'$_2$); 127.65 (C$_b$, C$_b$'); 133.67 (–CH=**C**H$_2$, –C'H'=**C'**H'$_2$); 135.59 (C$_a$, C$_a$'); 150.42 (C$_d$, C$_d$'); 164.08 (–C(O)O–, –C'(O)O–). **LR ESI-MS:** m/z calcd for $C_{32}H_{35}O_{15}$ [M+H]$^+$ 659.2, found 659.3 $[\alpha]_D^{25}$ +58 (DMSO , *c* 1.0)

2.2. Synthetic procedure for DAT-2[4]



Scheme S2 Synthetic pathway of crosslinker **DAT-2**.

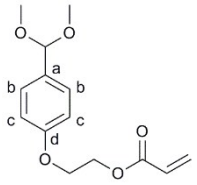
p-(2-hydroxyethoxy)benzaldehyde dimethyl acetal (3)



Compound **3** was synthetized based on the reported procedure.[3] To a solution of *p*-(2-hydroxyethoxy)benzaldehyde (0.078 mol) and lithium tetrafluoroborate (0.004 mol) in methanol (40 mL) trimethyl orthoformate (0.117 mol) were added, and the solution was stirred under reflux for 3h. After cooling, saturated aqueous $NaHCO_3$ (250 mL) were added, and the mixture were extracted twice with ethyl acetate (360 mL, 120 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (120 mL), dried over $MgSO_4$ and concentrated under vacuum to afford **3** as an orange syrup (15.92 g, 96%).

^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm]: 2.28 (t, 1H, J = 6.2 Hz, –OH); 3.31 (s, 6H, $–CH_3$); 3.93-3.97 (m, 2H, $–CH_2OH$); 4.06-4.10 (m, 2H, $–OCH_2–$); 5.35 (s, 1H, –OCHO–); 6.89-6.93 (m, 2H, H_c); 7.35-7.39 (m, 2H, H_b). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ [ppm]: 52.74 ($–CH_3$); 61.59 ($-CH_2OH$); 69.36 ($–OCH_2–$); 103.12 (–OCHO–); 114.33 (C_c); 128.14 (C_b); 131.04 (C_a); 158.86 (C_d). **LR ESI-MS:** m/z calcd for $C_{11}H_{16}O_4Na$ $[M+Na]^+$ 235.1, found 235.2

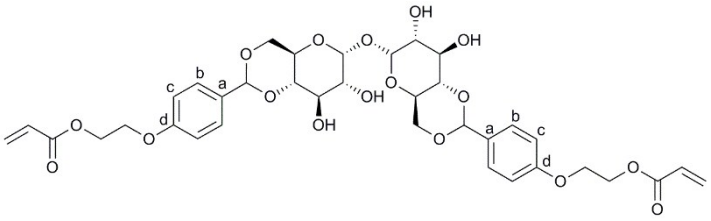
***p*-(2-acryloyloxyethoxy)benzaldehyde dimethyl acetal (4)**



To a solution of **3** (0.068 mol) and TEA (0.205 mol) in THF (300 mL) cooled on ice water bath, acryloyl chloride (0.102 mol) in THF (30 mL) was added dropwise. The mixture was allowed to warm up to room temperature and left overnight. Precipitated triethylamine hydrochloride was separated on a pad of Celite 501, and filtrate was concentrated under vacuum to afford **4** as a brown syrup (17.86 g) that was taken forward without further purification.

^{1}H NMR ($CDCl_3$, 600 MHz) δ [ppm]: 3.31 (s, 6H, $–CH_3$); 4.20-4.24 (m, 2H, –OCH2–); 4.50-4.53 (m, 2H, $–CH_2OC(O)–$); 5.35 (s, 1H, –OCHO–); 5.86 (dd, 1H, J = 10.5, 1.4 Hz, –CH=C**H**$_2$ *cis*); 6.16 (dd, 1H, J = 17.3, 10.5 Hz, –C**H**=CH_2); 6.44 (dd, 1H, J = 17.3, 1.4 Hz, -CH=C**H**$_2$ *trans*); 6.89-6.93 (m, 2H, H_c); 7.35-7.39 (m, 2H, H_b). **^{13}C NMR** ($CDCl_3$, 150 MHz) δ [ppm]: 52.72 ($–CH_3$); 63.03 ($–OCH_2–$); 66.07 ($–CH_2OC(O)–$); 103.07 (–OCHO–); 114.40 (C_c); 128.14 (C_b); 128.21 (–**C**H=CH_2); 131.10 (C_a); 131.45 (–CH=**C**H_2); 158.71 (C_d); 166.20 (–OC(O)–). **LR ESI-MS:** m/z calcd for $C_{14}H_{18}O_5Na$ $[M+Na]^+$ 289.1, found 289.2

4,6:4',6'-di-*O*-[*p*-(2-acryloyloxyethoxy)benzylidene]-α,α'-D-trehalose (DAT-2)



To a suspension of anhydrous trehalose (0.012 mol) in anhydrous DMF (45 mL), **4** (~0.030 mol), hydroquinone (0.240 mmol) and a catalytic amount of *p*-toluenesulfonic acid were added. The solution was stirred for 3 h at 80 °C and then reaction was quenched with triethylamine. The solvent was evaporated under reduced pressure and the residue was treated with ethyl ether (3 x 50 mL). The crude product was purified by silica gel flash chromatography ($CHCl_3$: MeOH : TEA 97 : 2 : 1) to afford **DAT-2** as a white solid (3.91 g, 44%).

^{1}H NMR (DMSO-d_6, 600 MHz) δ [ppm]: 3.36 (~t, 2H, J = 9.4 Hz, H-4, H-4'); 3.42 (ddd, 2H, J = 9.6, 6.0, 3.8 Hz, H-2, H-2'); 3.63 (~t, 2H, J = 9.9 Hz, H-6a, H-6'a); 3.76 (~td, 2H, J = 9.3, 5.1 Hz, H-3, H-3'); 4.00-4.06 (m, 2H, H-5, H-5'); 4.07 (dd, 2H, J = 9.7, 4.9 Hz, H-6b, H-6'b); 4.22-4.25 (m, 4H, $–OCH_2–$, $–OC'H'_2–$); 4.42-4.45 (m, 4H, $–CH_2OC(O)–$, $-C'H'_2OC'(O)–$); 4.93 (d, 2H, J = 3.8 Hz, H-1, H-1'); 5.19 (d, 2H, J = 5.1 Hz, C-3-OH, C-3'-OH'); 5.25 (d, 2H, J = 6.0 Hz, C-2-OH, C-2'-OH'); 5.51 (s, 2H, –OCHO–, –OCH'O–); 5.96 (dd, 2H, J = 10.3, 1.5 Hz, –CH=C**H**$_2$, $–C'H'=C'$**H'**$_2$ *cis*); 6.22 (dd, 2H, J = 17.3, 10.3 Hz, –C**H**=CH_2, $–C'$**H'**$=C'H'_2$); 6.35 (dd, 2H, J = 17.3, 1.5 Hz, –CH=C**H'**$_2$, $–C'H'=C'$**H'**$_2$ *trans*); 6.92-6.96 (m, 4H, H_c, H_c'); 7.35-7.39 (m, 4H, H_b, H_b'). **^{13}C NMR** (DMSO-d_6, 150 MHz) δ [ppm]: 62.75 ($–OCH_2–$, $–OC'H'_2–$); 65.72 (–**C**$H_2OC(O)–$, -**C'**$H'_2OC'(O)–$); 62.56, 68.19, 69.42, 72.01, 81.39 (C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6, C-6'); 94.94 (C-1, C-1'); 100.68 (–OCHO–,–OC'H'O–); 113.92 (C_c, C_c'); 127.73 (C_b, C_b'); 128.05 (–**C**H=CH_2, –**C'**$H'=C'H'_2$); 130.60 (C_a, C_a'); 131.96 (–CH=**C**H_2, $–C'H'=$**C'**H'_2); 158.39 (C_d, C_d'); 165.43 (–C(O)O–, –C'(O)O–). **LR ESI-MS:** m/z calcd for $C_{36}H_{43}O_{17}$ $[M+H]^+$ 747.2, found 747.6. $[\alpha]_D^{25}$ +51 ($CHCl_3$, *c* 1.0)

2.3. ^{1}H NMR study on acidic hydrolysis of DAT-1 and DAT-2

The susceptibility of **DAT-1** and **DAT-2** for acidic hydrolysis was assessed at 25 °C using ^{1}H NMR spectroscopy. The crosslinker (0.028 mmol) was dissolved in 480 µL of DMSO-d_6, followed by the addition of 90 µL of D_2O. To start hydrolysis, 30 µL of 200 mM TFA solution in D_2O was added. Hydrolysis was monitored by recording the spectra at predetermined time intervals.

Degree of hydrolysis (DH%) was calculated based on the integrations of signals corresponding to anomeric protons of diacetal (DAT), monoacetal (MAT) and trehalose (TRE), according to the equation:

$$DH\% = 100 - \frac{2 \cdot n_D}{2 \cdot n_D + n_M + (2 \cdot n_T + n_{M'})}$$

n_M – integration of signal from one anomeric proton of monoacetal

$2 \cdot n_D$ – integration of signals from two anomeric protons of diacetal

$(2 \cdot n_T + n_{M'})$ – integration of signals from two anomeric protons of trehalose and one anomeric proton of monoacetal

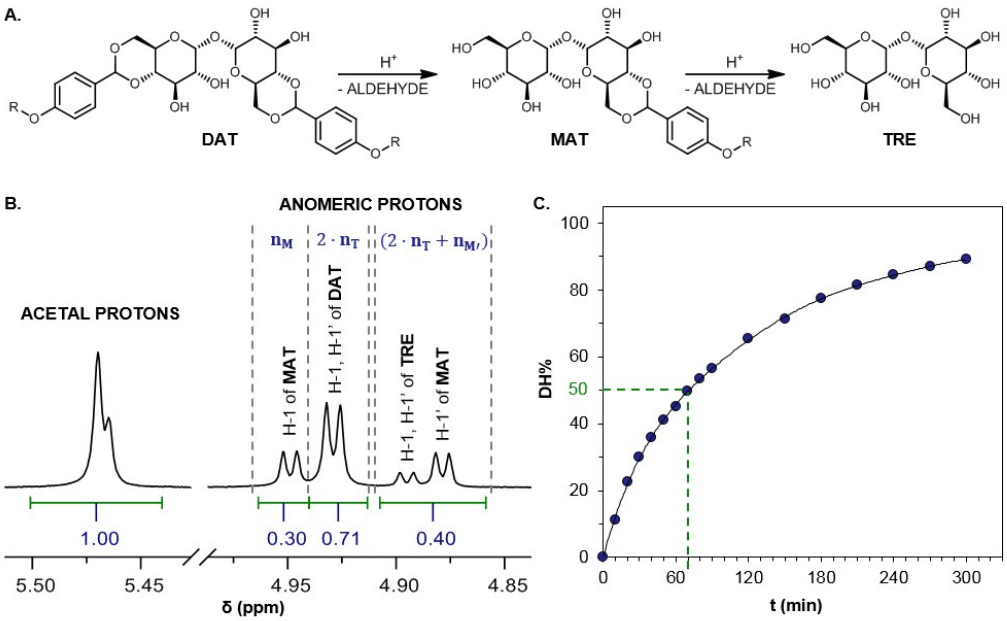


Fig. S1 A. Two step acidic hydrolysis of DAT; **B.** Selected sections of ^{1}H NMR spectrum of **DAT-2** after 70 min. of acidic hydrolysis representing signals of acetal (5.40–5.50 ppm) and anomeric protons (4.85–5.00 ppm) with depiction of anomeric signals used for calculation of degree of hydrolysis; **C.** Degree of hydrolysis over time for **DAT-2**.

3. Synthesis and study on hydrogels

3.1. Synthetic procedure for hydrogels[4]

Hydrogels were synthesized by redox-initiated free-radical polymerization at 25 °C in silicone tubes with an internal diameter of 8 mm. The detailed feed compositions and sample codes are given in **Table S1**. In all entries a constant crosslinker to monomer ratio of 0.1 mmol/g was used. Briefly, acrylamide-type monomer: HEAM, AM or DMAM (0.105 g) was dissolved in 940 µL of DI water,

followed by the addition of 460 µL of DMF containing trehalose-based crosslinker: **DAT-1**, **DAT-2** or **DET-1** (0.011 mmol). Then, 40 µL of TMEDA aqueous solution (345 mM, 0.0138 mmol) were added and the whole was argon flushed for 10 min. The polymerization was initiated by adding 40 µL of APS aqueous solution (230 mM, 0.0092 mmol). After 24h hydrogels were cut into 7 equal discs, the two external were discarded, and the others were transferred into DI water for 4 days, with DI water replacement every 12 h. After purification, hydrogels were air dried for one week at room temperature (Yields: 64–82%). Dried discs were stored in a vacuum desiccator for further use.

Table S1 Monomer feed compositions and hydrogel yields

Entry	Sample code	Acrylamide-type monomer [mg] ([mmol])	Trehalose-based crosslinker [mg] ([mmol])	Crosslinker to monomer ratio [mmol/g]	Yield [%]
1	**DAT-1-HEAM**	105 (0.912)	7.5 (0.011)	0.1	74
2	**DAT-2-HEAM**	105 (0.912)	8.5 (0.011)	0.1	68
3	**DAT-2-AM**	105 (1.477)	8.5 (0.011)	0.1	81
4	**DAT-2-DMAM**	105 (1.059)	8.5 (0.011)	0.1	82
5	**DET-1-HEAM**	105 (0.912)	5.1 (0.011)	0.1	64

3.2. Hydrogels characterization

3.1.1. Determination of trehalose content in hydrogels

To determine trehalose content in hydrogels, it was released into solution through acidic (hydrogels crosslinked by **DAT-1** or **DAT-2**) or alkaline (hydrogels crosslinked by **DET-1**) hydrolysis by immersing dry hydrogel disc in appropriate volume of 0.1 M HCl or NaOH solution, respectively to get final concentration of 1 mg/mL. After one night of magnetic stirring at 25 °C, 800 µL of degradation solution was withdrawn, neutralized with 200 µL of 0.4 M NaOH or HCl solution and subjected to enzymatic determination of trehalose.

Control solutions containing a fixed concentration of trehalose in 0.1 M HCl or NaOH, were treated the same way to confirm that it is stable under degradation conditions.

Trehalose was determined enzymatically using Trehalose Assay Kit (Megazyme International Ireland) in microplate assay procedure, based on standard curve. The absorbance was recorded using microplate absorbance reader (Sunrise, TECAN). Each determination was made in triplicate.

According to procedure, trehalose is hydrolyzed to D-glucose by trehalase, then released D-glucose is phosphorylated by hexokinase and adenosine-5'-triphosphate (ATP) to glucose-6-phosphate, which is oxidized by nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH). The amount of NADPH formed in this reaction is measured by the increase in absorbance at 340 nm.

3.1.2. Degradation study

Degradation study was performed at 37 °C in buffered saline solution with constant magnetic stirring at 200 rpm. To maintain desired pH, citrate (pH 3.0), phosphate (pH 5.0, 7.4 or 8.0) or glycine (pH 9.0) buffer was applied. Dry hydrogel disc (12.0 ± 1mg) was immersed in 5 mL of saline solution, and at

predetermined time intervals 240 µL of degradation solution was withdrawn and replaced with fresh buffer. Samples from acidic hydrolysis were subjected directly to enzymatic determination of trehalose. Samples from alkaline hydrolysis were acidify with 24 µL of 1 M HCl solution, incubated for 30 min. at 60 °C, neutralized with 24 µL of 1 M NaOH solution and then subjected to enzymatic determination of trehalose.

The dissolution time (t_{diss}) was estimated as time necessary to the degradation of the hydrogel into clear solution.

^{1}H NMR spectra of degradation products were recorded for frieze-dried degradation solutions dissolved in D_2O.

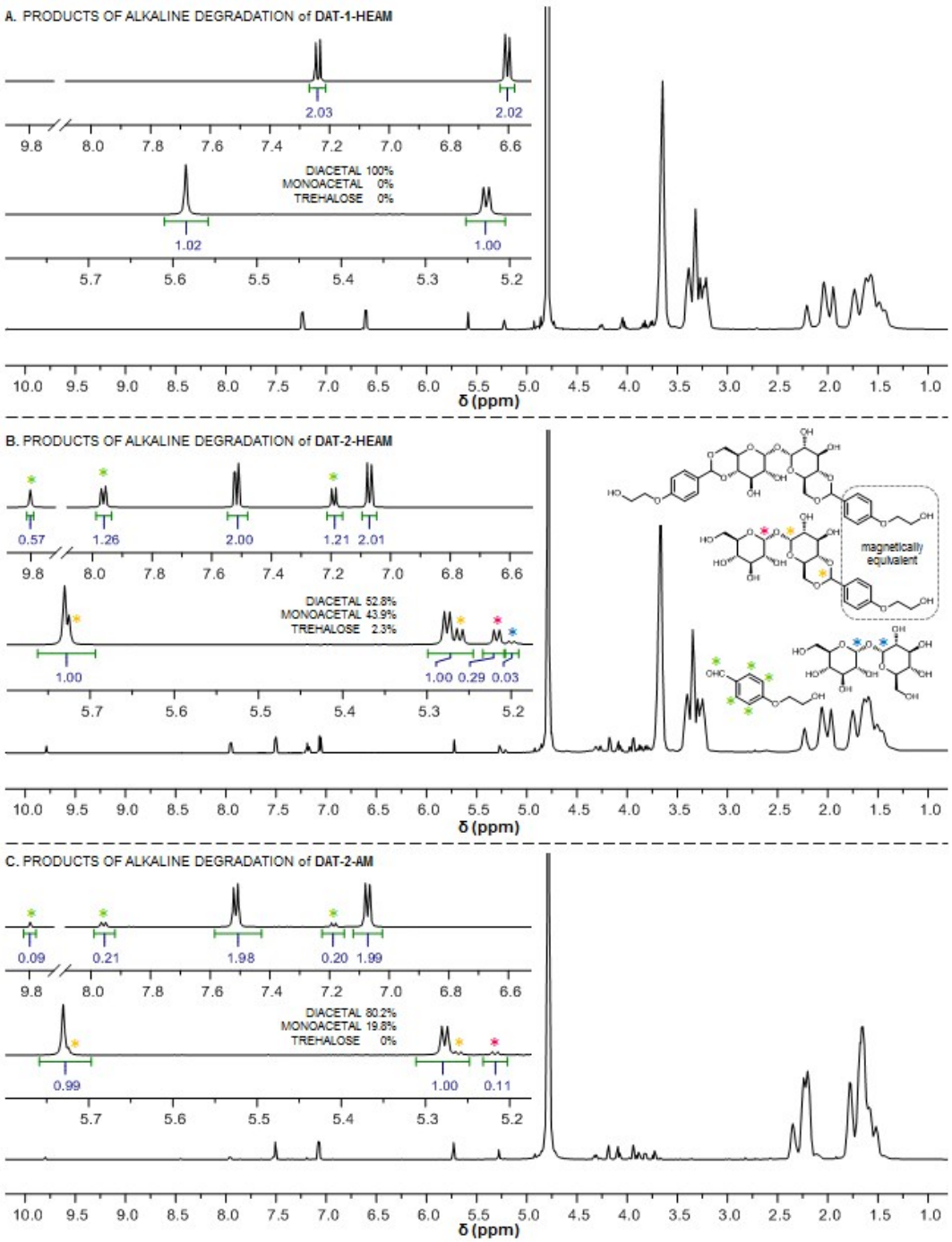


Fig. S2 ^{1}H NMR spectra of DAT-1-HEAM (A), DAT-2-HEAM (B) and DAT-2-AM (C) and after alkaline degradation.

Otherwise to ^{1}H NMR spectrum of the degradation products of hydrogel **DAT-1-HEAM**, in the spectra of the degradation products of **DAT-2-HEAM** and **DAT-2-AM**, there are present additional signals at the region of anomeric, acetal, aromatic and aldehyde shifts (indicated by asterisks), which are attributed to the products of acetal cleavage. Since acetal moiety remains intact in alkaline conditions, it indicates to partial hydrolysis of **DAT-2** crosslinks during hydrogel purification after synthesis, as the washings were carried out in deionized water, which may have pH down to 5.5. Based on the integration of anomeric signals, it was calculated that in **DAT-2-AM** about 20% of **DAT-2** crosslinks were hydrolyzed to monoacetal. The integration of its anomeric signals fits the integration of aromatic signals of corresponding free aldehyde. In **DAT-2-HEAM**, monoacetal content reaches almost 45% as well as just over 2% of free trehalose was determined. However, the integration of aromatic signals of aldehyde is significantly higher than that, which should correspond to the integration of anomeric signals of monoacetal and trehalose. The excess of aldehyde leads to the conclusion that part of trehalose from **DAT-2** hydrolysis was removed with washings. It explains significantly lower content of trehalose determined in hydrogel **DAT-2-HEAM**.

3.1.3. Equilibrium Swelling Ratio (ESR)

To determine ESR, dry hydrogel disc (10 – 12 mg) was immersed in 10 mL of PBS pH 7.0 at 25 °C and weighted after removing surface water every 12 hours until reaching equilibrium swelling. ESR was calculated from the following equation:

$$ESR = (W_E - W_D)/W_D$$

where: W_E is the weight of the equilibrium swollen hydrogel and W_D is the weight of dry hydrogel. ESR was taken as an average value of three independent measurements.

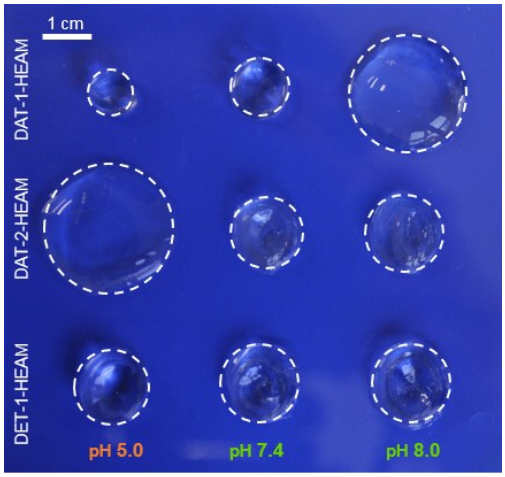


Fig. S3 Photographs of HEAM-based hydrogels crosslinked by **DAT-1**, **DAT-2** or **DET-1** after 30 h at pH 5.0. 7.4 or 8.0.

4. *In vitro* cytotoxicity study of post-acidic and post-alkaline degradation products

Selected hydrogels at concentration of 2.5 mg/mL were degraded in PBS solution buffered to pH 5.0 or 8.0. Preliminary cytotoxicity study of post-acidic and post-alkaline degradation products of hydrogels was performed with Normal Human Dermal Fibroblasts cell line (NHDF) (LONZA) using Cell Counting Kit-8 (CCK-8, DOJINDO). NHDF cells were grown in DMEM medium supplemented with 10% FBS in a humidified atmosphere containing 5% CO_2 at 37 °C. The cells were seeded overnight in 96-well plate (5000 cells per well) and then treated with appropriate volume of hydrogel post-degradation solution to get final concentration of degradation products of 0.25 or 0.50 mg/mL. After 24 and 48 h, CCK-8 reagent was added, and after 2 h of incubation cell viability was evaluated by measuring the absorbance at 450 nm. All experiments were performed in triplicate. Statistical analyses were performed using Student's *t*-test.

5. References

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[2] H. Taguchi, H. Sunayama, E. Takano, Y. Kitayama, T. Takeuchi, *Analyst*, 2015, **140**, 1448.

[3] N. Hamada, K. Kazahaya, H. Shimizu, T. Sato, *Synlett,* 2004, **6**, 1074.

[4] Synthetic procedures of DAT-2 crosslinker and hydrogels crosslinked by DAT-2 are a subject of polish patent application P.423711, December 4, 2017 (M. Burek, K. Kubic, I. Nabiałczyk, S. Waśkiewicz, I. Wandzik).

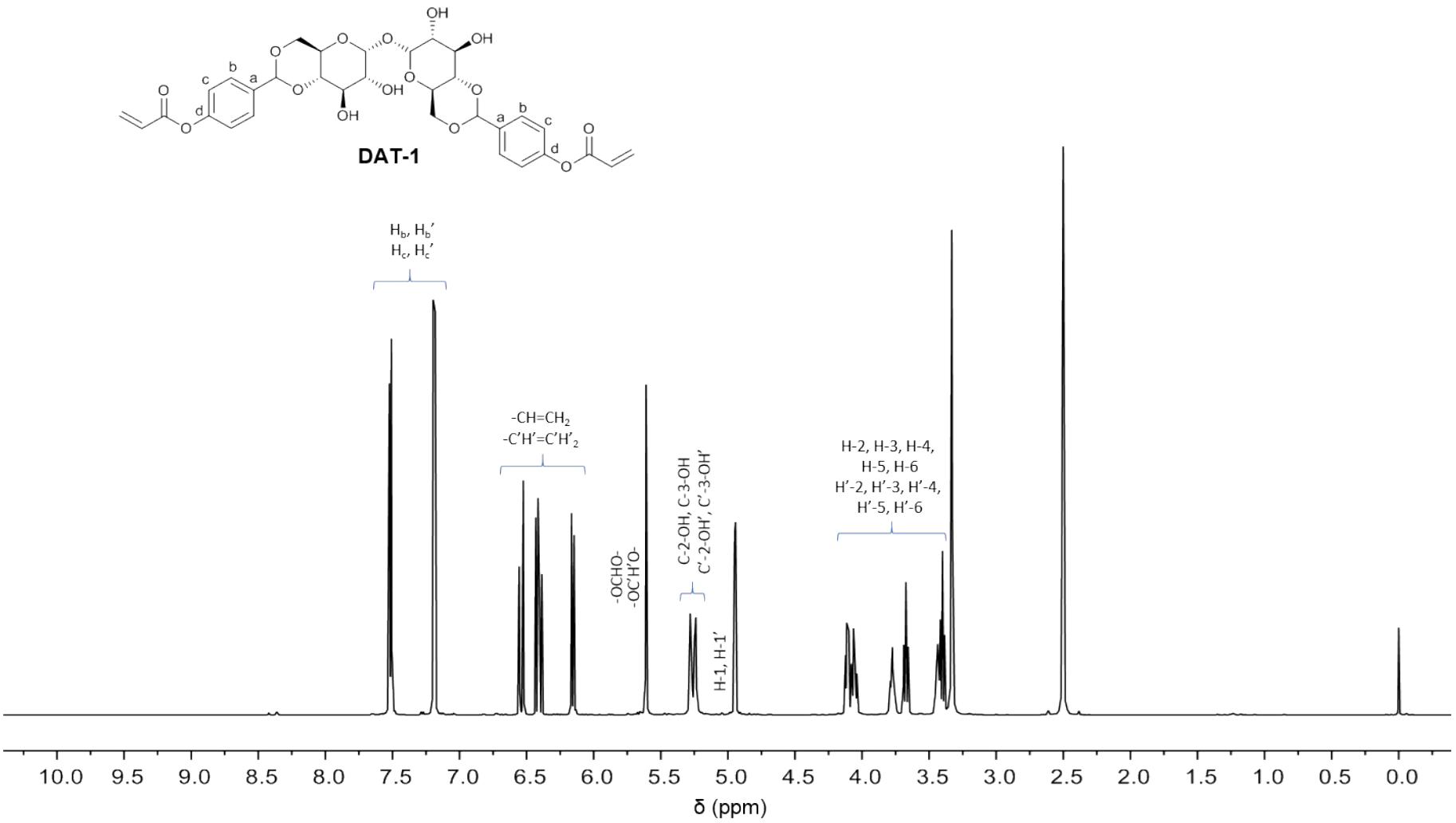

Fig. S4 ^{1}H NMR spectrum of **DAT-1** (DMSO-d_6, 600 MHz).

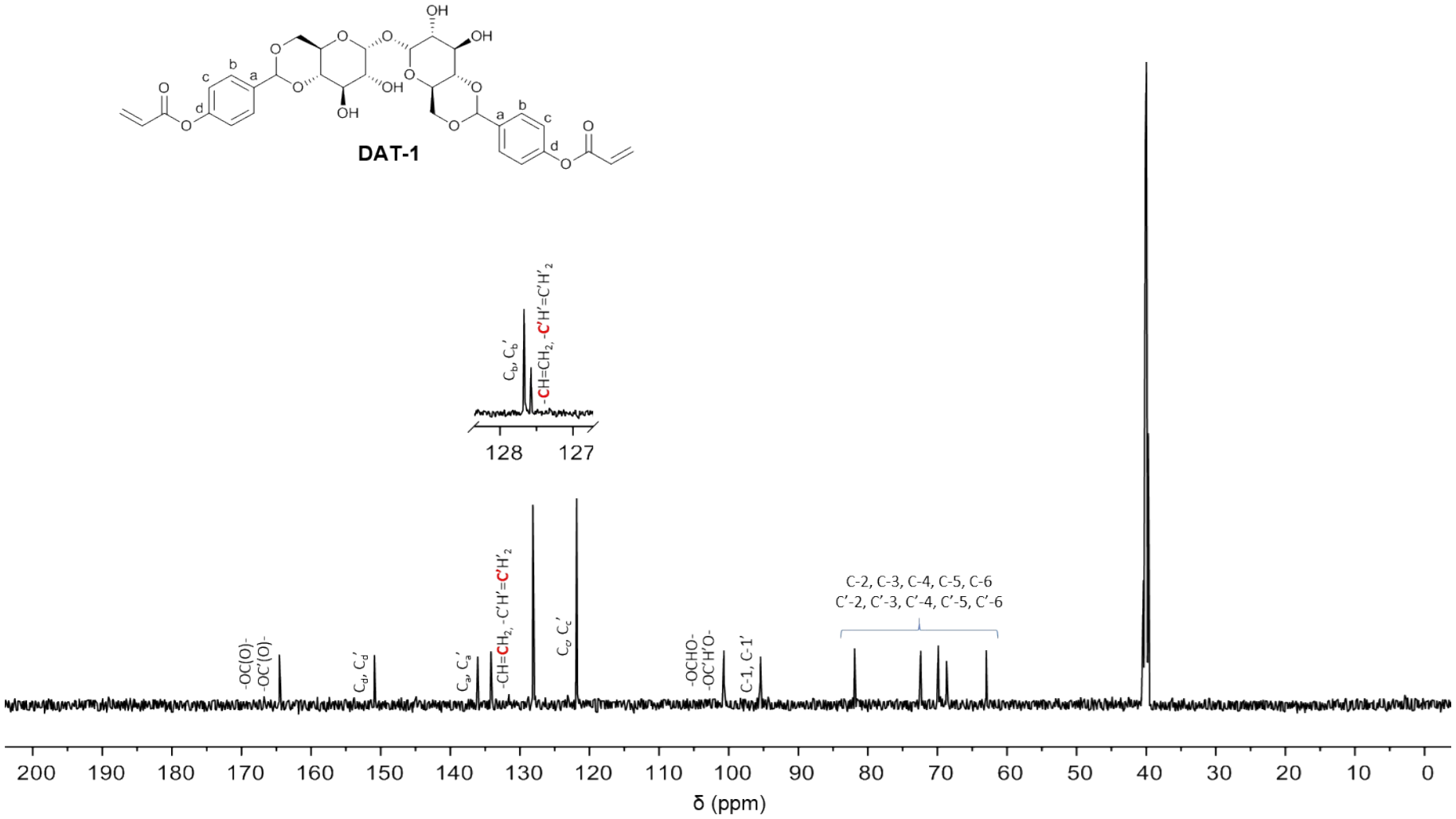

Fig. S5 ^{13}C NMR spectrum of **DAT-1** (DMSO-d_6, 150 MHz).

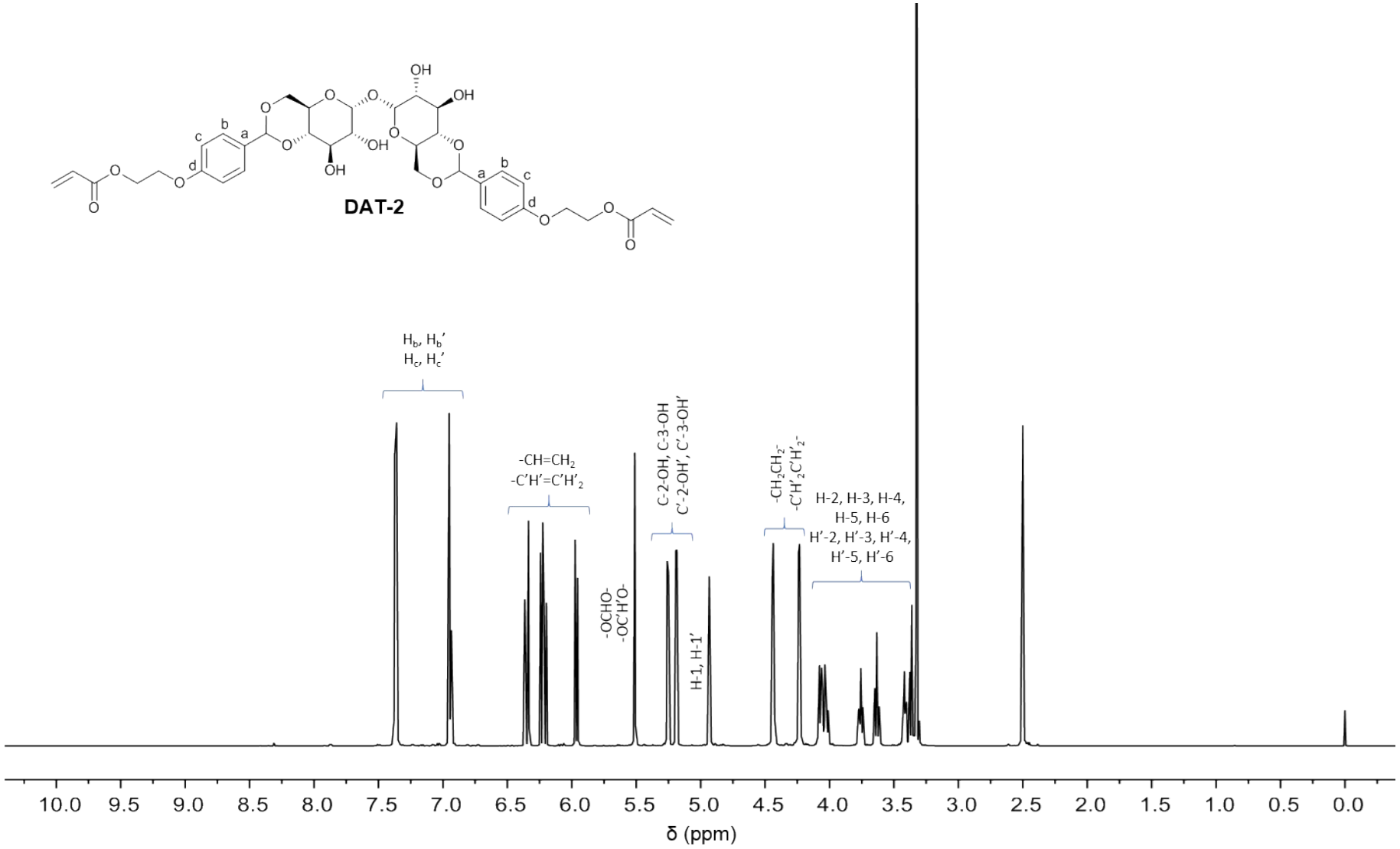

Fig. S6 ^{1}H NMR spectrum of **DAT-2** (DMSO-d_6, 600 MHz).

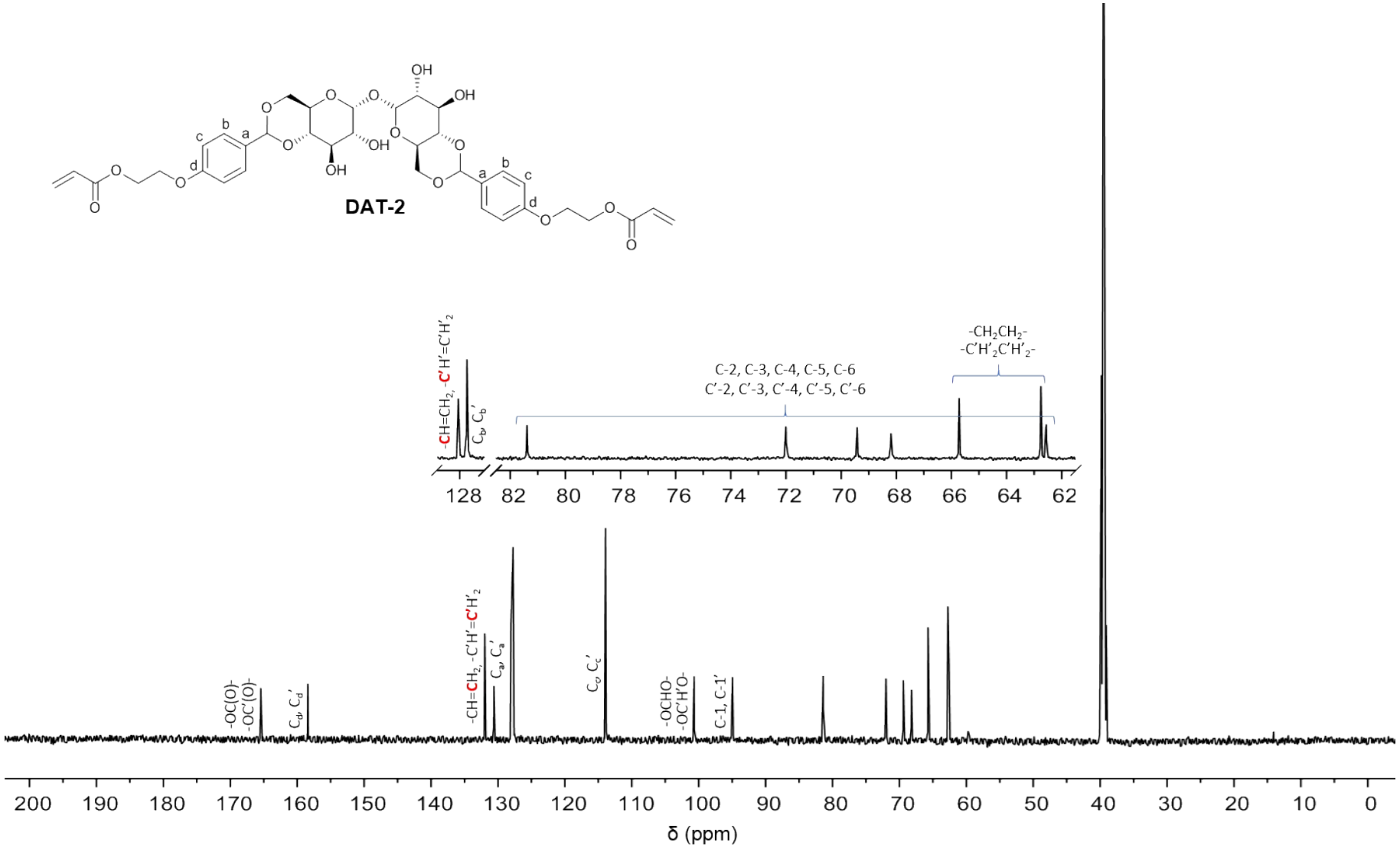

Fig. S7 ^{13}C NMR spectrum of **DAT-1** (DMSO-d_6, 150 MHz).