Supporting information

Synthesis of a mixture of sodium 3-hydroxypropyl-1-sulfonate (1) and unknown (2)

A mixture of sodium 3-hydroxypropyl-1-sulfonate **(1)** and unknown **(2)** was obtained after the controlled hydrolysis of PrS. To this extent, PrS was dissolved in 1M HCl_{aq} and stirred overnight at room temperature. Afterward, the solution was neutralized with $NaHCO_3$ and the solvent was evaporated. To remove all salts, the obtained white powder was thoroughly washed with MeOH. We suspect unknown compound **2** to be the already previously described[1] dimer disodium (3,3'-oxybis(propane-1-sulfonate)), however, further analysis (LC-MS and direct inlet MS) did not give conclusive results.

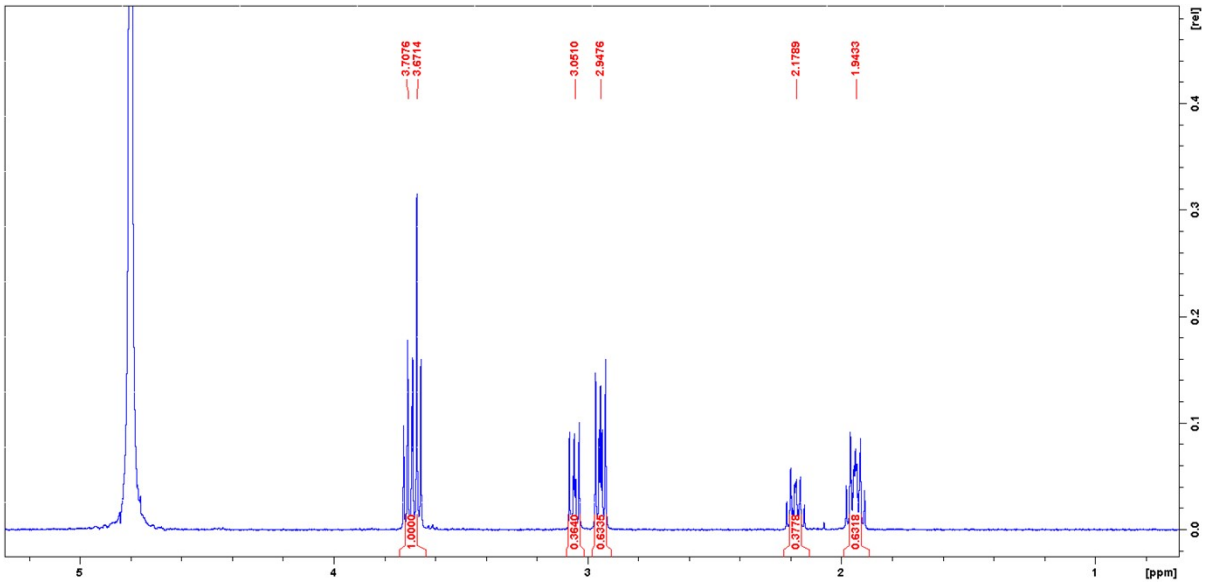


*Figure S1. ^1H NMR (400 MHz) of a mixture of compounds **(1)** and **(2)**.*

(1) sodium 3-hydroxypropane-1-sulfonate: ^1H NMR (1% d-TFA in D_2O, 400 MHz): δ 3.67 (t, 2H), 2.95(m, 2H), δ 1.94 (m, 2H). $^{13}C\{^1H\}$ NMR, (1% d-TFA in D_2O, 100.6 MHz): δ 60.7, 48.3, 27.4.

(2) Unknown ^1H NMR (1% d-TFA in D_2O, 400 MHz): δ 3.71 (t, 2H), 3.05 (m, 2H), 2.18 (m, 2H) $^{13}C\{^1H\}$ NMR, (1% d-TFA in D_2O, 100.6 MHz): δ 48.8, 44.3, 27.8

Control experiment to identify side product formation during mechanochemical synthesis

Figure S2a indicates that under the chosen conditions, no NH_2-substitution occurred (as indicated by the absence of the H_2-NHR signal indicated in green). However, signals which are identical to the signals present in the sample spiked with a mixture of **(1)** and **(2)** (Figure S2b) did occur around 3.6, 2.9 and 2 ppm. Hence, these signals can be ascribed to **(1)** and **(2)**. Subsequently, we can conclude that both side products are formed to a certain extent during the mechanochemical synthesis of *N*-sulfopropyl chitosan.

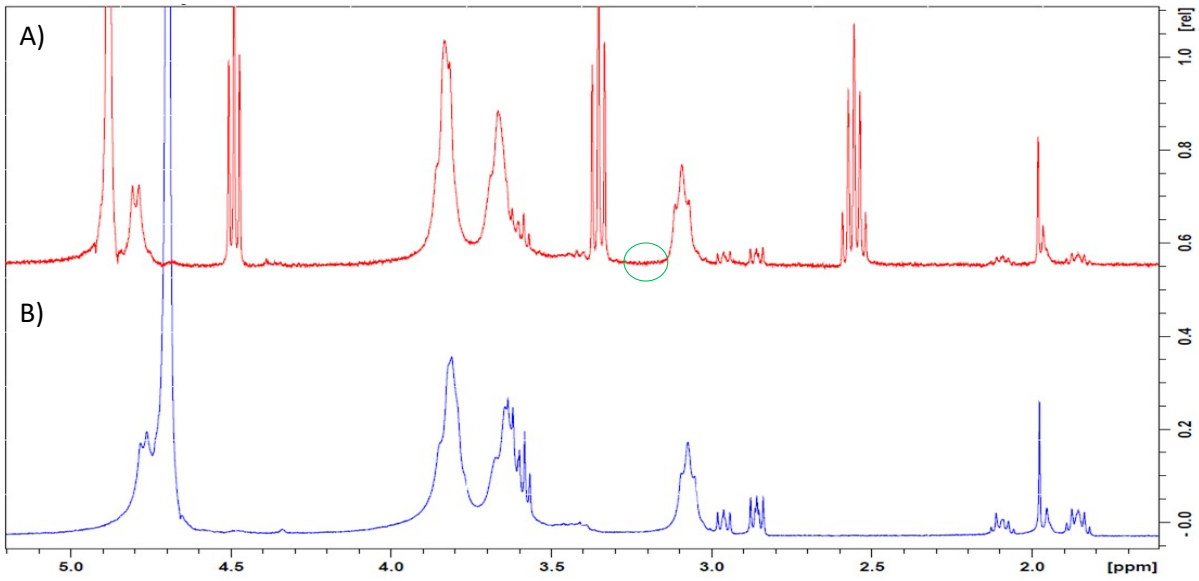


Figure S2. ***(A)*** *^{1}H NMR (400 MHz, 1% d-TFA in D_2O) of the mechanochemical reaction between PrS and chitosan after 5 minutes (25 mL SS jar, 1 12 mm SS ball, 30 Hz, 500 mg chitosan, 0.5 eq. PrS).* ***(B)*** *^{1}H NMR (400 MHz, 1% d-TFA in D_2O) of a chitosan sample spiked with a mixture of* ***(1)*** *and* ***(2).*** *Note: To aid visual clarity, the spectra were aligned according to the signals of* ***(1)*** *and* ***(2)****. The absence of the H_2-NHR signal is indicated in green.*

The chitosan, DS10, DS20 and DS40 samples after two days in basic aqueous (pH = 12) solution

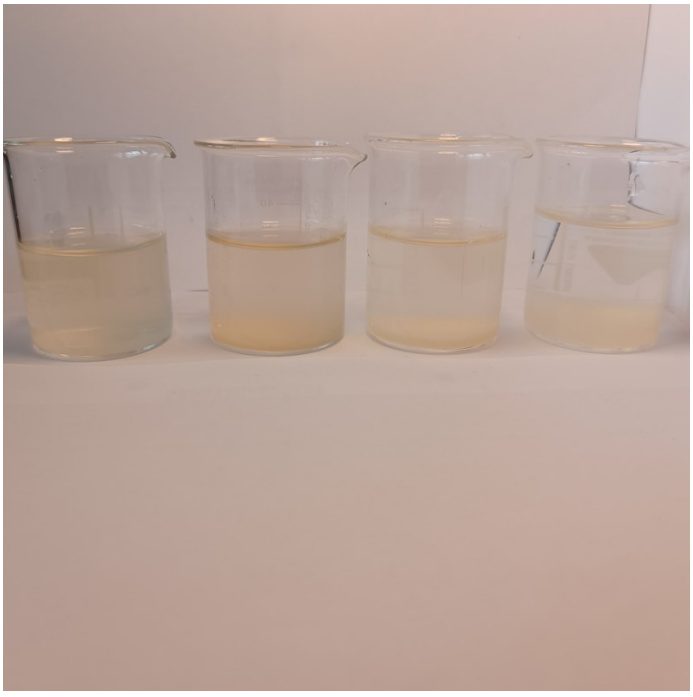


Figure S3. From left to right: the DS40, DS20, DS10 and chitosan samples after two days in a basic aqueous solution (pH = 12).

Control experiments regarding the mechanochemical reaction

To verify whether the reaction between chitosan and PrS is truly mechanochemical, several control experiments were performed. Firstly, in most cases, a certain induction period was observed when monitoring the reaction. This induction period, which decreased when the energy supplied within the system increased, is a widely observed and accepted phenomenon regarding mechanochemical transformations. This phenomenon might be explained by the storage of internal energy within a certain cohesive state, which in its turn exponentially increases the reaction rate once formed. During sampling, this change in rheology was observed.[2, 3] Next, the temperature directly after extended milling was measured utilizing an EBRO TFI260 infrared thermometer as heat is often a point of controversy within the field of mechanochemistry. To this extent, 500 mg of chitosan without PrS was continuously milled for 1 hour under certain conditions selected from Table 3, specified in Table S1, after which the jar was opened and the temperature inside the jar ($T_{internal}$) was directly measured. This was done without PrS addition in order to avoid any possible exothermic influences of the PrS reacting. When comparing the internal temperatures with the rates obtained in Figure 4, several conclusions can be made. Firstly, temperature effects might be accelerating reactivity, as the conditions in Figure 4 which gave the highest reaction rates also generate the most heat as is reflected by the temperatures ranging from 44-65 °C for runs 1, 10 and 11. However, temperature might not be the only factor that is driving this reaction, as for runs 7 and 8 a similar temperature of about 30 °C was reached, despite these runs 7 and 8 showing the crucial difference between having any form of reactivity or not as can be seen in Figure 4.

Table S1. The internal temperature ($T_{internal}$) the system reached under the selected conditions of Table 3.

Run	Jar (25 mL)	Milling balls	Frequency (Hz)	$T_{internal}$ (°C)
1	TC	1 x 12 mm TC	30	44
6	SS	2 x 12 mm SS	10	24
7	SS	14 x 5 mm SS	30	30
8	SS	5 x 7 mm SS	30	30
10	SS	2 x 15 mm SS	30	60
11	SS	1 x 20 mm SS	30	65

Additionally, the reaction was reevaluated under our previously selected conditions, now trying to avoid any form of temperature effects. Therefore, we performed the reaction stepwise in intervals of five minutes with 10 minutes of rest in between, to let the sample cool down to room temperature. The results are presented in Figure S4 and clearly indicate that even under these conditions reaction occurs.

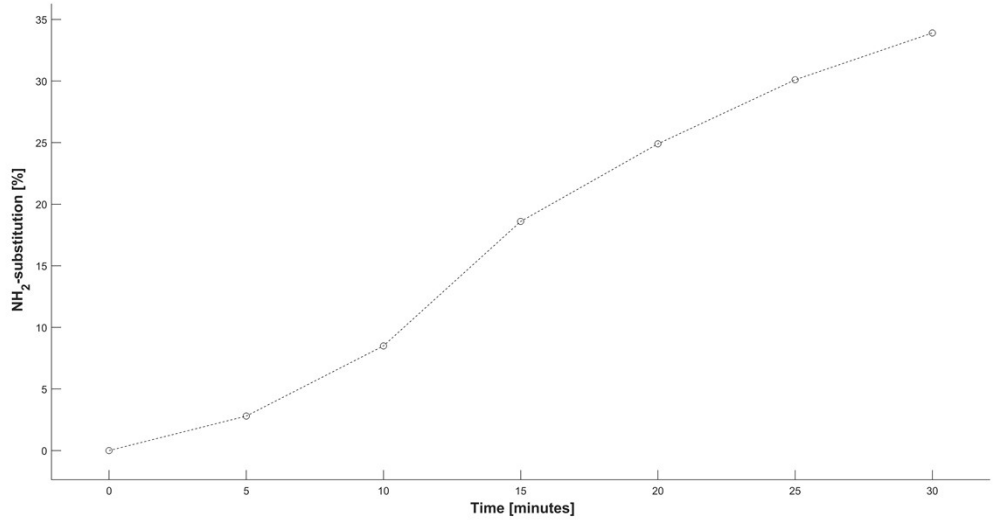


Figure S4. Percentage NH_2-substitution in function of the milling time utilizing cycles of five minutes milling times followed by 10 minutes of rest. (25 mL SS jar, 2 15 mm SS balls, 30 Hz, 500 mg chitosan, 0.5 eq. PrS)

Additionally, as the melting point of PrS is only 31 °C we tried a solventless reaction at 60 °C with both a premixed sample and crude sample of PrS and chitosan under argon. This premixed sample was milled for 1 min to thoroughly mix both reagents. However, only a trace amount of sulfopropylation could be observed in both cases after 48h (Figure S5). From the above, there are indications that temperature is not the only factor influencing the observed reaction rates and that there is an additional different form of activation at play, which is most likely mechanochemical in nature.

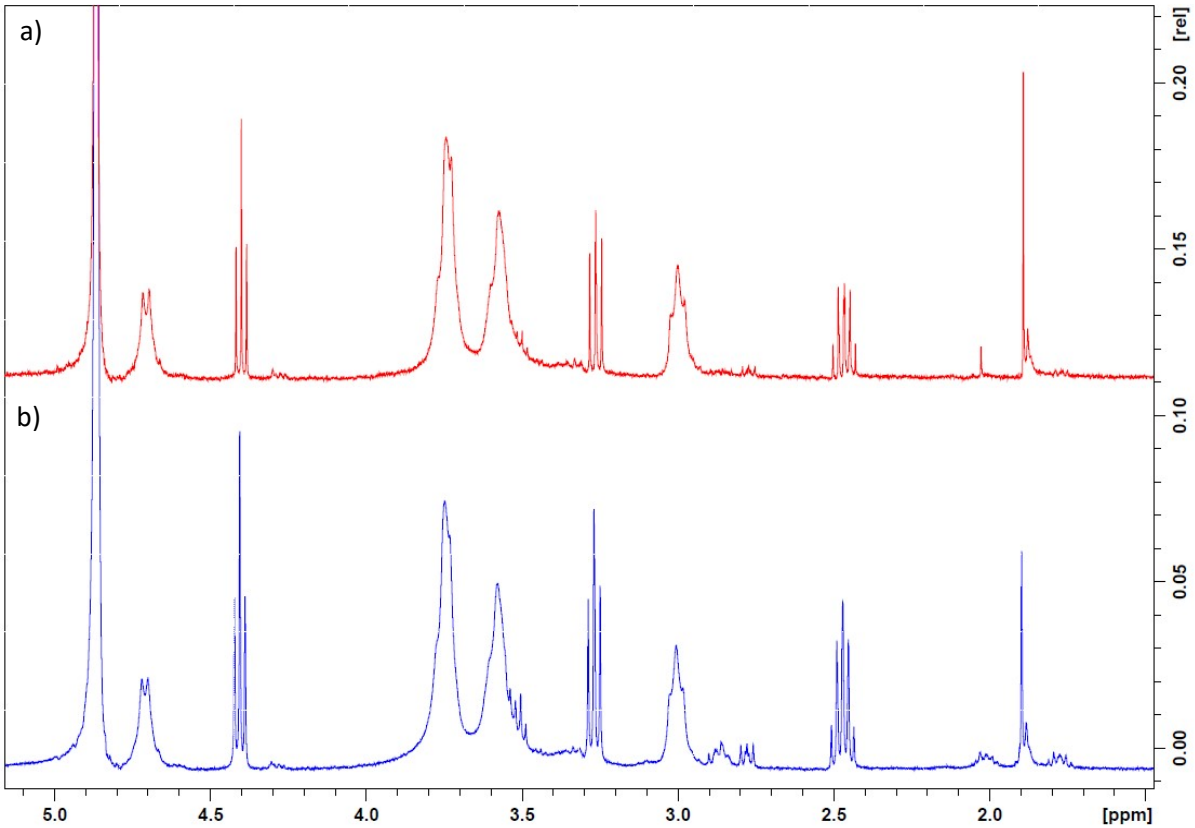


*Figure S5. ^1H NMR (400 MHz, 1% d-TFA in D_2O) of the premixed **(a)** and crude **(b)** solventless reaction between PrS and chitosan at 60 °C under argon*

Impact of the milling system on the chitosan's molecular weight

To check the impact of our mixer milling system on the molecular weight of the utilized chitosan, SEC/LC ELSD analyses were conducted. Blank chitosan samples were milled (25 mL SS jar, 2 15 mm SS balls, 30 Hz, 500 mg chitosan) for 40 and 80 min respectively without PrS to get an idea of the "worst case" degradation as now all the supplied energy will be directly transferred to the chitosan chains. To our knowledge, this was the only way we could directly compare the obtained relative molecular weights at different milling times as derivatized chitosan will have different behavior in solution compared to native chitosan. This results in different hydrodynamic volumes despite having similar molecular weights. This makes it very hard to decouple the effect of the milling by itself on the molecular weight reduction from the change in molecular weight due to the applied *N*-sulfopropylation. The relative molecular weights of these samples to pullulan standards are depicted in Table S1. Several conclusions can be drawn from these results. Firstly, from the first three results, it appears that extended milling does indeed lower chitosan's molecular size while creating less disperse samples, as already previously observed.[4, 5] Additionally, lower molecular weights, which are more disperse, are observed for the *N*-sulfopropylated samples compared to native chitosan samples that underwent the same milling time. This behavior might be explained by the observed internal salt formation, which significantly reduces the measured relative hydrodynamic volumes.

Table S2. Measured relative molecular weight distributions.

Sample	Mn (Da)	Mw (Da)	Đ	T_m (min)
Chitosan	108279,5	167235,3	1,544478	0
Chitosan	86399,29	120339,2	1,392826	40
Chitosan	62411,72	82221,07	1,317398	80
DS10	74999,96	104896,1	1,398616	10
DS20	73093,71	81422,73	1,11395	20
DS40	48355,71	86149,46	1,781578	40
DS60	37901,28	58062,1	1,53193	60
DS80	ND**	ND**	ND**	80

*Tm = Milling time

**ND = Non-determined as the observed signal was not strong enough because only very limited amounts of sample dissolved in the applied solvent system (0.1 % TFA).

Calculation of the degree of substitution of the DS20 sample based on ^{1}H NMR analysis

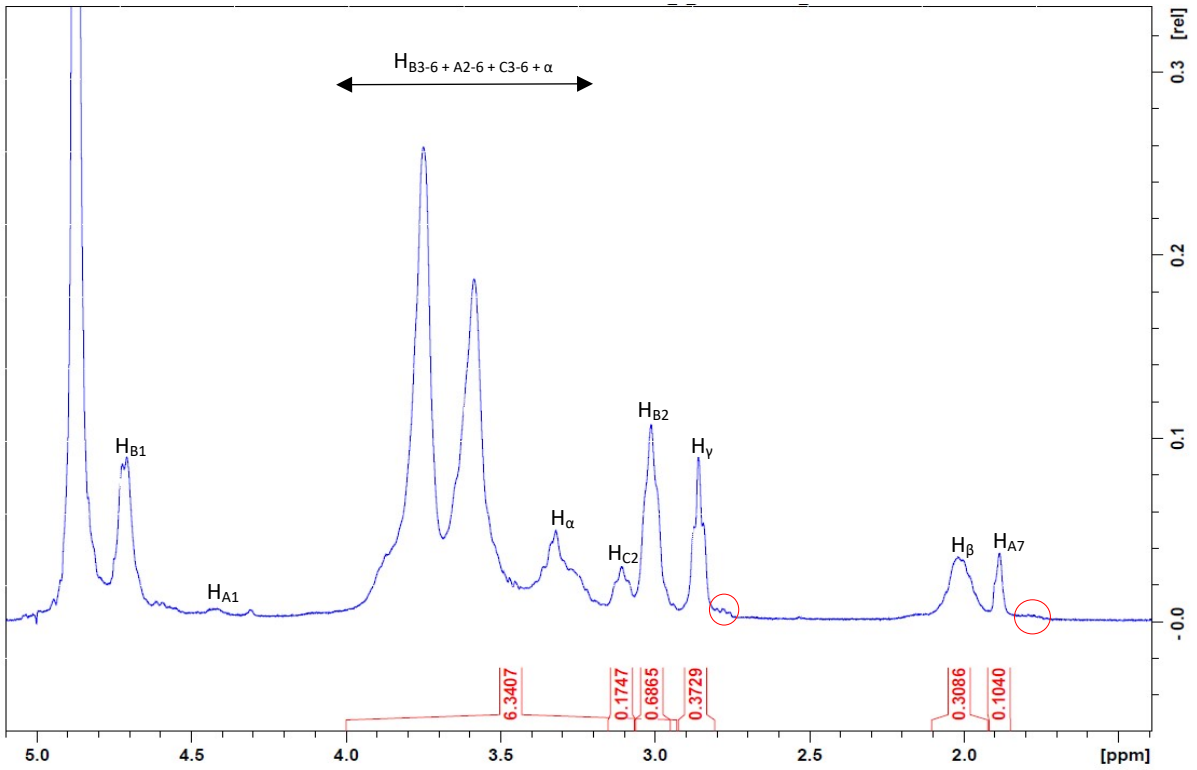


Figure S6. ^{1}H NMR (400 MHz, 1% d-TFA in D_2O, 50 mg/mL) of the DS20 sample. Minor 3-HPS impurities are indicated in red.

The following iterative procedure was applied to approximate the total amount of hydrogens present in the $H_{B3\text{-}6 + A2\text{-}6 + C3\text{-}6 + \alpha}$ region. The integral of the $H_{B3\text{-}6 + A2\text{-}6 + C3\text{-}6 + \alpha}$ region was first calibrated at 6 protons and subsequently, the H_β and H_γ regions were integrated. Afterward, the total amount of protons present in the $H_{B3\text{-}6 + A2\text{-}6 + C3\text{-}6 + \alpha}$ region were corrected and set to $6 + \frac{H_\beta + H_\gamma}{4}$. This process was repeated until there was no significant change for the corrected integral of the $H_{B3\text{-}6 + A2\text{-}6 + C3\text{-}6 + \alpha}$ region

Assuming the DA (= 0.08) did not change, the average degree of substitution was calculated as follows:

$$DS_\beta = \frac{0.3086}{2} = 0.15$$

$$DS_\gamma = \frac{0.3729}{2} = 0.19$$

$$DS_{H_2} = \frac{0.1747}{0.1747 + 0.6865} * 0.92 = 0.19$$

$$DS_{average} = \frac{0.15 + 0.19 + 0.19}{3} = 0.18$$

Assigned COSY, HSQC, ^{13}C and DEPT-135 NMR spectra of the DS20 sample[6-8]

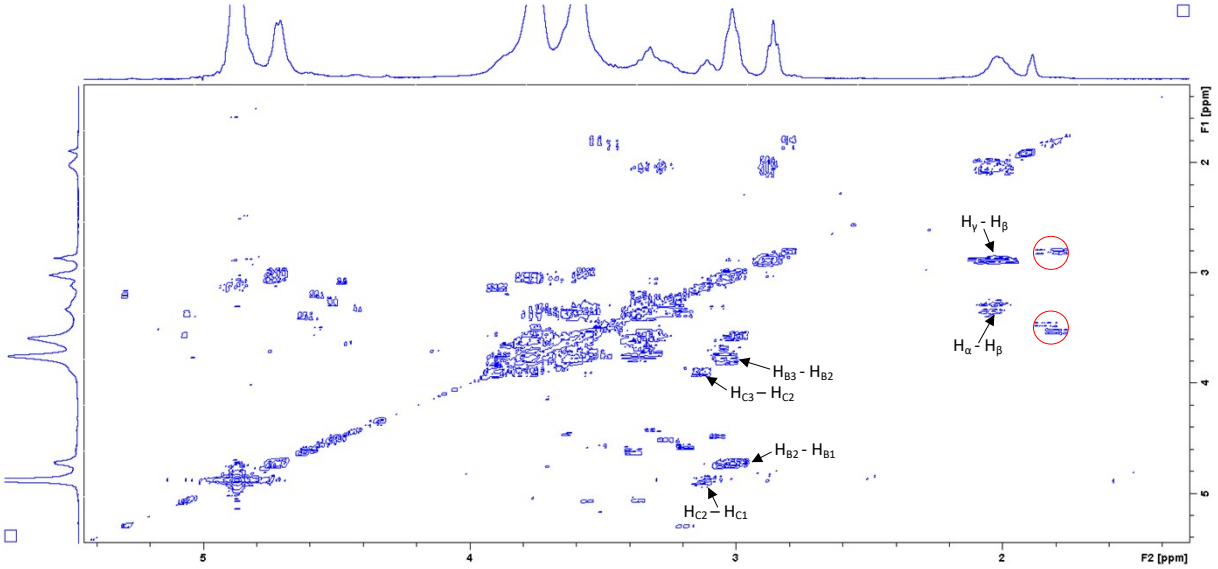


Figure S7. ^{1}H-^{1}H COSY NMR (400 MHz, 1% d-TFA in D_2O, 50 mg/mL) of the DS20 sample. Minor 3-HPS impurities are indicated in red.

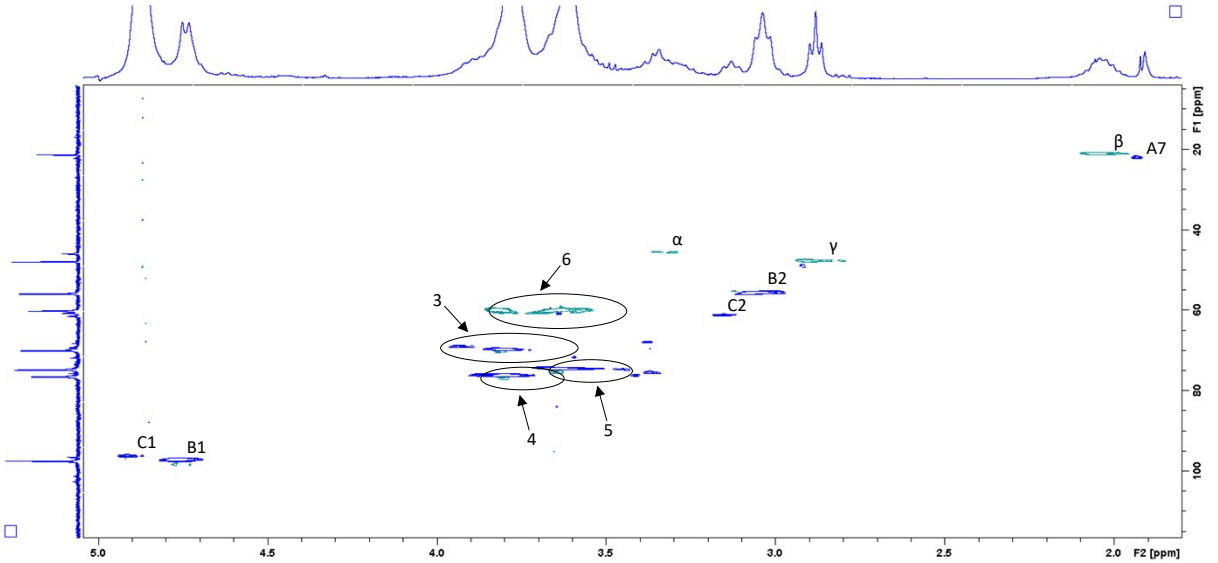


Figure S8. ^{1}H-^{13}C HSQC NMR (400 MHz, 1% d-TFA in D_2O, 50 mg/mL) of the DS20 sample. Note: Green = CH_2 Blue = CH/CH_3

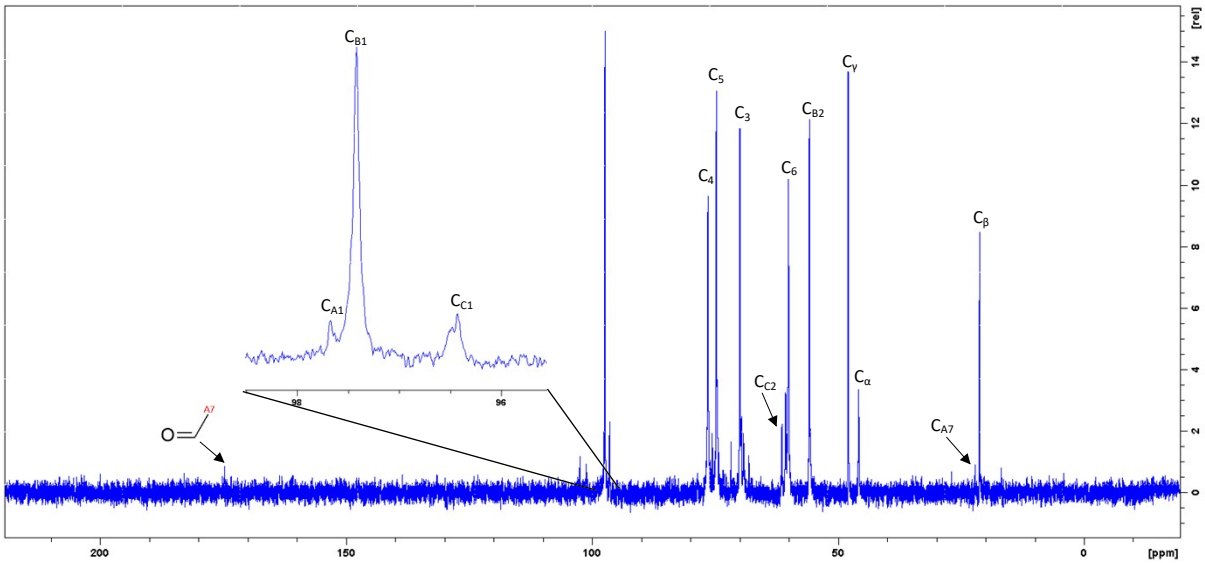


Figure S9. ^{13}C NMR (100.6 MHz, 1% d-TFA in D_2O, 50 mg/mL) of the DS20 sample

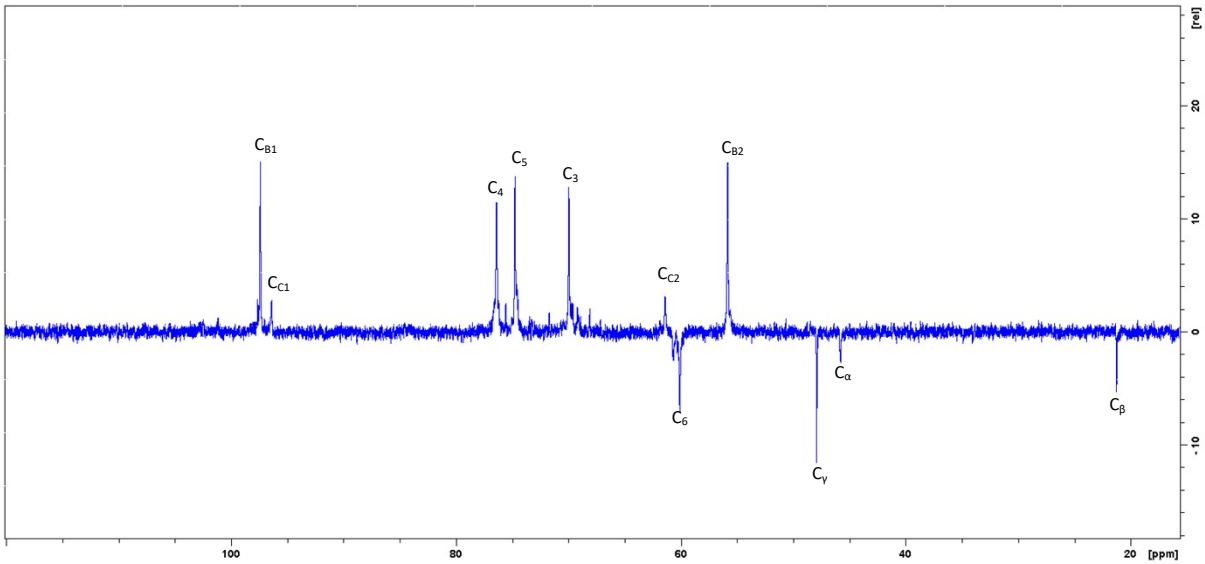


Figure S10. ^{13}C DEPT-135 NMR (100.6 MHz, 1% d-TFA in D_2O, 50 mg/mL) of the DS20 sample. Note: CH_2 = down CH/CH_3 = up

Calculation of the degree of substitution of the DS80 sample based on Figure 10

Assuming the DA did not change, the average degree of substitution was calculated as follows:

The integral of the $H_{B3\text{-}6 + A2\text{-}6 + C3\text{-}6 + D3\text{-}D6}$ region was set to 5.08 (5 + DA).

$$DS_{\alpha+\gamma} = \frac{H_\gamma + H_\alpha}{4} = \frac{3.1858}{4} = 0.80$$

$$DS_\beta = \frac{H_\beta}{2} = \frac{1.5780}{2} = 0.79$$

$$DS_{H1} = \frac{H_{D1} + H_{C1}}{H_{D1} + H_{C1} + H_{B1}} = \frac{0.4133 + 0.1276}{0.1583 + 0.4133 + 0.1276} * 0.92 = 0.75$$

$$DS_{average} = \frac{0.80 + 0.79 + 0.75}{3} = 0.78$$

Assigned COSY, HSQC, ^{13}C and DEPT-135 NMR spectra of the DS80 sample[6, 7]

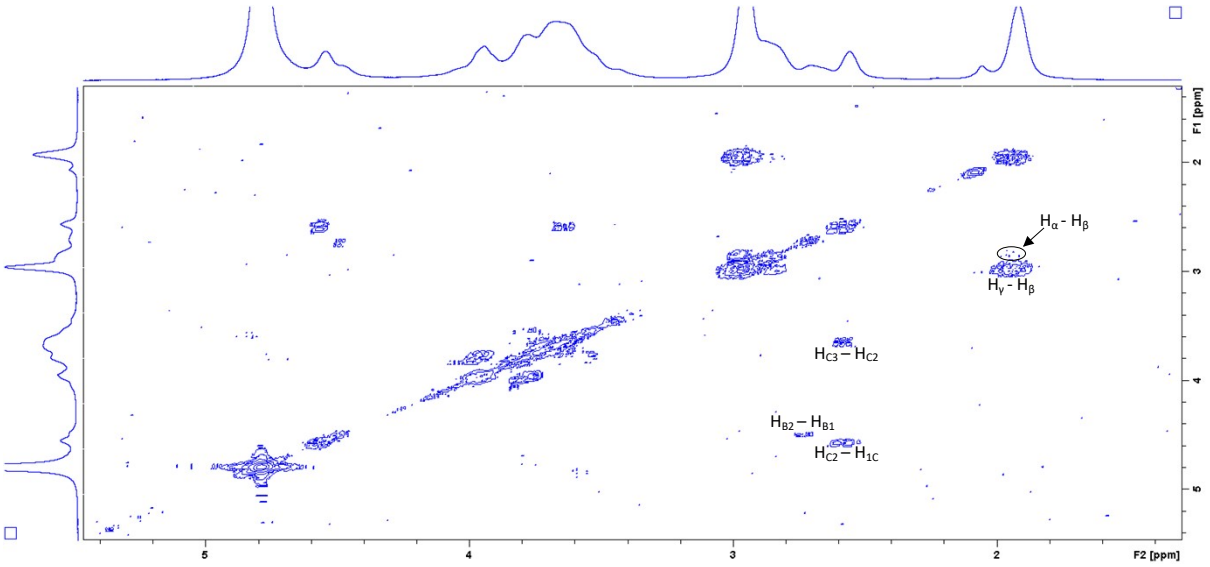


Figure S11. ^1H-^1H COSY NMR (400 MHz, D_2O, 50 mg/mL) of the DS80 sample.

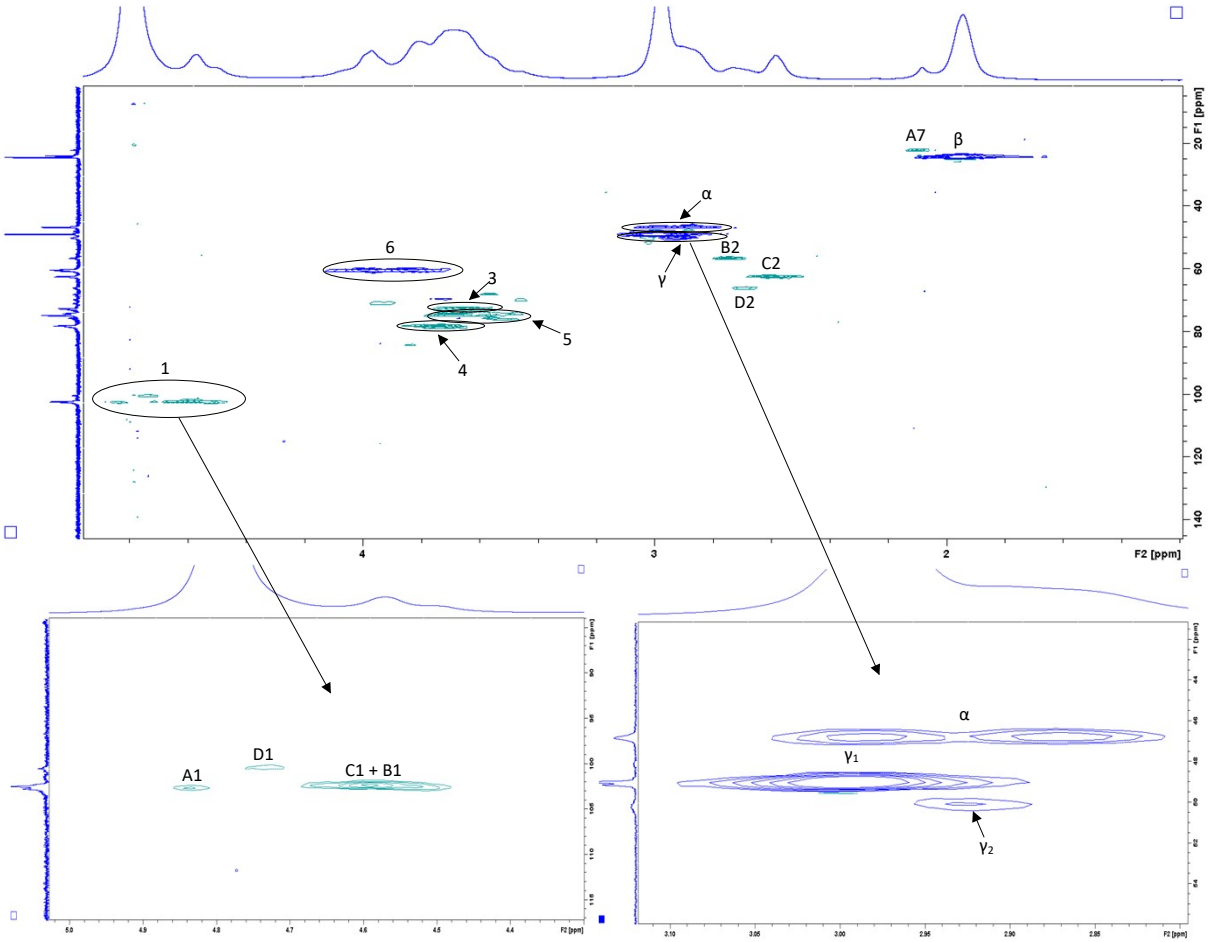


Figure S12. ^{1}H-^{13}C HSQC NMR (400 MHz, D_2O, 50 mg/mL) of the DS80 sample. Note: Blue = CH_2 Green = CH/CH_3

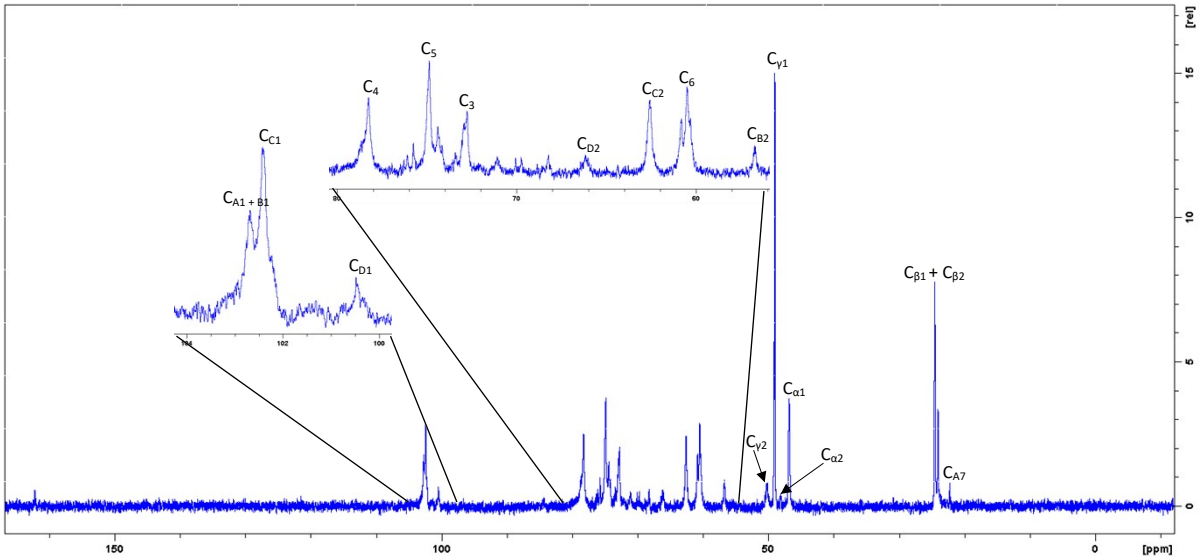


Figure S13. ^{13}C NMR (100.6 MHz, D_2O, 50 mg/mL) of the DS80 sample.

Note: If one would assume that the *N*-sulfopropyl chain is covalently attached to the polymer backbone, the more mobile carbon atoms would be situated further from the main polymer chain and the intensity of the ^{13}C NMR signal would increase along the chain from α to γ. This is indeed the case as can be seen in Figure S13.

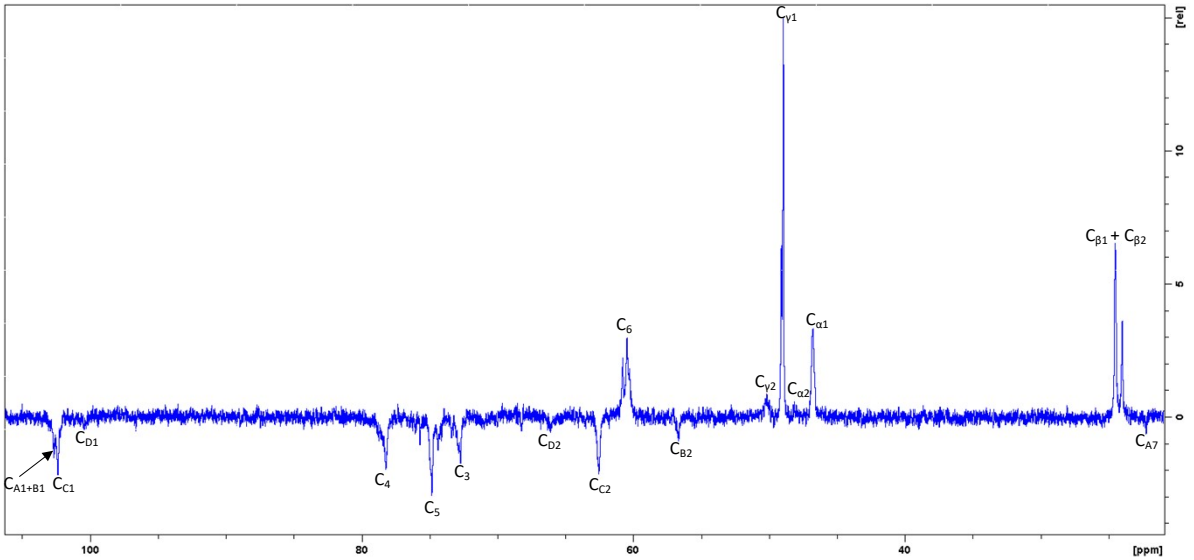
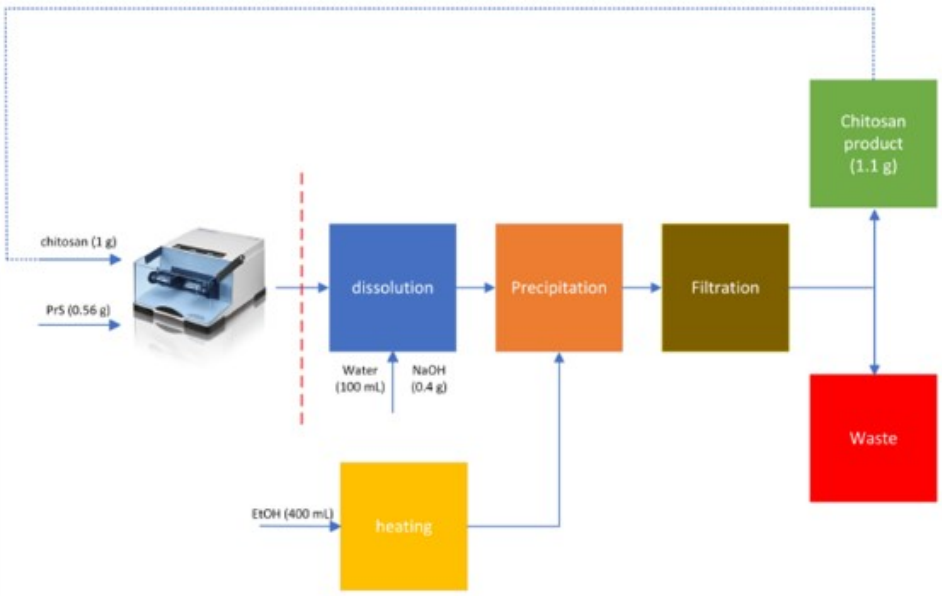
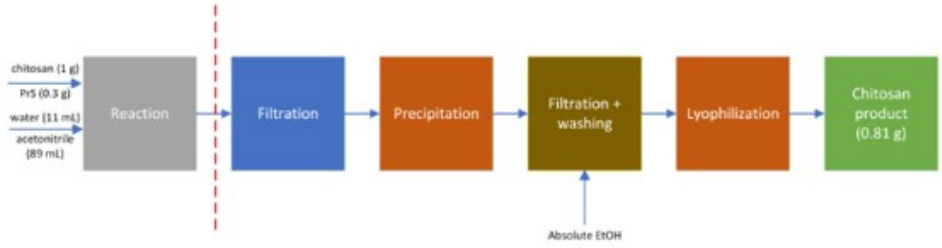


Figure S14. ^{13}C DEPT-135 NMR (100.6 MHz, D_2O, 50 mg/mL) of the DS80 sample. Note: CH_2 = down CH/CH_3 = up

This work



Byung-OK et al.



Heydari et al.

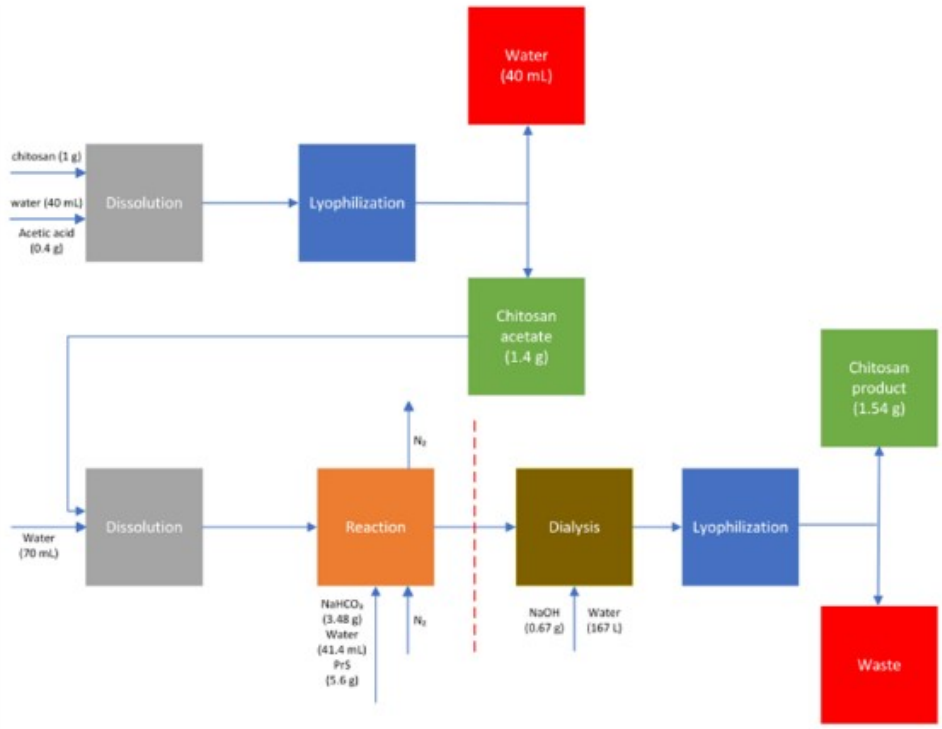


Figure S15. Overview of all three processes utilized to calculate their respective PMI to derivatize 1 gram of chitosan towards N-sulfopropyl chitosan with a given DS. Processes related to reaction and isolation are separated by the red dotted line. These processes were reconstructed from their respective reference.

Kinetic study via ^{1}H NMR of the crude mechanochemical reaction mixture

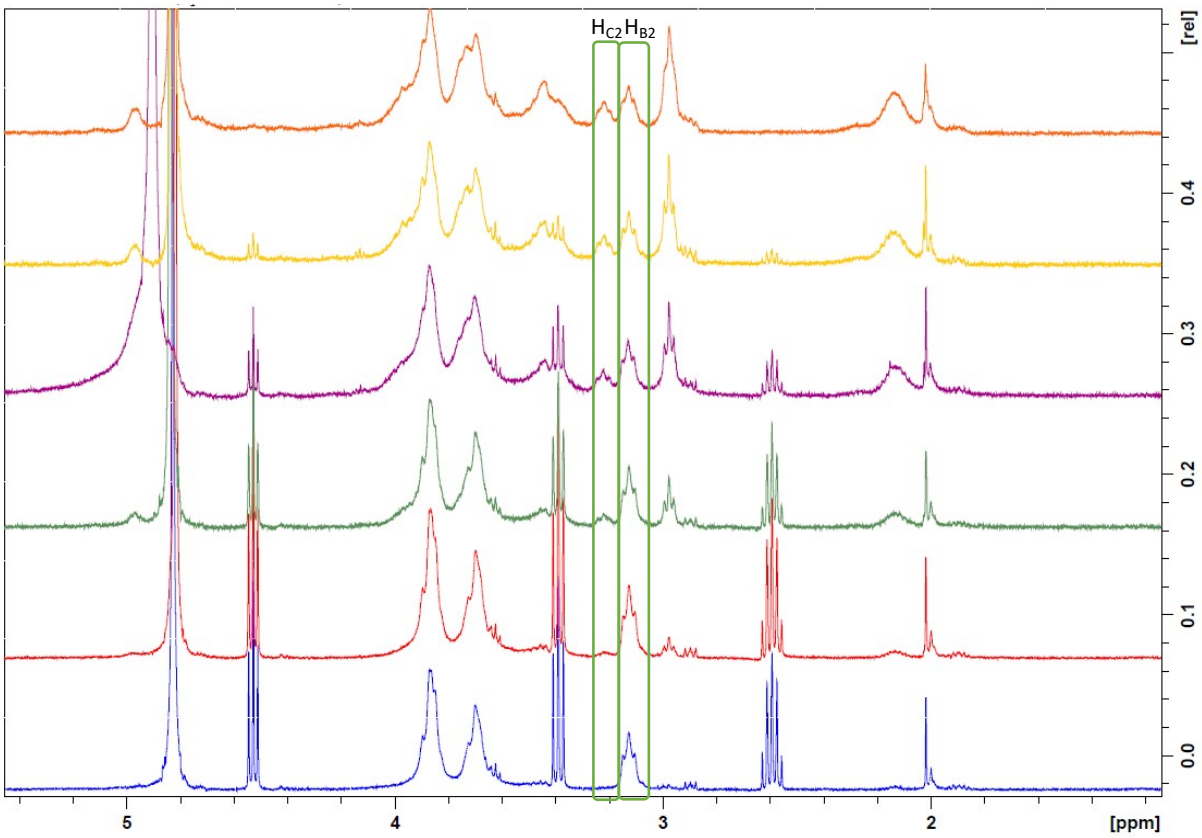


Figure S16. Evolution in time of the mechanochemical reaction between PrS and chitosan monitored by ^{1}H NMR (400 MHz, 1% d-TFA in D_2O). Crude samples were taken at 1,5,15,30, 40 and 60 minutes and directly analyzed. (25 mL SS jar, 2 15 mm SS balls, 30 Hz, 500 mg chitosan, 0.5 eq. PrS)

$$NH_2 - substitution\ (\%) = \frac{H_{C2}}{H_{C2} + H_{B2}}$$

Note: A value of 0 % for the NH_2-substitution only reflects that the observed H_{C2} integral is not defined enough in ^{1}H NMR to be accurately integrated, trace amounts of N-sulfopropylation might still be present.

Elemental analysis of the purified compounds

Table S3. Elemental analysis of the obtained products.

Sample	N (wt.%)	C (wt.%)	H (wt.%)	S (wt.%)
DS80	3.655101	31.079260	5.626658	7.017521
DS60	4.562052	35.278320	6.550825	5.425583
DS40	4.807348	34.089200	6.653444	4.559502
DS20	5.086029	33.268440	6.521273	2.012184
DS10	5.308331	33.381780	6.686409	1.188639
chitosan	6.572807	41.204270	7.355913	0

The pH values after the aqueous dissolution of the different chitosan derivatives obtained by Wang et al.[9]

Table S4. pH values after the aqueous dissolution of several 3-HPS chitosan salt derivatives (recreated from Wang et al.[9]).

Sample Concentration (mg/mL)	Sample 1 (23 % DS)	Sample 2 (48 % DS)	Sample 3 (61 % DS)	Sample 4 (76 % DS)
0.007813	6.36	6.31	6.29	6.31
0.015625	6.28	6.26	6.26	6.25
0.03125	6.24	6.21	6.22	6.02
0.0625	5.84	5.83	5.84	5.32
0.125	4.98	4.59	4.56	4.51
0.25	4.52	4.38	4.31	4.22
0.5	4.04	3.99	3.94	3.96
1	3.94	3.84	3.71	3.62
2	3.85	3.64	3.49	3.47

Note: As the concentration of the 3-HPS chitosan salt increases the pH of the solution clearly decreases, a similar trend can be observed as the DS increases.

Assumption linked to the PMI calculation

All chitosan mass was recovered via dialysis and subsequent lyophilization within the work of **Heydari *et al.***[8]

Within the work of **Byung-OK *et al.*[10]** the same chitosan wt.% was recovered as within our work. Because both processes involved an initial precipitation step.

All excess PrS is neutralized via the addition of an aqueous 1M $NaHCO_3$ solution within the work of **Heydari *et al.***[8]

N_2 consumption was neglected for the PMI calculations.

Only the degree of substitution was taken into account for the RME calculations, while the losses in chitosan mass were neglected. Hence the obtained value of 97.5 % (78/80) for our RME, despite the 5 % loss of PrS throughout the reaction, as also a part of the chitosan mass was lost during the reaction.

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