Supplementary information for:

Shaking up conjugates between chitosan and aldehydes via mechanochemistry

Casper Van Poucke*, Sven Mangelinckx and Christian V. Stevens

Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium.

*Correspondence to:
E-mail address: casper.vanpoucke@ugent.be (C. Van Poucke)
Telephone number: +32 9 264 5860 (Belgium)

FTIR data of the synthesized chitosan/citronellal imine adducts

For all samples, after soaking in EtOH, the C=N imine stretch at 1666 cm^{-1} in FTIR remained while the C=O stretch at 1724 cm^{-1} and H-C=O stretch at 2716 cm^{-1} of any leftover aldehyde disappeared. All equivalents are based on the number of NH_2 groups in a gram of material (5.27 mmol NH_2/g). When looking at the peak intensity at 1666 cm^{-1}, the same pattern as observed via elemental analysis (see Figure 2) can be observed in which the DS increases up to 0.5 eq. and drops down again at 1 eq. of citronellal.

0.125 eq. of citronellal:

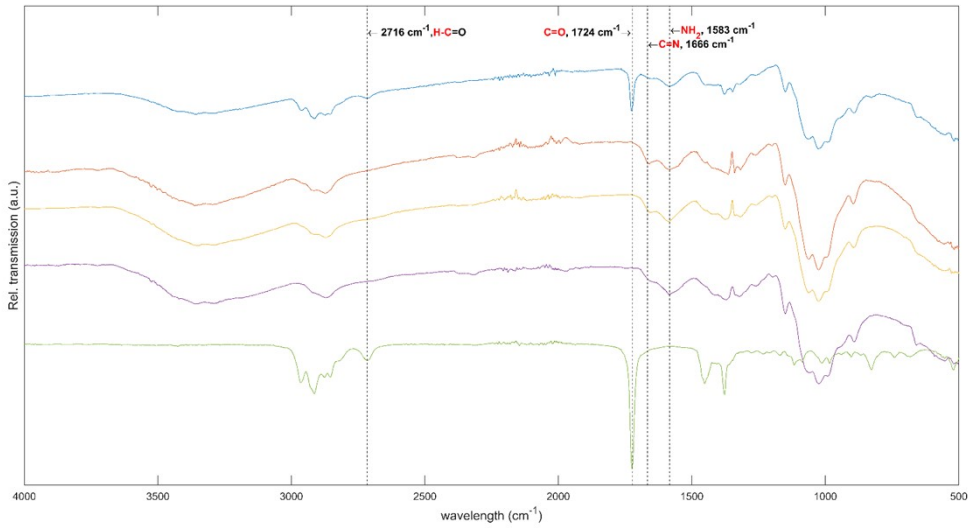


Figure S1. Comparison between the FTIR spectrum of citronellal (green), chitosan (purple), chitosan + 0.125 eq. of citronellal after 30 seconds of milling (blue), chitosan + 0.125 eq. of citronellal after 10 minutes of milling (orange) and chitosan + 0.125 eq. of citronellal after 10 minutes of milling + EtOH washing (yellow). The C=O stretch at 1724 cm^{-1} and H-C=O stretch at 2716 cm^{-1} of the original aldehyde, the NH_2 scissoring vibration of chitosan at 1583 cm^{-1} and the newly formed C=N imine stretch at 1666 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

0.250 eq. of citronellal:

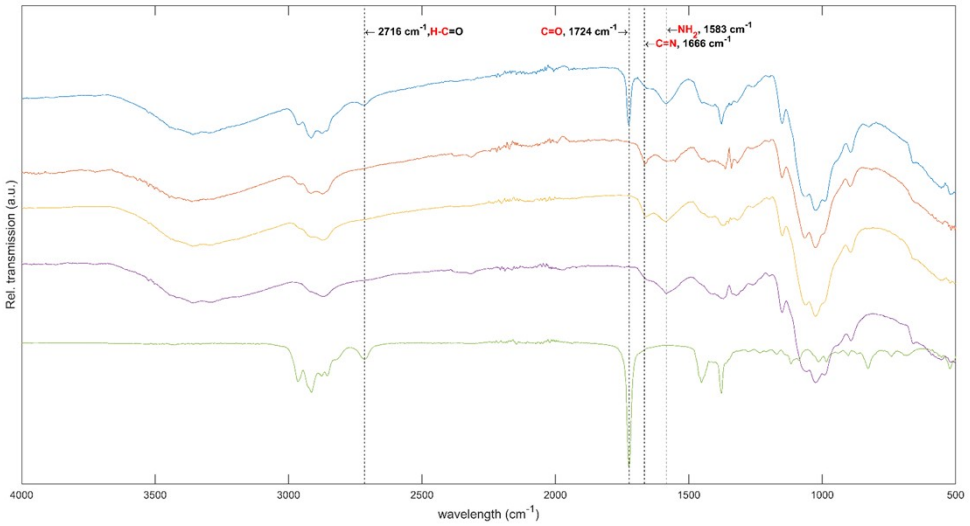


Figure S2. Comparison between the FTIR spectrum of citronellal (green), chitosan (purple), chitosan + 0.250 eq. of citronellal after 30 seconds of milling (blue), chitosan + 0.250 eq. of citronellal after 10 minutes of milling (orange) and chitosan + 0.250 eq. of citronellal after 10 minutes of milling + EtOH washing (yellow). The C=O stretch at 1724 cm^{-1} and H-C=O stretch at 2716 cm^{-1} of the original aldehyde, the NH_2 scissoring vibration of chitosan at 1583 cm^{-1} and the newly formed C=N imine stretch at 1666 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

0.500 eq. of citronellal:

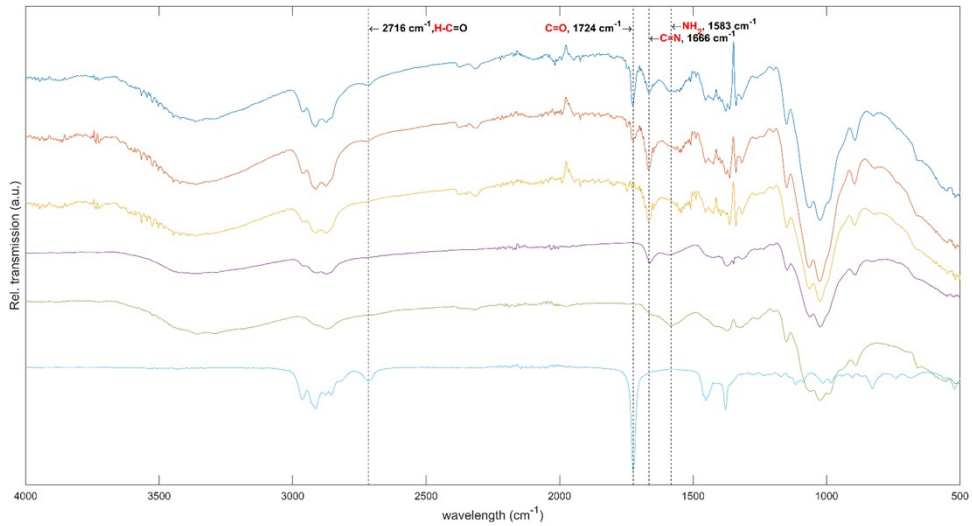


Figure S3. Comparison between the FTIR spectrum of citronellal (light blue), chitosan (green), chitosan + 0.500 eq. of citronellal after 10 minutes of milling (blue), chitosan + 0.500 eq. of citronellal after 20 minutes of milling (orange), chitosan + 0.500 eq. of citronellal after 30 minutes of milling (yellow) and chitosan + 0.500 eq. of citronellal after 30 minutes of milling + EtOH washing (purple). The C=O stretch at 1724 cm^{-1} and H-C=O stretch at 2716 cm^{-1} of the original aldehyde, the NH_2 scissoring vibration of chitosan at 1583 cm^{-1} and the newly formed C=N imine stretch at 1666 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

1 eq. of citronellal:

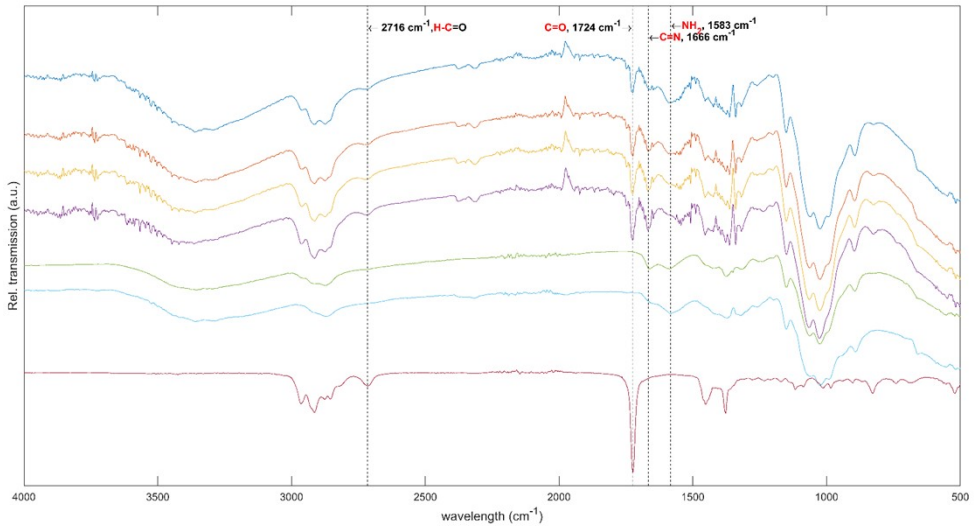


Figure S4. Comparison between the FTIR spectrum of citronellal (red), chitosan (light blue), chitosan + 1 eq. of citronellal after 10 minutes of milling (blue), chitosan + 1 eq. of citronellal after 20 minutes of milling (orange), chitosan + 1 eq. of citronellal after 30 minutes of milling (yellow), chitosan + 1 eq. of citronellal after 40 minutes of milling (purple) and chitosan + 1 eq. of citronellal after 40 minutes of milling + EtOH wash (green). The C=O stretch at 1724 cm^{-1} and H-C=O stretch at 2716 cm^{-1} of the original aldehyde, the NH_2 scissoring vibration of chitosan at 1583 cm^{-1} and the newly formed C=N imine stretch at 1666 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

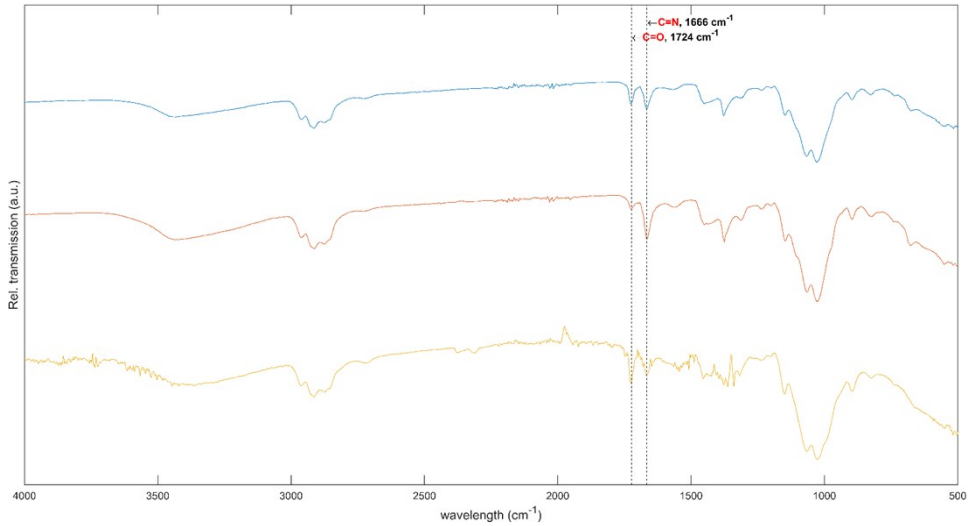


Figure S5. Comparison between the FTIR spectrum of chitosan + 1 eq. of citronellal after 40 minutes of milling (yellow), chitosan + 1 eq. of citronellal + 500 mg NaCl after 40 minutes of milling (blue) and chitosan + 1 eq. of citronellal after 40 minutes of milling (added in four portions, every 10 minutes)(orange). The C=O stretch at 1724 cm^{-1} and the newly formed C=N imine stretch at 1666 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

Note: NaCl was selected as a solid grinding auxiliary as it is a cheap and relatively soft material (≈ 200 MPa)[39] that does not erode the stainless steel grinding equipment (≈ 2000 MPa) while being unreactive towards the utilized substrates.

Note: both methods impact the overall texture and rheology of the material during milling as the free aldehyde tends to act as a lubricant which severely lowers the transfer of mechanical energy to the reaction mixture. On the one hand, the addition of NaCl increases the total solid content which transforms the slurry into a powder. On the other hand, the addition of the aldehyde in portions lowers the total free aldehyde content at any stage during the reaction.

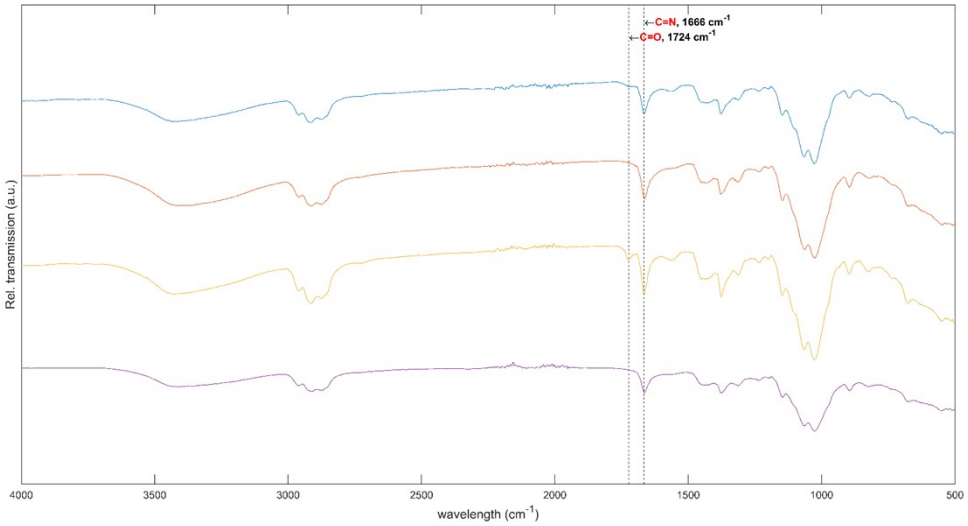


Figure S6. Comparison between the FTIR spectrum of chitosan + 1 eq. of citronellal + 500 mg NaCl after 60 minutes of milling (blue), chitosan + 1 eq. of citronellal + 500 mg NaCl after 60 minutes of milling + EtOH wash (orange), chitosan + 1 eq. of citronellal after 60 minutes of milling (added in four portions, every 10 minutes)(yellow) and chitosan + 1 eq. of citronellal after 60 minutes of milling (added in four portions, every 10 minutes) + EtOH wash (purple). The C=O stretch at 1724 cm^{-1} and the newly formed C=N imine stretch at 1666 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

Note: the kinetics are slower with the addition of unreactive NaCl as you “dilute” your reaction mixture. This lowers the probability of having both reactive species present during one collision, which in turn lowers the overall total functional collisions in which both chitosan and the aldehyde are involved, which lowers the overall reaction rate. However, a virtually total conversion was obtained after a total of one hour of milling time.

FTIR data of the synthesized *N*-octyl chitosan derivatives

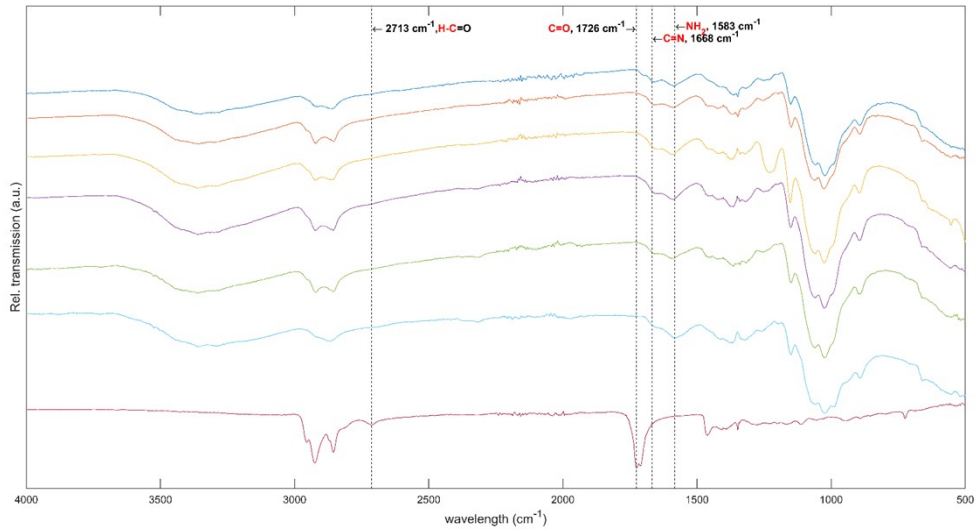


Figure S7. Comparison between the FTIR spectrum of octanal (red), chitosan (light blue), chitosan + 0.125 eq. of octanal after imine formation, reduction and product isolation (blue), chitosan + 0.250 eq. octanal after imine formation, reduction and product isolation (orange), chitosan + 0.375 eq. octanal after imine formation, reduction and product isolation (yellow), chitosan + 0.500 eq. octanal after imine formation, reduction and product isolation (purple) and chitosan + 0.625 eq. octanal after imine formation, reduction and product isolation (green). The C=O stretch at 1726 cm^{-1} and H-C=O stretch at 2713 cm^{-1} of the original aldehyde, the NH_2 scissoring vibration of chitosan at 1583 cm^{-1} and the newly formed C=N imine stretch at 1668 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

FTIR data of the synthesized chitosan/citronellal amine adduct

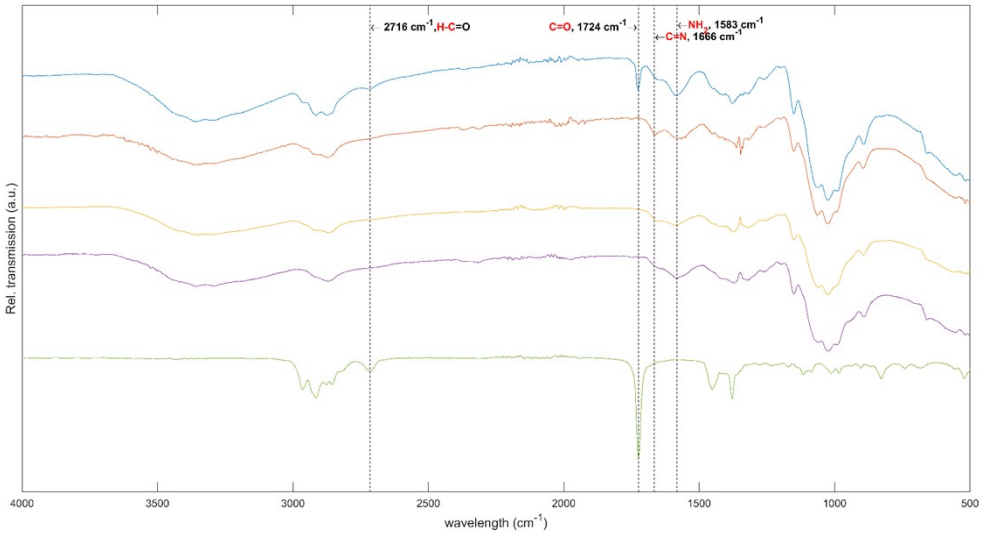


Figure S8. Comparison between the FTIR spectrum of citronellal (green), chitosan (purple), chitosan + 0.125 eq. citronellal after 30 seconds of milling (blue), chitosan + 0.125 eq. citronellal after 10 minutes of milling (orange) and chitosan + 0.125 eq. citronellal after imine formation, reduction and product isolation (yellow). The C=O stretch at 1724 cm^{-1} and H-C=O stretch at 2716 cm^{-1} of the original aldehyde, the NH_2 scissoring vibration of chitosan at 1583 cm^{-1} and the newly formed C=N imine stretch at 1666 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

Monitoring of the Schiff base formation between chitosan and several different furfural derivatives via FTIR

1 eq. of furfural:

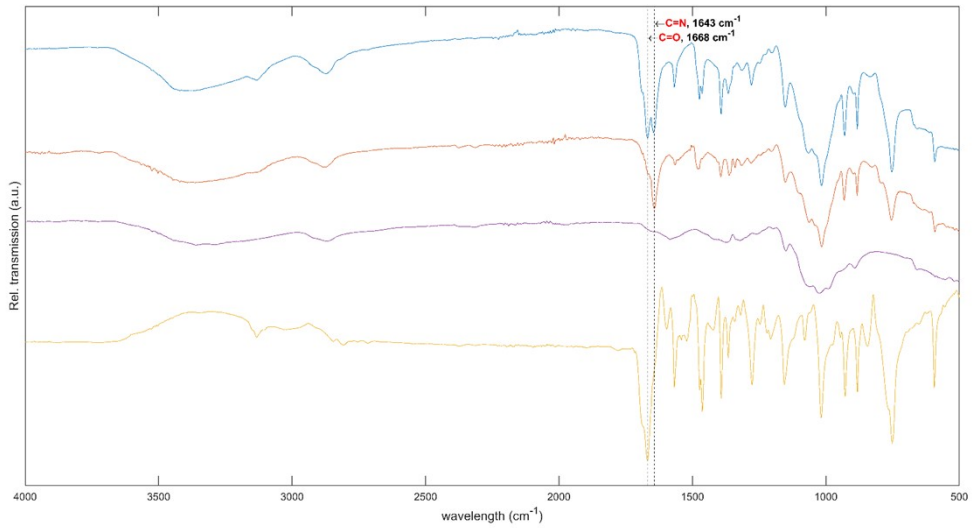


Figure S9. Comparison between the FTIR spectrum of furfural (yellow), chitosan (purple), chitosan + 1 eq. furfural after 10 minutes of milling (blue) and chitosan + 1 eq. furfural after 30 minutes of milling (orange). The C=O stretch at 1668 cm^{-1} and the newly formed C=N imine stretch at 1643 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

Note: washing with water and ethanol did not completely remove leftover unreduced imine as can be seen in Figure S10. To reduce the imine to below 1 %, a dissolution in 1 % (v/v) acetic acid, followed by precipitation in acetone was required. Moreover, some aldehyde signal can be observed in Figure S10. This is most likely formed in situ during the NMR analysis under aqueous acidic conditions as only a strong imine signal at 1643 cm^{-1} was observed in the FTIR spectrum (see Figure S11).

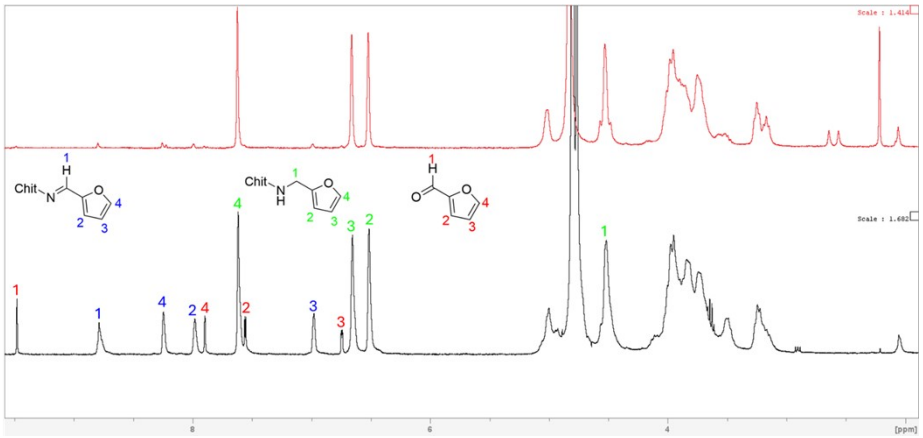


Figure S10. ^{1}H NMR (400 MHz) spectrum of the obtained N-(furan-2-yl)methyl chitosan product after purification by washing with water and dissolution in 1% (v/v) acetic acid (red) and the obtained N-(furan-2-yl)methyl chitosan product after purification by washing with water and ethanol (black) at 20 mg/mL in 1 vol% d-TFA in D_2O. The different impurities are indicated in red and blue, as well as the obtained product in green.

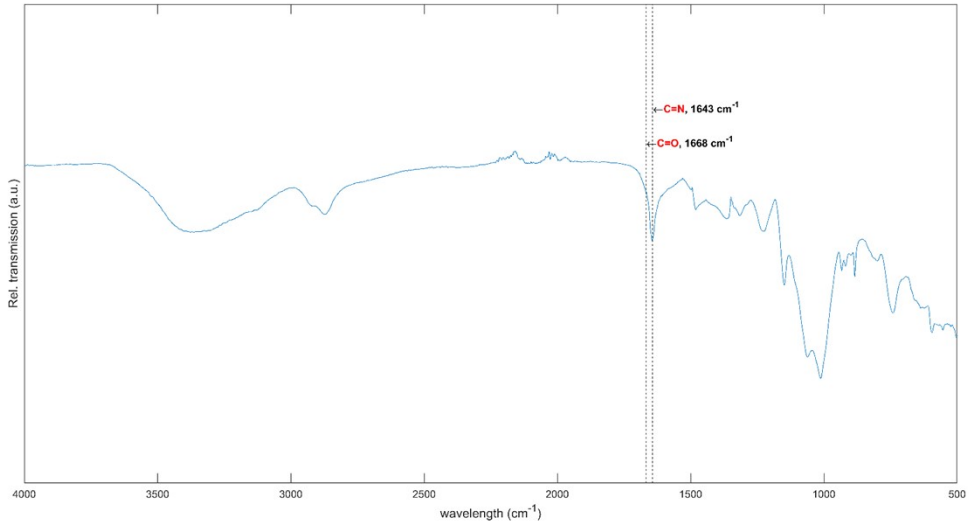


Figure S11. The FTIR spectrum of chitosan + 1 eq. furfural after 60 minutes of milling, reduction and washing with water and ethanol. The C=O stretch at 1668 cm^{-1} and the C=N imine stretch at 1643 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

1 eq. of 5-methylfurfural:

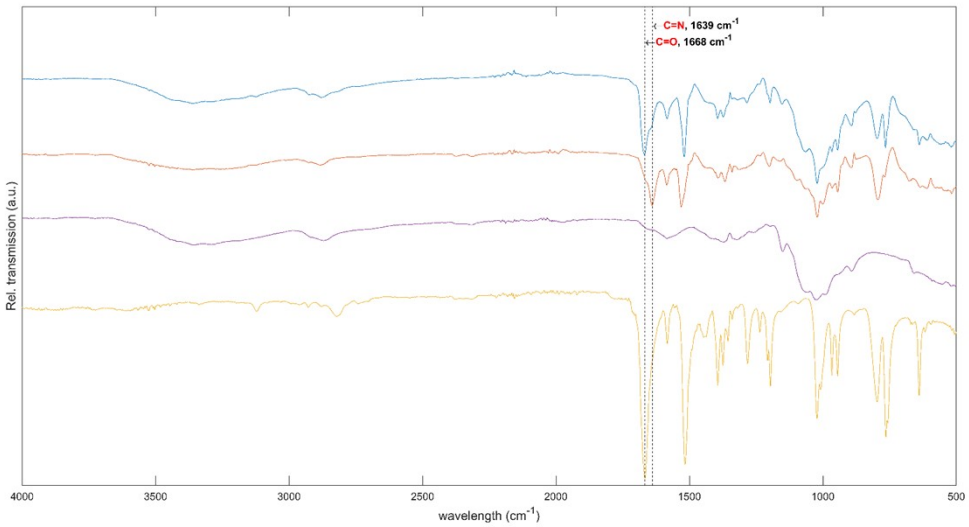


Figure S12. Comparison between the FTIR spectrum of 5-methylfurfural (yellow), chitosan (purple), chitosan + 1 eq. 5-methylfurfural after 10 minutes of milling (blue) and chitosan + 1 eq. 5-methylfurfural after 30 minutes of milling (orange). The C=O stretch at 1668 cm^{-1} and the newly formed C=N imine stretch at 1639 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

1 eq. of 5-bromofurfural:

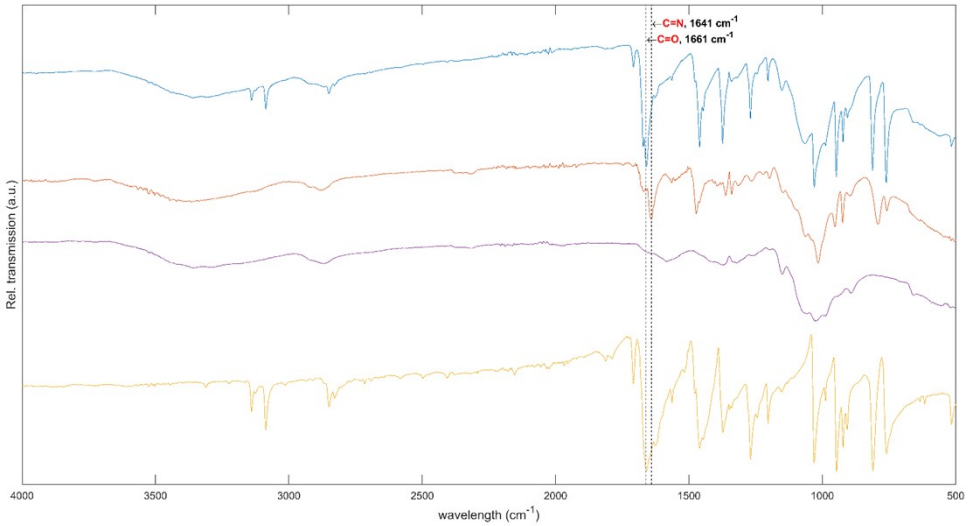


Figure S13. Comparison between the FTIR spectrum of 5-bromofurfural (yellow), chitosan (purple), chitosan + 1 eq. 5-bromofurfural after 10 minutes of milling (blue) and chitosan + 1 eq. 5-bromofurfural after 30 minutes of milling (orange). The C=O stretch at 1661 cm^{-1} and the newly formed C=N imine stretch at 1641 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

Note: the sharp peaks around 3000 cm^{-1} are most likely explained by the high crystallinity of the material. These peaks remain after 10 minutes of milling, but after 30 minutes, the observed crystallinity is gone. This phase transfer is accompanied by a sharp increase of the imine stretch at 1641 cm^{-1}. This could indicate that the reactivity of the added aldehyde increases over time as the material becomes more and more amorphous.

1 eq. of (5-formylfuran-2-yl)boronic acid

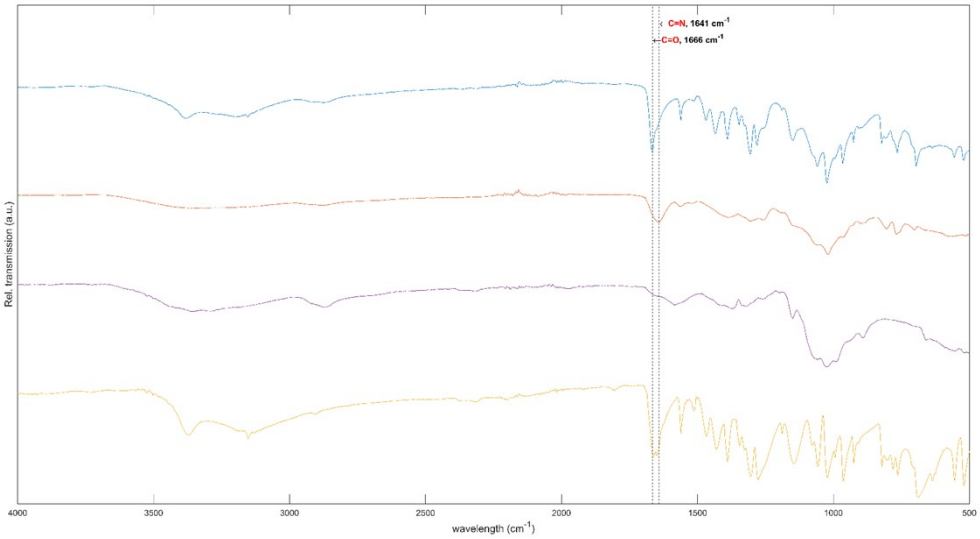


Figure S14. Comparison between the FTIR spectrum of (5-formylfuran-2-yl)boronic acid *(yellow), chitosan (purple), chitosan + 1 eq.* (5-formylfuran-2-yl)boronic acid *after 10 minutes of milling (blue) and chitosan + 1 eq.* (5-formylfuran-2-yl)boronic acid *after 30 minutes of milling (orange). The C=O stretch at 1666 cm^{-1} and the newly formed C=N imine stretch at 1641 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.*

Note: two different aldehyde signals are present, as well as sharp peaks around 3000 cm^{-1}, which are most likely explained by the high crystallinity of the starting material. After 10 minutes of milling, as the starting material becomes more amorphous, only one aldehyde signal remains at 1666 cm^{-1} and the crystalline phase disappears. Moreover, the appearance of a new C=N signal at 1641 cm^{-1} indicates imine formation. Unfortunately, as indicated by Figure S15, the obtained product was deborylated after reduction and purification.

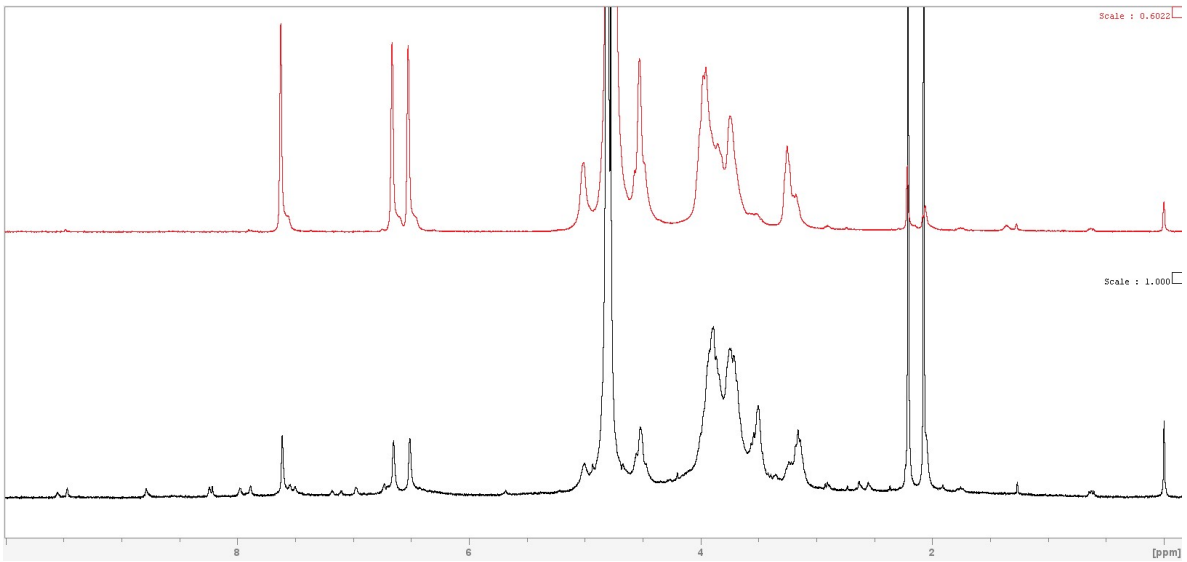


Figure S15. ^1H NMR (400 MHz) spectrum of N-(furan-2-yl)methyl chitosan (red) and the product obtained with (5-formylfuran-2-yl)boronic acid *after reduction and isolation (black) at 20 mg/mL in 1 vol% d-TFA in D_2O.*

1 eq. of 5-hydroxymethylfurfural[40]:

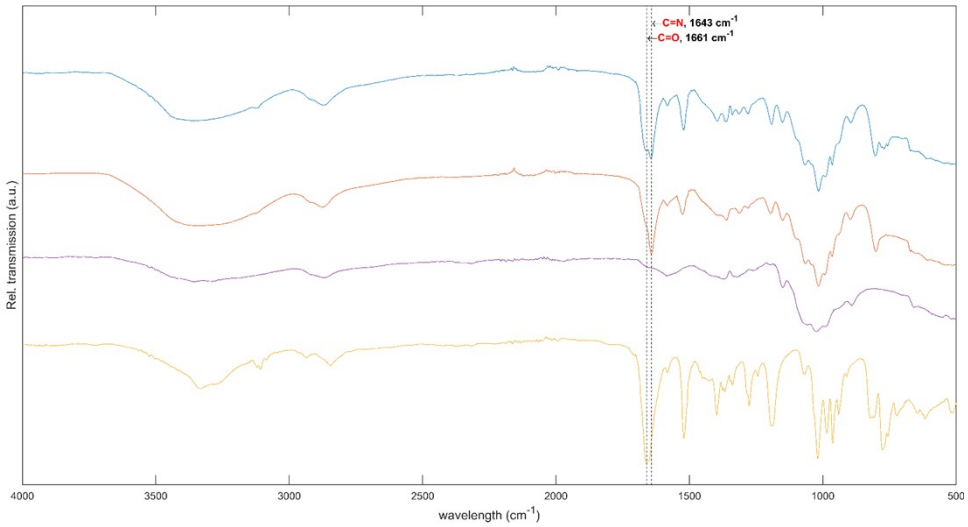


Figure S16. Comparison between the FTIR spectrum of 5-hydroxymethylfurfural (yellow), chitosan (purple), chitosan + 1 eq. 5-hydroxymethylfurfural after 10 minutes of milling (blue) and chitosan + 1 eq. 5-hydroxymethylfurfural after 30 minutes of milling (orange). The C=O stretch at 1661 cm^{-1} and the newly formed C=N imine stretch at 1643 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

1 eq. 5-nitrofurfural

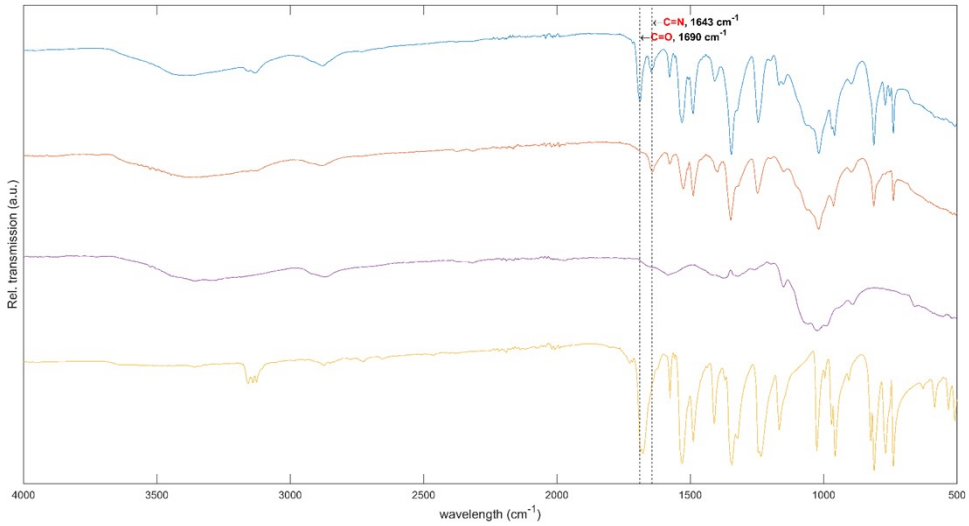


Figure S17. Comparison between the FTIR spectrum of 5-nitrofurfural (yellow), chitosan (purple), chitosan + 1 eq. 5-nitrofurfural after 10 minutes of milling (blue) and chitosan + 1 eq. 5-nitrofurfural after 30 minutes of milling (orange). The C=O stretch at 1690 cm^{-1} and the newly formed C=N imine stretch at 1643 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

Note: Schiff base formation was successful, as indicated by the C=N signal at 1643 cm^{-1} in Figure S17. However, during the subsequent reduction a strong exotherm occurred and a complex, insoluble and black reaction mixture was obtained which strongly resembled charcoal. Unfortunately, no product could be isolated from this mixture.

1 eq. of 5-(4-nitrophenyl)furfural:

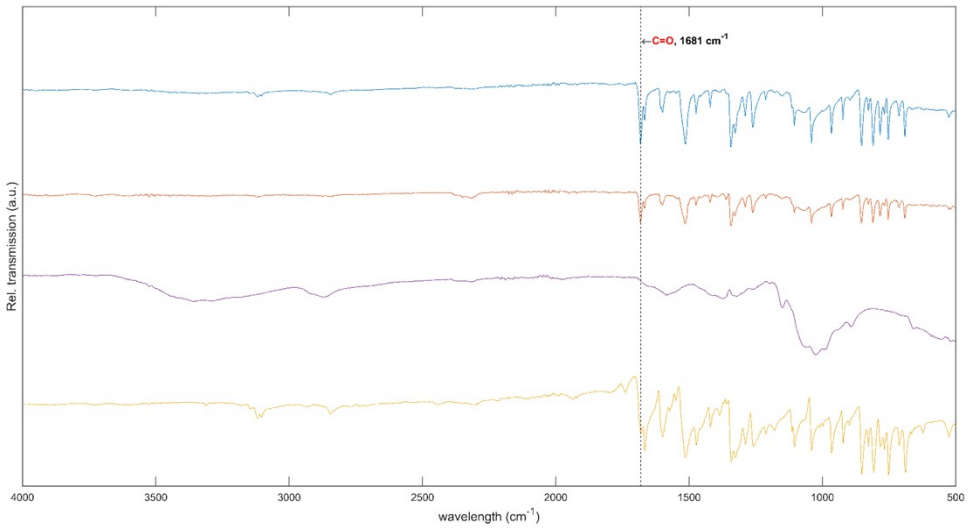


Figure S18. Comparison between the FTIR spectrum of 5-(4-nitrophenyl)furfural (yellow), chitosan (purple), chitosan + 1 eq. 5-(4-nitrophenyl)furfural after 10 minutes of milling (blue) and chitosan + 1 eq. 5-(4-nitrophenyl)furfural after 30 minutes of milling (orange). The C=O stretch at 1681 cm^{-1} is indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

1 eq. of thiophene-2-carboxaldehyde[41]:

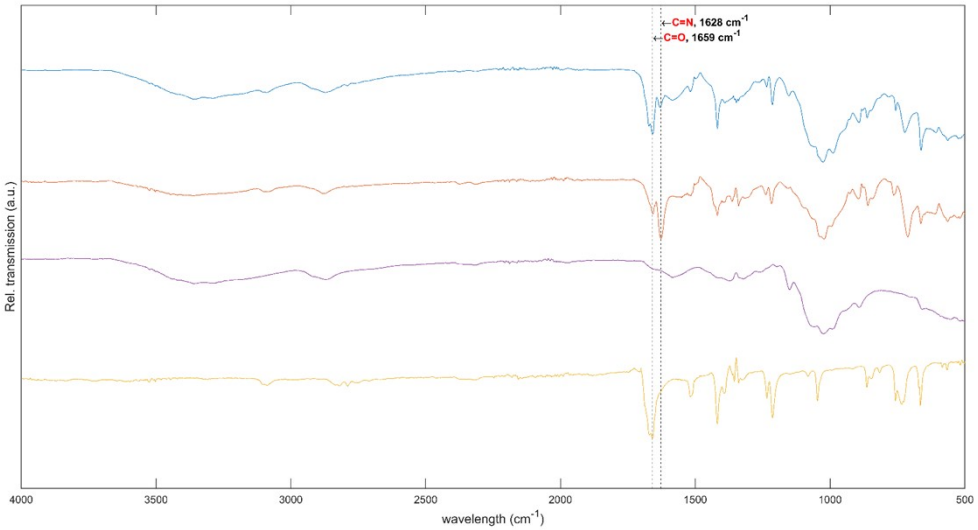


Figure S19. Comparison between the FTIR spectrum of thiophene-2-carboxaldehyde (yellow), chitosan (purple), chitosan + 1 eq. thiophene-2-carboxaldehyde after 10 minutes of milling (blue) and chitosan + 1 eq. thiophene-2-carboxaldehyde after 30 minutes of milling (orange). The C=O stretch at 1659 cm^{-1} and the newly formed C=N imine stretch at 1628 cm^{-1} of the product are indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

1 eq. of 1*H*-pyrrole-2-carboxaldehyde:

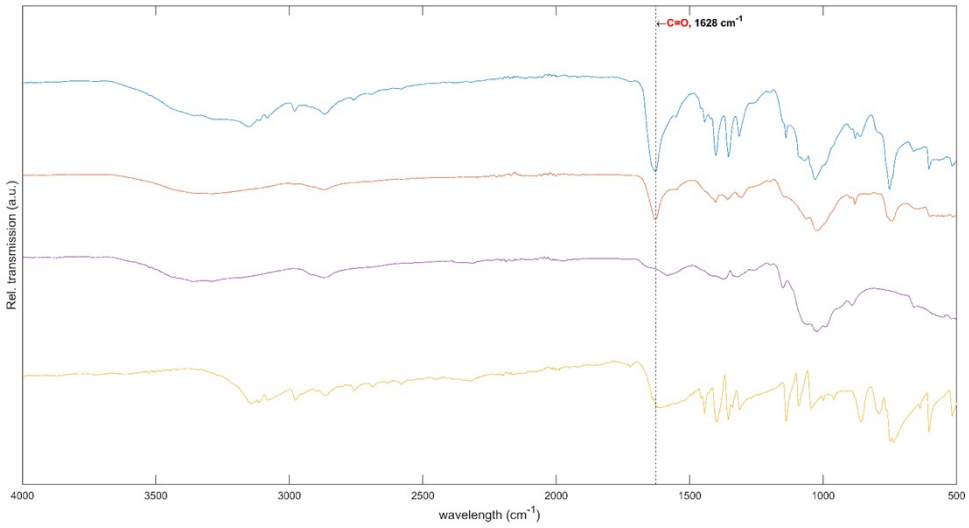


Figure S20. Comparison between the FTIR spectrum of 1H-pyrrole-2-carboxaldehyde (yellow), chitosan (purple), chitosan + 1 eq. 1H-pyrrole-2-carboxaldehyde after 10 minutes of milling (blue) and chitosan + 1 eq. 1H-pyrrole-2-carboxaldehyde after 30 minutes of milling (orange). The C=O stretch at 1628 cm^{-1} is indicated with a dotted line. The chitosan mass within the system was 500 mg. A 25 mL SS jar was utilized together with two 15 mm SS balls at a frequency of 30 Hz.

Relative molecular weight of the synthesized chitosan derivatives

Table S1. The measured relative molecular weight to pullulan standards of the different synthesized chitosan derivatives.

Sample*	Milling time (h)	DS_{NMR} (%)	M_n (kDa)	M_w (kDa)	$Đ_M$
Chitosan	0	0	158.0	279.2	1.77
$Chitosan_{blank}$	2	0	94.6	123.2	1.30
OA (0.125 eq.)	1	5.2	108.1	148.8	1.38
OA (0.125 eq.)	2	5.5	95.8	131.0	1.37
OA (0.125 eq.)	3	6.5	77.9	102.0	1.31
OA (0.125 eq.)	4	8.2	74.6	86.9	1.17
OA (0.250 eq.)	2	8.9	92.1	147.5	1.60
OA (0.375 eq.)	2	9.5	92.8	134.4	1.45
OA (0.5 eq.)	2	11.6	98.0	154.4	1.58
CA (0.125 eq.)	2	8.5	92.4	130.0	1.41

*OA = *N*-octyl chitosan; CA = chitosan/citronellal amine adduct

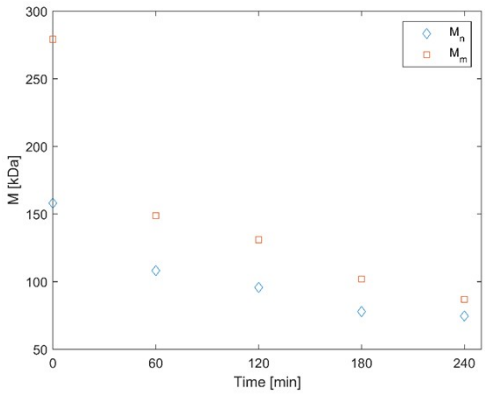


Figure S21. The measured relative molecular weight of the synthesized N-octyl chitosan derivatives (OA (0.125 eq.)) in function of the milling time.

The elemental composition of the different synthesized chitosan derivatives

Table S2. The measured elemental composition of the different synthesized chitosan derivatives.

Sample*	N (wt.%)	C (wt.%)	H (wt.%)	S (wt.%)	C/N	DS_{EA} (%)
$Chitosan_{blank}$	8.204476	42.68207	7.060194	0	5.20	0
OA (0.125 eq.)	8.295039	45.54444	7.418899	0	5.49	4.2
OA (0.250 eq.)	7.647139	42.72137	6.596095	0	5.59	5.6
OA (0.375 eq.)	8.249341	46.42193	7.381345	0	5.63	6.2
OA (0.500 eq.)	8.165513	48.03813	7.731237	0	5.88	9.9

OA (0.625 eq.)	7.515051	46.91236	7.737752	0	6.24	15.2
CA (0.125 eq.)	7.881576	45.04473	7.477432	0	5.72	6.0
CI (0.125 eq.)	7.897922	44.1387	7.438632	0	5.59	4.5
CI (0.250 eq.)	7.376898	45.80653	7.460869	0	6.21	11.7
CI (0.500 eq.)	6.756249	53.2048	8.191872	0	7.87	31.2
CI (1 eq.)	7.339363	47.3278	7.428164	0	6.45	14.5
CI (1 eq.)*2	4.181766	57.99820	8.604356	0	13.87	101.1
CI (1 eq.)*3	5.073248	67.63914	10.050729	0	13.33	94.8

*OA = *N*-octyl chitosan; CA = chitosan/citronellal amine adduct; CI = chitosan/citronellal imine adduct; all products were milled for 2 hours. Except the chitosan/citronellal imine adducts (CI (0.125 eq. – 1 eq.)) these were milled for 10, 10, 30, 40, 60 or 60 minutes, respectively.

*2 = An additional 500 mg of NaCl was added to the sample.

*3 = The aldehyde was added in portions of 0.25 equivalents every 10 minutes

Example: $DS_{EA,\ OA(0.125\ eq.)} = \frac{\frac{M_{C}}{N},\ S - \frac{M_{C}}{N},\ chit}{\frac{n * M_{C}}{M_{N}}} * 100 = \frac{5.49 - 5.20}{8 * \frac{12.011}{14.0067}} = 4.2\ \%$

Calculation of the degree of substitution (DS_{NMR}) via relative ^{1}H NMR integration

Note: in all cases, the NMR peak assignment is based upon a combination of 1D (^{1}H, ^{13}C DEPT135 and ^{13}C) and 2D (^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{13}C H2BC) NMR analysis. The DS_{NMR} was calculated utilizing the formula derived and explained in our previous publication.[22] Two examples are depicted below:

N-octyl chitosan:

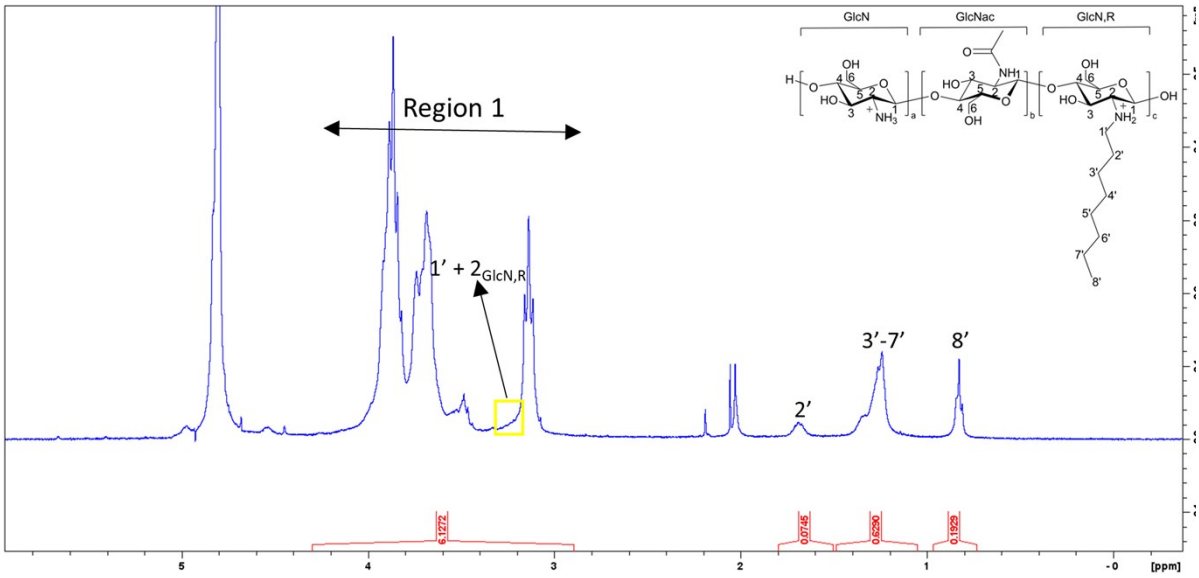


Figure S22. ^{1}H NMR (400 MHz) spectrum of N-octyl chitosan at 20 mg/mL in 1 vol% d-TFA in D_2O. The different ^{1}H integral regions important for the DS determination are indicated in black.

The average degree of substitution was calculated as follows:

$$DS_{NMR} = \frac{6\,x\left(I_{H_{2'}} + I_{H_{3'-7'}} + I_{H_{8'}}\right)}{15\,x\,(I_{region\,1}) - 2\,x\left(I_{H_{2'}} + I_{H_{3'-7'}} + I_{H_{8'}}\right)} = \frac{6\,x\,(0.(}{15\,x\,(6.1272) -}$$
$$= 0.060 = 6.0\ \%$$

Chitosan/citronellal amine adduct:

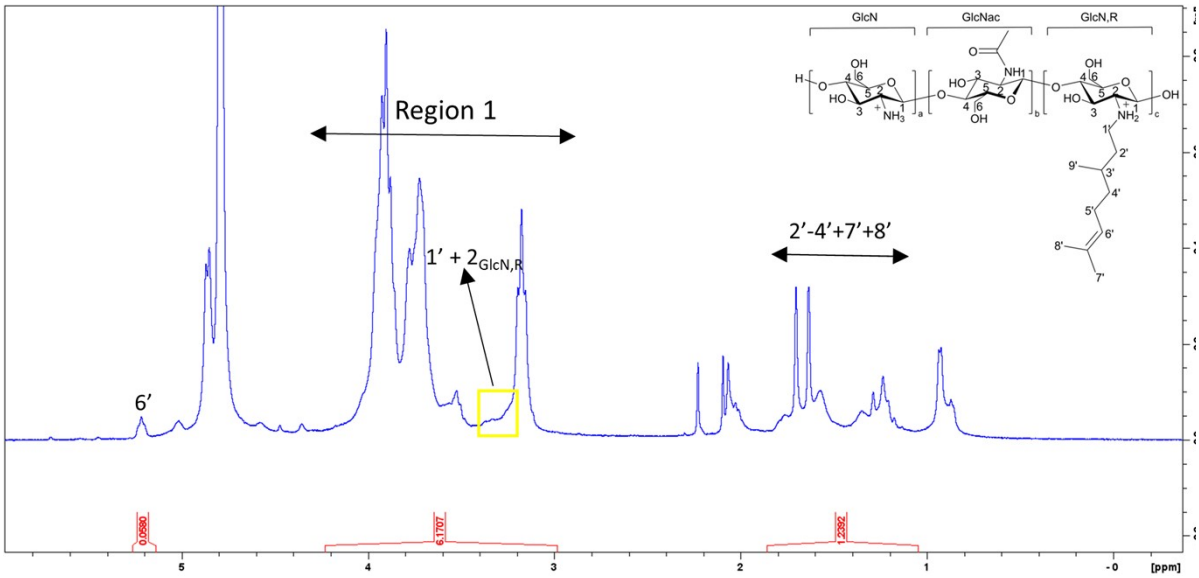


Figure S23. ^{1}H NMR (400 MHz) spectrum of the chitosan/citronellal amine adduct at 20 mg/mL in 1 vol% d-TFA in D_2O. The different ^{1}H integral regions important for the DS determination are indicated in black.

$$DS_{NMR} = \frac{6\,x\left(I_{H_{2'-4'+7'+8'}} + I_{H_{6'}}\right)}{12\,x\,(I_{region\,1}) - 2\,x\left(I_{H_{2'-4'+7'+8'}} + I_{H_{6'}}\right)} = 0.109 = 10.9\ \%$$

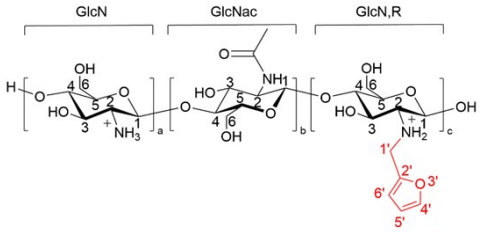
Notes:

- The integral of the protons on the 5' position overlapped with the remaining acetyl and acetic acid $\underline{CH_3}$ signals and the protons on the 9' position overlapped with the $\underline{CH_3}$ signals of ethanol. Therefore, these positions could not be integrated and were omitted in the calculation.

- The exact position of the $H_{2'}$ and $H_{5'}$ protons is still up for debate and will be the subject of further study within our group. One of two possibilities is displayed and utilized for the calculation as the exact position is not required as both positions have two protons.

^{1}H and ^{13}C peak tables of all the *N*-(furan-2-yl)methyl chitosan derivatives

_N_-(furan-2-yl)methyl chitosan:



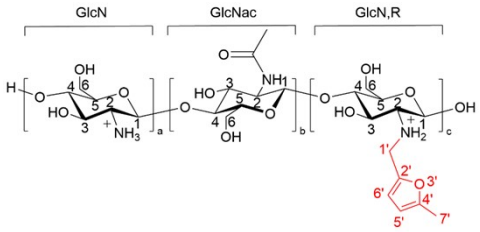
^{1}H

Unit	H1	H2	H3	H4	H5	$H6_a$	$H6_b$	H1’	H4’	H5’	H6’
GlcN	4.83	3.17	3.89	3.90	3.69	3.84	3.96				
GlcN,R	5.02	3.26	4.00	3.96	3.74	3.84	3.96	4.53	7.62	6.66	6.52

^{13}C

Unit	C1	C2	C3	C4	C5	C6	C1’	C2’	C4’	C5’	C6’
GlcN	100.3	58.6	72.7	79.5	77.5	62.6					
GlcN,R	99.7	63.5	72.2	79.7	77.2	63.3	45.9	146.7	147.8	116.2	113.9

_N_-(5-methylfuran-2-yl)methyl chitosan:



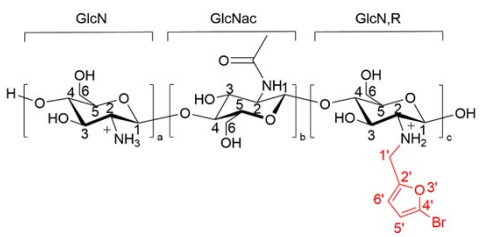
^{1}H

Unit	H1	H2	H3	H4	H5	$H6_a$	$H6_b$	H1’	H5’	H6’	H7’
GlcN	4.84	3.17	3.89	3.90	3.69	3.75	3.92				
GlcN,R	5.02	3.26	4.02	3.96	3.73	3.85	3.97	4.46	6.11	6.53	2.29

^{13}C

Unit	C1	C2	C3	C4	C5	C6	C1’	C2’	C4’	C5’	C6’	C7’
GlcN	100.3	58.6	72.8	79.1	77.4	62.6						
GlcN,R	99.7	63.3	72.2	79.4; 79.8	77.2	63.3	46.0	144.8	158.0	109.7	117.2	15.4

_N_-(5-bromofuran-2-yl)methyl chitosan:



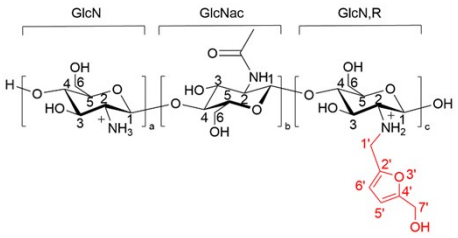
^{1}H

Unit	H1	H2	H3	H4	H5	$H6_a$	$H6_b$	H1′	H5′	H6′
GlcN	4.87	3.19	3.89	3.93	3.73	3.77	3.95			
GlcN,R	5.03	3.26	4.04	3.98	3.77	3.89	3.97	4.52	6.70	6.54

^{13}C

Unit	C1	C2	C3	C4	C5	C6	C1′	C2′	C4′	C5′	C6′
GlcN	100.4	58.6	72.8	79.1	77.5	62.7					
GlcN,R	99.7	63.5	72.4	79.5;79.9	77.2	63.5	46.0	148.9	126.8	119.3	116.0

N-(5-(hydroxymethyl)furan-2-yl)methyl chitosan:



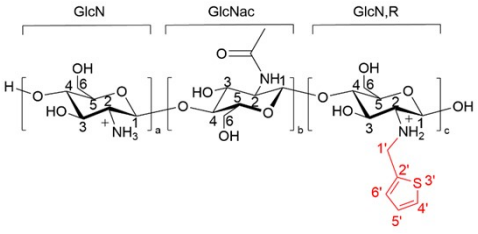
^{1}H

Unit	H1	H2	H3	H4	H5	$H6_a$	$H6_b$	H1′	H5′	H6′	H7′
GlcN	4.87	3.20	3.91	3.93	3.73	3.77	3.96				
GlcN,R	5.05	3.29	4.05	3.99	3.78	3.87	3.99	4.55	6.64	6.46	4.61

^{13}C

Unit	C1	C2	C3	C4	C5	C6	C1′	C2′	C4′	C5′	C6′	C7′
GlcN	100.4	58.6	72.8	79.1	77.5	62.7						
GlcN,R	99.7	63.5	72.3	79.4;79.6;79.8	77.3	63.3	45.9	146.9	158.5	117.1	112.4	58.5

N-(thiophen-2-yl)methyl chitosan:



^{1}H

Unit	H1	H2	H3	H4	H5	$H6_a$	$H6_b$	H1′	H4′	H5′	H6′
GlcN	4.85	3.17	3.89	3.90	3.70	3.75	3.92				
GlcN,R	5.03	3.24	4.01	3.96	3.77	3.82	3.95	4.71	7.59	7.31	7.14

Note: the aromatic signals are broadened due to pi-pi stacking in water (see Figure S24).

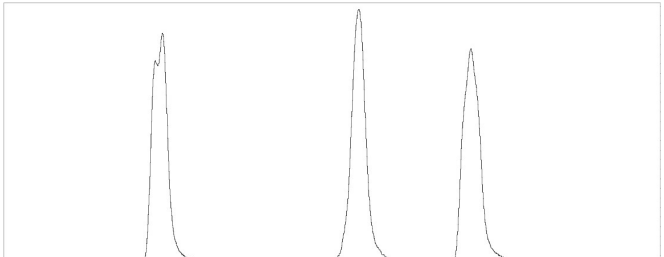
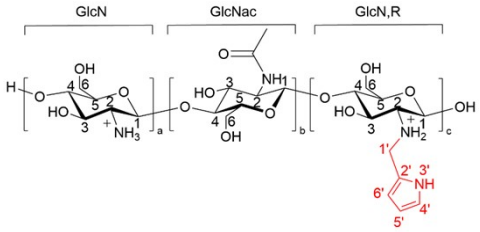


Figure S24. Zoom of the aromatic region of the ^{1}H NMR (400 MHz) spectrum of N-(thiophen-2-yl)methyl chitosan at 20 mg/mL in 1 vol% d-TFA in D_2O.

^{13}C

Unit	C1	C2	C3	C4	C5	C6	C1'	C2'	C4'	C5'	C6'
GlcN	100.3	58.5	72.8	79.1	77.4	62.6					
GlcN,R	99.7	62.7	72.3	79.3	77.3	63.3	47.7	133.6	131.9	134.5	130.7

_N_-(1_H_-pyrrol-2-yl)methyl chitosan:



^{1}H

Unit	H1	H2	H3	H4	H5	$H6_a$	$H6_b$	H1'	H4'	H5'	H6'
GlcN	4.86	3.17	3.89	3.91	3.72	3.77	3.94				
GlcN,R	5.00							4.51	6.98	6.40	6.26

Note: no new ^{13}C signals could be observed, possibly due to extensive overlap as well as the low DS_{NMR}. However, new H1 and H1' signals could be observed, which were in a similar range as the other obtained compounds, confirming the substitution.

Green metric calculations

Note: for all subsequent calculations, the loss of mass due to the water formed during the imine formation will be neglected, to preserve clarity while having a minimal impact on the obtained results.

RME:

$$RME = \frac{\sum total\ reactant\ mass\ that\ is\ attached\ to\ chitosan}{\sum total\ reactant\ mass}$$

For the work of Sela *et al.*[22]:

Assumptions:

- The reagents required for the carboxymethylation were ignored as this is not required for the reaction, but rather for the subsequent functionality of the obtained products.
- Degradation of the aldehyde reaction media (oxidation/aldol condensation, …) over time due to the applied heat and acidic conditions was neglected.
- The minimal reaction volume required was set at half the initial volume.
- Changes in the reaction volume due to the imine formation (reaction water and aldehyde consumption) were neglected.
- Every cycle 6.7 vol.% of the reaction media is lost.
- The molar mass of the utilized K-CMC is 252 g/mol.
- The obtained DS is equal to 3.1 % for every cycle.

Added reagents	amount

K-CMC	0.250 g
Citral (ρ = 0.893 $\frac{g}{mL}$, M = 152.24 $\frac{g}{mol}$)	5 mL
	4.465 g
Acetic acid (ρ = 1.05 $\frac{g}{mL}$)	0.125 mL
	0.13125 g

Number of cycles until half of the initial volume is leftover:

$2.5625\ mL\ =\ 5.125\ mL * 0.933^{x}$

$x = 10$

Amount of K-CMC modified after 10 cycles:

$$\sum_{n=0}^{10} 0.250 *\ 0.933^{n}\ = 1.99\ g$$

Amount of citral added onto the chitosan chain:

$$\frac{1.99\ g}{252\ \frac{g}{mol}} * 0.031 * 152.24\ \frac{g}{mol} = 0.037\ g$$

$$RME\ [\%] = \frac{0.037}{4.465} * 100 = 0.84$$

For the work of Marin *et al.*[14]:

An amino conversion of 62 % is reported at an amine:aldehyde ratio of 1/1. So the RME is also equal to 62 % with an obtained DS of 52.7 % $(62 * DDA\ (\ = 0.85))$.

For the work of Lin *et al.*[23]*:*

Assumptions:

- The DS is equal to 85.68 % as reported by Lin *et al.*[23]

Added reagents	amount
Chitosan (DDA ≈ 100 %, M = 161.16)	5 g
Citronellal (M = 154.25 $\frac{g}{mol}$)	20 g

Amount of citronellal added onto the chitosan chain:

$$\frac{5\ g}{161.16\ \frac{g}{mol}} * 0.8568 * 154.2 = 4.1\ g$$

$$RME\ [\%] = \frac{4.1\ g}{20\ g} = 20.1$$

For this work:

Assumptions:

- The extensive milling (60 min) completely deacetylated the product (DDA = 100 %) (DS_{EA} = % amino conversion)

$$RME\ [\%] = \frac{\%\ amino\ conversion/100}{amine{:}aldehyde\ ratio} * 100 = \frac{\frac{DS_{EA}}{100}}{added\ equivalents} * 100 = \frac{1}{1} * 100 = 100$$

PMI:

$$PMI = \frac{\sum total\ process\ mass}{mass\ of\ isolated\ product}$$

For the work of Desbières *et al.*[9]

Assumptions:

- The required amount of base to reach a pH of 5.1 was not reported, so this was neglected.
- The amount of ethanol that was required to dissolve the octanal and add it to the reaction was not reported, so it was neglected.
- All the synthesized product could be recovered afterwards.

Synthesis:

Added reagents	amount
Chitosan (DDA = 98 %, M = 161.16 * 0.98 + 0.02 * 203.2 = 162 $\frac{g}{mol}$)	4 g
Water (ρ = 1 $\frac{g}{mL}$)	217.48 mL
	217.48 g
Acetic acid (ρ = 1.05 $\frac{g}{mL}$)	2.52 mL
	2.64 g
Ethanol (ρ = 0.789 $\frac{g}{mL}$)	150 mL
	118.35 g
Octanal (ρ = 0.789 $\frac{g}{mL}$, $M = 128.212\ \frac{g}{mol}$)	0.312 g ($\frac{4\ g}{162\ \frac{g}{mol}} * 0.1 * 128.212\ \frac{g}{mol}$)
$NaCNBH_3$ (M = 62.84 $\frac{g}{mol}$)	4.65 g ($\frac{4\ g}{162\ \frac{g}{mol}} * 3 * 62.84\ \frac{g}{mol}$)

Obtained product mass:

The obtained DS was 12.5 %, which is higher than the expected 10 %, so all octanal mass was added onto the chitosan chain.

$4 + 0.321 = 4.321\ g$

$$PMI_{reaction} = \frac{4\ g + 217.48\ g + 2.64\ g + 118.35\ g + 0.321\ g + 4.65\ g}{4.321\ g} = 80.4$$

Isolation: No data reported.

For our previous work.

Assumptions:

- The amount of HCl and water required for the acidification up to pH = 3 is neglected.

Synthesis:

Added reagents	amount
Chitosan (DDA = 92 %, $\frac{5.27\ mmol\ NH_2}{g}$)	2 g
Water (ρ = 1 $\frac{g}{mL}$)	198 mL
	198 g
Acetic acid (ρ = 1.05 $\frac{g}{mL}$)	2 mL
	2.1 g
Cetylpyridinium chloride	0.1 g
Octanal (ρ = 0.789 $\frac{g}{mL}, M = 128.212\ \frac{g}{mol}$)	0.169 g ($\frac{5.27\ mmol\ NH_2}{gram} * 2\ g * 0.125\ * 128.212\ \frac{g}{mol}/1000$)
PICB (M = 106.96 $\frac{g}{mol}$)	0.113 g ($\frac{5.27\ mmol\ NH_2}{gram} * 2\ g * 0.1\ * 106.96\ \frac{g}{mol}/1000$)

Measured obtained product mass:

2.07 g

$$PMI_{reaction} = \frac{2\ g + 198\ g + 2.1\ g + 0.1\ g + 0.169\ g + 0.113\ g}{2.07\ g} = 97.8$$

Isolation:

Added reagents	amount
Methanol (ρ = 0.792 $\frac{g}{mL}$)	20 mL
	15.84 g
Acetone (ρ = 0.784 $\frac{g}{mL}$)	600 mL
	470.4 g

$$PMI_{isolation} = \frac{15.84\ g + 470.4\ g}{2.07\ g} = 234.9$$

$$PMI = PMI_{reaction} + PMI_{isolation} = 97.8 + 234.9 = 332.7$$

For this work:

Assumptions:

- The added drop of acetic acid during product isolation is neglected.

Synthesis:

Added reagents	amount
Chitosan (DDA = 92 %, $\frac{5.27\ mmol\ NH_2}{g}$)	0.5 g
Octanal (ρ = 0.789 $\frac{g}{mL}$, $M = 128.212\ \frac{g}{mol}$)	0.04 g ($\frac{5.27\ mmol\ NH_2}{gram} * 0.5\ g * 0.125\ * 128.212\ \frac{g}{mol}/1000$)
$NaBH_4$ (M = 37.83 $\frac{g}{mol}$)	0.01 g ($\frac{5.27\ mmol\ NH_2}{gram} * 0.5\ g * 0.1\ * 37.83\ \frac{g}{mol}/1000$)

Measured obtained product mass:

0.32 g

$$PMI_{reaction} = \frac{0.5\ g + 0.04\ g + 0.01\ g}{0.32\ g} = 1.73$$

Isolation:

Added reagents	amount
Water (ρ = 1 $\frac{g}{mL}$)	25 mL
	25 g
Ethanol (ρ = 0.789 $\frac{g}{mL}$)	25 mL
	19.725 g

$$PMI_{isolation} = \frac{25\ g + 19.725\ g}{0.32\ g} = 139.8$$

$$PMI = PMI_{reaction} + PMI_{isolation} = 1.73 + 139.8 = 141.5$$

For the small-scale synthesis of *N*-(furan-2-yl)methyl chitosan within this work:

Assumptions:

- The mass of the cotton utilized to filter the material was neglected.

Synthesis:

Added reagents	amount
Chitosan (DDA = 92 %, $\frac{5.27\ mmol\ NH_2}{g}$)	0.5 g
Furfural (ρ = 1.1601 $\frac{g}{mL}$, $M = 96.085\ \frac{g}{mol}$)	0.25 g ($\frac{5.27\ mmol\ NH_2}{gram} * 0.5\ g * 1\ * 96.085\ \frac{g}{mol}/1000$)
$NaBH_4$ (M = 37.83 $\frac{g}{mol}$)	0.1 g ($\frac{5.27\ mmol\ NH_2}{gram} * 0.5\ g * 1\ * 37.83\ \frac{g}{mol}/1000$)

Measured obtained product mass:

0.306 g

$$PMI_{reaction} = \frac{0.5\ g + 0.25\ g + 0.1\ g}{0.306\ g} = 2.78$$

Isolation:

Added reagents	amount
Water (ρ = 1 $\frac{g}{mL}$)	24.85 mL
	24.85 g
Acetone (ρ = 0.784 $\frac{g}{mL}$)	50 mL
	39.2 g
Acetic acid (ρ = 1.05 $\frac{g}{mL}$)	0.15 mL
	0.1575 g

$$PMI_{isolation} = \frac{24.85\ g + 39.2\ g + 0.1575\ g}{0.306\ g} = 209.8$$

$$PMI = PMI_{reaction} + PMI_{isolation} = 2.77 + 209.8 = 212.6$$

For the big-scale synthesis of *N*-(furan-2-yl)methyl chitosan within this work:

Assumptions:

- The mass of the cotton utilized to filter the material was neglected.

Synthesis:

Added reagents	amount
Chitosan (DDA = 92 %, $\frac{5.27\ mmol\ NH_2}{g}$)	2.5 g
Furfural (ρ = 1.1601 $\frac{g}{mL}$, $M = 96.085\ \frac{g}{mol}$)	1.266 g ($\frac{5.27\ mmol\ NH_2}{gram} * 2.5\ g * 1\ * 96.085\ \frac{g}{mol}/1000$)
$NaBH_4$ (M = 37.83 $\frac{g}{mol}$)	0.5 g ($\frac{5.27\ mmol\ NH_2}{gram} * 2.5\ g * 1\ * 37.83\ \frac{g}{mol}/1000$)

Measured obtained product mass:

1.571 g

$$PMI_{reaction} = \frac{2.5\ g + 1.266\ g + 0.5\ g}{1.571\ g} = 2.72$$

Isolation:

Added reagents	amount
Water (ρ = 1 $\frac{g}{mL}$)	114.35 mL
	114.35 g
Acetone (ρ = 0.784 $\frac{g}{mL}$)	100 mL
	78.4 g
Acetic acid (ρ = 1.05 $\frac{g}{mL}$)	0.65 mL
	0.6825 g

$$PMI_{isolation} = \frac{114.35\ g + 78.4\ g + 0.6825\ g}{1.571\ g} = 123.1$$

$$PMI = PMI_{reaction} + PMI_{isolation} = 2.72 + 123.1 = 125.8$$