

Supplementary Materials

Synthesis of bifunctional molecules for the production of polymers based on unsaturated fatty acids as bioderived raw materials

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General experimental information

Chemicals used

Material for cell cultivation and aldoxime dehydratase (Oxd) expression was purchased by Carl Roth (Antibiotics, LB- and TB-premixed medium, D-glucose, D-lactose). Buffer salts (KH_2PO_4 and K_2HPO_4) and hydroxylamine hydrochloride and sodium carbonate were obtained from VWR chemicals. Unsaturated fatty acids were purchased from Sigma Aldrich, TCI Chemicals or Alfa Aesar, respectively. $\text{Rh}(\text{acac})(\text{CO})_2$ was purchased from Alfa Aesar and TPPTS and TPP were purchased from TCI Chemicals.

Analytical methods

NMR spectra were recorded on a Bruker Avance III 500 at a frequency of 500 MHz (^1H) or 125 MHz (^{13}C). The chemical shift δ is given in ppm and referenced to the corresponding solvent signal. Conversions were determined by comparison of significant protons of the product and reactant (alkenes: double bond protons; aldehydes: CHO-protons, aldoximes, NHOH-protons and nitriles: protons in neighborhood to nitrile-functionality).

Accurate mass nano-ESI measurements are performed using a Q-IMS-TOF mass spectrometer Synapt G2Si (Waters GmbH, Manchester, UK) in resolution mode, interfaced to a nano-ESI ion source. Nitrogen serves both as the nebulizer gas and the dry gas for nano-ESI. Nitrogen is generated by a nitrogen generator NGM 11. Helium 5.0 is used as buffer gas in the IMS entry cell, nitrogen 5.0 is used for IMS separations. 1,3-Dicyanobenzene is used as an electron-transfer reagent in ETD experiments. Samples were dissolved in acetonitrile and introduced by static nano-ESI using in-house pulled glass emitters.

Characterization of aldehydes

Hydroformylation of 5-hexenoic acid. Hydroformylation was carried out at 100 °C for 4 h resulting in >99% conversion towards a *n-iso*-mixture of 1:0.51 in a yield of 94%. 7-Oxoheptanoic acid: $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) δ/ppm : 11.98 (s, 1H), 9.65 (t, 1H), 2.40 (m, 2H), 2.20 (m, 2H), 1.49 (m, 4H), 1.26 (m, 2H).

The $^1\text{H-NMR}$ spectrum is in accordance with the literature.[1]

Hydroformylation of 7-octenoic acid. Hydroformylation was carried out at 100 °C for different reaction times resulting in 95% conversion within 23 h towards a *n-iso*-mixture of 1:0.41 in a yield of 93%. 9-Oxononanoic acid: $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) δ/ppm : 11.98 (s, 1H), 9.65 (t, 1H), 2.40 (m, 2H), 2.20 (m, 2H), 1.49 (m, 4H), 1.26 (m, 2H).

The $^1\text{H-NMR}$ spectrum is in accordance with the literature.[2]

Hydroformylation of 9-decenoic acid. Hydroformylation was carried out at 100 °C for different reaction times resulting in 95% conversion within 23 h towards a *n-iso*-mixture of 1:0.33 in a yield of 92%. 11-Oxoundecanoic acid: $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) δ/ppm : 11.98 (s, 1H), 9.76 (t, 1H), 2.42 (m, 2H), 2.35 (m, 2H), 1.63 (m, 2H), 1.56 (m, 2H), 1.30 (m, 10H).

The $^1\text{H-NMR}$ spectrum is in accordance with the literature.[3]

Hydroformylation of oleic acid using Rh-TPP in a neat approach. 9-Formylstearic acid was obtained as a colourless oil with an isolated yield of 98%. ¹H-NMR (CDCl₃, 500 MHz) δ/ppm: 11.40 (s, 1H), 9.54 (d, 1H), 2.35 (t, 2H), 2.24 (m, 1H), 1.65 (m, 2H), 1.45 (m, 2H), 1.28 (m, 22H), 0.88 (t, 3H); ¹³C-NMR (CDCl₃, 125 MHz) δ/ppm: 205.96, 180.13, 52.12, 34.17, 30.02, 29.85, 29.75, 29.58, 29.34, 29.28, 29.17, 29.08, 29.04, 27.23, 27.16, 24.74, 22.80, 14.24.

Characterization of aldoximes

7-*E*-Aldoxime heptanoic acid. The *E*-aldoxime was isolated by filtration as a colourless solid with an isolated yield of 60%. ¹H-NMR (DMSO-d₆, 500 MHz) δ/ppm: 11.98 (s, 1H), 10.71 (s, 1H), 6.62 (t, 1H), 2.20 (m, 4H), 1.50 (p, 2H), 1.40 (p, 2H), 1.29 (m, 2H); ¹³C-NMR (DMSO-d₆, 125 MHz) δ/ppm: 174.43, 150.25, 33.54, 28.37, 25.34, 24.42, 24.23; HRMS (ESI, negative ions) *m/z*: [M-H]⁻ calcd for C₇H₁₂NO₃⁻ 158.0823, found 158.0827.

9-*E*-Aldoxime nonanoic acid. The *E*-aldoxime was isolated by filtration as a colourless solid with an isolated yield of 62%. ¹H-NMR (DMSO-d₆, 500 MHz) δ/ppm: 11.97 (s, 1H), 10.70 (s, 1H), 6.62 (t, 1H), 2.20 (m, 4H), 1.48 (p, 2H), 1.39 (p, 2H), 1.26 (m, 6H); ¹³C-NMR (DMSO-d₆, 125 MHz) δ/ppm: 174.47, 150.34, 33.64, 28.72, 28.46, 28.44, 25.57, 24.52, 24.45; HRMS (ESI, negative ions) *m/z*: [M-H]⁻ calcd for C₉H₁₆NO₃⁻ 186.1136, found 168.1140.

11-*E*-Aldoxime undecanoic acid. The *E*-aldoxime was isolated by filtration as a colourless solid with an isolated yield of 68%. ¹H-NMR (DMSO-d₆, 500 MHz) δ/ppm: 11.96 (s, 1H), 10.69 (s, 1H), 6.62 (t, 1H), 2.20 (m, 4H), 1.47 (p, 2H), 1.38 (p, 2H), 1.25 (m, 10H); ¹³C-NMR (DMSO-d₆, 125 MHz) δ/ppm: 174.49, 150.36, 33.66, 28.93, 28.84, 28.80, 28.70, 28.53, 25.61, 24.53, 24.49; HRMS (ESI, negative ions) *m/z*: [M-H]⁻ calcd for C₁₁H₂₁NO₃⁻ 216.1594, found 216.1594.

9-Formyloxime stearic acid. The *E*/*Z*-aldoxime mixture (~1:1) was isolated by filtration as a colourless solid with an isolated yield of 95%. ¹H-NMR (CDCl₃, 500 MHz) δ/ppm: 9.04 (s, 1H), 7.21 (d, 1H, *Z*-Aldoxime-H), 6.44 (d, 1H, *E*-Aldoxime-H), 3.08 (m, 1H, *Z*), 2.31 (t, 2H), 2.19 (m, 1H, *E*), 1.60 (m, 2H), 1.30 (m, 26H), 0.88 (t, 3H); ¹³C-NMR (CDCl₃, 125 MHz) δ/ppm: 179.58, 156.49 (*Z*), 155.79 (*E*) 39.96, 35.22, 34.49, 33.19, 33.02, 32.03, 29.83, 29.75, 29.60, 29.41, 29.17, 29.07, 27.18, 24.94, 22.80, 14.25; HRMS (ESI, positive ions) *m/z*: [M-H]⁺ calcd for C₁₉H₃₇NO₃H⁺ 328.2846, found 328.2839.

Characterization of nitriles

6-Cyanohexanoic acid. The nitrile was obtained as a yellowish oil from the Cu^{II}-acetate synthesis in an isolated yield of 71%. ¹H-NMR (DMSO-d₆, 500 MHz) δ/ppm: 2.38 (m, 4H), 1.69 (m, 4H), 1.53 (m, 2H).

The ¹H-NMR spectrum is in accordance with the literature.[1]

8-Cyanooctanoic acid. The nitrile was obtained as a yellowish oil from the Cu^{II}-acetate synthesis in an isolated yield of 85%. ¹H-NMR (DMSO-d₆, 500 MHz) δ/ppm: 2.35 (m, 4H), 1.66 (m, 4H), 1.46 (m, 2H), 1.36 (m, 4H).

The ¹H-NMR spectrum is in accordance with the literature.[4]

10-Cyanodecanoic acid. The nitrile was obtained as a yellowish oil from the Cu^{II}-acetate synthesis in an isolated yield of 89%. ¹H-NMR (DMSO-d₆, 500 MHz) δ/ppm: 2.34 (m, 4H), 1.64 (m, 4H), 1.44 (m, 2H), 1.31 (m, 8H).

The ¹H-NMR spectrum is in accordance with the literature.[5]

9-Cyanostearic acid. The nitrile was obtained as a yellowish oil from the Cu^{II}-acetate synthesis in an isolated yield of 91%. ¹H-NMR (DMSO-d₆, 500 MHz) δ/ppm: 11.71 (s, 1H), 2.48 (m, 1H), 2.40 (m, 2H), 1.58 (m, 8H), 1.29 (m, 20H) 0.87 (t, 3H); ¹³C-NMR (CDCl₃, 125 MHz) δ/ppm: 180.94, 130.44, 61.49, 34.24, 32.01, 31.93, 31.84, 29.45, 29.24, 29.09, 29.01, 28.87, 28.79, 27.15, 27.04, 24.54, 22.62, 21.03, 14.04; HRMS (ESI, positive ions) *m/z*: [M-H]⁺ calcd for C₁₉H₃₅NO₃H⁺ 310.2741, found 310.2737.

Oxd sequences

Aldoxime dehydratase from *Bacillus* sp. OxB-1 (OxdB) (Accession number: GenBank: AP013294.1)

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ATGAAAAATATGCCGGAAAATCACAATCCACAAGCGAATGCCTGGACTGCCGAATTTCC
TCCTGAAATGAGCTATGTAGTATTTGCGCAGATTGGGATTCAAAGCAAGTCTTTGGATCA
CGCAGCGGAACATTTGGGAATGATGAAAAAGAGTTTCGATTTGCGGACAGGCCCCAAA
CATGTGGATCGAGCCTTGCATCAAGGAGCCGATGGATACCAAGATTCCATCTTTTTAGC
CTACTGGGATGAGCCTGAAACATTTAAATCATGGGTTGCGGATCCTGAAGTACAAAAGT
GGTGGTCGGGTAAAAAATCGATGAAAATAGTCCAATCGGGTATTGGAGTGAGGTAACG
ACCATTCCGATTGATCACTTTGAGACTCTTCATTCCGGAGAAAATTACGATAATGGGGTT
TCACACTTTGTACCGATCAAGCATAACAGAAGTCCATGAATATTGGGGAGCAATGCGCGA
CCGCATGCCGGTGTCTGCCAGTAGTGATTTGGAAAGCCCCCTTGGCCTTCAATTACCG
GAACCCATTGTCCGGGAGTCTTTCGGAAAACGGCTAAAAGTCACGGCGCCGGATAATAT
TTGCTTGATTGGAACCGCTCAAATTGGTCTAAATGTGGTAGCGGGGAAAGGGAAACGT
ATATAGGACTAGTGGAACCGACCCTCATAAAAGCGAATACGTTTCTTCGTGAAAATGCTA
GTGAAACAGGCTGTATTAGTTCAAATTAGTCTATGAACAGACCCATGACGGCGAAATA
GTAGATAAATCATGTGTCATCGGATATTATCTCTCCATGGGGCATCTTGAACGCTGGAC
GCATGATCATCCAACACATAAAGCGATCTACGGAACCTTTTATGAGATGTTGAAAAGGCA
TGATTTTAAGACCGAACTTGCTTTATGGCACGAGGTTTCGGTGCTTCAATCCAAAGATAT
CGAGCTTATCTATGTCAACTGCCATCCGAGTACTGGATTTCTTCCATTCTTTGAAGTGAC
AGAAATTCAAGAGCCTTTACTGAAAAGCCCTAGCGTCAGGATCCAGTGA
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Oxd expression

E.coli BL21-CodonPlus(DE3)-RIL cells harboring the pUC18 plasmid including with the OxdB-gene were stored as glycerol stocks at -80 °C. A sample from the glycerol stocks for each Oxd was plated on LB-agar containing 34 µg/mL Chloramphenicol and 100 µg/mL Carbenicillin and incubated for 12 to 18 h at 37 °C. Pre-cultures were prepared in 5 mL LB-medium containing 50 µg/mL Kanamycin and 34 µg/mL Chloramphenicol (OxdRE) or 100 µg/mL Carbenicillin (OxdB) using a single colony from the LB-agar plate. The cultures were incubated for 12 to 18 h at 37 °C and 180 rpm. Main cultures for Oxd expression were performed using TB-autoinduction medium (40 mL 20 g/L lactose solution, 4 mL 50 g/L D-glucose solution and 356 mL TB-medium (purchased from Carl Roth) in a 500 mL Erlenmeyer flask). 34 µg/mL Chloramphenicol (OxdRE) and 100 µg/mL Carbenicillin were added to the medium. Main cultures were inoculated with 1 % (4 mL) of the relating pre-cultures and incubated for 2 h at 37 °C and 150 rpm. After 1 h incubation at 37 °C OxdB-cultures were cultivated at 30 °C for 72 h and 150 rpm. Cell harvest was performed at 4,000 xg for 15 min at 4 °C. Cells were washed three times with PPB (50 mM, pH

7) and afterwards resuspended in 2x mass of the bio wet weight (bww) of the cells PPB (50 mM, pH 7) and stored at 4 °C until usage. Expression success was analyzed by SDS-PAGE using a 12 % separation gel in comparison to a protein marker (*PageRuler Prestained* (Thermo Fisher)).

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NMR-Spectra













