Supporting information

Crystallization-integrated mandelate racemase-catalyzed

dynamic kinetic resolution of racemic mandelic acid

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1. Exemplary chromatograms of chiral HPLC analysis



Figure S1: Exemplary chromatograms from the chiral HPLC of mandelic acid. **I.** Standard of racemic mandelic acid with addition of enantiopure (R)-mandelic acid (2:1); **II.** DKR fed-batch after 96 hours, 150 mM DPEN, 200 mM racMA, 10 U/mI mandelate racemase, aqueous phase. **III.** DKR fed-batch 50 ml product salt after 96 h, enantiomeric excess determination (*ee* 94.9%).

2. NMR

All spectra were recorded with the Bruker Biospin Avance III (400 MHz) and Avance Neo (600 MHz) instruments. 1H-NMRs were recorded at 400 MHz and 13C-NMRs were recorded at 100.6 MHz.

2.1 Diastereomeric salts for solubility screening and XRPD analysis

(R)-MA (1R,2R)-DPEN diamine salt

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.43-7.12 (m, 20 H Aryl), 6.29 (s, R-OH), 4.81 (s, 2H,R-CHOH-R), 4.20 (s, 2H, R-CHNR₃-R). Peaks at 3.75, 3.16 and 1.69-1.43 ppm belong to residual CPME.



¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 175.35 (s, 2C, R-COO⁻), 142.57 (s, 2C, Aryl**C**-CHOHR), 139.60 (s, 2C, Aryl**C**-CHNH₃R), 128.59-126.94 (m, 20 C, Aryl), 73.52 (s, 2C, R-CHOH-R), 60.23 (s, 2C, R-CHNH₃-R). Peaks at 82.46, 56.03, 31.89 and 23.60 ppm belong to residual CPME.



(R)-MA (1R,2R)-DPEN monoamine salt

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.41-7.11 (m, 15 H Aryl), 4.61 (s, 1H,R-CHOH-R), 4.11 (s, 2H, R-CHNR₃-R).



¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 175.18 (s, 1C, R-COO⁻), 143.91 (s, 1C, Aryl**C**-CHOHR), 140.73 (s, 2C, Aryl**C**-CHNH₃R), 128.48-126.66 (m, 15 C, Aryl), 73.90 (s, 1C,R-CHOH-R), 60.80 (s, 2C, R-CHNH₃-R).



(S)-MA (1R,2R)-DPEN diamine salt

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.42-7.14 (m, 20 H Aryl), 5.66 (s, R-OH), 4.79 (s, 2H,R-CHOH-R) , 4.19 (s, 2H, R-CHNR₃-R)



¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 175.13 (s, 2C, R-COO⁻), 142.60 (s, 2C, Aryl**C**-CHOHR), 139.82 (s, 2C, Aryl**C**-CHNH₃R), 128.59-126.93 (m, 20 C, Aryl), 73.49 (s, 2C, R-CHOH-R), 60.32 (s, 2C, R-CHNH₃-R)



(S)-MA (1R,2R)-DPEN monoamine salt

 $^1\text{H-NMR}$ (DMSO-d_6, 400 MHz) δ [ppm]: 7.41-7.12 (m, 15 H Aryl), 4.61 (s, 1H,R-CHOH-R) , 4.13 (s, 2H, R-CHNR_3-R)



¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 175.21 (s, 1C, R-COO⁻), 143.92 (s, 1C, Aryl**C**-CHOHR), 140.76 (s, 2C, Aryl**C**-CHNH₃R), 128.48-126.65 (m, 15 C, Aryl), 73.91 (s, 1C,R-CHOH-R), 60.81 (s, 2C, R-CHNH₃-R)



(R)-MA (R)-122TPEA

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.60-6.94 (m, 15H, Aryl), 5.14-4.44 (dd, 2H, J=11.67 Hz, J_{H-H} =253.39 Hz, Aryl-CHR-R), 4.73 (s, 1H, R-CHOH-R)



¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 174.70 (s, 1C, R-COO⁻), 142.98-142.31 (3s, 3C, Aryl**C**-CHR₂), 140.93 (s, 1C, Aryl**C**-CHOH-R), 129.20-126.45 (m, 20C, Aryl), 73.54 (s, 1C, R-CHOH-R), 57.89 (s, 1C, R-CHNH₃-R), 57.77 (s, 1C, R-CH-Aryl₂)



(S)-MA (R)-122TPEA

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.62-6.94 (m, 15H, Aryl), 5.15-4.48 (dd, 2H, J=11.18 Hz, J_{H-H}=243.18 Hz, Aryl-C**H**R-R), 4.73 (s, 1H, R-C**H**OH-R)



¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 174.79 (s, 1C, R-COO⁻), 143.10-142.27 (3s, 3C, Aryl**C**-CHR₂), 140.78 (s, 1C, Aryl**C**-CHOH-R), 129.21-126.46 (m, 20C, Aryl), 73.60 (s, 1C, R-CHOH-R), 57.86 (s, 1C, R-CHNH₃-R), 57.77 (s, 1C, R-CH-Aryl₂)



(R)-MA (S)-1PEA

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.47-7.16 (m, 10H, Aryl), 4.60 (s, 1H, R-CHOH-R), 4.32-4.27 (q, 1H, J=6.82 Hz, R-CHNH₃-R), 1.45-1.43 (d, 3H, J=6.79 Hz, R-CH₃)



¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 175.58 (s, 1C, R-COO⁻), 144.05 (s, 1C, Aryl**C**-CHNH₃-R), 140.86 (s, 1C, Aryl**C**-CHOH-R), 129.01-126.61 (m, 10C, Aryl), 73.99 (s, 1C, R-CHOH-R), 50.29 (s, 1C, R-CHNH₃-R), 21.63 (s, 1C, R-CH₃)





(S)-MA (S)-1PEA

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.47-7.16 (m, 10H, Aryl), 4.58 (s, 1H, R-CHOH-R), 4.31-4.26 (q, 1H, J=6.85 Hz, R-CHNH₃-R), 1.45-1.43 (d, 3H, J=6.81 Hz, R-CH₃)





¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 175.36 (s, 1C, R-COO⁻), 144.00 (s, 1C, Aryl**C**-CHNH₃-R), 140.84 (s, 1C, Aryl**C**-CHOH-R), 129.03-126.61 (m, 10C, Aryl), 73.91 (s, 1C, R-CHOH-R), 50.26 (s, 1C, R-CHNH₃-R), 21.65 (s, 1C, R-CH₃)



(*R*)-MA (1*R*,2*S*)-ADPE

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.42-7.06 (m, 15H, Aryl), 5.09-5.07 (d, 1H, J=3.86 Hz, R-CHOH-R ADPE), 4.62 (s, 1H, R-CHOH-R MA), 4.31-4.29 (d, 1H, J=3.98 Hz, R-CHNH₃-R)



¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 175.50 (s, 1C, R-COO⁻), 143.94 (s, 1C, Aryl**C**-CHNH₃-R), 142.01 (s, 1C, Aryl**C**-CHOH-R), 137.02 (s, 1C, Aryl**C**-CHOH-R), 129.09-126.63 (m, 15C, Aryl), 73.96 (s, 1C, R-CHOH-R ADPE), 73.83 (s, 1C, R-CHOH-R MA), 60.43 (s, 1C, R-CHNH₃-R)



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(S)-MA (1*R*,2S)-ADPE

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.43-7.06 (m, 15H, Aryl), 5.10-5.08 (d, 1H, J=4.11 Hz, R-CHOH-R ADPE), 4.64 (s, 1H, R-CHOH-R MA), 4.33-4.32 (d, 1H, J=3.96 Hz, R-CHNH₃-R)



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¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 175.56 (s, 1C, R-COO⁻), 143.79 (s, 1C, Aryl**C**-CHNH₃-R), 141.94 (s, 1C, Aryl**C**-CHOH-R), 136.79 (s, 1C, Aryl**C**-CHOH-R), 129.11-126.70 (m, 15C, Aryl), 73.93 (s, 1C, R-CHOH-R ADPE), 73.73 (s, 1C, R-CHOH-R MA), 60.36 (s, 1C, R-CHNH₃-R)



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2.2 Preparative reaction

Rac-Na⁺MA⁻

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.37-7.28 (m, 5H, Aryl), 4.91(s, 1H, R-OH), 4.70 (s, 1H, R-CHOH-R)



(1R,2R)-DPEN • 2HCI

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.38-7.29 (m, 6H, Aryl), 7.18-7.14 (m, 4H, Aryl), 5.00 (s, 2H, R-CHNH₃-R), 4.69 (s, 6H, R-NH₃⁺)



Product salt, preparative 50 ml reaction

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 7.42-7.11 (m, Aryl), 4.60 (s, R-CHOH-R), 4.12 (s, R-CHNH₃-R)



Purity mandelic acid from product salt

¹**H-NMR** (DMSO-d₆, 400 MHz) δ [ppm]: 12.61 (s, 1H, R-COOH), 7.44-7.27 (m, 5H, Aryl), 5.08 (s, 1H, R-CHOH-R Isopropyl ester), 5.04 (s, 1H, R-CHOH-R), 4.90 (sept, 1H, J=6.17 Hz, R-OCH-R Isopropyl ester), 1.19-1.06 (dd, 6H, J=6.27 Hz, JH-H= 43.25 Hz, R-CH₃ Isopropyl ester)



¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 174.56 (s, 1C, R-COOH), 172.54 (s, 1C, R-COO-R Isopropyl ester), 140.67 (s, 1C, Aryl**C**-CHOHR), 140.17 (s, 1C, Aryl**C**-CHOHR Isopropyl ester), 128.66-127.00 (m, 10C, Aryl, Mandelic acid and Isopropyl ester), 72.95 (s, 1C,R-CHOH-R Isopropyl ester), 72.83 (s, 1C,R-CHOH-R), 68.30 (s, 1C, R-OCH-R Isopropyl ester), 21.98-21.75 (2s, 2C, R-CH₃ Isopropyl ester)



Purity (1*R*,2*R*)-DPEN from product salt

8

 $^{1}\text{H-NMR}$ (DMSO-d_6, 400 MHz) δ [ppm]: 7.17-7.07 (m, 10H Aryl), 3.83 (s, 2H, R-CHNH_2-R) , 1.91 (s, 4H, R-NH_2)



15

[ppm]

¹³**C-NMR** (DMSO-d₆, 100.6 MHz) δ [ppm]: 145.32 (s, 2C, Aryl**C**-CHNH₂R), 128.31-126.63 (m, 10C, Aryl), 62.93 (s, 2C, R-**C**HNH₂-R)



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150			10	00				50				[ppm]

3. XRPD data for determination of the crystalline product phase of the mandelate resolution experiments



Figure S2: XRPD data comparison of a crystallized product salt sample from the DKR-based approaches (blue line, reaction conditions: 200 mM racemic mandelic acid, 150 mM (1R,2R)-DPEN, 10 U/ml mandelate racemase, 96 h) to (R)- and (S)-MA (1R,2R)-DPEN monoamine salts (red/green lines respectively) and the (S)-MA (1R,2R)-DPEN monoamine salt (orange line). The (R)-MA (1R,2R)-DPEN diamine salt was excluded from the comparison and can be seen in **Figure S3**. The comparison of the positions of the major reflections (dotted lines) shows that the dominant phase of the reaction product salt clearly corresponds to the (R)-MA (1R,2R)-DPEN monoamine salt. Reflections marked with an asterisk (*) can be attributed to residual amounts of NaCl in the product salt.



Figure S3: XRPD pattern of the (R)-MA (1R,2R)-DPEN diamine salt, precipitated from ether. The salt shows very low crystallinity including a typical background signal of amorphous phases and it was therefore excluded from the comparison in **Figure S2**.

4. Miscellaneous

4.1 Chemicals

All required chemicals and solvents were commercially available and purchased from BldPharm, Sigma-Aldrich, Merck, Acros Organics, VWR, Thermo Fisher scientific, TCI Chemicals, Fluka and Deutero and were used without further purification. racMA, (*R*)-MA and (*S*)-MA were purchased from Sigma. (1*R*,2*R*)-DPEN was purchased from BLD-Pharm.

4.2 Product salt from preparative reaction

