Supporting Information

For

Synthetic Lignin-like and Degradable Nanocarriers

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Experimental Section and Additional Data

Materials

Carbonyldiimidazol, benzoic acid, acetovanillone, bromine and horseradish peroxidase were bought from Sigma Aldrich. Vanillin, isoeugenol and the $LiAlH_4$/THF solution (10%, ca. 2.5 mol/L) were obtained from TCI Chemicals. All materials were used without further purification.

Poly[(ethylene-*co*-butylene)-*b*-(ethylene oxide)] (P(E/B-*b*-EO)) consisted of a poly(ethylene-*co*-butylene) block with a molecular weight of (M_w=3700 g/mol) and poly(ethylene oxide) block of (M_w = 3600 g/mol). The surfactant was synthesized according to the protocol of Schlaad *et al*.[1]

Methods

Nuclear magnetic resonance (NMR) spectroscopy

^1H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy was performed at a Bruker AVANCE (USA) system at 300 MHz. The NMR samples contained ca. 50 mg of the respective substance which was dissolved in 600 µL of either DMSO-d_6 or $CDCl_3$.

Dynamic Light Scattering (DLS)

The hydrodynamic diameters of the nanoparticles was measured by DLS with a NICOMP 380 submicron particle sizer (Nicomp Particle Sizing systems, USA) at a fixed angle of 90° and a laser diode running at 635 nm. The sample was diluted to a concentration of 0.01 wt% with water or cyclohexane before measurement.

Fourier transform infrared (FTIR) spectroscopy

To prove the covalent crosslinking of the nanocarriers, the washed dispersions were freeze dried and then analyzed with a Nicolet iS10 FTIR spectrometer with Vertical ATR Accessory. Spectra were recorded between 600 and 4000 cm^{-1}.

Scanning electron microscopy (SEM)

The nanocarrier morphologies were examined with a Gemini 1530 (Carl Zeiss AG, Oberkochem, Germany) scanning electron microscope (SEM) operating at 0.35 kV. The samples were prepared by casting diluted and purified nanocarrier dispersions on silicon wafers.

Germination assay

4 and **6**, each 25 mg, were dissolved in DMSO (5 mL). 20 mL of a 0.1 M HCl solution containing 1 mg mL^{-1} Lutensol AT50 was added dropwise over 30 min. Afterward, the suspension was dialyzed against MilliQ water (3 x 8 L for 12 h) to remove DMSO and HCl. The washed monomer particles were centrifuged at (4000 rpm, 30 min) and the clear supernatant was separated. To the pellet, a Lutensol AT50 solution (1 mg mL^{-1}) was added and the solid was resuspended. Each sample had a solid-content of 0.2 wt%.

Conidia of *Phaeomoniella chlamydospora* (Pch) and *Phaeoacremonium minimum* (Pmi) from 18-day-old agar plate cultures were harvested. After centrifugation at 4000 rpm for 10 minutes, the conidia were resuspended in YMG-medium to a concentration of $1 \cdot 10^5$ spores per milliliter. The degradation test was carried out in 96-well microtiter plates (Greiner Bio-One GmbH, Frickenhausen). 1 µL of the monomer dispersion (10 mg mL^{-1}) was then added to 200 µL MM1G medium containing 10.000 spores. After an incubation time of 72 hours at 27°C, the optical density was measured at a wavelength of 600 nm (Benchmark Plus Microplate reader, BioRad, Munich). Tests were conducted in triplicates. As references, the germination of Conidia in YMG medium (ideal growth conditions), in MM1G medium (minimal growth conditions) and the germination of spores in MM1G medium containing additionally 1 µL Lutensol (1 mg mL^{-1}) solution was taken.

The media had the following composition: YMG: 10.00 $g \cdot L^{-1}$ malt extract, 10.00 $g \cdot L^{-1}$ glucose, 4.00 $g \cdot L^{-1}$ yeast extract, pH 5.5; MM1G: 1 $g \cdot L^{-1}$ glucose, trace elements, pH 5.5.

Syntheses

4-acetyl-2-methoxyphenyl benzoate (1)

Carbonyldiimidazol (14.6 g, 90.3 mmol, 1 eq.) was dissolved in 100 mL tetrahydrofurane (THF). To the turbid solution, benzoic acid (11.0 g, 90.3 mmol, 1 eq.) was added under vigorous stirring while keeping the reaction vessel open to compensate for the fast increasing pressure due to the release of carbon dioxide. After the solution became clear, we added acetovanillone (15.0 g, 90.3 mmol, 1 eq.) and the reaction was allowed to proceed overnight at 50°C. 4-acetyl-2-methoxyphenyl benzoate was obtained as a white powder by precipitation in 500 mL water. The solid was filtered off and was dried at 40°C in a vacuum oven (21.6 g, 88%).

^{1}H NMR (300 MHz, 298 K, DMSO-d_6): δ 8.14-7.40 (m), 3.84 (s), 2.62 (s). **^{13}C NMR (300 MHz, 298 K, DMSO-d_6):** δ 196.97, 163.63, 151.02, 143.26, 135.84, 134.21, 129.86, 129.03, 123.24, 121.69, 111.67, 55.98, 26.73.

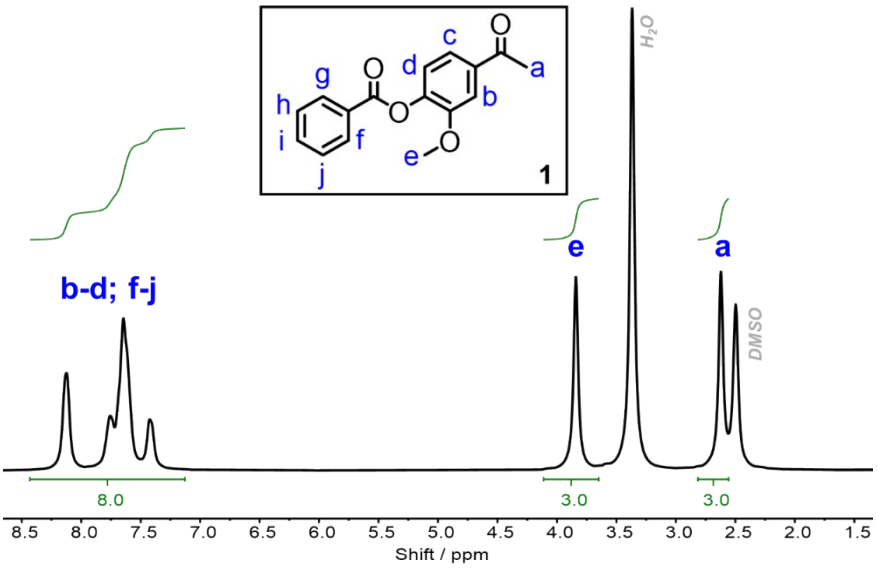

Figure S1: ^{1}H NMR (300 MHz, 298 K, DMSO-d_6) spectrum of 4-acetyl-2-methoxyphenyl benzoate **1** and the respective chemical shifts.

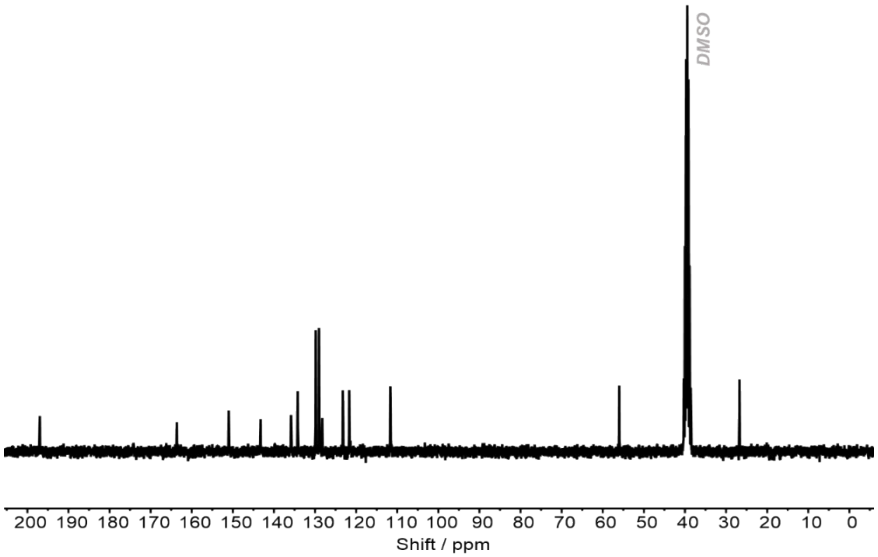

Figure S2: ^{13}C NMR (300 MHz, 298 K, DMSO-d_6) spectrum of 4-acetyl-2-methoxyphenyl benzoate **1** and the respective chemical shifts.

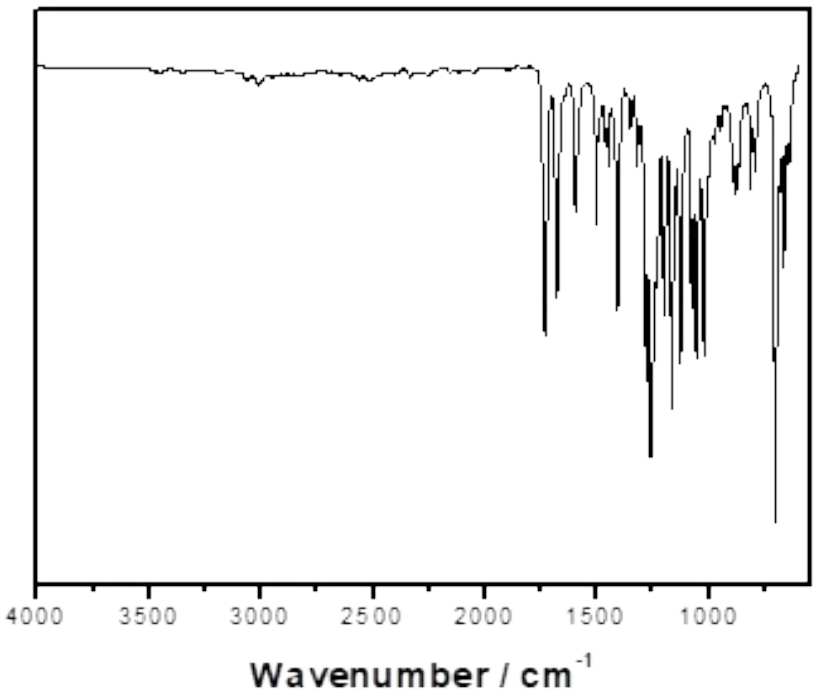

Figure S3: FTIR spectrum of 4-acetyl-2-methoxyphenyl benzoate **1**.

4-(2-bromoacetyl)-2-methoxyphenyl benzoate (2)

1 (16.2 g, 60.0 mmol, 1 eq.) was dissolved in a dioxane/diethyl ether mixture (dry, 3/4 vol.). Under an inert gas atmosphere, bromine (3.08 mL, 120.0 mmol, 2 eq.) was added dropwise via a syringe pump over 2 h under ice-cooling and vigorous stirring. The mixture was stirred for 60 min until 100 mL of diethyl ether was added. Subsequently, the solution was washed with ice-cold water (4 x 150 mL). In the last washing step, 4-(2-bromoacetyl)-2-methoxyphenyl benzoate precipitated as white solid, which was separated by filtration and finally dried at 40°C in a vacuum oven (10.7 g, 51%).

^{1}H NMR (300 MHz, 298 K, DMSO-d_6): δ 8.14-7.66 (m), 4.99 (s), 3.85 (s). **^{13}C NMR (300 MHz, 298 K, DMSO-d_6):** δ 190.82, 163.60, 151.18, 143.75, 134.29 132.97, 129.88, 129.07, 123.48, 122.21, 112.44, 56.14, 34.11.

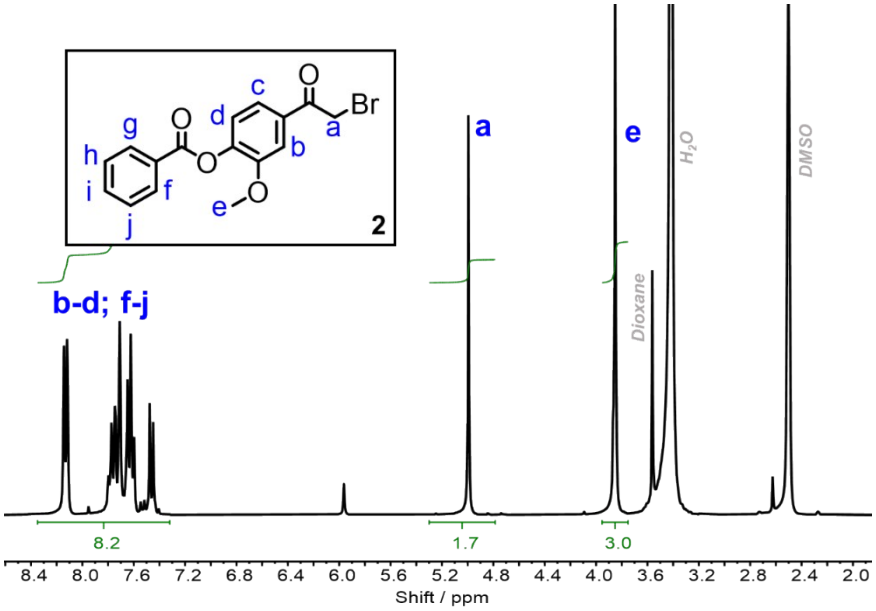

Figure S4: ^{1}H NMR (300 MHz, 298 K, DMSO-d_6) spectrum of 4-(2-bromoacetyl)-2-methoxyphenyl benzoate **2** and the respective chemical shifts.

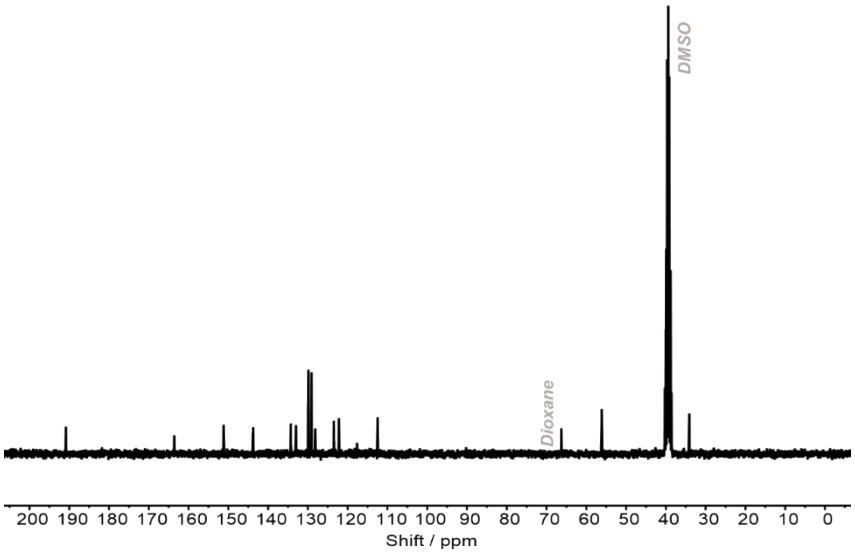

Figure S5: ^{13}C NMR (300 MHz, 298 K, DMSO-d_6) spectrum of 4-(2-bromoacetyl)-2-methoxyphenyl benzoate **2** and the respective chemical shifts.

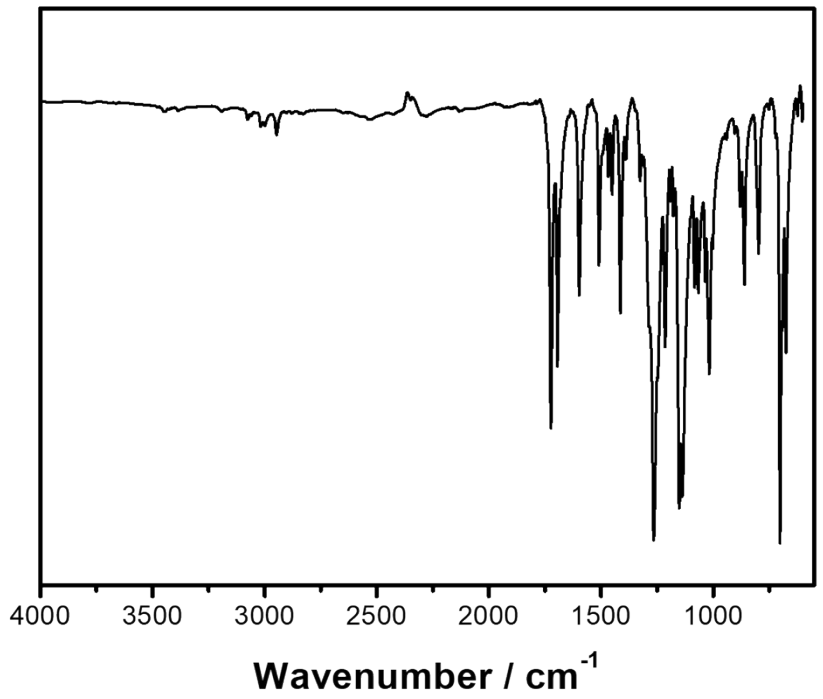

Figure S6: FTIR spectrum of 4-(2-bromoacetyl)-2-methoxyphenyl benzoate **2**.

4-(2-(4-formyl-2-methoxyphenoxy)acetyl)-2-methoxyphenyl benzoate (3)

To 30 mL dry THF, vanillin (2.2 g, 14.3 mmol, 1 eq.), potassium carbonate (3.0 g, 21.5 mmol, 1.5 eq.) and **2** (5.0 g, 14.3 mmol, 1 eq.) were added. The reaction was performed at 50°C under stirring and inter gas atmosphere. After two days, the formation of precipitate was observed and 50 mL water was added. The yellowish solid was separated by filtration and was washed with water (3 x 50 mL) to remove KBr and excess K_2CO_3. Impurities were removed from the crude product by column chromatography using at first pure dichloromethane (DCM) as an eluent. Finally, the column was flushed with a DCM methanol mixture (2 vol.%) to separate the product. The solutions were dried with $MgSO_4$ first, and the solvent was removed under reduced pressure to yield **3** as a yellowish powder (5.5 g, 92%).

^{1}H NMR (300 MHz, 298 K, DMSO-d_6): δ 9.84 (s), 8.15-7.11 (m) 5.81 (s), 3.89-3.87 (2s). **^{13}C NMR (300 MHz, 298 K, DMSO-d_6):** δ 192.84, 191.40, 163.64, 152.74, 149.15, 143.80, 133.12, 129.89, 129.07, 128.17, 125.52, 112.68, 111.72, 70.59, 56.14, 55.61.

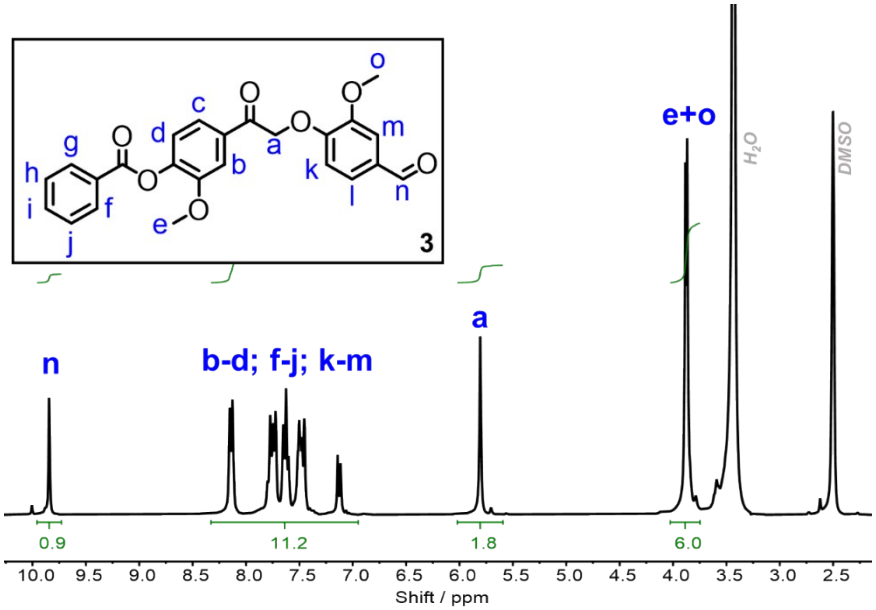

Figure S7: ^{1}H NMR (300 MHz, 298 K, DMSO-d_6) spectrum of 4-(2-(4-formyl-2-methoxyphenoxy)acetyl)-2-methoxyphenyl benzoate **3** and the respective chemical shifts.

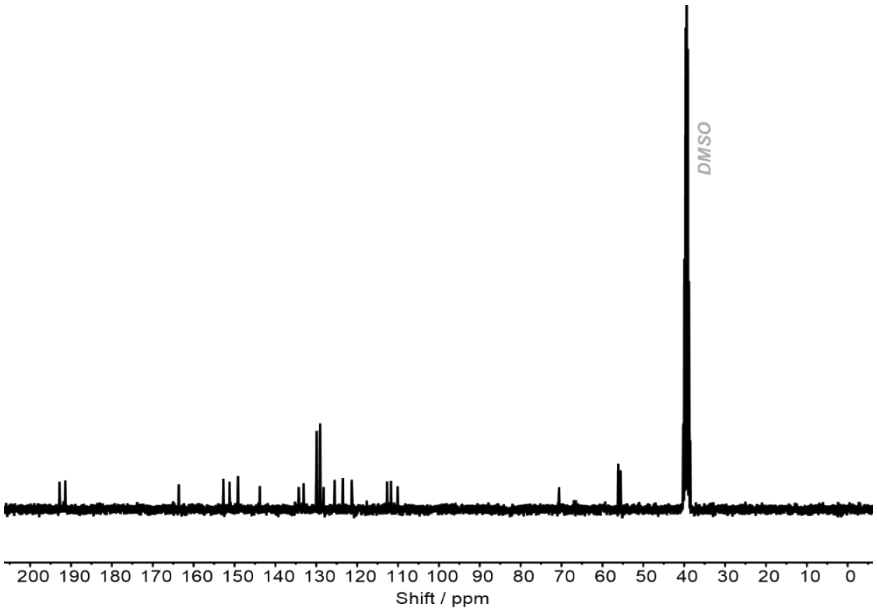

Figure S8: ^{13}C NMR (300 MHz, 298 K, DMSO-d_6) spectrum of 4-(2-(4-formyl-2-methoxyphenoxy)acetyl)-2-methoxyphenyl benzoate **3** and the respective chemical shifts.

4-(1-hydroxy-2-(4-(hydroxymethyl)-2-methoxyphenoxy)ethyl)-methoxyphenol (4)

To cleave the benzoyl protection moiety and to reduce the carbonyl groups, **3** (2.1 g, 5.0 mmol, 1 eq.) was dissolved in 90 mL THF and 13.75 mL of a $LiAlH_4$/THF solution (2.5 mol/L, 5.5 mmol, 1.1 eq.) was added dropwise while cooling with a water bath. After complete addition, the mixture was refluxed for 1 h at 70°C and then cooled down to room temperature under stirring overnight. Afterwards, ice was added to the solution until the evolution of gas stopped completely, leading to the formation of a gel-like solid. After having quenched unconverted $LiAlH_4$, 100 mL of a 10% H_2SO_4 solution was added and the mixture was washed with diethyl ether (3 x 100 mL). The combined organic phases were dried with $MgSO_4$ and were than evaporated under reduced pressure yielding a brownish oil. The crude product was dissolved in 200 mL of a saturated Na_2CO_3 solution and was then washed with diethyl ether

(3 x 100 mL) to remove the benzyl alcohol. The aqueous phase was collected and was set with a 10% HCl solution to a pH of 3. Again, the mixture was washed with diethyl ether (3 x 200 mL). The organic phase was dried with $MgSO_4$ until **4** was obtained as a brownish oil under reduced pressure (0.85 g, 52%).

^{1}H NMR (300 MHz, 298 K, DMSO-d_6): δ 8.83 (s), 7.01-6.70 (m), 5.41(d), 5.06 (t), 4.80 (m), 4.40 (d), 3.98-3.86 (m), 3.75 (2s). **^{13}C NMR (300 MHz, 298 K, DMSO-d_6):** δ 148.84, 147.23, 146.94, 145.66, 135.33, 133.50, 118.65, 118.62, 114.92, 113.30, 110.92, 110.63, 74.31, 70.84, 62.77, 55.46.

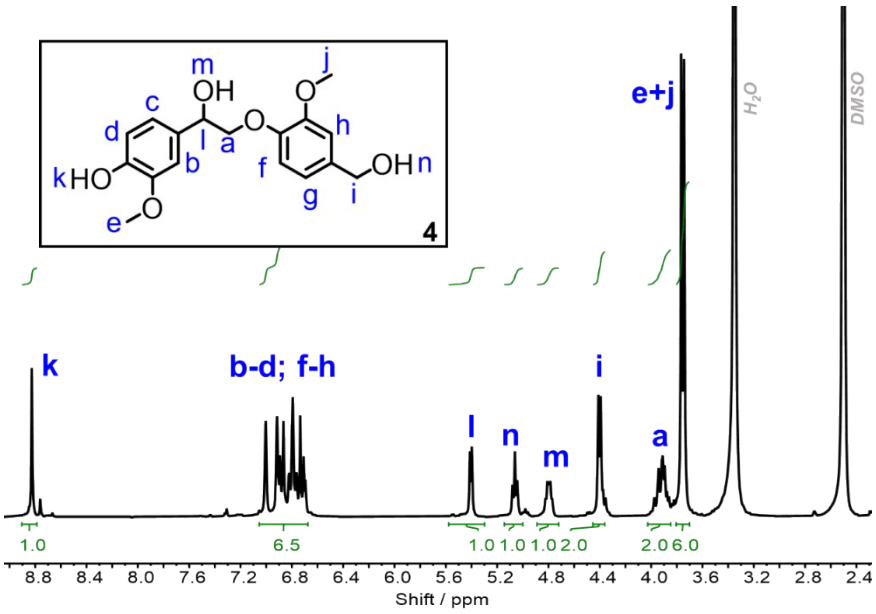

Figure S9: ^{1}H NMR (300 MHz, 298 K, DMSO-d_6) spectrum of 4-(1-hydroxy-2-(4-(hydroxymethyl)-2-methoxyphenoxy)ethyl)-methoxyphenol **4** and the respective chemical shifts.

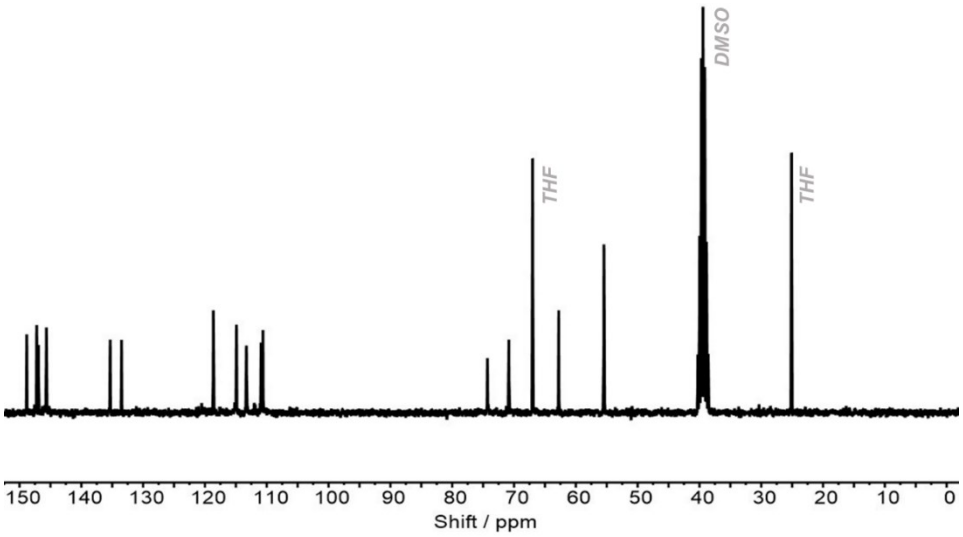

Figure S10: ^{13}C NMR (300 MHz, 298 K, DMSO-d_6) spectrum of 4-(1-hydroxy-2-(4-(hydroxymethyl)-2-methoxyphenoxy)ethyl)-methoxyphenol **4** and the respective chemical shifts.

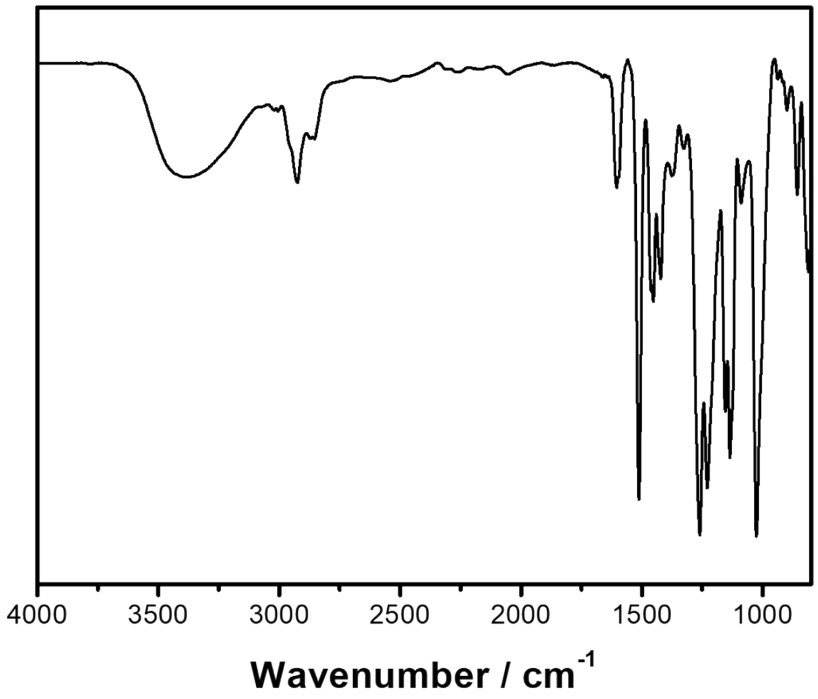

Figure S11: FTIR spectrum of 4-(1-hydroxy-2-(4-(hydroxymethyl)-2-methoxyphenoxy)ethyl)-methoxyphenol **4**.

(E)-2-methoxy-4-(7-methoxy-3-methyl-5-(prop-1-en-1-yl)-2,3-dihydrobenzofuran-2-yl)phenol (Dehydro-isoeugenol, 5)

Dehydro-isoeugenol was prepared by oxidative dimerization according to the method of Sarkanen *et al*.[2] Briefly, isoeugenol (5.0 g, 30.45 mmol) and horseradish peroxidase (10 mg) were dissolved in a mixture of acetone (125 mL) and water (200 mL). To initiate the reaction, a hydrogen peroxide solution (3.14%, 17.4 mL) was added over 45 min while cooling the vessel with a water bath. Afterwards, the mixture was stored at 4°C overnight to precipitate the product from the reaction mixture as slightly yellow crystals. The solid was filtered off and recrystallized in ethanol, yielding **5** as a white crystalline solid (3.4 g, 68%).

^{1}H NMR (300 MHz, 298 K, $CDCl_3$): δ 6.98-6.77 (m), 6.37 (d), 6.17-6.05 (m), 5.65 (s), 5.10 (d), 3.89 (2s), 3.50-3.40 (m), 1.89 (d), 1.38 (d). **^{13}C NMR (300 MHz, 298 K, $CDCl_3$):** δ 146.69, 146.59, 145.80, 144.17, 133.28, 132.21, 132.09, 130.94, 123.51, 119.99, 114.09, 113.32, 109.24, 108.94, 93.81, 45.64, 18.39, 17.57.

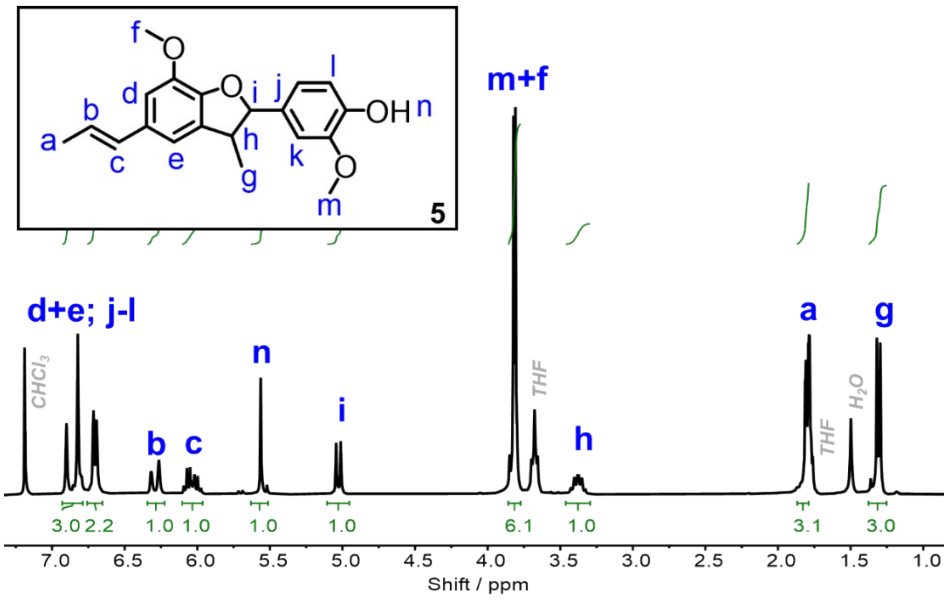

Figure S12: ^{1}H NMR (300 MHz, 298 K, $CDCl_3$) spectrum of (E)-2-methoxy-4-(7-methoxy-3-methyl-5-(prop-1-en-1-yl)-2,3-dihydrobenzofuran-2-yl)phenol (dehydro-isoeugenol, **5**) and the respective chemical shifts.

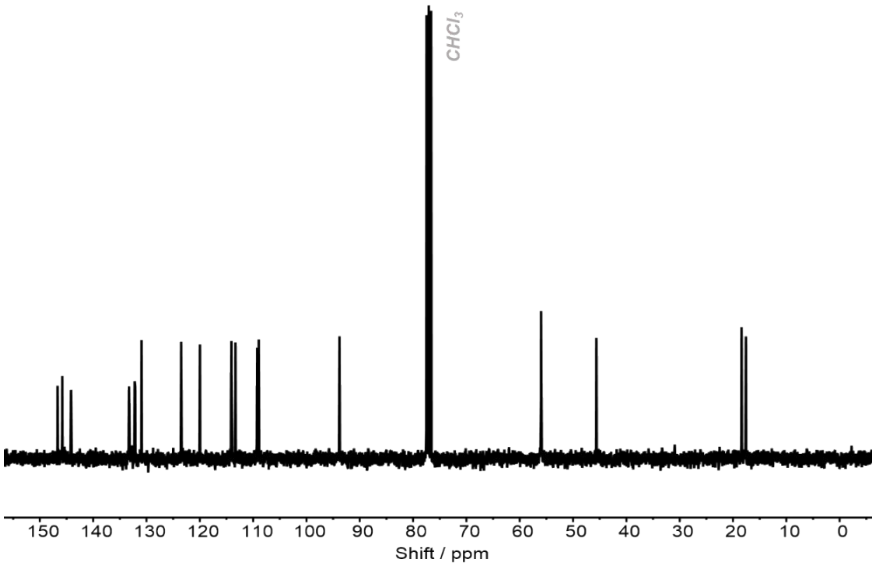

Figure S13: ^{13}C NMR (300 MHz, 298 K, $CDCl_3$) spectrum of (E)-2-methoxy-4-(7-methoxy-3-methyl-5-(prop-1-en-1-yl)-2,3-dihydrobenzofuran-2-yl)phenol (dehydro-isoeugenol, **5**) and the respective chemical shifts.

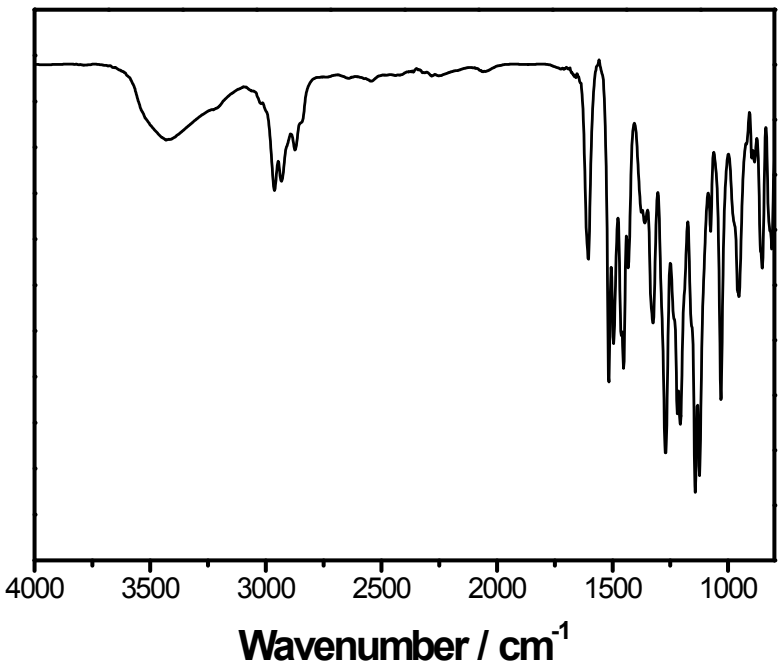

Figure S14: FTIR spectrum of (E)-2-methoxy-4-(7-methoxy-3-methyl-5-(prop-1-en-1-yl)-2,3-dihydrobenzofuran-2-yl)phenol **5**.

4-(5-(1-hydroxypropyl)-7-methoxy-3-methyl-2,3-dihydrobenzofuran-2-yl)-2-methoxyphenol (6)

To a solution of **5** (0.5 g in 5 mL THF, 1.53 mmol, 1 eq.) 9-borabicyclo[3.3.1]nonan (9-BBN, 0.5 M in THF, 6.12 mL, 3.1 mmol, 2 eq.) was added dropwise and the reaction was refluxed at 65°C for 4 h. Afterwards, sodium hydroxide (3.0 M in water, 0.77 mL, 2.3 mmol, 1.5 eq.) and then hydrogen peroxide (30%, 0.92 mL, 7.7 mmol, 5 eq.) was added to the mixture. After one hour, we diluted the solution with 20 mL brine and then extracted with ethyl acetate (3 x 30 mL). The crude product was isolated under reduced pressure from the organic phase and then purified by column chromatography using ethyl acetate and petrol ether (1:3) as eluent. Compound **5** had an R_f value of 0.68 whereas for **6** an R_f value of 0.15 was determined. After solvent evaporation, **6** was obtained as a yellowish solid (0.30 g, 60%).

^{1}H NMR (300 MHz, 298 K, $CDCl_3$): δ 6.90-6.68 (ml), 5.66 (s, n), 5.02 (d), 4.47 (t), 3.81 (2s), 3.38 (m), 1.72 (m), 1.30 (d), 0.86 (t). **^{13}C NMR (300 MHz, 298 K, $CDCl_3$):** δ 146.71, 145.80, 144.13, 144.09, 138.41, 133.11, 132.02, 119.96, 114.15, 113.36, 109.52, 109.90, 93.82, 76.34, 45.69, 32.06, 117.48, 10.41.

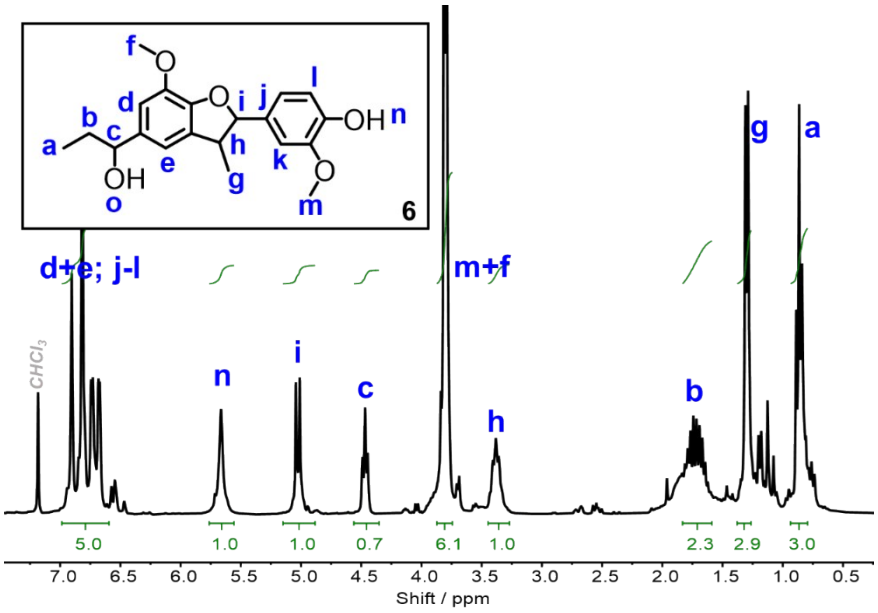

Figure S15: ^{1}H NMR (300 MHz, 298 K, $CDCl_3$) spectrum of 4-(5-(1-hydroxypropyl)-7-methoxy-3-methyl-2,3-dihydrobenzofuran-2-yl)-2-methoxyphenol **6** and the respective chemical shifts.

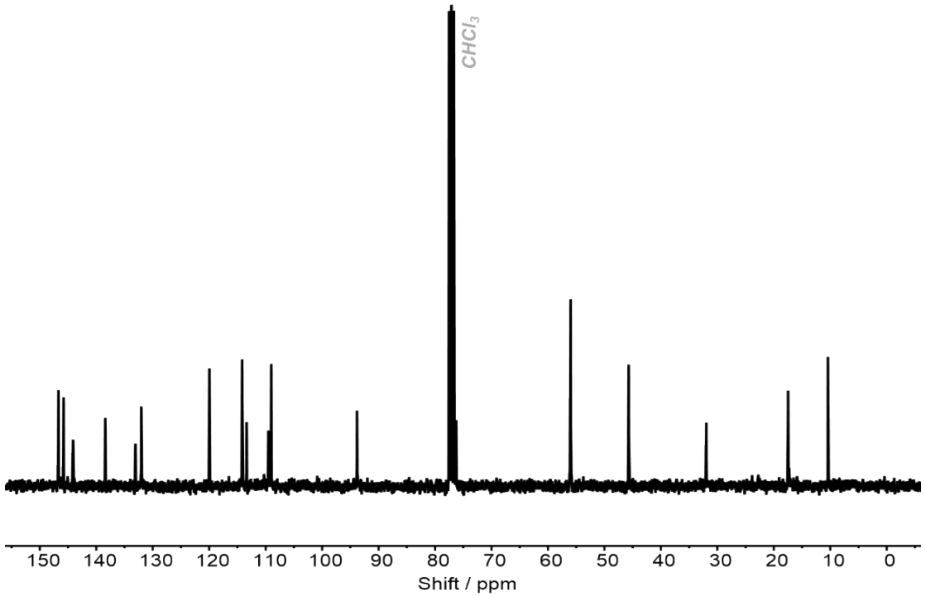

Figure S16: ^{13}C NMR (300 MHz, 298 K, $CDCl_3$) spectrum of 4-(5-(1-hydroxypropyl)-7-methoxy-3-methyl-2,3-dihydrobenzofuran-2-yl)-2-methoxyphenol **6** and the respective chemical shifts.

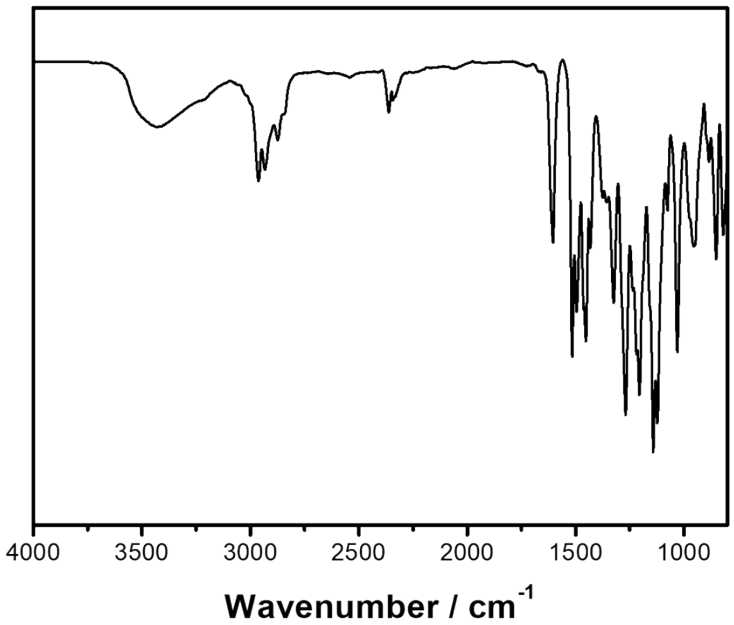

Figure S17: FTIR spectrum of 4-(5-(1-hydroxypropyl)-7-methoxy-3-methyl-2,3-dihydrobenzofuran-2-yl)-2-methoxyphenol **6**.

Preparation of nanocarrier dispersions

4 (0.1 g, 0.30 mmol) or **6** (0.1 g, 0.29 mmol) were dissolved separately in 1.5 mL DMSO and then added to each 9.6 mL cyclohexane containing 80 mg of the surfactant P(E/B-*b*-EO). To generate a miniemulsion, the two-phase mixture was ultrasonicated (Branson Digital Sonifier W450-D, 1/ 2″ tip, 70% amplitude, 3 min, 20 s ultrasound followed by 10 s pauses) while cooling with water. Afterwards, toluene diisocyanate (123 µL, 0.86 mmol) was dissolved in a P(E/B-*b*-EO)-cyclohexane solution (57 mg in 6.4 mL) and was added dropwise to the miniemulsion under vigorous stirring (1000 rpm). The reaction was allowed to process overnight at 30°C. Unreacted TDI and excess of surfactant were removed by centrifugation and redispersion of the nanocarriers in fresh cyclohexane (twice). For transferring the nanocarriers in an aqueous dispersion, two-times redispersed nanocarrier dispersion in cyclohexane was added dropwise to a 0.1 wt% SDS-solution under sonication and shaking. The cyclohexane was evaporated by stirring overnight. The dispersions were characterized by DLS and SEM.

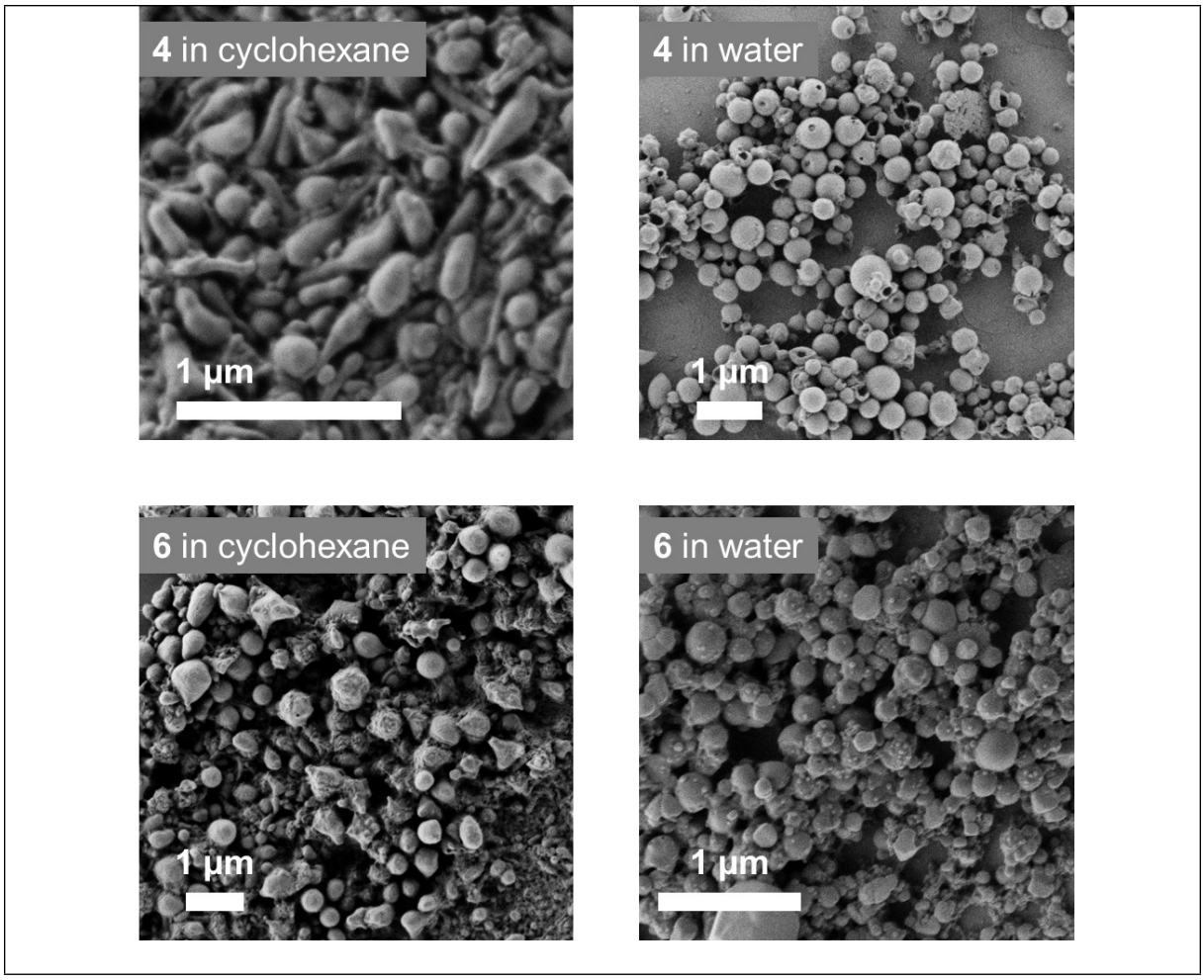

Figure S18: SEM images of nanocarriers obtained after interfacial crosslinking of **4** or **6** with TDI.

Additional References

1. H. Schlaad, H. Kukula, J. Rudloff and I. Below, *Macromolecules*, 2001, **34**, 4302-4304.

2. K. V. Sarkanen and A. F. A. Wallis, *Journal of the Chemical Society, Perkin Transactions 1*, 1973, DOI: 10.1039/P19730001869, 1869-1878.