Supplementary Information

Multi-responsive cellulose nanocrystal-rhodamine conjugates – An advanced structure study by solid-state dynamic nuclear polarization (DNP) NMR

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Experimental section

Materials

Microcrystalline cellulose (MCC) with granule size of 50 μm, rhodamine B base (97%), 1,1'-carbonyldiimdazole (CDI) (CAS: 530-62-1) and ethylenediamine were purchased from Sigma-Aldrich (Steinheim, Germany). 2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO) was obtained from Acros (Geel, Belgium). Dialysis membrane from Spectrum Laboratories Inc. (Rancho Dominquez, USA) has an approximate molecular weight cut-off of 1000 Daltons. AMUPol was purchased from Aix-Marseille University. All chemicals were used without further purification.

Preparation of cellulose nanocrystals (CNC)

Cellulose nanocrystals were prepared according to literature procedures.[1] Microcrystalline cellulose (MCC) (5 g) was swollen in water (50 ml) for 96 h. Then, MCC was separated *via* centrifugation and re-dispersed in 250 ml water under vigorous stirring. TEMPO (0.0781 g) and NaBr (0.5144 g) were added to the cellulose suspension and the mixture was stirred for further 30 min. An aqueous NaClO solution (14 wt.%, 10.89 ml) was progressively added to the mixture at pH 10. After total addition of the NaClO solution, the pH value was further maintained using 1 M aqueous NaOH solution until the pH value did not change. After 15 min of ultrasonic treatment of the reaction mixture at ambient temperature, additional aqueous NaClO solution (14 wt.%, 10.89 ml) was progressively added to the mixture and the oxidation was repeated until the pH value was stable. After adjustment of the pH value to 7.5 using 1 M aqueous HCl solution, the oxidized cellulose was isolated by centrifugation (15 min, 4500 U/min), re-dispersed in water and placed into the ultrasonic bath for 1 h at room temperature. Finally, the suspension was dialyzed until the conductivity was ≈1 μS/cm and the total volume was adjusted to 200 ml.

Conductive titration

The ratio of the surface-exposed carboxylic to carbonyl groups of CNC was determined via electric conductivity titration. In order to evaluate the content of carboxylic groups, 3 ml of aqueous CNC suspension was pipetted in a beaker and the pH value was adjusted to 2 with 0.1 M HCl. The solution was titrated using 665 Dosimat (Metrohm) at the dosing rate of 0.01 ml/s, while the conductivity was recorded using 865 Conductivity Module (Metrohm) with an

interval of 2 s. In order to determine the content of aldehyde groups, the CNC suspension was oxidized with $NaClO_2$ at RT and pH value of 4-5. Then, the total carboxyl groups were titrated again and the difference between pre-oxidized and oxidized CNC demonstrated the content of aldehyde groups.

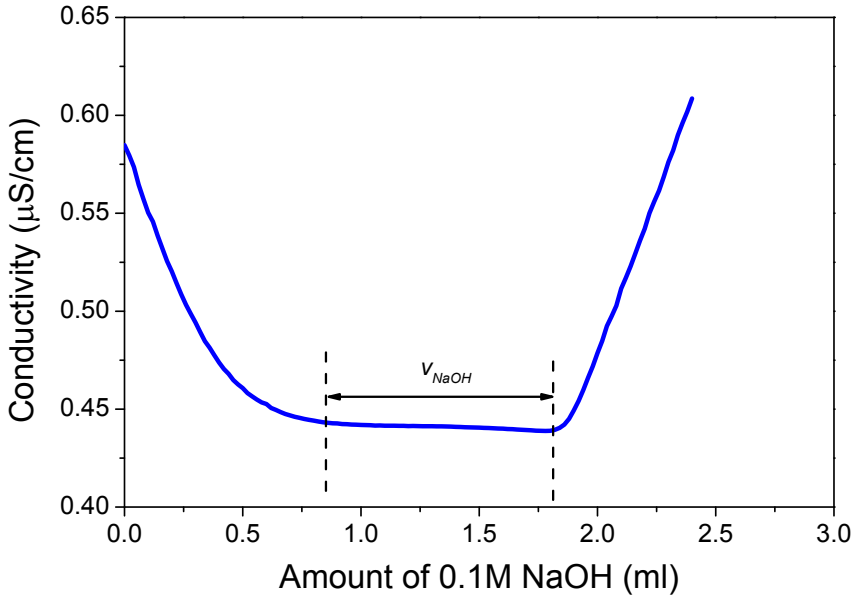
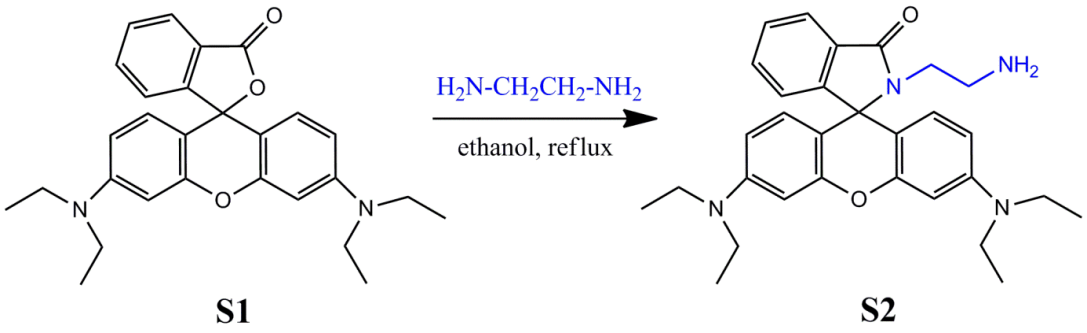


Figure S1. Conductive titration curve of CNC.

Synthesis of rhodamine methacrylamide

Amino ethyl rhodamine (S2) was synthesized as described before with a few modifications. Rhodamine**, S1** (1.15 g, 2.6 mmol) and ethylenediamine (2.03 g, 33.8 mmol) were dissolved in ethanol (100 ml) and refluxed for 16 h. The solvent was removed by evaporation, and the residue was dissolved in an aqueous HCl solution (1M, 100 ml). Aqueous NaOH solution (1 M) was added to the solution under stirring until the beginning of an appearance of pink precipitate. Then, the mixture was filtered; the solid aminoethyl rhodamine **S2** was washed with water, and dried in vacuum. Yield: 52.5%.



Scheme S1. Schematic representation of the synthesis of amino ethyl rhodamine (S2) from rhodamine (S**1**).

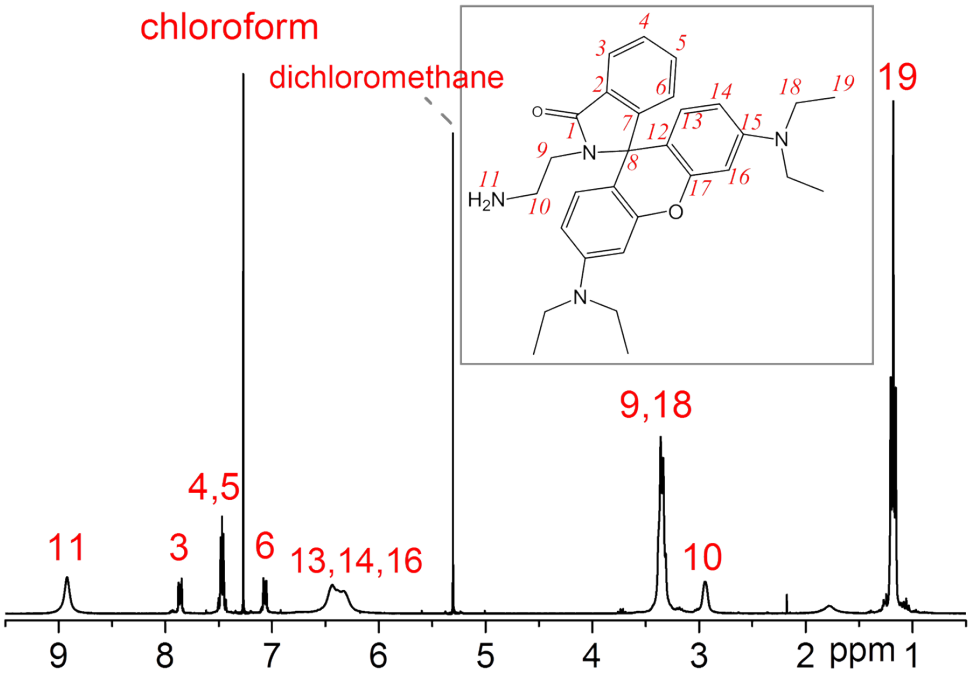


Figure S2. [1]H NMR spectrum of aminoethyl rhodamine **S2**.

Synthesis of CNC-aminoethyl rhodamine (CNC-RhB)

CNC (150 mg) was washed with acetone (20 ml x 2) and with N,N-dimethylformamide (DMF, 20 ml x 3), before it was dispersed in 8 ml DMF. Then, CDI (34 mg) was added to the **CNC** suspension. After stirring for 10 min, 50 mg aminoethyl rhodamine, synthesized as reported in the literature [2] (Scheme S1), was added and the mixture was stirred at 60℃ for 20 h. The product was separated via centrifugation and washed with DMF (25 ml each), until the supernatant became colorless. Finally, the product was dispersed in DMF and the concentration was adjusted to 8.9 mg/ml.

For the non-fluorescent **CNC-RhB** with expected close-ring rhodamine spiroamide, the DMF suspension was treated with aqueous NaOH (5 wt.%) solution at first. Then, **CNC-RhB** was separated by centrifugation and washed with acetone before drying at RT (**CNC-RhB-close**). The fluorescent **CNC-RhB** with expected open-ring RhB spiroamide was obtained after the treatment of the DMF suspension of **CNC-RhB** with aqueous HCl solution (5 wt.%) and separation via centrifugation (**CNC-RhB-open**). Heat-treated **CNC-RhB** was received after complete removal of DMF by washing with acetone, drying at RT and followed heat-treatment at 130°C for 1 h (**CNC-RhB-130°C**).

FTIR spectroscopy

FTIR spectroscopy was conducted on a Spectrum One FTIR Spectrometer (PerkinElmer, Massachusetts, USA) at RT between 4000 and 600 cm^{-1} with a step size of 4 cm^{-1}. The samples were measured twice per 32 scans and an average spectrum was then generated for each sample. Baseline correction was conducted using the method 'concave rubber band algorithm' with 200 baseline points and 5 iterations.

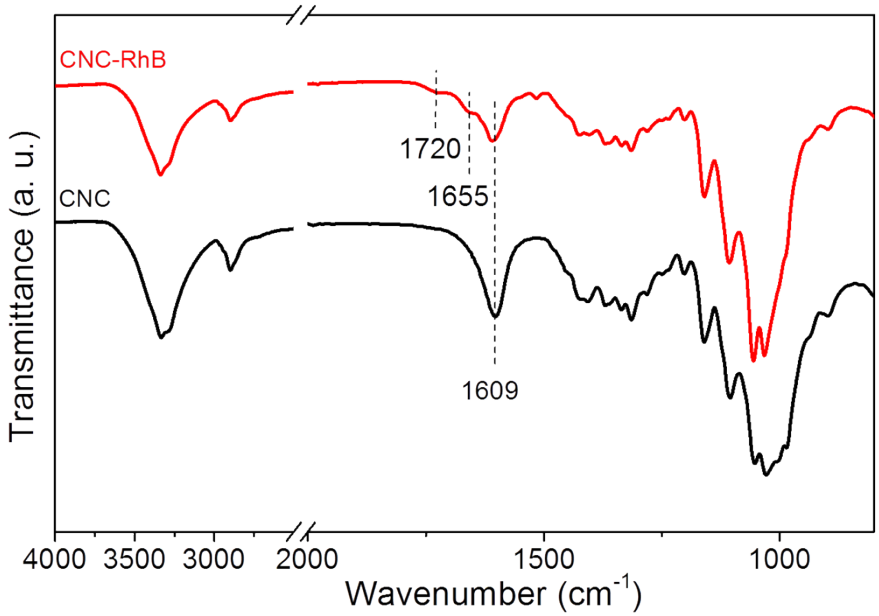


Figure S3. FTIR spectrum of CNC and CNC-RhB at room temperature.

The CNC-RhB showed slightly lower transmittance signals than CNC, which is probably due to the lower amount of CNC-RhB used for the FTIR measurement and the absorbance of the rhodamine spiroamide groups at CNC surface.

UV illumination and heat-treatment

The UV illumination of the samples was carried out at room temperature in a UV irradiation chamber (Bio-link 365 from Vilber Lourmat Deutschland GmbH, Eberhardzell, Germany) employing an excitation wavelength of 365 nm for 10 min. The heat-treatment was performed in a closed oven (TypKendro T12 from Heraeus Holding GmbH, Hanau, Germany) at 130°C.

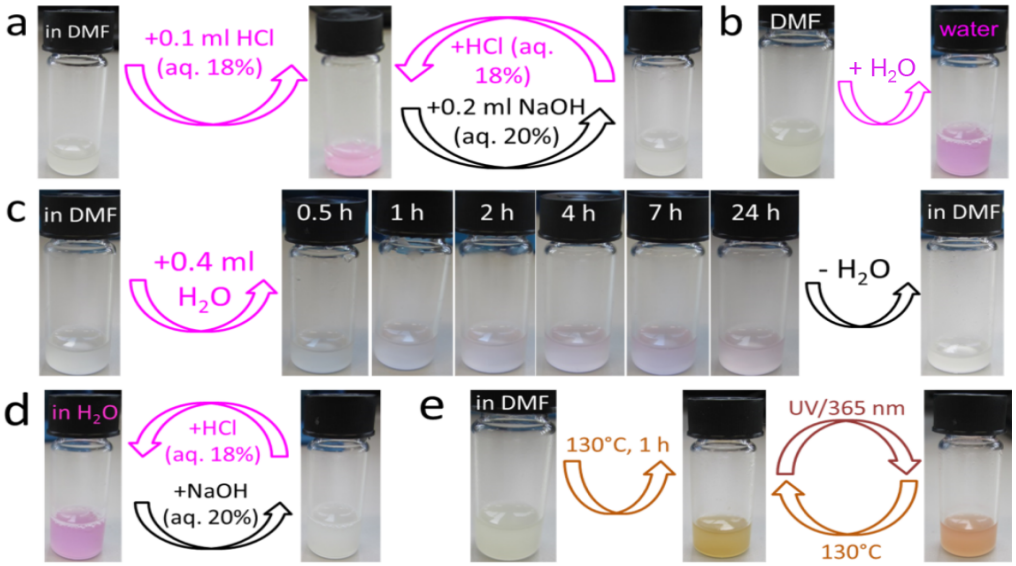


Figure S4. Representative photos representing the color change of **CNC-RhB**. The **CNC-RhB** in DMF after the synthesis has a neutral pH value. (a) Color changing of **CNC-RhB** in DMF by the addition of aqueous HCl (referred as **CNC-RhB-open**) or aqueous NaOH solution (referred as **CNC-RhB-close**). (b) Exchange of solvent from DMF into water leading

to the color change. (c) Time-dependent color development after the addition of 0.4 ml water to 1 ml suspension of **CNC-RhB** in DMF and the extraction of water from the suspension leading to disappearance of the magenta color. (d) Color-switching of **CNC-RhB** in water. (e) The heat-treatment of **CNC-RhB** in DMF (referred as **CNC-RhB-130°C**) with the brown color and followed color switching process.

Other characterizations

Elemental analysis

The contents of carbon, hydrogen and nitrogen of the samples were determined with the Elemental Analyser vario EL III CHN from Elementar (Hanau, Germany).

Atomic force microscopy (AFM)

Atomic force microscopic images were obtained using a scanning-force microscope (MFP-3D, Asylum research, Santa Babara, USA) with a tapping mode cantilever (Budget sensors, Sofia, Bulgaria, spring constant kc~48N/m, res. frequency ~190kHz) in tapping mode. The samples were deposited from aqueous solutions on a silicon wafer and dried overnight.

Fluorescence spectroscopy

Fluorescence measurements were carried out on a TIDAS S 700/ CCD UV/NIR fluorescence spectrometer linked with a monochromatic light source TIDAS LSM (J&M Analytik AG, Essingen, Germany). The whole system was processed using the TIDAS-DAQ software version 2.39 (J&M Analytik AG). Quartz cuvettes (Type 3, from Starna GmbH; Pfungstadt, Germany) were employed for each measurement. Typically, an excitation wavelength of 520 nm and an acquisition time of 400 ms were utilized. Spectra were accumulated with 100 scans.

Solid-state DNP measurements

For DNP measurements, samples were prepared according to reference.[3] In a typical procedure 15 mg of the sample was impregnated with 15 µL of a 20 mM AMUPol solution in a mixture of glycerol-d_8/D_2O/H_2O (60/30/10, v/v/v). Then, the slightly wet mixture was packed into a 3.2 mm sapphire rotor and capped with a zirconia drive cap.

It should be noted that **CNC-RhB-close** with rhodamine spiroamide in the closed-ring form is hydrophobic. The addition of the radical matrix to **CNC-RhB-close** led to the absence of signals for samples with an inhomogeneous mixed sample preparation (Figure S5). Thus, 10-15 µL THF (^{13}C NMR shifts at 24 ppm and 67 ppm) was added into the sample to increase the interaction between the radical matrix and **CNC-RhB-close**, so that signals of all carbons were obtained.

All spectra were recorded on a commercial 9.4T Bruker AVANCE III spectrometer system equipped with a 263 GHz gyrotron, a transmission line, and a Bruker triple-resonance 3.2 mm low temperature probe. All the CP MAS NMR spectra with and without microwave irradiation were recorded at a spinning rate of 8 kHz at sample temperatures of nominally 100 K. The CP field strengths were optimized with a linear ramp (75–100%).[4] The acquisition time in all experiments was set to 20 ms. All ^{13}C CP MAS NMR spectra resulted from the accumulation of 128 transients (*ca*. 20 min) with a recycle delay of 10 s and a contact time of 2 ms. ^{15}N CP MAS NMR spectra were recorded with 7400 scans, and with a repetition delay of 8 s according to a measurement time of about 16.5 h. A contact time of 3 ms was used. The ^{13}C chemical shifts were referenced to TMS, while ^{15}N chemical shifts were referenced to CH_3NO_2 employing $^{15}NH_4Cl$ (–341.168 ppm[5]) as external reference.

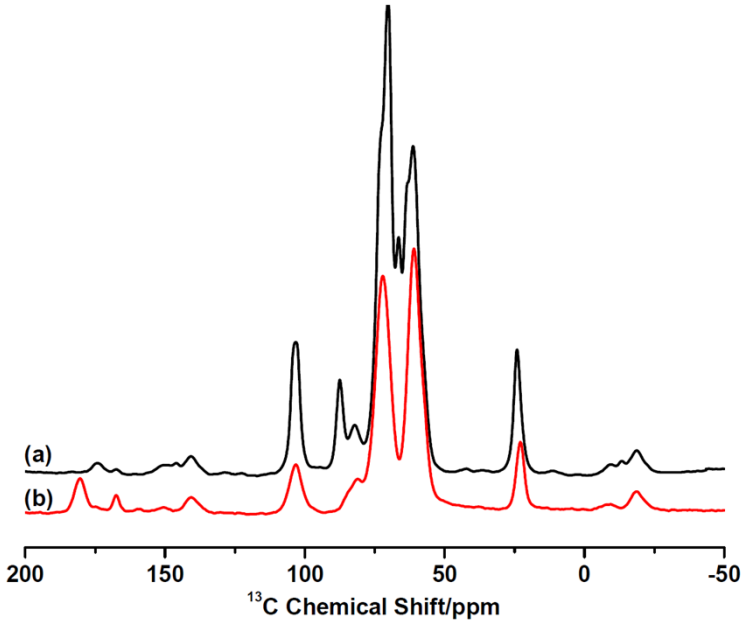


Figure S5. ^{13}C CP MAS DNP spectra of CNC-RhB-close as (a) homogeneously mixed sample, (b) inhomogeneously mixed sample.

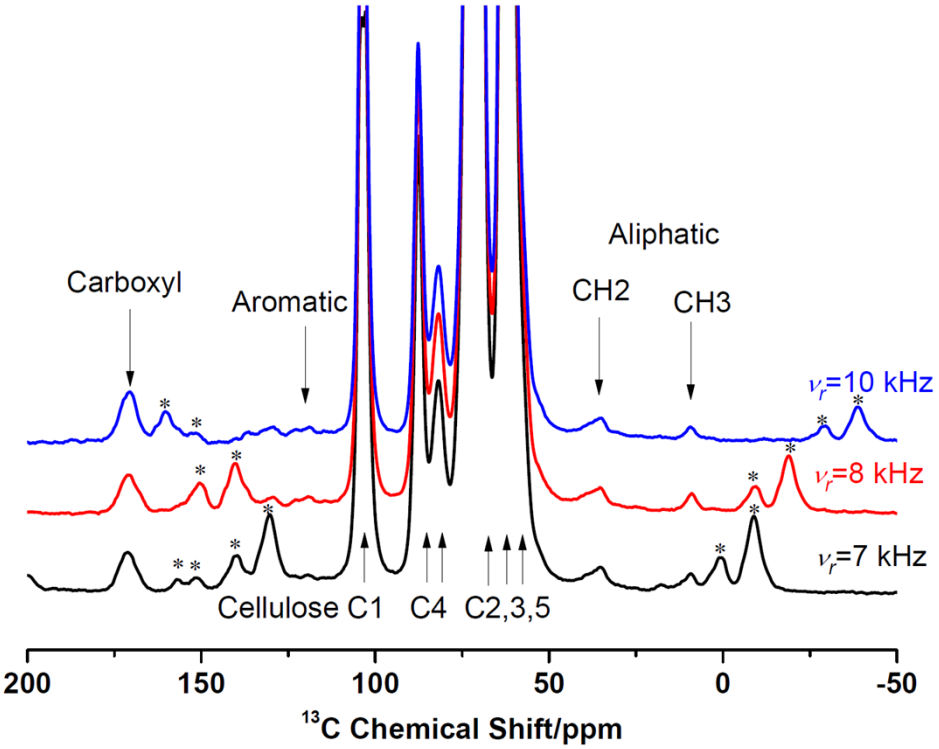


Figure S6. Representations of ^{13}C CP MAS spectra of CNC-RhB-open sample under different spinning rate (7, 8 and 10 kHz, with Sapphire rotor) leads to determination of the true signals, the asterisks (*) indicate spinning sidebands. The spectra recorded with microwave irradiation at 107 K. RF (^{1}H) = 55 kHz during CP and SPINAL64 decoupling. RF (^{13}C) = 67 kHz during CP. Other experimental conditions were: recycle delay = 10 s, contact time = 2.0 ms for ^{13}C. The spectra were normalized to compare intensities per scan.

In Figure S6, **CNC-RhB-open** sample was measured at different magic angel spinning rate (7, 8 and 10 kHz), so that expected signals and spinning sidebands can be differentiated. The ^{13}C chemical shifts can be assigned to expected characteristic groups, e.g. aliphatic groups (0 to 50 ppm), cellulose domain (50 to 110 ppm), aromatic groups (110 to 140 ppm), and carbonyl carbons (165 to 190 ppm).

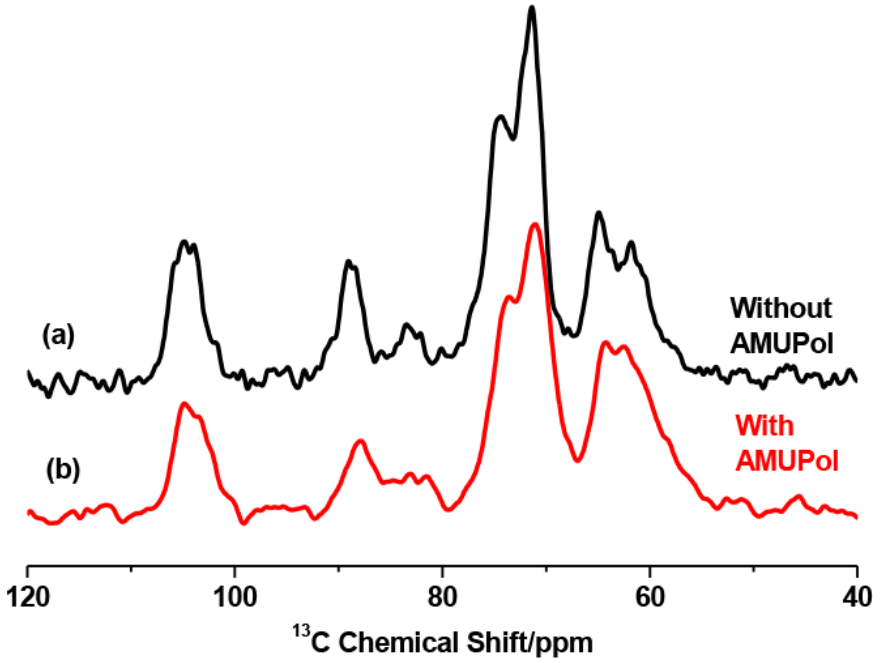


Figure S7. Solid-state ^{13}C CP MAS NMR spectra of **CNC-RhB** without microwave irradiation at 101 K with CP from ^{1}H at B_0 = 9.4 T without (a) and with (b) AMUPol impregnation, using sapphire rotors spinning at ν_r = 8 kHz. The spectra were normalized to compare intensities per scan.

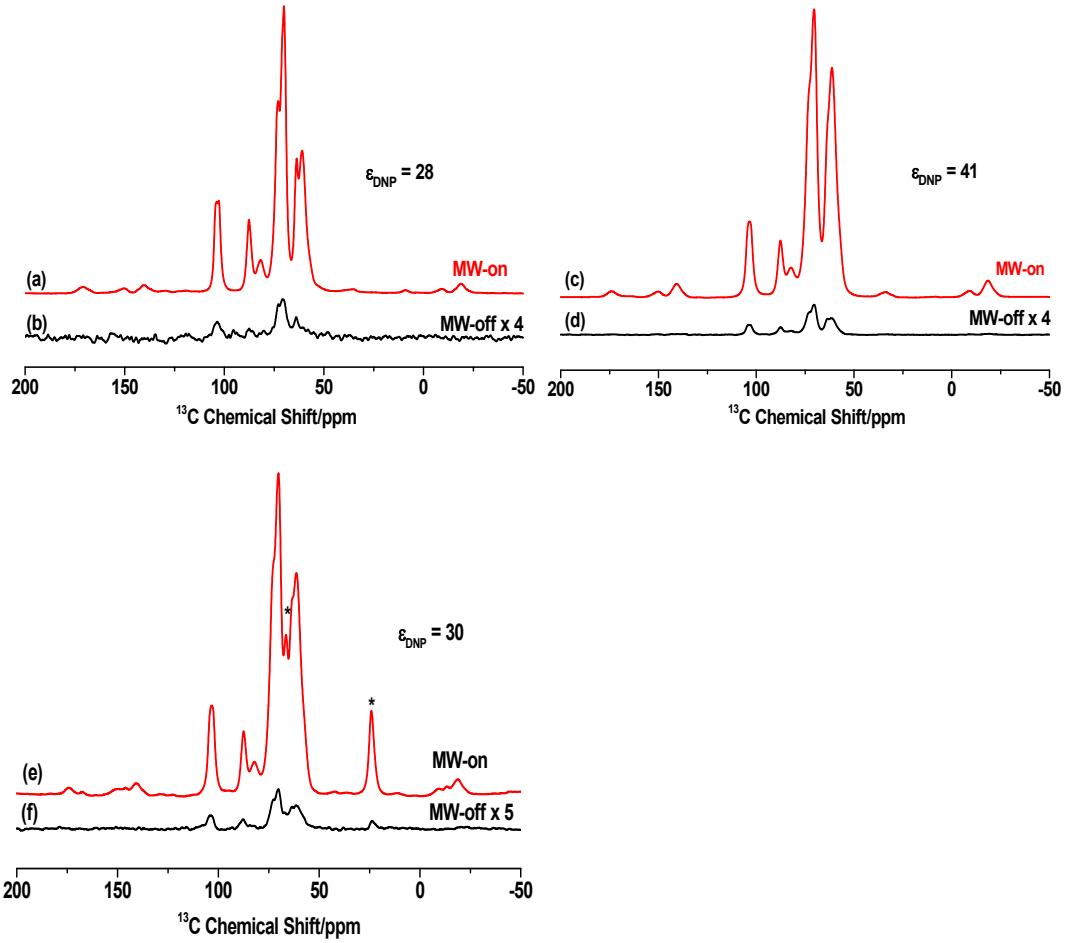


Figure S8. ^{13}C CP MAS NMR spectra of **CNC-RhB-open** (a, b), and **CNC-RhB-130°C** (c, d) and **CNC-RhB-close** (e, f) for DNP experiments at the temperature of 101 K with microwave off (b, d, f) and 107 K with microwave on (a, c, e). A DNP enhancement factor ε_{DNP} of 28, 41 and 30 was observed. Using sapphire rotors spinning at ν_r = 8 kHz. The spectra recorded with (red) and without (black) microwave irradiation at 107 /101 K, respectively. RF (^{1}H) = 55 kHz during CP and SPINAL64 decoupling. RF (^{13}C) = 67 kHz during CP. Other experimental conditions were: Recycle delay = 10 s, Contact time = 2.0 ms for ^{13}C. The spectra were normalized to compare intensities per scan. * THF-d_8.

CNC-RhB-open showed decreased relative signal intensities ascribed to methyl groups. This could be due to several reasons: (i) lower polarization efficiency. (ii) -NCH_2CH_3 could form a delocalized *pi* bond ($HNEt_2^+$), which could quench the vicinity carbons.

Reference

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2. Y. Shiraishi, R. Miyamoto, X. Zhang and T. Hirai, *Org. Lett.*, 2007, **9**, 3921-3924.
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