ELECTRONIC SUPPLEMENTARY INFORMATION

Critical Assessment of the Efficiency of Chitosan Biohydrogel Beads as Recyclable and Heterogeneous Organocatalyst for C–C Bond Formation

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1. Experimental details

General remarks

^{1}H and ^{13}C NMR spectra were recorded at 25 ºC on Bruker Avance 300 or 400 MHz spectrometers. Chemical shifts are denoted in δ (ppm) relative to tetramethylsilane (TMS δ = 0) as internal standard or relative to residual solvent peaks. Coupling constants *J* are given in Hertz. The following standard abbreviations are used for characterization of 1H NMR signals: s = singlet, d = doublet, t = triplet, m = multiplet. Error of reported values: chemical shift 0.01 ppm (^{1}H NMR), 0.1 (^{13}C NMR), coupling constant 0.1 Hz. The enantiomeric excess (ee) values were determined by chiral-phase HPLC using a Varian 920-LC Liquid Chromatograph and a column Phenomenex Lux Cellulose-1, 4.6 x 250 mm, 5 microm. Melting points (m.p.) are uncorrected and were measured in a Büchi 504392-S or Opti Melt MPA 100 equipment with a digital camera. IR spectra were recorded using a Diamond ATR (attenuated total reflection) accessory (Golden Gate) or in a VARIAN 1000 FT-IR (Scimitar™ Series). TGA measurements were carried out under nitrogen on a Perkin Elmer Thermographic TGA-7 instrument. The samples were measured with the following program heating rate: (1) equilibration step for 30 min @ 30 ºC; (2) heating profile from 30 ºC to 600 ºC @ 10 ºC/min; (3) 15 min @ 600 ºC. UV-vis measurements were performed on a Varian Cary 50 Instrument. DSC measurements were performed in a SETERAM TMA 92 16.18. The DSC thermograms were obtained under dynamic argon atmosphere (1 l h^{-1}, low-rate gas flow measured with a Brooks flowmeter equipped with a Tube R-2-15-AA P-073) at a heating rate of 5 ºC min^{-1}. Samples were placed an open Al_2O_3 crucible. In, Zn, Ag and Al metals were used to calibrate the DSC modulus in relation to temperature and enthalpy. An empty sample holder was used as reference and the runs were performed by heating the samples from 25 up to 400 ºC. Height of the peaks were measured by the difference between the heat flow at the peak and at the DSC curve baseline. The average diameter of the hydrogel beads was calculated by taking pictures of 20 beads under an optical microscope (Wild Makroskop M420 1.25x) equipped with a digital camera (Canon Power shot A640). pH values were determined using a HANNA instruments Microprocessor pH 211 Meter. In general, 30 mL of the corresponding filtrate were filtrated via a Rotilabo®-Spritzenfilter purchased from Carl Roth GmbH (diameter 33 mm, Nylon-Membran, 0.45 μm) in a Falcon tube and stirred slowly at RT until a constant pH value was adjusted. SEM images were obtained with a Zeiss DSM 950 scanning electron microscope operated at 10 kV. The samples were sputtered with Au prior to imaging by a SCD 040 Balzers Union. TLC was facilitated by the use of the following stains in addition to UV light (254 nm) with fluorescent-indicating plates (aluminium sheets precoated with silica gel 60 F_{254}, Merck): phosphomolybdic acid, vanillin, iodine. Atomic Absorption Spectroscopy was performed using Perkin-Elmer model A300 (USA) Atomic Absorption Spectrophotometry (AAS). Standard used for Na^+ determination was purchased from Qualigens Fine Chemicals (Fischer Scientific – Ireland). Na^+ AAS standard solution contains 1000 mg/L Na^+ in 0.5 M HNO_3. Subsequent dilutions of the standard solution provided AAS values of 5.26 and 10.83 for Na^+ 5 ppm and 10 ppm standard solutions respectively. Chitosan bead solutions for AAS studies were prepared as following: 10 beads of chitosan were taken from different batches prepared at different pH and dissolved in 1 mL of concentrate acetic acid and the clear solution was diluted to 50 mL with distilled water in a 50 mL standard flask, which was used for AAS measurements. Statistical validation of representative results from random experiments was performed by simple one-way analysis of variance

yielding overall significance ($p < 0.05$). The values in the text, tables and figures are expressed as mean ± standard deviation.

All reactions were carried out in a 5 mL round-bottom-flask under stirring at 500 rpm. Analytical grade solvents and commercially available reagents were purchased from TCI Europe or Aldrich and were used as received. Low molecular weight chitosan (Cat. No. 448869; Batch No. MKBB9037; CAS 9012−76−4; viscosity 20−200 cP, 1% in 1% acetic acid; actual DDA = 91.7%); medium molecular weight (MMW) chitosan (Cat. No. 448877; Batch No. MKBC3804; CAS 9012−76−4; viscosity 200−800 cP, 1% in 1% acetic acid; DDA = 75−85%) and high molecular weight (HMW) chitosan from crab shells (Cat. No. 48165; Batch No. BCBC2236V; CAS 9012−76−4; viscosity > 400 mPa.s, 1% in acetic acid; DDA = 75−85%) were purchased from Aldrich and used without further purification.

General preparation of chitosan hydrogel beads (CSHB)

0.64 g of LMW chitosan were placed in a beaker and dissolved in 0.1 M HCl (40 mL) during 1 h at RT. The as-prepared viscous clear solution was added drop wise into 0.1 M NaOH aqueous solution (600 mL) at RT using a dropping funnel (50 mL, diameter of the tip = 4 mm), resulting in immediate coagulation of droplets into beads. The distance between the dropping funnel tip and solution surface was adapted between 1.0 and 1.5 cm to ensure almost uniform beads with a spherical like geometry (4.0 ± 0.1 mm). The obtained beads were matured in this solution for 1 h without stirring. After this time, the hydrogel beads were collected on a Buchner funnel (without filter paper) and washed with 250 mL portions of Milli-Q water until the pH value of the filtrate was found to be in the desired range. The hydrogel beads were placed on a filter paper to remove the excess of water before using them in the catalytic experiments. The beads were found to be stable, in terms of aspect and reactivity, for at least one month when stored at RT. For the preparation of hydrogel beads of 2.2 ± 0.2 mm in diameter, a 20 mL syringe with a needle of 0.8 mm in diameter was used instead the dropping funnel. A similar protocol was used to prepare the beads from MMW and HMW chitosan. Different number of washings during the isolation of the beads usually provided batches with different basicity (Table S1).

Table S1 Representative set of various CSHB batches submitted to different number of washings

Entry	Batch number	Water used in each washing (mL)	Number of washings	pH[a]
1	**1**	250	12	7.34
2	**1**	250	18	6.57
3	**2**	250	12	7.62
4	**2**	250	15	7.00
5	**2**	250	21	6.61
6	**3**	250	< 20	7.87
7	**3**	250	20	7.32
8	**3**	250	28	6.87

[a] Slight variations in the pH values number of washings could be observed depending on the suction capacity of the filter pump.

Determination of number of accessible amine groups

The number of accessible amine groups of the CSHB catalyst was calculated by analyzing its reaction with salicylaldehyde (SA). 20 CSHB units were mixed with a solution of SA (2.5 mL from a 0.16 M stock solution in EtOH) and nitrobenzene (NB) (0.4 mmol) as GC internal standard. After 1 h, the formation of the Schiff base complex between SA and accessible $–NH_2$ groups of the CSHB was quantified by analyzing the GC peak area of the remaining unreacted salicylaldehyde.[1] A master calibration curve (*i.e.* NB/SA areas plotted against NB/SA concentrations) was used to determine the actual concentrations in solution. The number of accessible $–NH_2$ groups was further determined taking into consideration the actual DDA of the chitosan used for the preparation of the hydrogel beads: DDA (degree of deacetylation) = 91.7%, M_d (molar mass of deacetylated unit) = 161.16 g/mol, M_a (molar mass of acetylated unit) = 203.19 g/mol, m (weight of 20 dried beads) = 28 mg, loading = 6.093 mmol/g. Accessible $–NH_2$ groups = [(1/161.16 g/mol × 0.917) + (1/203.19 g/mol × 0.083)] × 0.0028 g = 0.0017 mol. (Note: This method does not consider some degree of data artefact caused by potential adsorption/absorption of unreacted SA and/or NB on/in the beads occurred during the formation of the salicylaldimine Schiff base complex).

Procedure for the preparation of cross-linked CSHB

60 units of chitosan hydrogel beads (mean pH 6.9, 10.2 mmol of $–NH_2$ groups) were placed into a 20 mL glass vial and mixed with 10 mL of aqueous glutaraldehyde solution (25 wt.%, c = 25 mM; molar ratio glutaraldehyde/NH_2 = 2.45). This mixture was shaken for 48 h at room temperature. After this time, the beads were washed/shaken 10 times with 10 mL of water during 5 min every time to remove the unreacted glutaraldehyde as indicated by TLC analysis.

General procedure for aldol model reaction I

To a solution of 4-nitrobenzaldehyde (115.1 mg, 1.0 mmol) and acetone (1 mL, 13.6 mmol) in DMSO (3 mL), 20 hydrogel bead units (corresponding to 17 mol% of free amine groups with respect to aldehyde) were added in one portion. The resulting reaction mixture was gently stirred (500 rpm)[2] under the specified conditions of time and temperature. TLC was used to monitor the reaction. After the indicated reaction time, the work-up was initiated by decanting the supernatant. The hydrogel beads were resuspended in DMSO (2 mL), stirred for 5 min and decanted. This process was repeated 3 times and the organic phases combined. Water (5 mL) was added to the solution and extracted with EtOAc (3 × 10 mL). The combined organic layers were further washed with water (3 × 15 mL), dried over Na_2SO_4 (20 min) and evaporated under reduced pressure. Conversion was determined by ^{1}H NMR spectroscopy of the crude product. The use of different stirring rate during the reaction might cause small deviations on the kinetic and therefore on the conversion.

General procedure for aldol model reaction II

To a mixture of 20 hydrogel bead units (corresponding to 17 mol% of free amine groups with respect to aldehyde) in the corresponding solvent system (3 mL), a solution

[1] T. G. Waddell, D. E. Leyden, M. T. Debello, *J. Am. Chem. Soc.*, 1981, **103**, 5303–5307.
[2] In general, slow break down of the beads could be observed during more intense physical stirring.

of 4-nitrobenzaldehyde (115.1 mg, 1.0 mmol) in cyclohexanone (1.4 mL, 13.6 mmol) was added. The resulting reaction mixture was gently stirred (500 rpm) for 48 h at RT. TLC was used to monitor the reaction. After the indicated reaction time, the supernatant was diluted with EtOAc (2 mL) and decanted. The hydrogel beads were resuspended in DMSO (2 mL), stirred for 5 min and decanted. This process was repeated 3 times and the organic phases combined. Water (5 mL) was added to the solution and extracted with EtOAc (3 × 10 mL). The combined organic layers were further washed with water (3 × 15 mL), dried over Na_2SO_4 (20 min), filtrated and evaporated under reduced pressure. Conversion and diastereomeric ratio was determined by ^{1}H NMR spectroscopy of the crude product.

General procedure for Knoevenagel condensation reaction

To a solution of the appropriate aldehyde (1.0 mmol) and malonitrile or ethyl-2-cyanoacetate (1.1 mmol, 72.7 mg or 118 μL respectively) in DMSO (3 mL), 20 hydrogel bead units (corresponding to 17 mol% of free amine groups with respect to aldehyde) were added in one portion. The resulting reaction mixture was gently stirred (500 rpm) at RT until no more aldehyde was observed (TLC). After this time, the supernatant was decanted and the remaining hydrogel beads were resuspended in DMSO (2 mL), stirred for 5 min and decanted. This process was repeated 3 times. Water (5 mL) was added to the solution and extracted with EtOAc (3 × 10 mL). The combined organic layers were further washed with water (3 × 15 mL), dried over Na_2SO_4 and evaporated under reduced pressure. Conversion was determined by ^{1}H NMR spectroscopy of the crude product. When malononitrile was used as donor, the water employed during the work-up was replaced by brine.

General procedure for nitro-aldol (Henry) reaction

A mixture of the appropriate aldehyde (1.0 mmol) and nitromethane (0.54 mL, 10.0 mmol) or nitroethane (0.72 mL, 10.0 mmol) was dissolved in the corresponding solvent (emulgated in the case of water) (3.0 mL) under stirring. To this mixture, 20 units of chitosan hydrogel beads (corresponding to 17 mol% of free amine groups with respect to aldehyde) were added in one portion. The resulting reaction mixture was gently stirred (500 rpm) under the specified conditions of time and temperature. For the work-up, the supernatant was decanted and the remaining hydrogel beads were washed with the solvent used in the reaction (2 mL) (*i.e.* EtOH, MeOH) and the filtrate evaporated. In the case of water, EtOAc (3 mL) was used to wash the beads by stirring during 5 min and further decantation. This process was repeated 3 times. The combined aqueous layers were extracted with EtOAc (3 × 10 mL), dried over Na_2SO_4 and evaporated under reduced pressure. In the case of DMSO, water (5 mL) was added to the organic layers followed by extraction with EtOAc (3 × 10 mL). The combined organic layers were further washed with water (3 × 15 mL), dried over Na_2SO_4, filtrated and evaporated under reduced pressure. Conversion was determined by ^{1}H NMR spectroscopy of the crude product.

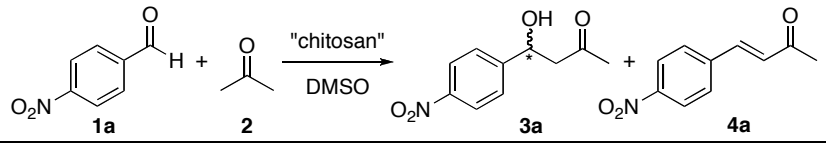
General procedure for Michael addition

A mixture of benzylidenemalonitrile (**17**) (154 mg, 1.0 mmol), cyclohexanone (**5**) (0.11 mL, 1.0 mmol) or resorcinol (**18**) (110 mg, 1.0 mmol) and chitosan catalyst (LMW PCS = 36 mg or CSHB = 26 beads, 22 mol% of free amine groups with respect to 17) in

water (5 mL) was refluxed during 3 h. After this time, the yellow precipitate was filtered off and wash with water (2 × 5 mL). The crude residue was resuspended in absolute EtOH and heated until boiling. The hot mixture was filtered and the procedure repeated three times with the isolated chitosan catalyst. In spite of the probable diffusion of EtOH into the CSHB during this procedure, the spherical beads could be recovered and reused in a second run without significant loss of catalytic activity. Aminopyrancarbonitrile **19** and chromene **20** were finally isolated by filtration upon recrystallization from the hot ethanolic solution.

2. Additional tables

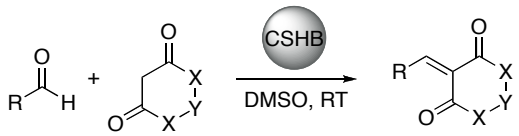
Table S2 Aldol model reaction I between 4-nitrobenzaldehyde (**1**) and acetone (**2**) performed in DMSO under different conditions[a]

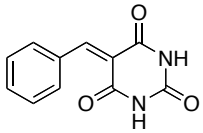
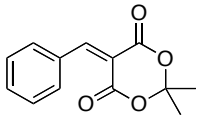


Entry	Catalyst	Catalyst loading (mol%)	T (± 2ºC)	Conversion (%)[b]	Selectivity **3a**:**4a**[c]
1	-	-	23	0	-
2	DGB[d]	17	23	0	-
3	PCS	17	23	0	-
4	CSHB	17	23	4	99:1
5	CSHB	51	23	2	99:1
6	CSHB	51	40	5	99:1

[a] Reation conditions: **1a** (1.0 mmol), **2** (1 mL, 13.6 mmol), DMSO (3 mL), 24 h, mean pH = 6.8. Beads number: Entry 1 = 20; entry 2 = entry 3 = 60 (corresponding to 17 and 51 mol%, respectively, of free amino groups with respect to aldehyde). [b] Determined by ^{1}H NMR of the crude product based on the aldehyde proton. Batch-to-batch estimated relative error for entries 3–5 = ± 0.5%. [c] Based on ^{1}H NMR analysis. [d] Dried gel beads (DGB) were used instead CSHB.

Table S3 Knoevenagel condensation reaction of barbituric acid and Meldrum's salt catalyzed by CSHB in DMSO at RT[a]

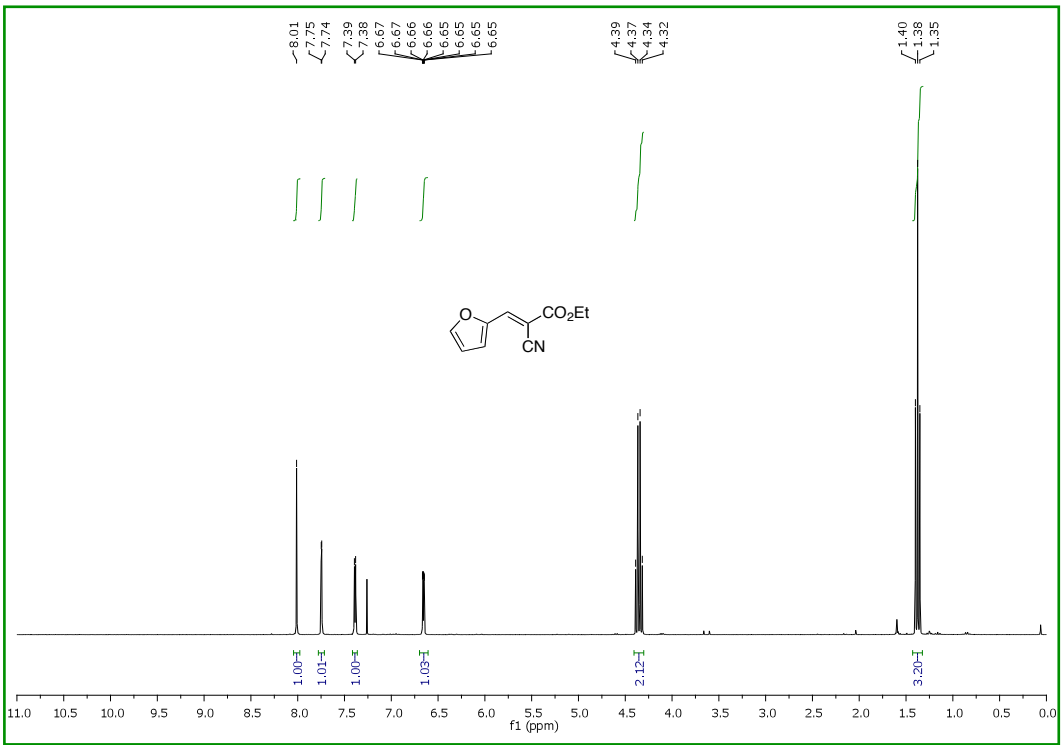


Entry	RCHO	Product		Time (min)	Conversion (%)[b]
1	**1l**		**10l**	5	2
2	**1m**		**10m**	5	30

[a] Reaction conditions: **1** (1.0 mmol), **donor** (1.1 mmol), DMSO (3 mL), mean pH = 6.9, beads number = 20 (corresponding to 17 mol% of free amine groups with respect to aldehyde), RT. [b] Determined by ^{1}H NMR spectroscopy of the crude product based on aldehyde proton.

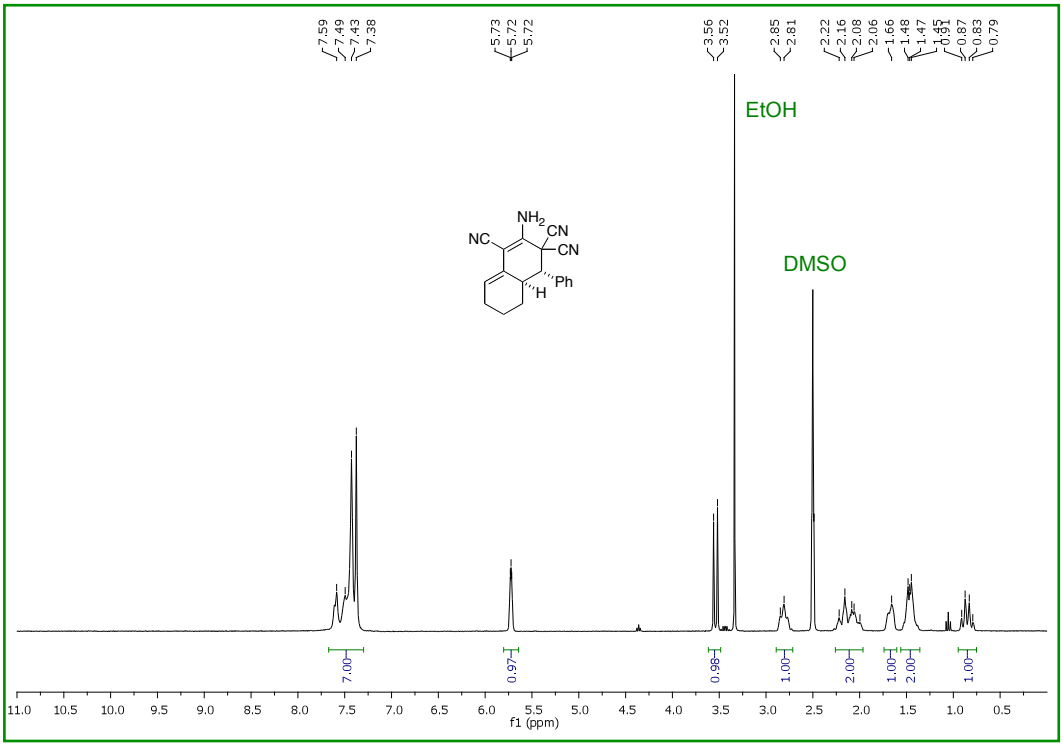
3. Selected ^{1}H NMR spectra

♦ Isolated compound **10k**

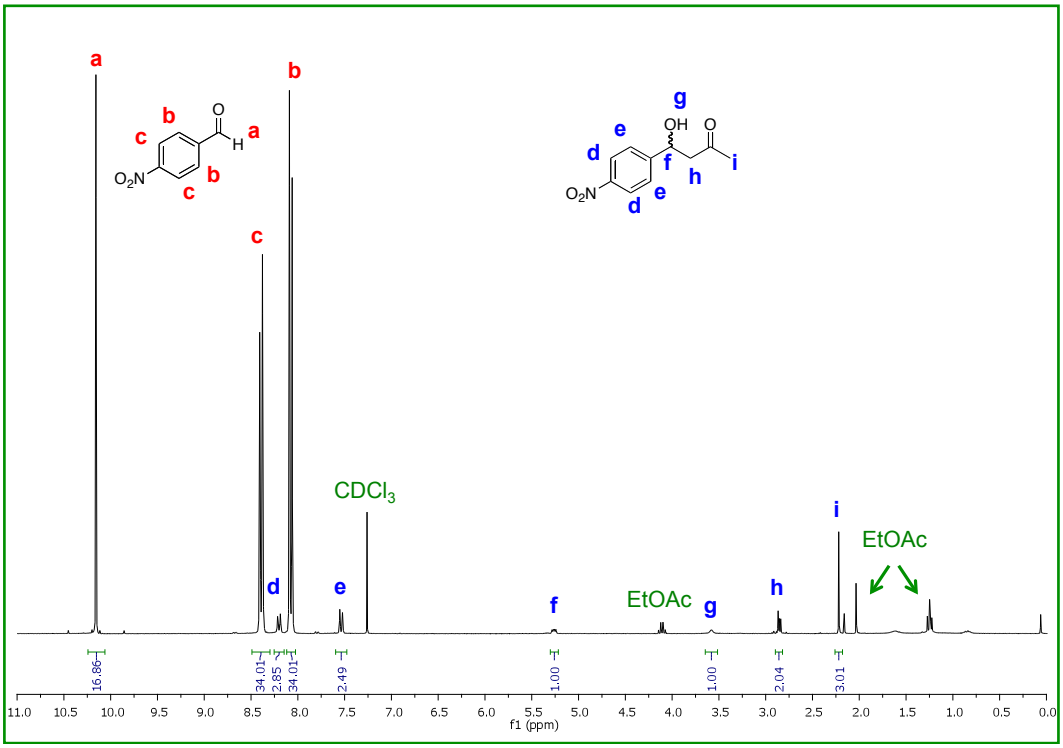


^{1}H NMR (300 MHz, $CDCl_3$) δ/ppm = 8.02 (s, 1 H), 7.75 (d, *J* = 1.6 Hz, 1 H), 7.39 (d, *J* = 3.7 Hz, 1 H), 6.70–6.62 (m, 1 H), 4.36 (q, *J* = 7.1 Hz, 2 H), 1.38 (t, *J* = 7.1 Hz, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ/ppm = 162.6, 148.7, 148.2, 139.4, 121.7, 115.3, 113.8, 98.6, 62.5, 14.1

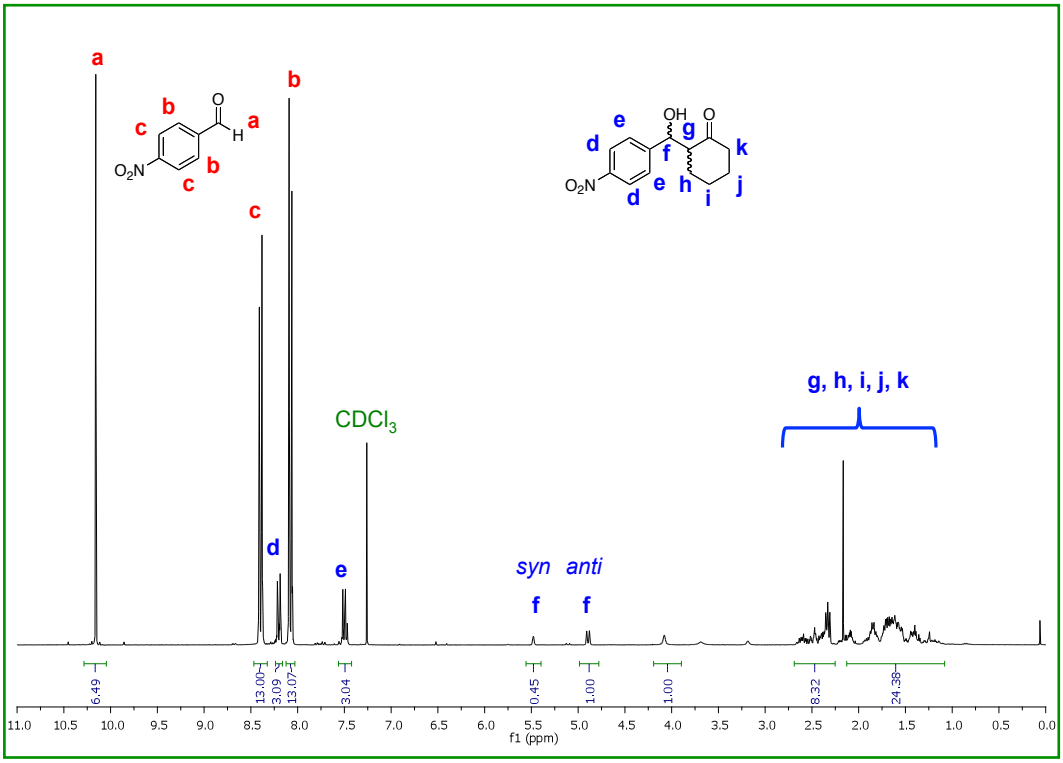
♦ Isolated compound **19**



♦ Table S1, entry 5



♦ Table 2, entry 6



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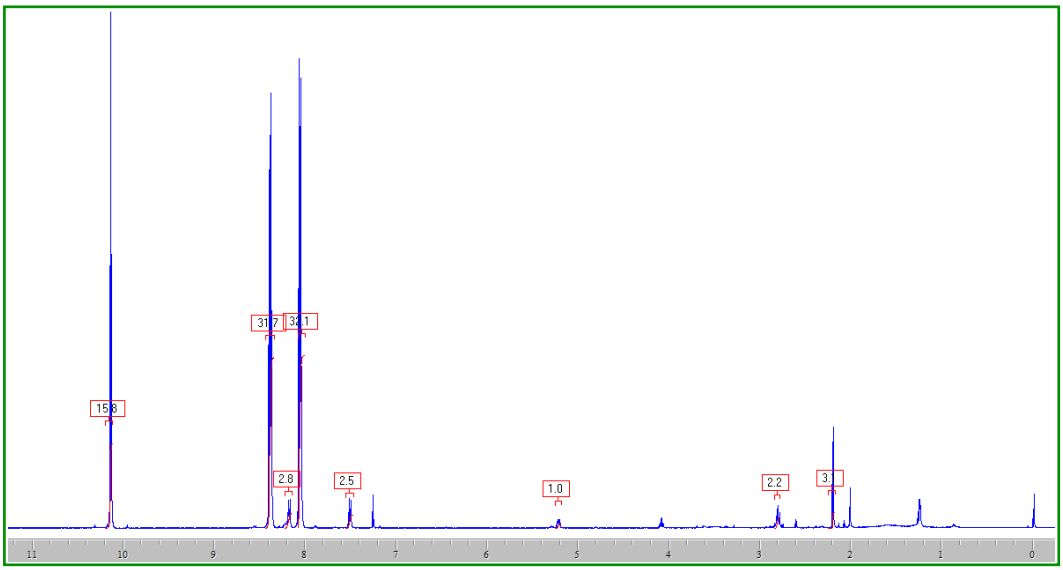

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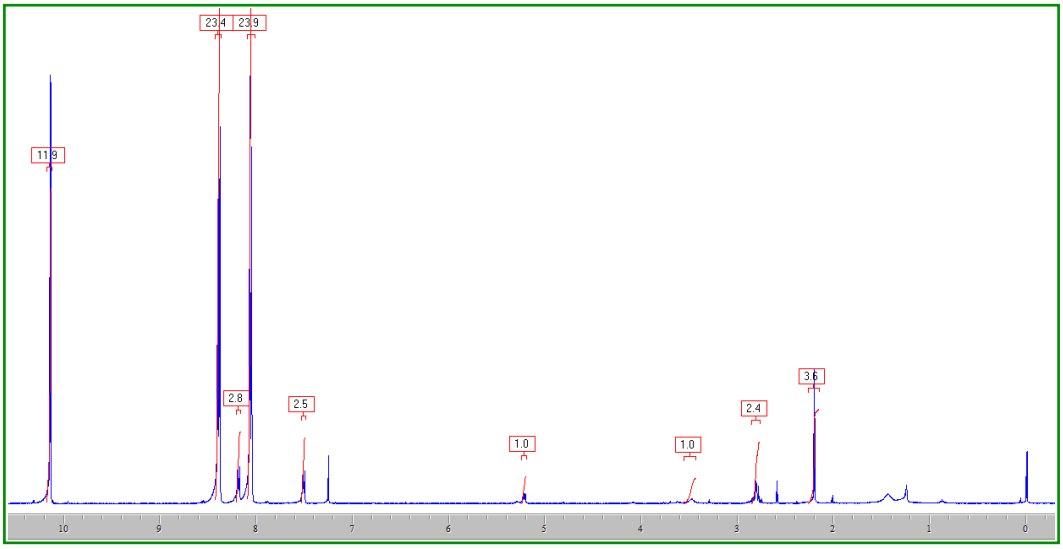

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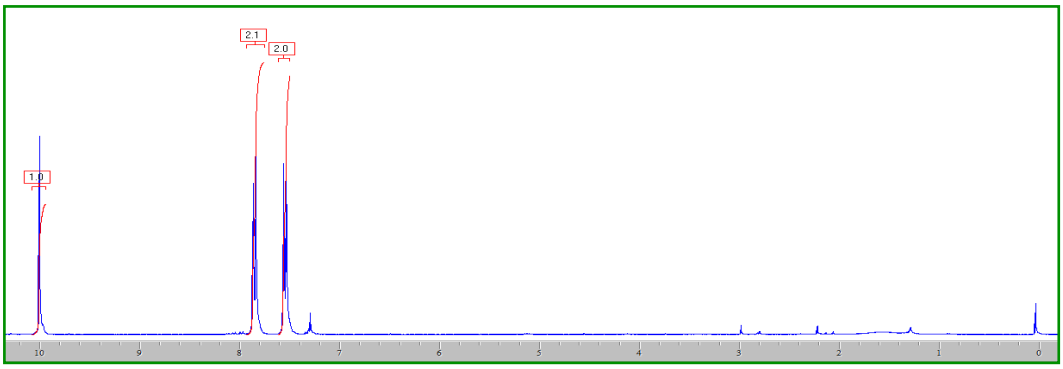

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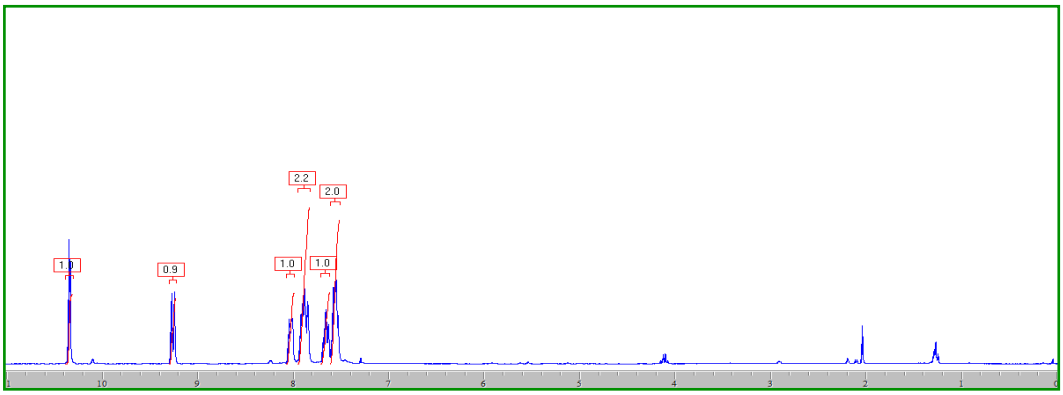

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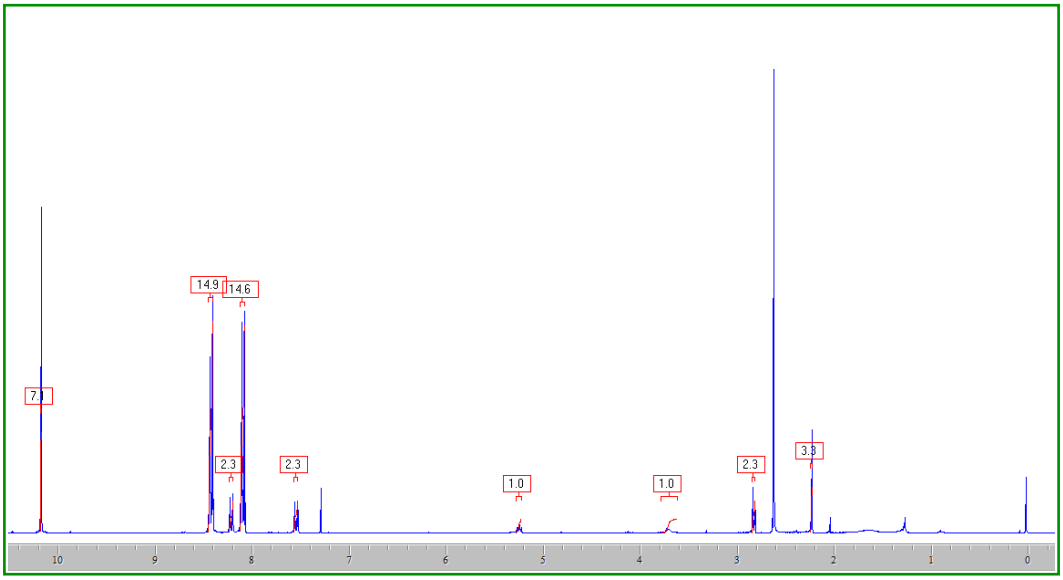

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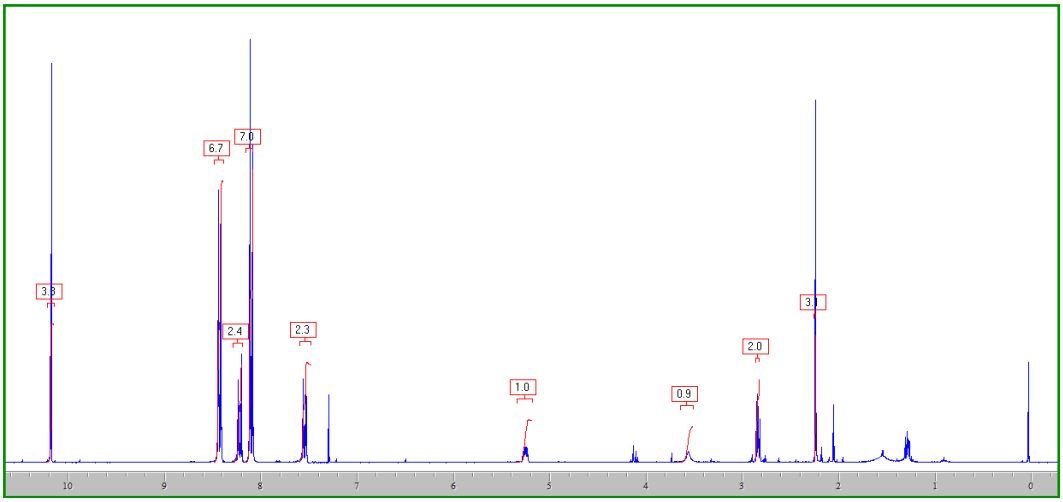

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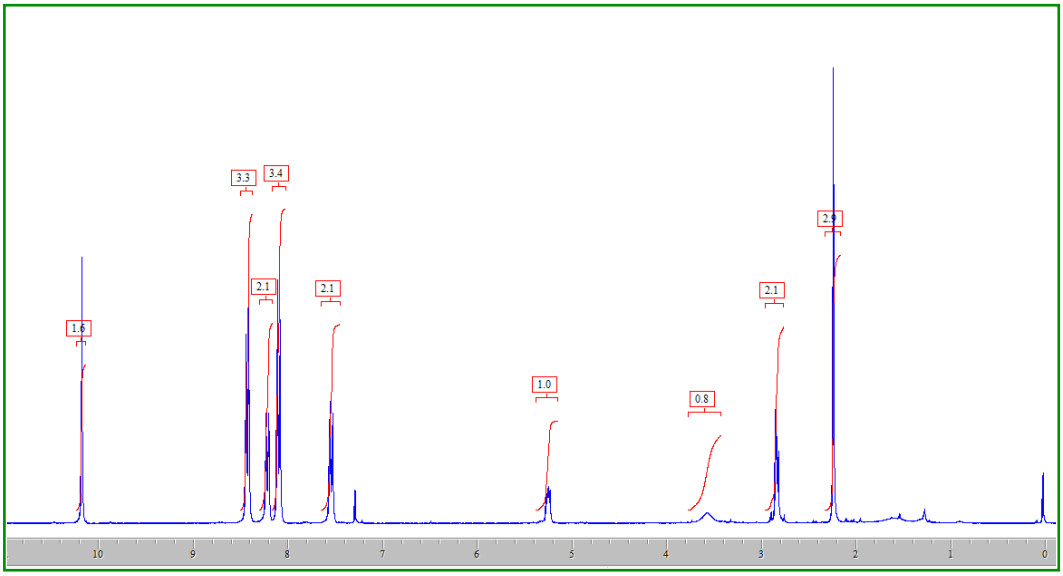

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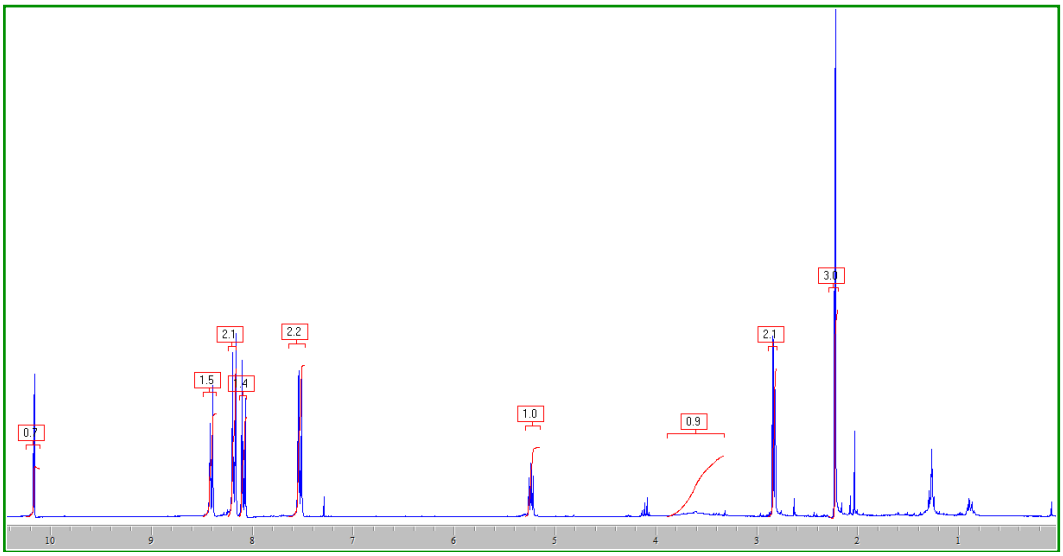

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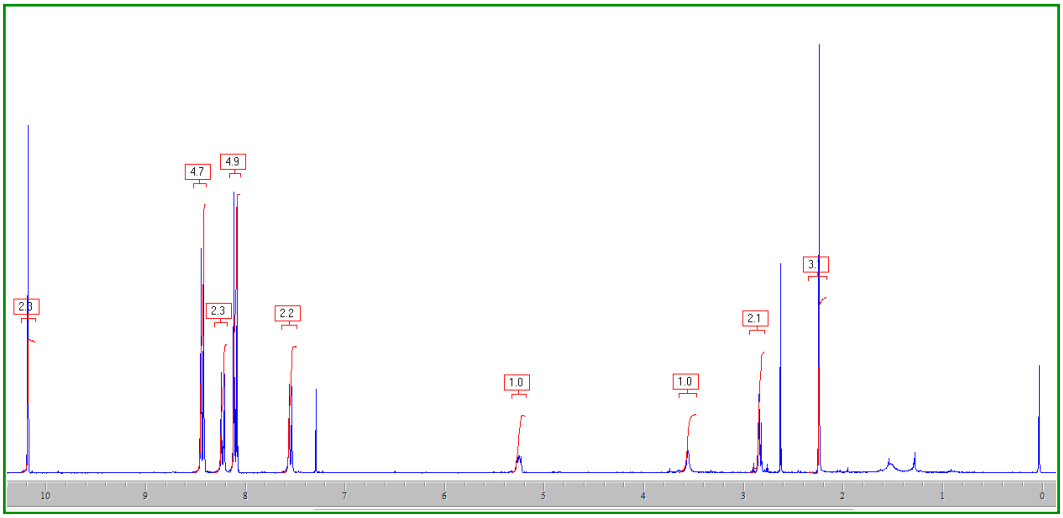

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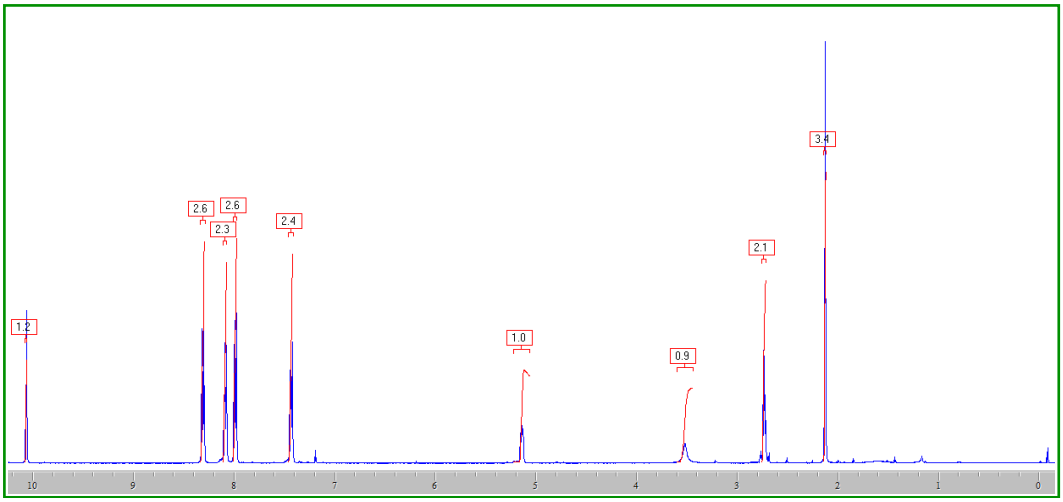

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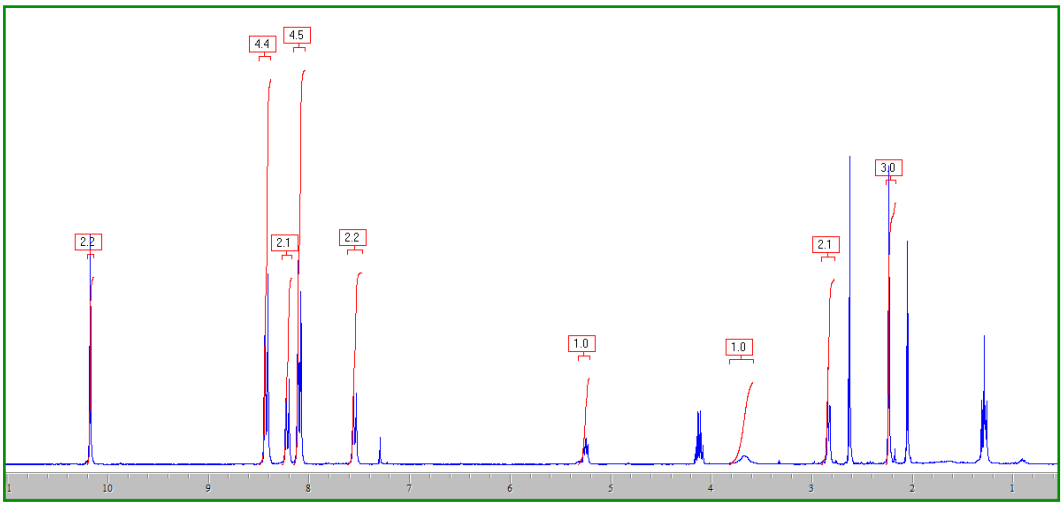

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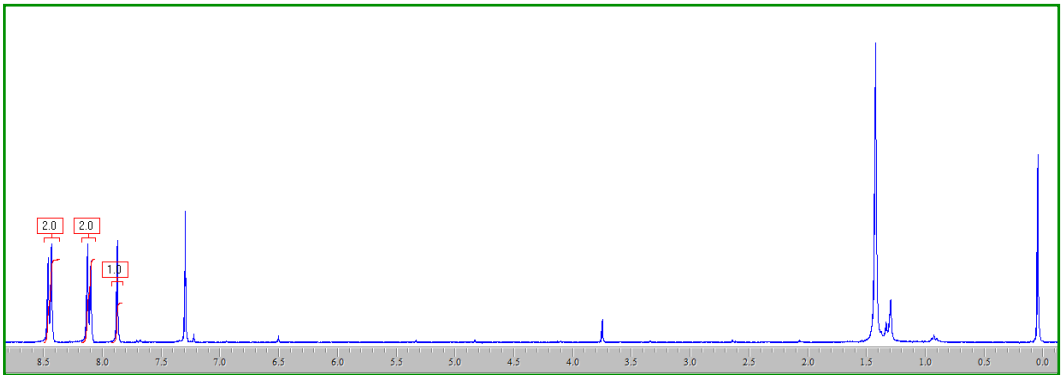

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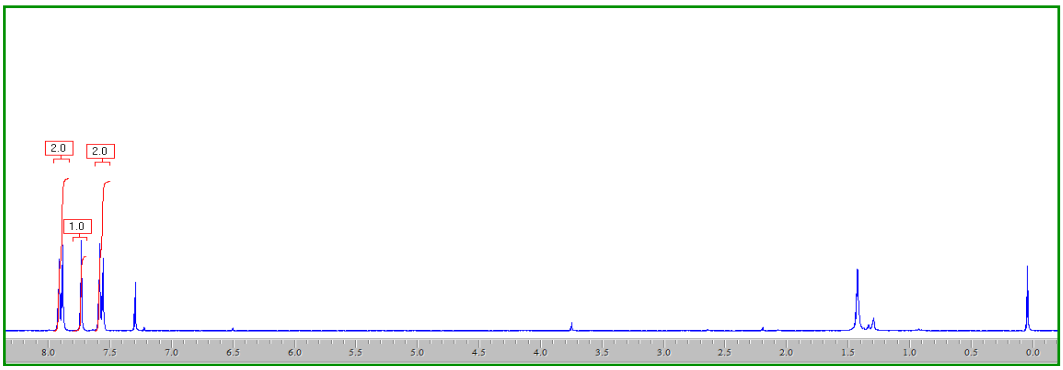

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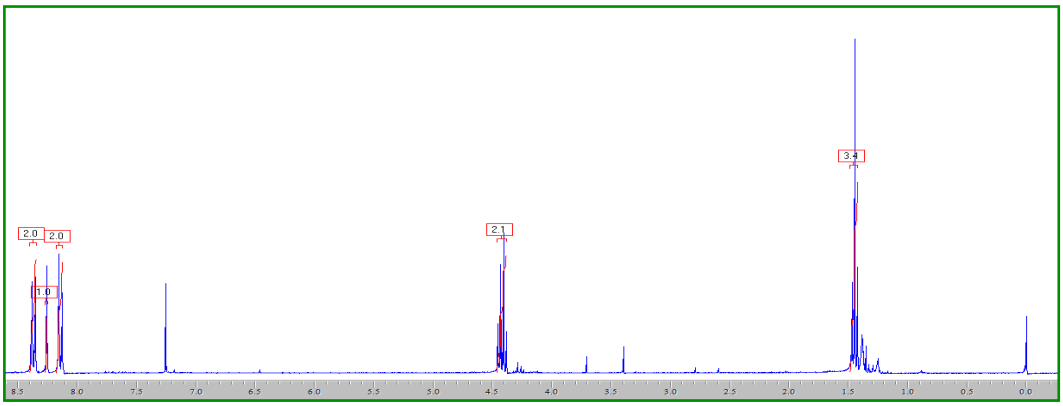

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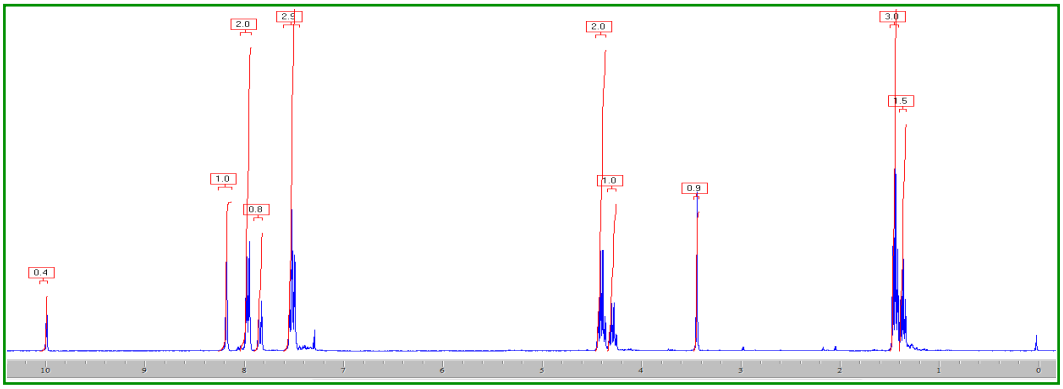


4. Additional photographs

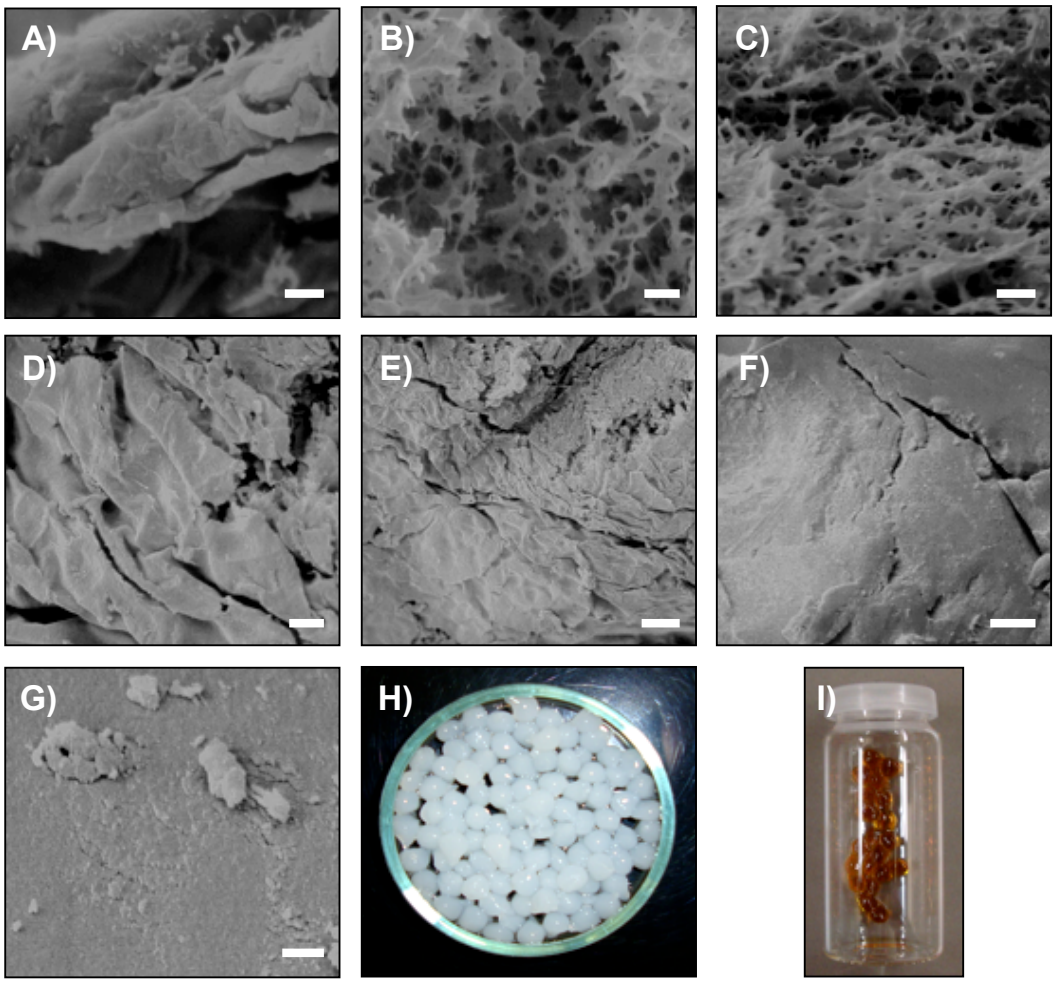


Fig. S1 A) SEM image of LMW PCS (scale bar 2 μm; magnification 5000X). B–C) SEM images of the xerogels made from CSHB (scale bars 2 μm; magnification 5000X). D–E) SEM images of different surface areas of freeze-dried CSHB (D: scale bar 10 μm; magnification 1000X; C: scale bar 50 μm; magnification 200X). F) SEM image of xerogel made from the freeze-dried CSHB after the 3rd cycle in the model nitroaldol reaction between **1a** and **11** in H_2O (scale bar 20 μm; magnification 500X). G) SEM image of xerogel made from the freeze-dried glutaraldehyde cross-linked CSHB after the model Knoevenagel reaction between **1f** and **9** in DMSO (scale bar 2 μm; magnification 5000X). H) Freshly prepared 4 mm-diameter CSHB. I) CSHB after 4th cycle in the model Knoevenagel reaction between **1a** and **7** in H_2O. After each cycle the beads acquire a brownish color due to non-specific adsorption of reactants. However, both the mechanical integrity and catalytic activity of the beads towards the Knoevenagel reaction remain intact.

5. TGA plots

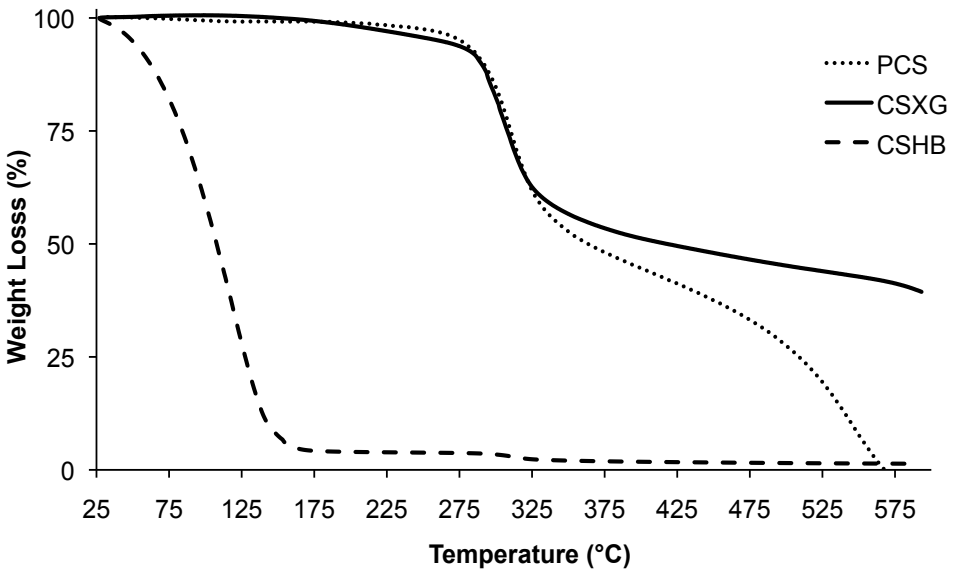
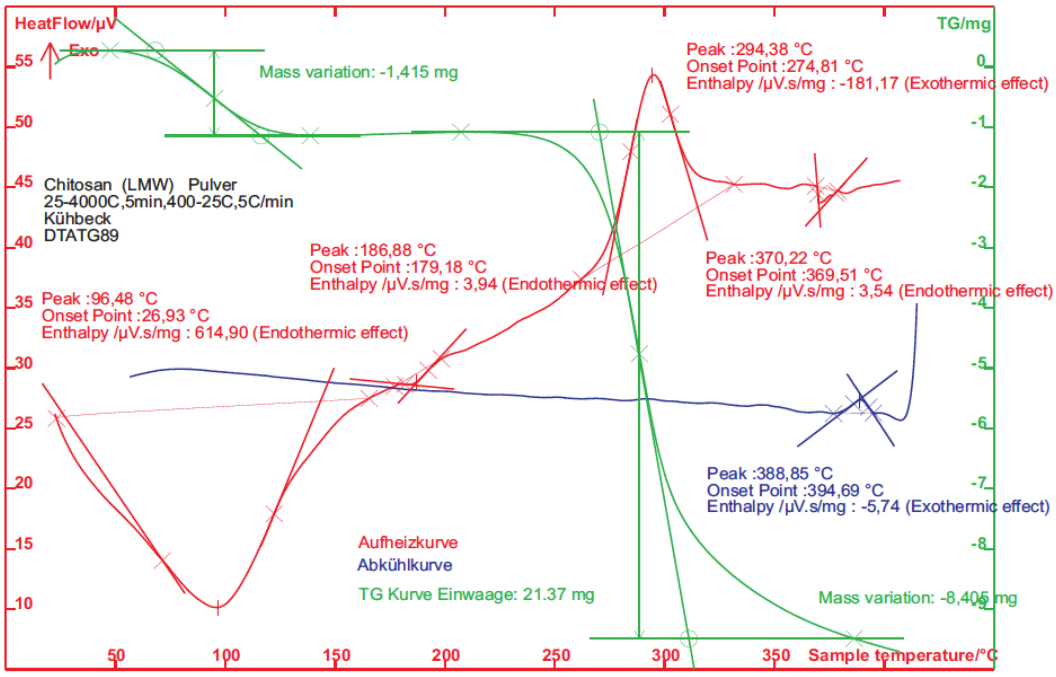


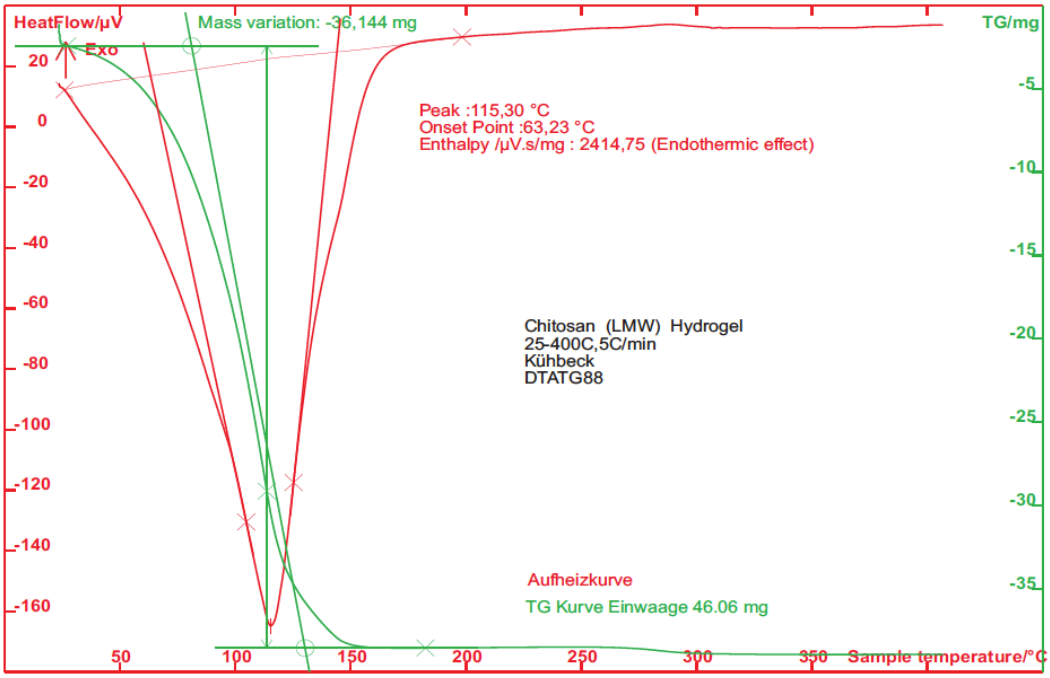
Fig. S2 TGA spectra of PCS, CSHB (4 mm diameter) and air-dried chitosan beads (CSXG).

6. DSC thermograms

(a)



(b)



(c)

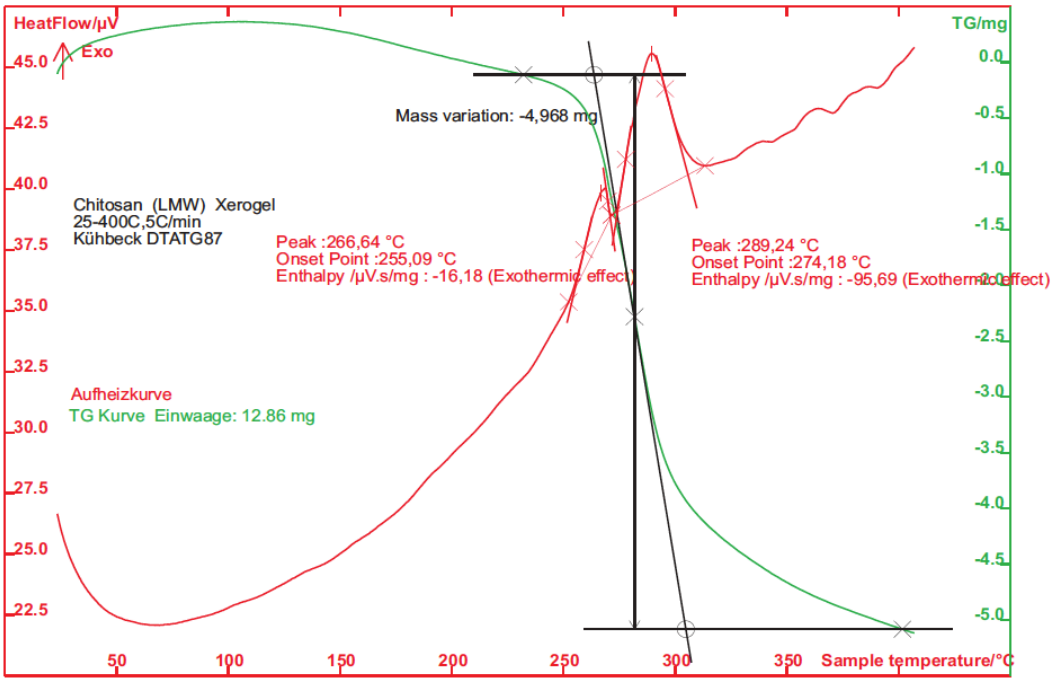


Fig. S3 DSC curves for (a) PCS, (b) CSHB and (c) CSXG under argon atmosphere (low-rate flow, 1 l h^{-1}) at 5 ºC min^{-1}. Temperature range: 25-400 ºC. Sample mass: 21.37 mg (PCS); 46.06 mg (CSHB); 12.86 mg (CSXG). Note: The enthalpic contributions shown in the plots are related to the sample mass input at the beginning of the measurement. Due to the high water uptake ability of the CS, water normalization of the curves would be necessary in order to strictly compare the thermodynamic contributions of the different samples.

7. UV-vis studies

Absorption of 2-nitrobenzaldehyde in DMSO was investigated at λ = 260 nm. The extinction coefficient (ε) was estimated in 60605 ± 1844 $M^{-1}cm^{-1}$ from the corresponding calibration curve made from 5 independent measurements at each

concentration value (Fig. S4, A). 20 units of CSHB (17 mol% of free amine groups respect to aldehyde) were added to a 3 mL solution of 2-nitrobenzaldehyde (from a 0.3 M stock solution) and the absorption (A) values were measured over time (Fig. S4, B). The reported values (<A>) correspond to the average of 5 independent measurements. The concentration (c) of 2-nitrobenzaldehyde was determined via the Beer-Lambert law (A = εcl; l = length of the light path).

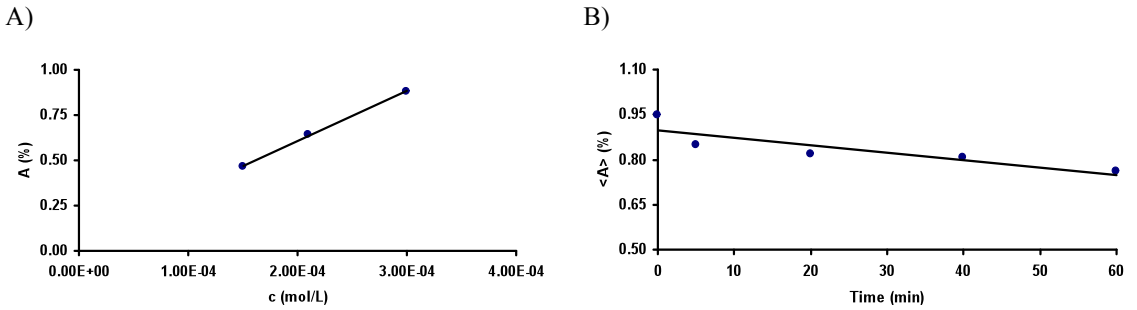


Fig. S4 A) UV-vis calibration curve of 2-NBA in DMSO. B) UV-vis absorption of 2-nitrobenzaldehyde in DMSO over time.

8. Model kinetic study

The kinetic study of a model Knoevenagel condensation between 2-nitrobenzaldehyde (2-NBA) and ethylcyanoacetate in DMSO at room temperature catalyzed by CSHB was carried out within a reaction time range of 40 min (Fig. S5). The yields were determined by NMR analysis using an internal standard (*i.e.* dimethylacetamide), which was added to the isolated crude product.

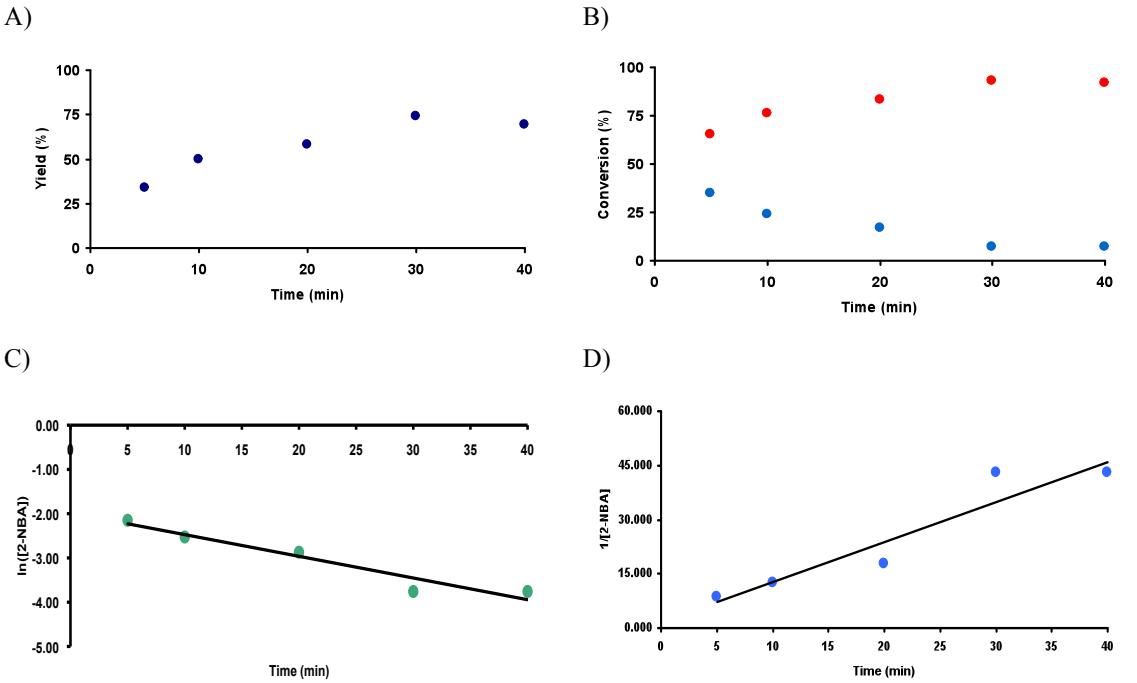


Fig. S5 A) Plot of product yield (%) *vs.* time (min). B) Plot of reaction conversion (%) *vs.* time (min). Red dots: Disappeared 2-nitrobenzaldehyde during reaction. Blue dots: Remained 2-nitrobenzaldehyde during reaction. C) Plot of natural logarithm of the remained concentration of 2-nitrobenzaldehyde during

reaction *vs.* time. D) Plot of reciprocal fraction of the remained concentration of 2-nitrobenzaldehyde during reaction *vs.* time.