Supplementary Information

Effect of room temperature ionic liquids on selective biocatalytic hydrolysis of chitin *via* sequential or simultaneous strategies

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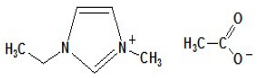
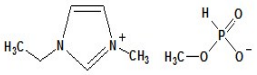
1. Selected room temperature ionic liquids

Table S1. Chemical structure and corresponding physico-chemical properties of the two distinct room temperature imidazolium-based ionic liquids used in this study [a].

Ionic Liquid	Structure	Abbreviation	Molar mass ($g.mol^{-1}$)	Melting Point	Density	Viscosity (cP at 25°C)	Kamlet-Taft parameters at 25°C		
							α	β	π*
1-ethyl-3-methylimidazolium acetate		$[C_2mim][OAc]$	170.22	< -20°C	1.03	93	0.47[b]	1.11[b]	1.04[b]
1-ethyl-3-methylimidazolium methylphosphonate		$[C_2mim][MeO(H)PO_2]$	206.18	ND	ND	107	0.52[c]	1.00[c]	1.06[c]

[a] Data from Solvionic SA (Verniolle, France) index; [b] From C. Froschauer, M. Hummel, M. Iakovlev, A. Roselli, H. Schottenberger, and H. Sixta, *Biomacromolecules* 2013, *14*: 1741-1750; [c] From Y. Fukaya, K. Hayashi, M. Wada and H. Ohno, Green Chem., 2008, 10: 44-46. ND: not determined.

2. Characteristic HPLC chromatograms

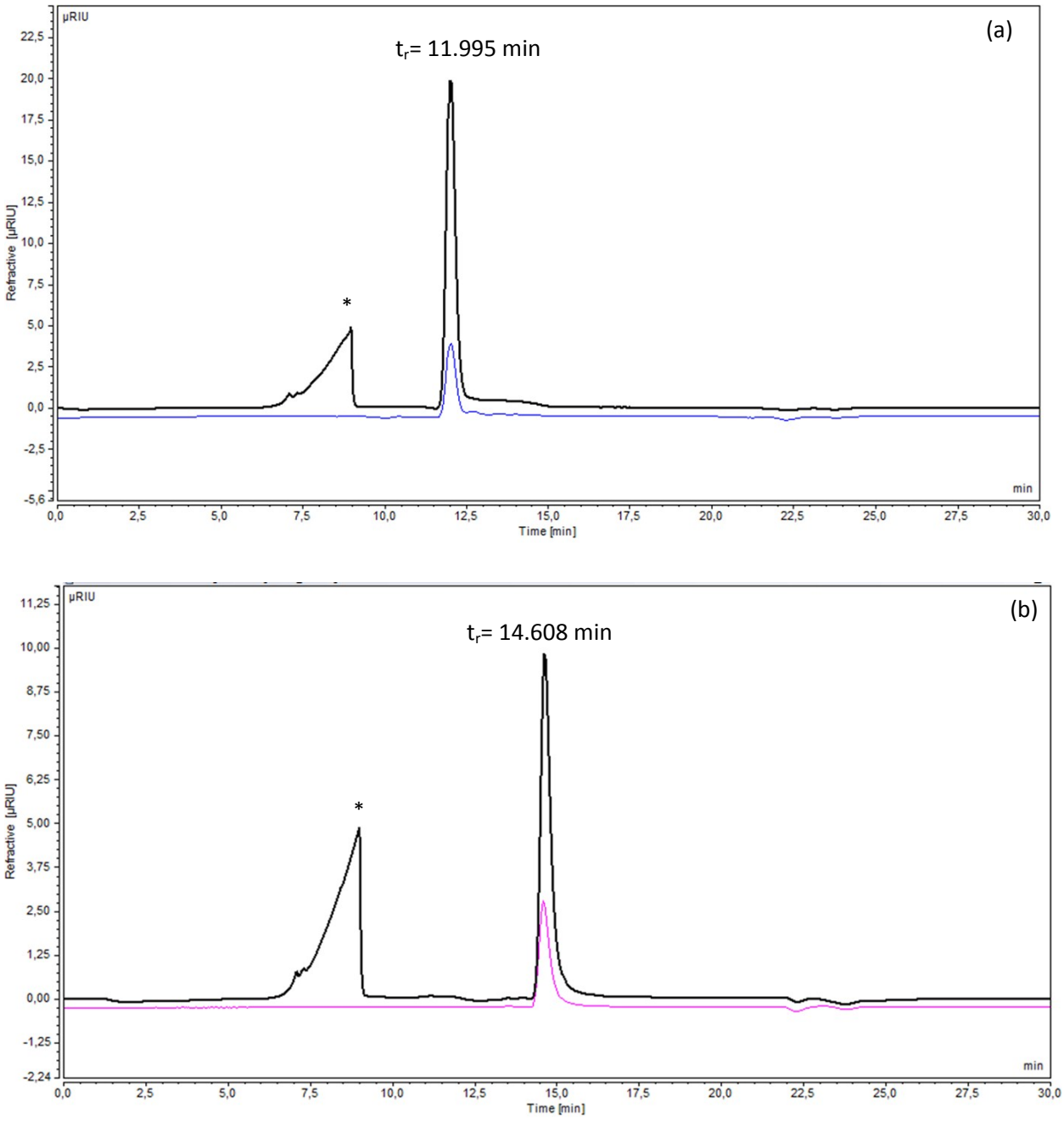


Figure S1. Characteristic HPLC chromatograms of: (a) standard *N,N'*-diacetylchitobiose (blue line) and supernatant of hydrolysate (black line) obtained by enzymatic hydrolysis of [C_2mim][OAc]-pretreated chitin in aqueous buffer catalyzed by the chitinase from *S. griseus* for 24h and (b) standard *N*-acetylglucosamine (pink line) and supernatant of hydrolysate (black line) obtained by enzymatic hydrolysis of [C_2mim][OAc]-pretreated chitin in aqueous buffer. This reaction was catalyzed by the chitinase from *S. griseus* for 24h then supplemented by the chitinase from *T. viride* for 12h. *identified as enzymes preparation and aqueous buffer.

3. SEM micrographs

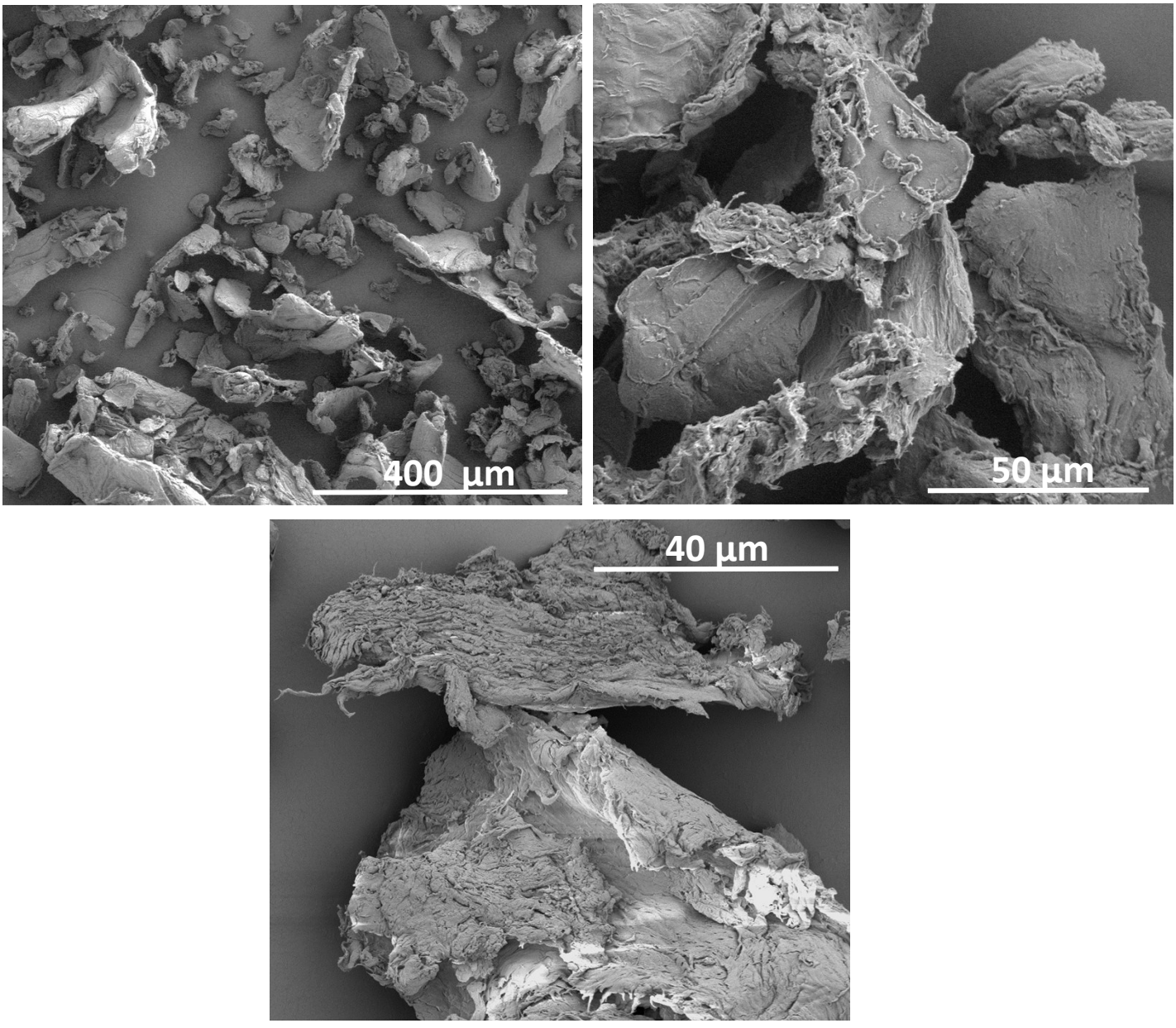


Figure S2. SEM images of untreated chitin.

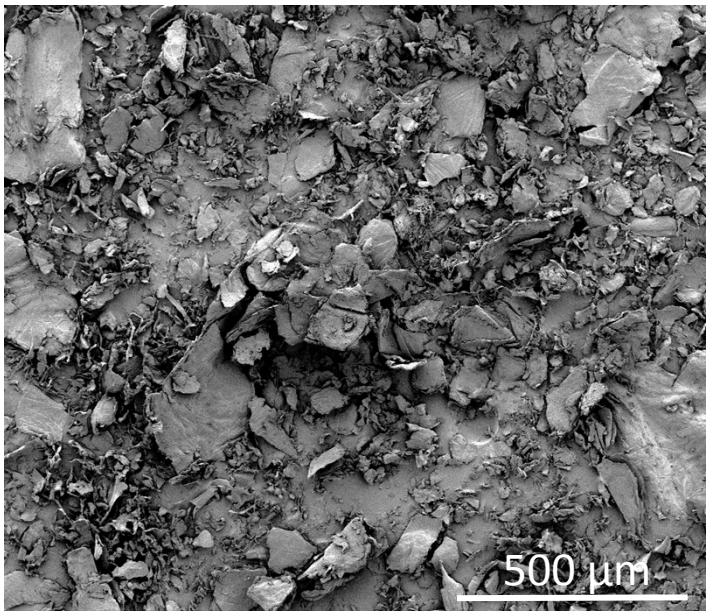


Figure S3. SEM images of $[C_2mim][MeO(H)PO_2]$-pretreated chitin.

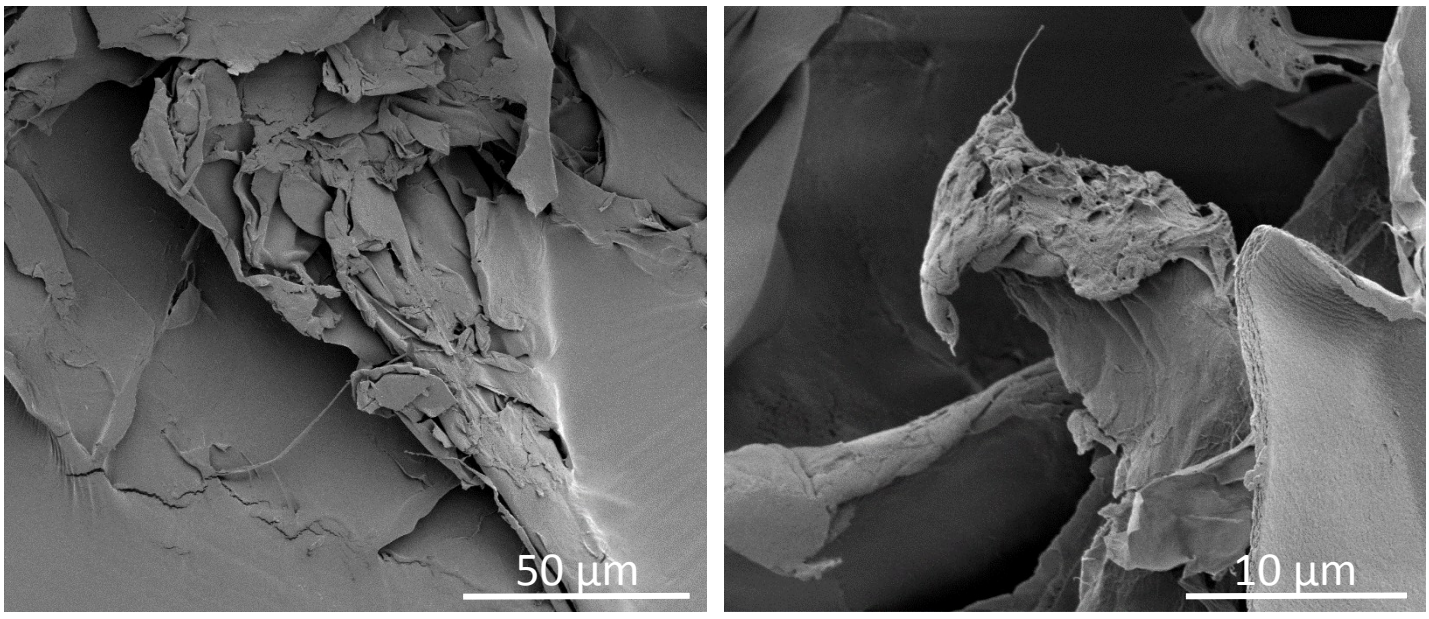


Figure S4. SEM images of $[C_2mim][OAc]$-pretreated chitin.

4. Solid-state ^{13}C NMR

Solid-state ^{13}C NMR spectra were obtained with 4 mm CP-MAS probe operating at 125.7452 MHz on a Bruker DRX 500 at 25 °C. The spinning rate was 5 kHz with 2 ms contact times and 5 s delay between scans (2048 scans). The degree of acetylation (DA) corresponds to the ratio of integrated methyl peak area to the sum of integrated carbons peaks. The chemical shifts were externally referred by setting the carbonyl resonance of glycine to 176.03 ppm.

Ref: Kasaai, M. *J. Agric. Food Chem.* 2009, *57*, 1667-1676. Younes, I.; Rinaudo, M. *Mar. Drugs* 2015, *13*, 1133-1174. Heux, L.; Brugnerotto, J.; Desbrière, J.; Versali, M.-F.; Rinaudo, M. Biomacromolecules 2000, *1*, 746-751. Duarte, M. L.; Ferreira, M. C.; Marvao, M. R.; Rocha, J. *Int. J. Biol. Macromolec.* 2001, *28*, 359-363.

CP-MAS ^{13}C NMR (25 °C, 125 MHz): δ_C 173.8 (C=O), 104.1 (C1), 83.0 (C4), 75.7 (C5), 73.3 (C3), 60.8 (C6), 55.2 (C2), 22.8 (CH_3).

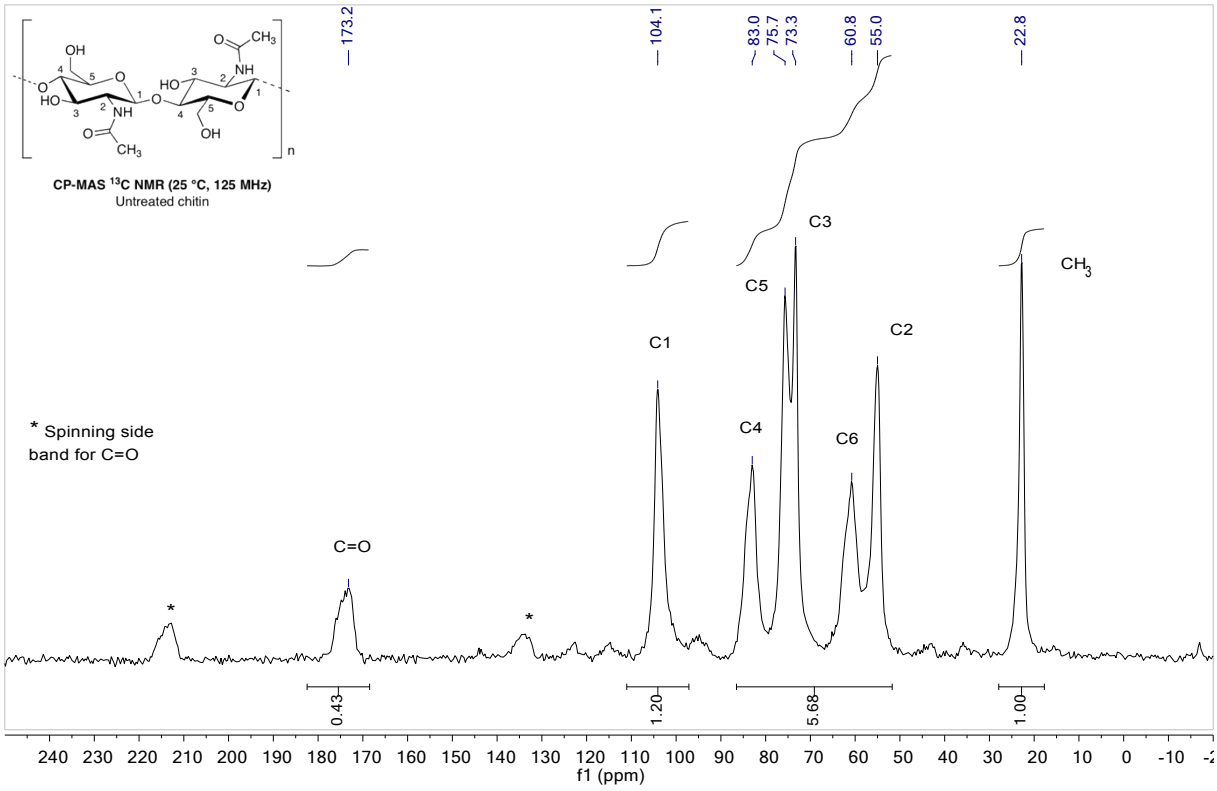


Figure S5. Solid-state CP-MAS ^{13}C NMR spectrum of untreated chitin (DA = 87%).

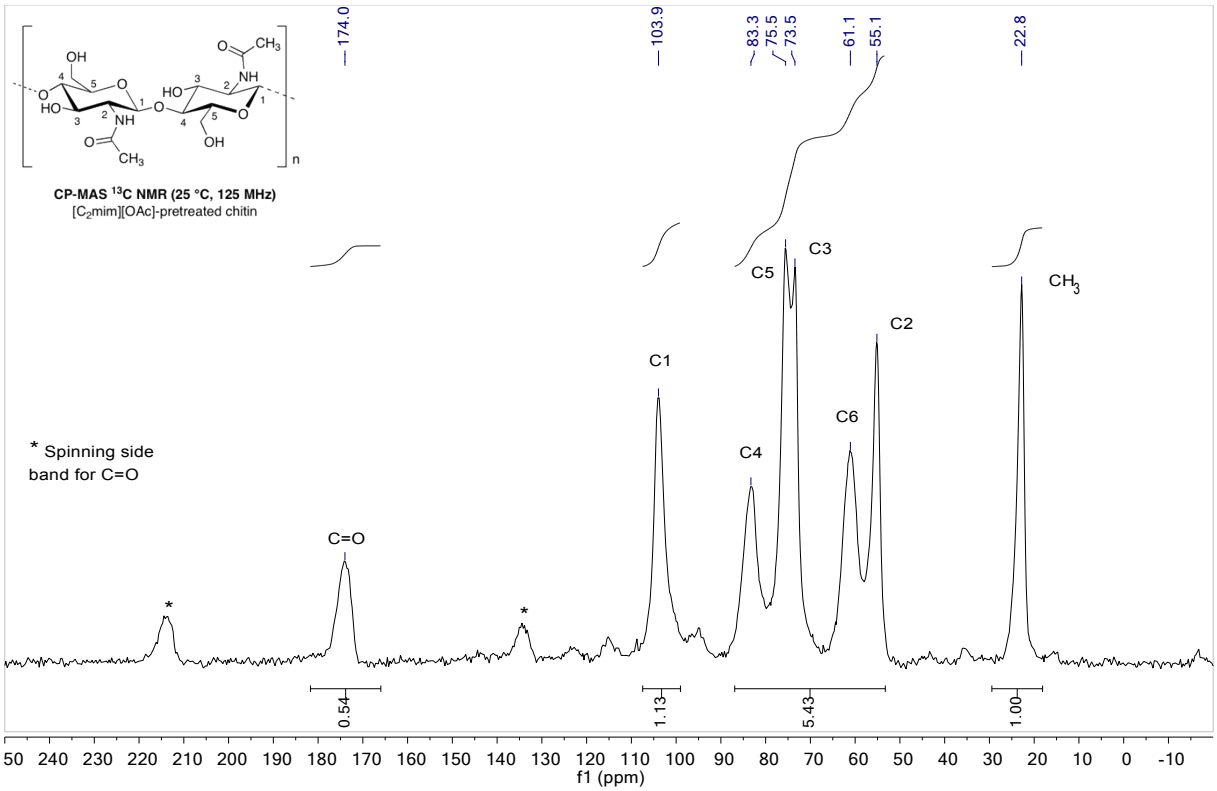


Figure S6. Solid-state CP-MAS ^{13}C NMR spectrum of [C_2mim][OAc]-pretreated chitin (DA = 91%).

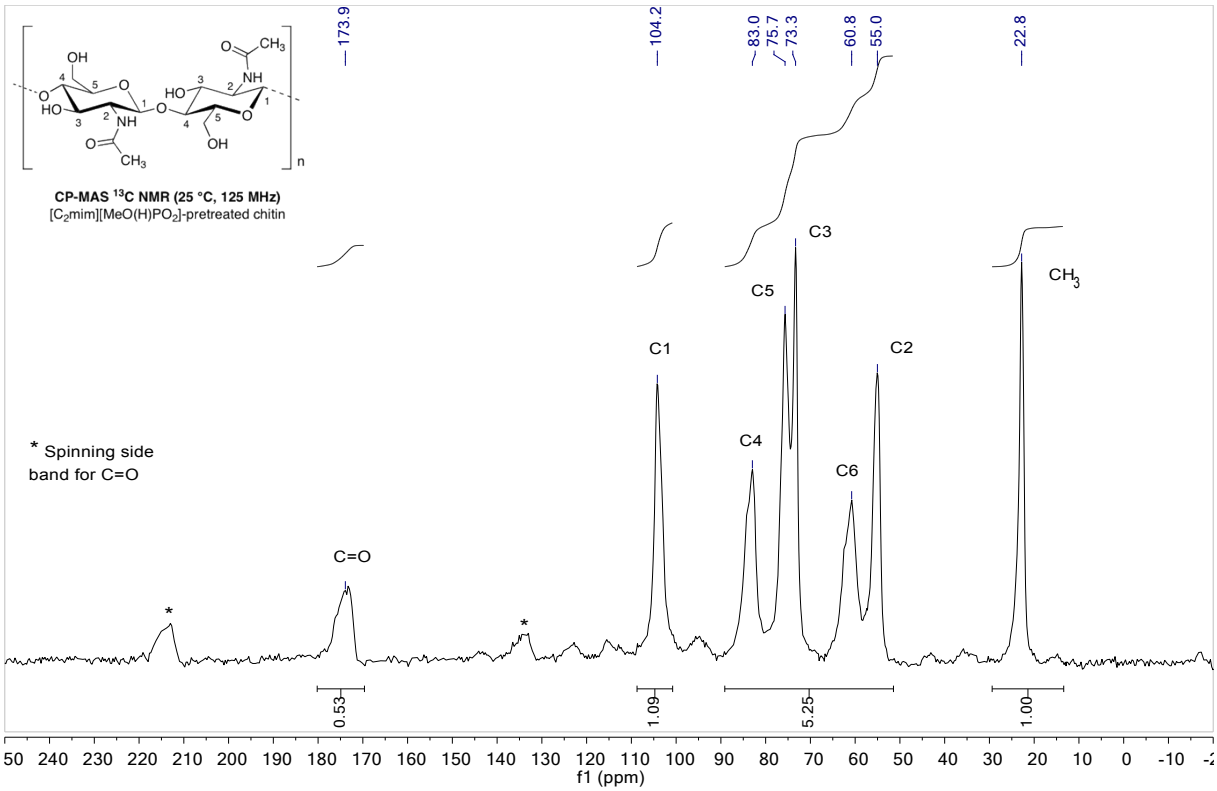


Figure S7. Solid-state CP-MAS ^{13}C NMR spectrum of [C_2mim][MeO(H)PO_2]-pretreated chitin (DA = 94%).

5. Infra-Red spectra

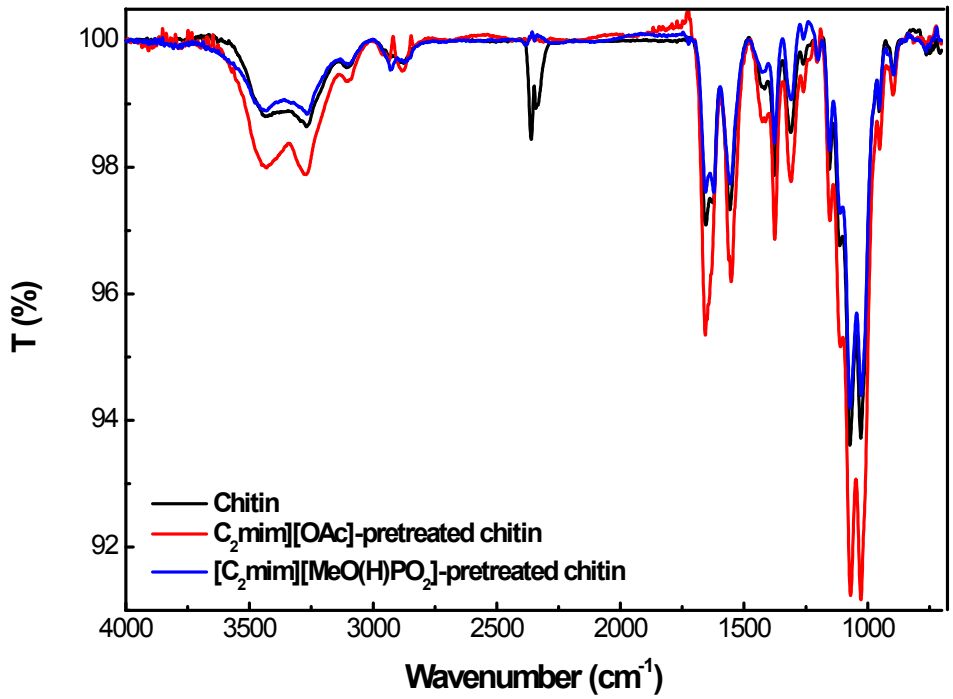


Figure S8. IR spectra of untreated chitin (black line), $[C_2mim][OAc]$-pretreated chitin (red line) and $[C_2mim][MeO(H)PO_2]$-pretreated chitin (blue line).

6. Mass spectra of isolated hydrolysis products

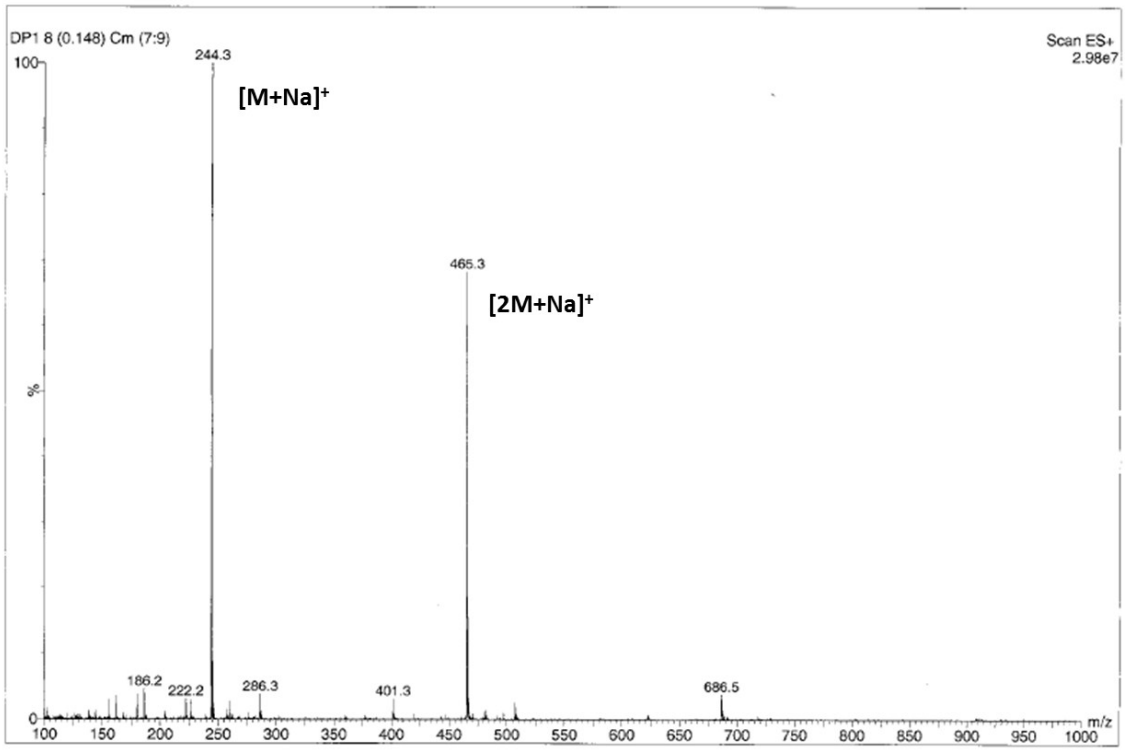


Figure S9. ESI-MS spectrum of the isolated *N*-acetylglucosamine.

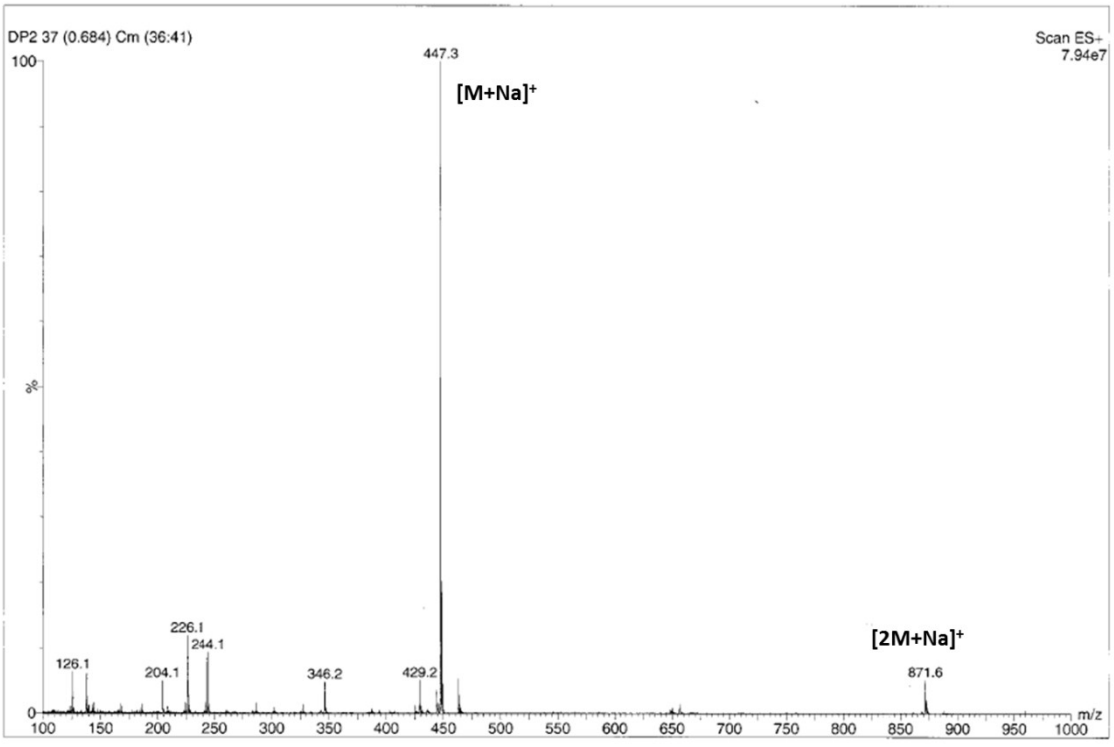


Figure S10. ESI-MS spectrum of the isolated *N,N'*-diacetylchitobiose

7. Solubility test of chitin in room temperature ionic liquids

The experimental procedure used for this solubility test was adapted from the one proposed by Fukaya et al about dissolution studies of recalcitrant polysaccharide in RTIL**.**
Suspensions of commercial chitin (0.05, 0.1, 0.2, 0.5, 1.0 and 1.5 and 2.0 % w/v) were incubated in [C_2mim][OAc] and [C_2mim][MeO(H)PO_2] at 110 °C for 40 min under vigorous stirring. The highest concentration leading to a clear solution was evaluated as the maximal solubility value. In [C_2mim][OAc], clear solutions were observed for 0.05 % and 0.1 % w/v of chitin whereas no clear solution was recorded for [C_2mim][MeO(H)PO_2] as depicted above.
Ref: Y. Fukaya, K. Hayashi, M. Wada and H. Ohno, Green Chem., 2008, 10, 44-46.

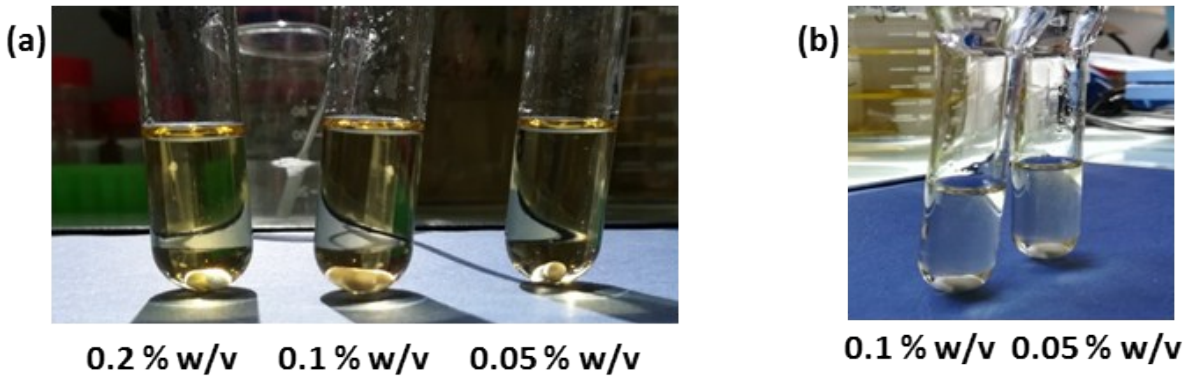


Figure S11. Solubilization test of chitin in [C_2mim][OAc] (a) and [C_2mim][MeO(H)PO_2] (b) at 110 °C for 40 min.

8. Best experimental procedure to efficiently produce *N*-acetylglucosamine

Chitin (2% w/v) was added to 10 mL of [C_2mim][OAc], and incubated in an oil bath at 110 °C with stirring for 40 min. After incubation, each chitin suspension was cooled in an ice bath. The pretreated chitin was precipitated by adding 20 mL of ultra-pure water (deionized water with a resistivity of 18.3 MΩ cm, Barnsted Easy Pure RF) to the mixture (v/v) with vigorous stirring for 30 min in order to increase the polarity of the medium. After a centrifugation step (10,733 g, 20 min, 4 °C with an Allegra 64R Beckman Coulter Rotor: F0850), the supernatant containing [C_2mim][OAc] and water was kept for future recycling. The pretreated chitin powder was collected by vacuum filtration thoroughly and washed with ultra-pure water. The resulting solid was then poured into ultra-pure water, sonicated for 5 minutes and collected again by vacuum filtration. This step was repeated 5 times before freeze drying the product.
10 mg of [C_2mim][OAc]-pretreated chitin were added to 0.9 mL of phosphate buffer (10 mM, pH 6.0) and incubated for 30 min (40 °C; 1000 rpm). After this pre-incubation step, hydrolysis was initiated by addition of 100 µL of 1 mg/mL chitinase from *S. griseus* preparation. After 24 h of reaction, the second chitinase from *T. viride* was added to the first one. After 12 h, enzymatic reaction was stopped by incubating at 90 °C for 20 min to deactivate both enzymes. Supernatant was diluted in ultra-pure water and filtered (0.2 µm) prior to analysis by HPLC. The conversion yield into *N*-acetylglucosamine was 760.0 ± 0.1 mg / g chitin.

9. Best experimental procedure to efficiently produce *N,N'*-diacetylchitobiose

Chitin (2% w/v) was added to 10 mL of [C_2mim][OAc], and incubated in an oil bath at 110 °C with stirring for 40 min. After incubation, each chitin suspension was cooled in an ice bath. The pretreated chitin was precipitated by adding 20 mL of ultra-pure water (deionized water with a resistivity of 18.3 MΩ cm, Barnsted Easy Pure RF) to the mixture (v/v) with vigorous stirring for 30 min in order to increase the polarity of the medium. After a centrifugation step (10,733 g, 20 min, 4 °C with an Allegra 64R Beckman Coulter Rotor: F0850), the supernatant containing [C_2mim][OAc] and water was kept for future recycling. The pretreated chitin powder was collected by vacuum filtration thoroughly and washed with ultra-pure water. The resulting solid was then poured into ultra-pure water, sonicated for 5 minutes and collected again by vacuum filtration. This step was repeated 5 times before freeze drying the product.
10 mg of [C_2mim][OAc]-pretreated chitin were added to 0.9 mL of phosphate buffer (10 mM, pH 6.0) and incubated for 30 min (40 °C; 1000 rpm). After this pre-incubation step, hydrolysis was initiated by addition of 100 µL of 1 mg/mL chitinase from *S. griseus* preparation. After 48 h of reaction, enzymatic reaction was stopped by incubating at 90 °C for 20 min to deactivate the enzyme. Supernatant was diluted in ultra-pure water and filtered (0.2 µm) prior to analysis by HPLC. The conversion yield into *N,N'*-diacetylchitobiose was 705.0 ± 5.0 mg / g.