Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Material

Development of an efficient and scalable bioprocess for the plant hormone 12-OPDA: Overcoming hurdles of nature´s biosynthesis

Tim Lukas Guntelmann[a], Karl-Josef Dietz[b] and Harald Gröger *[a]

[a.] *Chair of Industrial Organic Chemistry and Biotechnology, Faculty Chemistry, Bielefeld University.*
[b.] *Chair of Plant Biochemistry and Physiology, Faculty of Biology, Bielefeld University.*

Table of Contents

1 Experimental Information

1.1 Sequences and plasmid maps

In this work two allene oxide cyclase genes were used. Allene oxide cyclase 2 (wildtype and codon-optimized for *E. coli*) and 4 (wildtype) from *Arabidopsis thaliana*. The allene oxide synthase from Arabidopsis thaliana was used as wildtype gene on a pET28a and also on pET28a duet vector with AtAOC2 and 4. All plasmid maps and sequences are shown below. All constructs were ordered commercially from *TwistBioscience*, San Francisco and *ThermoFisher*.

1.1.1 pET28a(+)-AtAOS_wtΔchlrp-AtAOC4_wtΔchlrp_(N)-Strep-tag (Twist Bioscience)

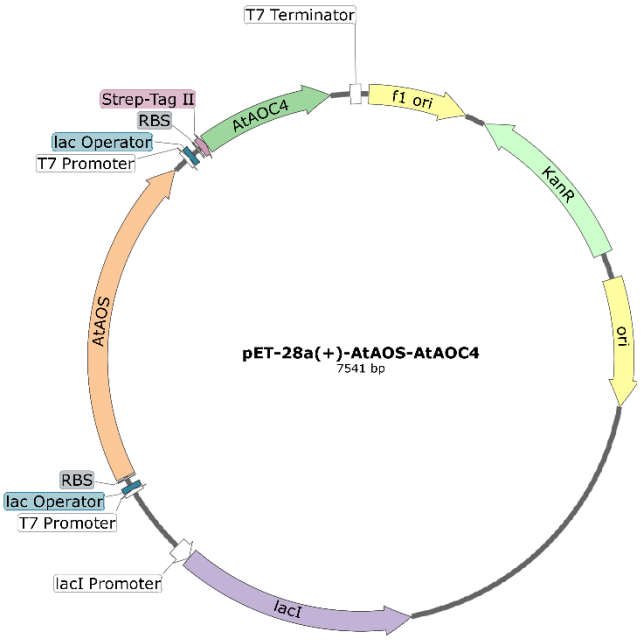


Figure S. 1: Plasmid map of the pET28a-AtAOS_AtAOC4 vector.

Allene oxide synthase (AOS) from *Arabidopsis thaliana* (56.1 kDa), wild type sequence, without chloroplast signaling sequence (chlrp) and with the solubility sequence AKKTS (Uniprot: Q96242)

ATGGCAAAAAAAACATCATCAGCATCAGGATCAGAAACACCAGACCTAACAGTAGCGACACGAACCGGATCCAAAGATCTCCCGATCCG
AAACATACCGGGAAACTACGGTTTACCAATCGTAGGACCAATCAAAGACCGTTGGGATTACTTTTACGACCAAGGAGCTGAAGAGTTCTT
CAAATCACGAATCCGTAAATACAACTCCACGGTGTACAGAGTCAACATGCCACCGGGAGCTTTTATCGCCGAGAATCCACAAGTCGTGGC
TTTACTCGACGGTAAAAGCTTCCCGGTTTTATTCGATGTCGATAAAGTCGAAAAGAAAGATCTTTTCACCGGTACTTACATGCCGTCAACG
GAACTAACCGGAGGCTACCGTATCCTCTCGTACCTCGATCCATCGGAGCCTAAACACGAAAAGCTCAAAAATCTCCTTTTCTTCCTCCTCA
AGTCATCTCGAAACCGGATCTTCCCTGAGTTTCAAGCTACTTACTCCGAGCTTTTCGATTCTTTGGAGAAAGAGCTTTCCCTTAAAGGGAA
AGCGGATTTCGGCGGTTCCAGCGACGGAACCGCCTTTAATTTCTTGGCTCGGGCTTTCTACGGGACGAATCCCGCAGATACAAAGCTCAA
AGCCGACGCTCCGGGTTTGATCACTAAATGGGTTTTATTCAATCTCCATCCATTACTCTCTATTGGTTTACCGAGAGTTATAGAAGAACCTC
TCATCCATACATTTAGTCTACCACCGGCGTTAGTCAAATCTGATTACCAGAGACTCTACGAGTTTTTCTTAGAATCCGCCGGTGAGATTCTC
GTTGAAGCCGATAAATTGGGTATCTCACGAGAAGAAGCTACTCACAATCTTCTCTTCGCCACGTGCTTCAACACGTGGGGTGGGATGAA
GATTTTGTTTCCGAATATGGTTAAACGTATCGGGCGGGCGGGTCATCAAGTTCATAACCGATTAGCGGAGGAGATTAGATCTGTGATTAA
ATCCAACGGCGGAGAACTCACGATGGGAGCGATTGAGAAAATGGAGTTAACCAAATCAGTGGTTTACGAATGTCTCCGGTTTGAACCAC
CGGTTACGGCTCAATACGGTAGAGCGAAGAAGGATCTGGTTATCGAAAGCCACGACGCGGCGTTTAAAGTCAAAGCCGGTGAAATGCT
TTACGGTTATCAACCGTTGGCGACGAGAGATCCGAAGATTTTTGATCGGGCGGATGAGTTTGTGCCGGAGAGATTCGTCGGAGAAGAA
GGAGAGAAGCTTTTGAGGCATGTGTTGTGGTCGAATGGACCGGAGACGGAGACTCCGACGGTGGGGAATAAACAATGCGCCGGTAAG
GATTTTGTTGTTTTGGTGGCGAGGTTGTTTGTGATTGAGATTTTCCGGCGATATGATTCGTTTGATATTGAGGTTGGTACGTCGCCGTTAG
GAAGCTCCGTTAATTTCTCGTCGTTAAGGAAAGCTAGCTTTGTCGACAAGCTTGCGGCgGCACTCGGGTAA

Amino acid sequence

MAKKTSSASGSETPDLTVATRTGSKDLPIRNIPGNYGLPIVGPIKDRWDYFYDQGAEEFFKSRIRKYNSTVYRVNMPPGAFIAENPQVVALLDGK
SFPVLFDVDKVEKKDLFTGTYMPSTELTGGYRILSYLDPSEPKHEKLKNLLFFLLKSSRNRIFPEFQATYSELFDSLEKELSLKGKADFGGSSDGTAF
NFLARAFYGTNPADTKLKADAPGLITKWVLFNLHPLLSIGLPRVIEEPLIHTFSLPPALVKSDYQRLYEFFLESAGEILVEADKLGISREEATHNLLFA
TCFNTWGGMKILFPNMVKRIGRAGHQVHNRLAEEIRSVIKSNGGELTMGAIEKMELTKSVVYECLRFEPPVTAQYGRAKKDLVIESHDAAFKV
KAGEMLYGYQPLATRDPKIFDRADEFVPERFVGEEGEKLLRHVLWSNGPETETPTVGNKQCAGKDFVVLVARLFVIEIFRRYDSFDIEVGTSPLG
SSVNFSSLRKASFVDKLAAALG-

Allene oxide cyclase 4 (AOC4) from *Arabidopsis thaliana* (23.6 kDa), wild type sequence, without chloroplast signaling sequence (chlrp) and with (N)-strep-tag (Uniprot: Q93ZC5)

ATGGCAAGCTGGAGCCACCCGCAGTTCGAAAAGAGCGCAGCAAGATCCACCACTTCCTCCACTGGAGGTTTCTTCAGAACCATTTGCAGC
AGCAGTAGCAATGACTACTCCAGACCAACTAAGATCCAAGAGCTTAACGTCTACGAGTTCAACGAAGGTGATCGAAACAGTCCAGCGGT
TTTAAAACTAGGGAAAAAACCAGATCAGCTTTGTCTCGGAGATCTTGTCCCCTTCACAAACAAACTCTACACAGGAGATCTCACAAAACG
CATAGGAATCACCGCAGGTCTCTGCGTTTTGATCCAACATGTTCCAGAGAAGAAAGGTGATCGCTTTGAAGCTTCTTACAGTTTCTACTTC
GGAGATTATGGTCACATATCAGTTCAAGGACCTTATCTCACTTACGAGGACACGTTTCTTGCTATCACCGGTGGCTCCGGCGTGTTTGAA
GGAGCTTATGGACAGGTCAAGCTTCGTCAGCTTGTGTATCCTACGAAGCTGTTTTACACTTTCTACTTGAAAGGTGTTGCTGCTGATTTAC
CTGTGGAGCTTACCGGAAAACATGTTGAGCCGTCGAAGGAAGTGAAACCGGCGGCTGAAGCTCAGGCGACTCAGCCCGGCGCTACGAT
CGCTAACTTTACTAATTGA

Amino acid sequence

MASWSHPQFEKSAARSTTSSTGGFFRTICSSSSNDYSRPTKIQELNVYEFNEGDRNSPAVLKLGKKPDQLCLGDLVPFTNKLYTGDLTKRIGITAG
LCVLIQHVPEKKGDRFEASYSFYFGDYGHISVQGPYLTYEDTFLAITGGSGVFEGAYGQVKLRQLVYPTKLFYTFYLKGVAADLPVELTGKHVEPS
KEVKPAAEAQATQPGATIANFTN-

1.1.2 pET28a-AtAOS(-)_AtAOC2_Δchlrp_(N)-His-tag (Twist Bioscience)

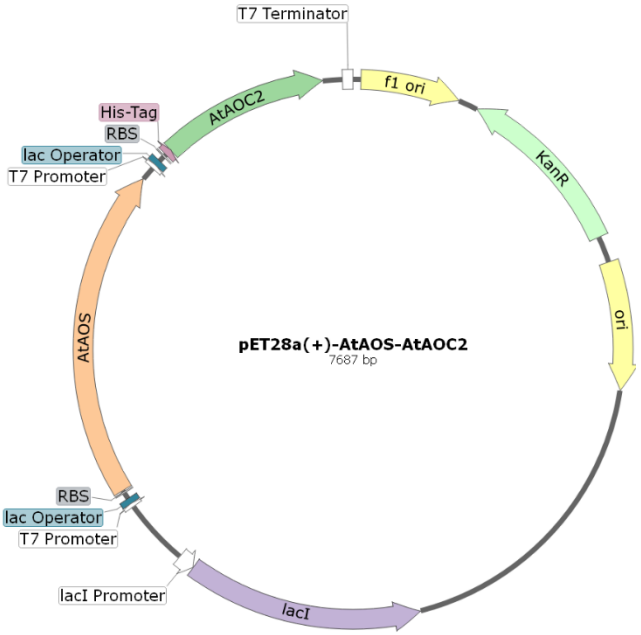


Figure S. 2: Plasmid map of the pET28a-AtAOS_AtAOC2 vector.

Allene oxide synthase (AOS) from *Arabidopsis thaliana* (56.1 kDa), wild type sequence, without chloroplast signaling sequence (chlrp) and with the solubility sequence AKKTS (Uniprot: Q96242)

ATGGCAAAAAAAACATCATCAGCATCAGGATCAGAAACACCAGACCTAACAGTAGCGACACGAACCGGATCCAAAGATCTCCCGATCCG
AAACATACCGGGAAACTACGGTTTACCAATCGTAGGACCAATCAAAGACCGTTGGGATTACTTTTACGACCAAGGAGCTGAAGAGTTCTT
CAAATCACGAATCCGTAAATACAACTCCACGGTGTACAGAGTCAACATGCCACCGGGAGCTTTTATCGCCGAGAATCCACAAGTCGTGGC
TTTACTCGACGGTAAAAGCTTCCCGGTTTTATTCGATGTCGATAAAGTCGAAAAGAAAGATCTTTTCACCGGTACTTACATGCCGTCAACG
GAACTAACCGGAGGCTACCGTATCCTCTCGTACCTCGATCCATCGGAGCCTAAACACGAAAAGCTCAAAAATCTCCTTTTCTTCCTCCTCA
AGTCATCTCGAAACCGGATCTTCCCTGAGTTTCAAGCTACTTACTCCGAGCTTTTCGATTCTTTGGAGAAAGAGCTTTCCCTTAAAGGGAA
AGCGGATTTCGGCGGTTCCAGCGACGGAACCGCCTTTAATTTCTTGGCTCGGGCTTTCTACGGGACGAATCCCGCAGATACAAAGCTCAA
AGCCGACGCTCCGGGTTTGATCACTAAATGGGTTTTATTCAATCTCCATCCATTACTCTCTATTGGTTTACCGAGAGTTATAGAAGAACCTC
TCATCCATACATTTAGTCTACCACCGGCGTTAGTCAAATCTGATTACCAGAGACTCTACGAGTTTTTCTTAGAATCCGCCGGTGAGATTCTC
GTTGAAGCCGATAAATTGGGTATCTCACGAGAAGAAGCTACTCACAATCTTCTCTTCGCCACGTGCTTCAACACGTGGGGTGGGATGAA
GATTTTGTTTCCGAATATGGTTAAACGTATCGGGCGGGCGGGTCATCAAGTTCATAACCGATTAGCGGAGGAGATTAGATCTGTGATTAA
ATCCAACGGCGGAGAACTCACGATGGGAGCGATTGAGAAAATGGAGTTAACCAAATCAGTGGTTTACGAATGTCTCCGGTTTGAACCAC
CGGTTACGGCTCAATACGGTAGAGCGAAGAAGGATCTGGTTATCGAAAGCCACGACGCGGCGTTTAAAGTCAAAGCCGGTGAAATGCT
TTACGGTTATCAACCGTTGGCGACGAGAGATCCGAAGATTTTTGATCGGGCGGATGAGTTTGTGCCGGAGAGATTCGTCGGAGAAGAA
GGAGAGAAGCTTTTGAGGCATGTGTTGTGGTCGAATGGACCGGAGACGGAGACTCCGACGGTGGGGAATAAACAATGCGCCGGTAAG
GATTTTGTTGTTTTGGTGGCGAGGTTGTTTGTGATTGAGATTTTCCGGCGATATGATTCGTTTGATATTGAGGTTGGTACGTCGCCGTTAG
GAAGCTCCGTTAATTTCTCGTCGTTAAGGAAAGCTAGCTTTGTCGACAAGCTTGCGGCGGCACTCGGGTAA

Amino acid sequence

MAKKTSSASGSETPDLTVATRTGSKDLPIRNIPGNYGLPIVGPIKDRWDYFYDQGAEEFFKSRIRKYNSTVYRVNMPPGAFIAENPQVVALLDGK
SFPVLFDVDKVEKKDLFTGTYMPSTELTGGYRILSYLDPSEPKHEKLKNLLFFLLKSSRNRIFPEFQATYSELFDSLEKELSLKGKADFGGSSDGTAF
NFLARAFYGTNPADTKLKADAPGLITKWVLFNLHPLLSIGLPRVIEEPLIHTFSLPPALVKSDYQRLYEFFLESAGEILVEADKLGISREEATHNLLFA
TCFNTWGGMKILFPNMVKRIGRAGHQVHNRLAEEIRSVIKSNGGELTMGAIEKMELTKSVVYECLRFEPPVTAQYGRAKKDLVIESHDAAFKV
KAGEMLYGYQPLATRDPKIFDRADEFVPERFVGEEGEKLLRHVLWSNGPETETPTVGNKQCAGKDFVVLVARLFVIEIFRRYDSFDIEVGTSPLG
SSVNFSSLRKASFVDKLAAALG*

Allene oxide cyclase 2 (AOC2) from *Arabidopsis thaliana* (29.0 kDa), codon optimized sequence, without chloroplast signaling sequence (chlrp) and with (N)-His_6 tag (Uniprot: Q9LS02)

ATGAGAGGATCGCATCACCATCACCATCACGGATCCATGGCTTCTTCAGCAGTGTCTCTCCAATCCATCTCTATGACAACTCTCAACAATCT
TTCATGTAATCAACAATTTCATCGAAGCTCTCTTCTTGGTTCCTCTAAATCCTTCCAAAATCTTGGTATCTCATCTAACGGTTCAGATTTCTC
CTATCCATCAAGTTTCACTGCCAAGAAGAACCTCACTGCTTCTCGAGCTCTCTCCCAAAACGGGAATATCGAAAACCCTAGACCAAGCAAA
GTTCAAGAACTGAGTGTGTACGAAATCAACGAATTAGATCGACACAGCCCCAAGATTCTTAAAAACGCATTCAGCTTAATGTTCGGTCTC
GGAGATCTCGTACCATTCACAAACAAACTCTACACAGGCGATCTCAAGAAACGCGTGGGCATCACGGCAGGTCTCTGCGTCGTCATCGA
ACACGTCCCAGAGAAGAAAGGTGAAAGATTCGAAGCTACTTATAGCTTCTACTTCGGAGACTATGGCCACTTGTCCGTTCAAGGACCATA
CTTGACTTACGAGGATTCGTTCCTCGCCATCACTGGTGGTGCTGGAATCTTTGAAGGTGCCTACGGACAGGTCAAGCTTCAACAGCTTGT
GTATCCGACAAAACTGTTCTACACTTTTTACCTTAAAGGGTTGGCTAATGATTTGCCGTTGGAGCTCACCGGAACACCGGTACCGCCGTCT
AAGGACATAGAGCCGGCGCCGGAAGCTAAGGCACTGGAGCCTAGCGGAGTTATAAGTAACTATACCAACTAA

Amino acid sequence

MRGSHHHHHHGSMASSAVSLQSISMTTLNNLSCNQQFHRSSLLGSSKSFQNLGISSNGSDFSYPSSFTAKKNLTASRALSQNGNIENPRPSKV
QELSVYEINELDRHSPKILKNAFSLMFGLGDLVPFTNKLYTGDLKKRVGITAGLCVVIEHVPEKKGERFEATYSFYFGDYGHLSVQGPYLTYEDSFL
AITGGAGIFEGAYGQVKLQQLVYPTKLFYTFYLKGLANDLPLELTGTPVPPSKDIEPAPEAKALEPSGVISNYTN*

1.1.3 pET28a-AtAOS(-)_Δchlrp_(C)-His-tag (ThermoFisher)

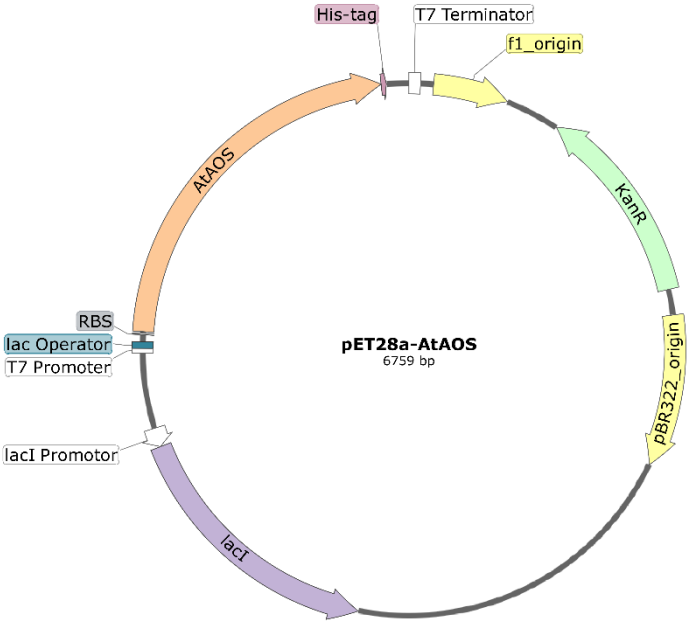


Figure S. 3: Plasmid map of the pET28a-AtAOS vector.

Allene oxide synthase (AOS) from *Arabidopsis thaliana* (57.0 kDa), wild type sequence, without chloroplast signaling sequence (chlrp) and with the solubility sequence AKKTS and C-terminal His-tag (Uniprot: Q96242)

ATGGCAAAAAAAACATCATCAGCATCAGGATCAGAAACACCAGACCTAACAGTAGCGACACGAACCGGATCCAAAGATCTCCCGATCCG
AAACATACCGGGAAACTACGGTTTACCAATCGTAGGACCAATCAAAGACCGTTGGGATTACTTTTACGACCAAGGAGCTGAAGAGTTCTT
CAAATCACGAATCCGTAAATACAACTCCACGGTGTACAGAGTCAACATGCCACCGGGAGCTTTTATCGCCGAGAATCCACAAGTCGTGGC
TTTACTCGACGGTAAAAGCTTCCCGGTTTTATTCGATGTCGATAAAGTCGAAAAGAAAGATCTTTTCACCGGTACTTACATGCCGTCAACG
GAACTAACCGGAGGCTACCGTATCCTCTCGTACCTCGATCCATCGGAGCCTAAACACGAAAAGCTCAAAAATCTCCTTTTCTTCCTCCTCA
AGTCATCTCGAAACCGGATCTTCCCTGAGTTTCAAGCTACTTACTCCGAGCTTTTCGATTCTTTGGAGAAAGAGCTTTCCCTTAAAGGGAA
AGCGGATTTCGGCGGTTCCAGCGACGGAACCGCCTTTAATTTCTTGGCTCGGGCTTTCTACGGGACGAATCCCGCAGATACAAAGCTCAA
AGCCGACGCTCCGGGTTTGATCACTAAATGGGTTTTATTCAATCTCCATCCATTACTCTCTATTGGTTTACCGAGAGTTATAGAAGAACCTC
TCATCCATACATTTAGTCTACCACCGGCGTTAGTCAAATCTGATTACCAGAGACTCTACGAGTTTTTCTTAGAATCCGCCGGTGAGATTCTC
GTTGAAGCCGATAAATTGGGTATCTCACGAGAAGAAGCTACTCACAATCTTCTCTTCGCCACGTGCTTCAACACGTGGGGTGGGATGAA
GATTTTGTTTCCGAATATGGTTAAACGTATCGGGCGGGCGGGTCATCAAGTTCATAACCGATTAGCGGAGGAGATTAGATCTGTGATTAA
ATCCAACGGCGGAGAACTCACGATGGGAGCGATTGAGAAAATGGAGTTAACCAAATCAGTGGTTTACGAATGTCTCCGGTTTGAACCAC
CGGTTACGGCTCAATACGGTAGAGCGAAGAAGGATCTGGTTATCGAAAGCCACGACGCGGCGTTTAAAGTCAAAGCCGGTGAAATGCT
TTACGGTTATCAACCGTTGGCGACGAGAGATCCGAAGATTTTTGATCGGGCGGATGAGTTTGTGCCGGAGAGATTCGTCGGAGAAGAA
GGAGAGAAGCTTTTGAGGCATGTGTTGTGGTCGAATGGACCGGAGACGGAGACTCCGACGGTGGGGAATAAACAATGCGCCGGTAAG
GATTTTGTTGTTTTGGTGGCGAGGTTGTTTGTGATTGAGATTTTCCGGCGATATGATTCGTTTGATATTGAGGTTGGTACGTCGCCGTTAG
GAAGCTCCGTTAATTTCTCGTCGTTAAGGAAAGCTAGCTTTGTCGACAAGCTTGCGGCGGCACTCGGGCACCACCACCACCACCACTAA

Amino acid sequence

MAKKTSSASGSETPDLTVATRTGSKDLPIRNIPGNYGLPIVGPIKDRWDYFYDQGAEEFFKSRIRKYNSTVYRVNMPPGAFIAENPQVVALLDGK
SFPVLFDVDKVEKKDLFTGTYMPSTELTGGYRILSYLDPSEPKHEKLKNLLFFLLKSSRNRIFPEFQATYSELFDSLEKELSLKGKADFGGSSDGTAF
NFLARAFYGTNPADTKLKADAPGLITKWVLFNLHPLLSIGLPRVIEEPLIHTFSLPPALVKSDYQRLYEFFLESAGEILVEADKLGISREEATHNLLFA
TCFNTWGGMKILFPNMVKRIGRAGHQVHNRLAEEIRSVIKSNGGELTMGAIEKMELTKSVVYECLRFEPPVTAQYGRAKKDLVIESHDAAFKV
KAGEMLYGYQPLATRDPKIFDRADEFVPERFVGEEGEKLLRHVLWSNGPETETPTVGNKQCAGKDFVVLVARLFVIEIFRRYDSFDIEVGTSPLG
SSVNFSSLRKASFVDKLAAALGHHHHHH*

1.2 Microbiology

1.2.1 Production of chemical competent cells

To LB media (200 mL) preculture (200 µL) was added and incubated until an OC_{600} of 0.4 - 0.5. The main culture was aliquoted (50 mL each), chilled on ice for 10 min ad get centrifuged (4 °C, 1750x g, 10 min). The supernatants were discarded. The pellets were carefully resuspended in FB buffer (14 mL) and chilled on ice for another 20 min before they get centrifuged (4 °C, 1750x g, 10 min). The supernatants were discarded. The pellets were carefully resuspended in FB buffer (3.4 mL). The suspension was aliquoted (50 µL each) and stored at -80 °C.

1.2.2 Transformation of competent cells with plasmid DNA

The aliquot of the desired competent cell chilled on ice for 15-30 min. The plasmid (1 µL) was added and chilled for another 15 - 30 min. The cells were incubated at 42 °C for 90 sec and was set on ice directly for 3 min. After LB media (700 µL) was added, the tube was set on ice for 3 min again. The culture was incubated at 37 °C and 800 rpm for 1 - 3 h. The tube was centrifuged (4 °C, 5000x g, 10 min) and 600 µL of the supernatant were removed. The pellet was resuspended in the remaining media and was cultured on an agar plate with suitable antibiotic overnight at 37 °C.

1.2.3 Heterologous expression

The plasmids were transformed into *E. coli* BL21-CodonPlus(DE3)-RIL strain. The cells were picked from a LB-agar plate or glycerol stock and added to a LB-media (10 mL) with antibiotics (kanamycin: 40 µg/mL; chloramphenicol: 80 µg/mL). The cultures were shaken overnight (37 °C, 180 rpm). For the main culture TB-medium was used (1 L in 5 L non-baffled Erlenmeyer flask with antibiotics (kanamycin: 40 µg/mL; chloramphenicol: 80 µg/mL)). They were inoculated with preculture (10 mL) and incubated until the OD_{600} was 0.5 - 0.7. Afterwards, IPTG (final concentration 0.05 - 1 mM) was added and the temperature was reduced to 25 - 30°C for 20 h. The suspension was centrifuged (4 °C, 4000x g, 30 min). The supernatant was removed, and the pellet was stored at -20°C.

The best expression and activity for the 12-OPDA (**4**) synthesis were found with the WCC1. Therefore, an expression temperature of 30 °C and an IPTG concentration of 0.05 mM are necessary.

The WCC with pET28a-AtAOS plasmid was expressed according to Löwe *et al.*[1]

Table S. 1: All used whole-cell catalysts with the containing plasmids. The strain every time *E. coli* BL21-CodonPlus(DE3)-RIL.

WCC	Plasmid	Antibiotics
1	pET28a-AtAOS-AtAOC4	Kan^R, Cm^R
2	pET28a-AtAOS_AtAOC2	Kan^R, Cm^R
3	pET28a-AtAOS	Kan^R, Cm^R

Table S. 2: Ingredients of media.

Media	Ingredients
Lysogeny broth (LB)-Agar	10 g/L Tryptone 5 g/L yeast extract 10 g/L NaCl 15 g/L Agar-Agar-Kobe I In ddH_2O; sterile
Lysogeny broth (LB)-Medium[a]	10 g/L Tryptone 5 g/L yeast extract 10 g/L NaCl In ddH_2O; pH 7.0; sterile
Terrific broth (TB)-Medium[a]	12 g/L Peptone from Casein 24 g/L Yeast extract 4 g/L Glycerol (>99.5 %) 2.31 g/L KH_2PO_4 12.54 g/L K_2HPO_4 In ddH_2O; pH 7.2; sterile

a commercial mixture from *Carl Roth GmbH & Co. KG.*

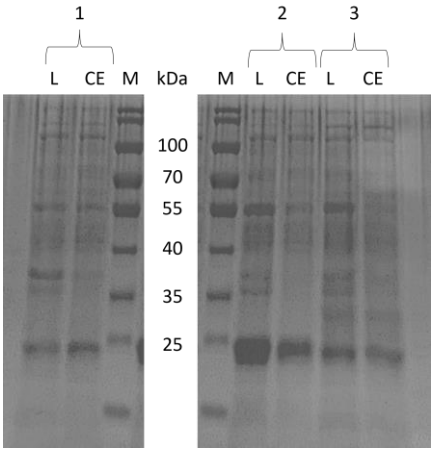


Figure S. 4: 12% SDS-PAGEs of different types of whole-cell catalyst expressed at 30 °C for 20 h with 0.05 mM ITPG except the reproduction of Löwe *et al*. (3)[1]. All plasmids were used in *E. coli* BL21-CodonPlus(DE3)-RIL. 1) pET28a-AtAOS_AtAOC2 (WCC2); 2) pET28a-AtAOS_AtAOC4 (WCC1); 3) pET28a-AtAOS + pQE30-AtAOC2 (reproduction of Löwe *et al.*)[1]. The used marker was PAGE Ruler Prestained Protein Ladder 10-250 kDa von *Thermo Fisher Scientific*. (L = lysate; CE = crude extract)

1.2.4 Fermentation of the optimized whole-cell catalyst

From the optimized whole-cell catalyst (*E. coli* BL21-CodonPlus(DE3)-RIL_pET28a-AtAOS_AtAOC4) a LB-media preculture (10 mL) with antibiotics (kanamycin: 40 µg/mL; chloramphenicol: 80 µg/mL) were inoculated and grown at 37 °C, 180 rpm for 8 h. The whole preculture was added to a sterile PAN-medium (100 mL) in a 1 L baffled flask, trace-element solution (100 µL) and antibiotics (kanamycin: 40 µg/mL; chloramphenicol: 80 µg/mL) were added. This culture was incubated overnight (37 °C, 140 rpm). A starting volume of 1 L (diluted with 100 mL H_2O to overcome loss of media during autoclaving process) was filled into a 2.5 L *Minifors* bench-top fermenter from *Infors HT*. The reactor was prepared for autoclaving and calibrated according to the manufacture's manual. After autoclaving, the oxygen electrode was polarized and calibrated, as well as trace-element cocktail (1 mL) and antibiotics (kanamycin: 50 µg/mL; chloramphenicol: 100 µg/mL) were added. Then, the reactor was heated up to 30 °C before it was inoculated with the overnight culture (100 mL). The initial stirring speed was 300 rpm. The stirrer was allowed to ramp the speed to maintain the dissolved oxygen (DO) setpoint to 20%. The glycerol feed (800 g/L stock solution) was initialized by the sequence after the initial glycerol was complete consumed a detected by a spike in DO. With an OD_{600} of 60-80, the IPTG (1 mM final concentration) was added. The temperature was still set to 30 °C and the expression was stopped by harvesting the cells after 20 h. The harvest was conducted by transferring the suspension into 800 mL centrifugation vessels and centrifuging these at 4000x g for 30 min at 4 °C. The supernatant was discarded and the cells were stored at -20 °C.

Table S. 3: Ingredients of media.

Media	Ingredients
PAN-Medium	1.6 g/L $Na_2HPO_4 \cdot H_2O$ 3.2 g/L KH_2PO_4 2.6 g/L K_2HPO_4 0.2 g/L NH_4Cl 2.0 g/L $(NH_4)_2SO_4$ 0.6 g/L $MgSO_4$ 0.2 g/L $CaCl_2$ 7.5 g/L Glycerol (>99.5%) In ddH_2O; pH 7.0; sterile
Trace-element solution	5 mL/L H_2SO_4 (conc.) 6 g/L $CuSO_4 \cdot 5\ H_2O$ 0.08 g/L KI 3 g/L $MnSO_4 \cdot H_2O$ 0.3 g/L Na_2MoO_4 0.02 g/L H_3BO_3 0.5 g/L $CoCl_2$ 20 g/L $ZnCl_2$ 65 g/L $FeSO_4 \cdot 7\ H_2O$

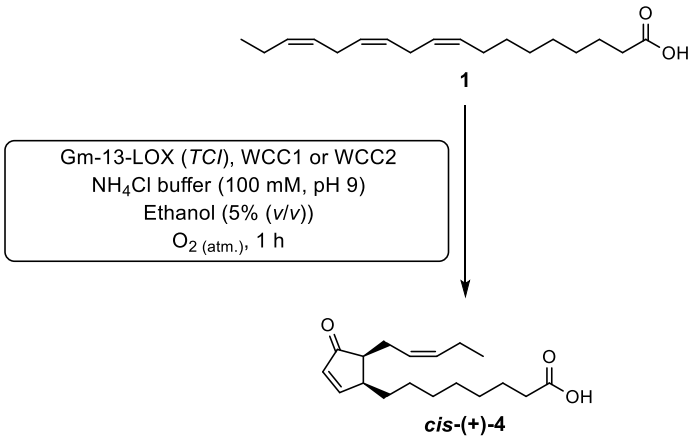
1.2.4.1 Control sequence for *Infors HT* IRIS software

```
//Creator: Tim Guntelmann 2023 (Based on the sequence developed and published by Montua et al.[2])
#0, Set up, 10
//set parameters (lower critical, lower alarm, target value, upper alarm, upper critical)
Limits(10,100,300,1250,1250,Stirrer)
Limits(5.5,5.75,6.8,7.25,7.5,pH)
Limits(28,29,30,31,32,temp)
Limits(5,10,20,120,125,pO2)
Feed_pump.sp=0
Feed_pump.bv=Feed_pump.sp
SEQ=1
#1, batchphase, 10
IF(pO2.v<=pO2.sp)(SEQ=2)
#2,CheckforO2spike,10
//After 06 hours and if the stirrer rpm is above 400 check for O2 spike and initiate feed control if no spike is detected feeding will commence after 10 hours; from here, the Stirrer is allowed to ramp up
IF(pO2.v>pO2.sp+25)AND(ELAPSED>21600) AND Feed_pump.sp=1.0} AND (Stirrer.sp>=400) {SEQ=3 AND Feed_pump.sp=1.0}
IF(ELAPSED > 36000){SEQ=3 AND Feed_pump.sp=1.0}
IF(pO2.v<pO2.sp){Stirrer.sp=Stirrer.sp+1}
IF(pO2.v>pO2.sp)AND(Stirrer.sp>=302){Stirrer.sp=Stirrer.sp-2}
#3,Feed,10
// three minute delay before feed decrease allows stirrer to catch up, When Stirrer reaches near max speed jump to max speed and dial back feed to prevent oxygen deprivation
IF(pO2.v<pO2.sp-5)AND(SEQ_TIME>     TIME(0:3))AND(Feed_pump.sp>1.0){Feed_pump.sp=Feed_pump.sp-0.1; SEQ=4}
IF(pO2.v>pO2.sp+5){Feed_pump.sp=Feed_pump.sp+0.1; SEQ=4}
IF(pO2.v<pO2.sp){Stirrer.sp=Stirrer.sp+10}
#4,Pause,10
// three minute delay to ensure that the feed isn't increased with excessive frequency; if Oxygen saturation drops too far after increasing/decreasing feedrate, it is reduced; If equilibrium is reached Stirrer is allowed to ramp
// After induction limits for temperature and oxygen should be adjusted, a higher pO2 setpoint provides less oxygen for oxphos --> less heat output from cells and thus easier cooling
IF(SEQ_TIME > TIME(0:3))AND(Stirrer.sp<1150) {SEQ=3}
IF(SEQ_TIME > TIME(0:3))AND(Stirrer.sp>1150) {SEQ=5}
IF(pO2.v<=pO2.la)AND(Feed_pump.sp>1.0){Feed_pump.sp=Feed_pump.sp-0.3}
IF(pO2.v<pO2.sp){Stirrer.sp=Stirrer.sp+5}
IF(pO2.v>pO2.sp-0.5)AND(pO2.v<pO2.sp+0.5){Stirrer.sp=Stirrer.sp+10}
#5, Feed_Stirrermax, 10
// three minute delay before feed decrease allows stirrer to catch up
IF(pO2.v<pO2.sp-5)AND(SEQ_TIME>     TIME(0:3))AND(Feed_pump.sp>1.0){Feed_pump.sp=Feed_pump.sp-0.1; SEQ=4}
IF(pO2.v>pO2.sp+5){Feed_pump.sp=Feed_pump.sp+1.0; SEQ=4}
IF(pO2.v<pO2.la)AND(Feed_pump.sp>1.0){Feed_pump.sp=Feed_pump.sp-0.5}
IF(pO2.v<pO2.sp){Stirrer.sp=Stirrer.sp+5}
IF[temp.v>temp.sp+2)AND(Feed_pump.sp>2.0){Feed_pump.sp=Feed_pump.sp-0.1}
```

2 Biotransformations

2.1 Synthesis of *cis*-(+)-12-OPDA starting from pure *α*-linolenic acid

2.1.1 Synthesis in NH_4Cl buffer with Ethanol



Scheme S. 1: Reaction scheme of the biocatalytic synthesis of *cis*-(+)-12-OPDA (***cis*-(+)-4**) starting from *α*-linolenic acid (**1**) by using commercially available Gm-13-LOX (*TCI*, L0059) and a whole-cell catalyst.

2.1.1.1 With magnetic stirrer and direct O_2 insertion

NH_4Cl buffer (47.5 mL, 100 mM, pH 9) was added to a three-necked-flask (100 mL). The buffer was saturated with oxygen by a O_2 balloon with syringe, which was put directly into the solution. The approaches 2 and 4 were cooled by an ice bath for min. 10 min. After 10 min of oxygen bubbling, the Gm-13-LOX (*TCI*, L0059) and the WCC1 were resuspended in the (cold) buffer. Ethanol (1.5 mL) was added. *α*-linolenic acid (*α*-LA, **1**) was dissolved in Ethanol (1 mL) and moved to the flask. The suspension was stirred with magnetic stirrer (600 rpm) for 1 h, while the flask is closed. Reaction mixture was transferred to two 50 mL centrifugation tubes (25 mL each). To each tube HCl (2 M, 1 mL) and EtOAc (25 mL) was added. The mixture was mixed properly and centrifuged (10000x g, 10 min) for phase separation. The organic layer was removed and dried over $MgSO_4$. The solvent was removed in vacuum. The product was dissolved in $CDCl_3$ and the conversion and ratios were determined ^{1}H-NMR spectroscopy.

Table S. 4: Shown are all amounts which were used fort he approaches 1 - 4.

Approach	Temperature	WCC1[a] / mg	13-LOX / mg	*α*-LA (1) / mg
1	rt	152.0	0.85	53.8
2	0 °C	154.0	0.85	50.7
3	rt	307.0	1.9	50.7
4	0 °C	308.3	1.9	51.1

a cell wet weight

Table S. 5: Results of the approaches 1 - 4.

Approach	*α*-LA (1) / %	13-HPOT (2) / %	12-OPDA (4) / %	*α*-ketol (5) / %	*cis-trans* of 4
1	23	4	68	6	99:1
2	20	2	71	7	>99:1
3	27	3	67	3	99:1
4	0	0	96	4	>99:1

2.1.1.2 With KPG stirrer and O_2 atmosphere

Room temperature

NH_4Cl buffer (50 mL, 100 mM, pH 9) was added to a three-necked-flask (100 mL). Gm-13-LOX (*TCI*, L0059; *Sigma Aldrich*, L7395) and the WCC1 or WCC2 were resuspended in the buffer.

0 °C

NH_4Cl buffer (47.5 mL, 100 mM, pH 9) was added to a three-necked-flask (100 mL) and cooled down to 0°C with an ice bath. Gm-13-LOX (*TCI*, L0059 or *Sigma Aldrich*, L7395) and the WCC1 or WCC2 were dissolved in the cold buffer and added to the flask.

Ethanol (1.5 mL) was added. *α*-linolenic acid (*α*-LA, **1**) was dissolved in Ethanol (2 x 0.5 mL) and moved to the flask. The suspension was stirred for 1 h under oxygen atmosphere (needle size: inlet = 0.9 x 50 mm; outlet = 0.5 x 40 mm). Reaction mixture was transferred to two 50 mL centrifugation tubes (25 mL each). To each tube HCl (2 M, 1 mL) and EtOAc (25 mL) was added. The mixture was mixed properly and centrifuged (10000x g, 10 min) for phase separation. The organic layer was removed and dried over $MgSO_4$. The solvent was removed in vacuum. The product was dissolved in $CDCl_3$ and the conversion and ratios were determined ^{1}H-NMR spectroscopy.

Table S. 6: Shown are all amounts which were used for the approaches 5 - 10.

Approach	Temperature	WCC1[c] / mg	13-LOX / mg	*α*-LA (1) / mg
5	rt	317.5	1.9	50.6
6	0 °C	320.1	1.7	52.9
7[a]	rt	310.1	1.9	53.9
8[a]	0 °C	309.4	1.7	51.9
9[b]	rt	326.6	1.9	53.2
10[b]	0 °C	302.3	1.6	53.2

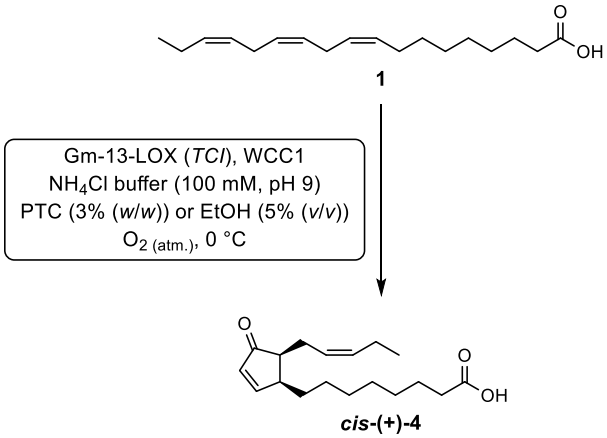
a Gm-13-LOX from *Sigma Aldrich* was used.
b WCC2: BL21-CodonPlus(DE3)-RIL_pET28a-AtAOS_AtAOC2
c cell wet weight

Table S. 7: Results of the approaches 5 - 10.

Approach	*α*-LA (1) / %	13-HPOT (2) / %	12-OPDA (4) / %	*α*-ketol (5) / %	*cis-trans* of 4
5	3	0	94	3	99:1
6	0	0	96	4	>99:1
7[a]	5	0	91	4	98:2
8[a]	0	0	96	4	>99:1
9[b]	4	0	79	17	98:2
10[b]	1	0	81	18	>99:1

a Gm-13-LOX from *Sigma Aldrich* was used.
b WCC2: BL21-CodonPlus(DE3)-RIL_pET28a-AtAOS_AtAOC2

2.1.2 Screening of different phase-transfer catalysts



Scheme S. 2: Reaction scheme of the biocatalytic synthesis of *cis*-(+)-12-OPDA (***cis*-(+)-4**) starting from *α*-linolenic acid (**1**) by using commercially available Gm-13-LOX (*TCI*, L0059) and a whole-cell catalyst.

NH_4Cl buffer (38 mL, 100 mM, pH 9) was added to a three-necked-flask (100 mL). The buffer was cooled down with an ice bath while stirring (500 rpm). Gm-13-LOX (*TCI*, L0059) was dissolved in the cold buffer and added to the flask. The WCC1 was resuspended in residual buffer (10 mL) and added to the flask. The additives were added. The phase-transfer catalysts were used as stock-solution (15 mg/mL). α-linolenic acid (*α*-LA, **1**) was moved to the flask last. In approaches with ethanol, the α-LA (**1**) was dissolved in ethanol before added. The suspension was stirred (KPG, 500 rpm) at 0 °C under oxygen atmosphere (needle size: inlet = 0.9x50 mm; outlet = 0.5x40 mm). The reaction progress was controlled *via* ^{1}H-NMR: The sample (1 mL) was taken, acidified with HCl (2 M, 40 µL) and extracted with $CDCl_3$ (850 µL). The mixture was vortexed and centrifuged (10.000x g, 5 min). The organic layer was separated and directly used for NMR. After full conversion, the reaction mixture was moved to separation funnel and diluted with saturated NaCl solution (25 mL) and acidified with HCl (2 M, 2 mL). Afterwards, the suspension was extracted with EtOAc (1 x 50 mL). The aqueous layer was separated, and the mixed layer was centrifuged (10.000x g, 10 min). The organic layer was dried over $MgSO_4$. The solvent was removed in vacuum.

Table S. 8: Shown are all amounts which were used for the approaches 11 - 22. (TEAB = tetraethylammonium bromide)

Approach	WCC[b] / g	13-LOX / mg	α-LA (1) / mg	Ethanol / mL	PTC (3% ($w/w_{substrate}$)) / mL (stock solution (15 mg/mL))
11	1.65	7.5	255.3	0	-
12	1.51	7.9	252.6	0	0.5 (Triton® CG-110)
13	1.52	7.6	250.2	0	0.5 (TEAB)
14	1.50	7.9	253.2	0	0.5 (TPGS-750[3])
15	1.54	7.5	252.2	1	0.5 (TEAB)
16	1.55	8.0	251.7	2.5	-
17[a]	1.55	8.2	256.1	2.5	-
18	3.01	15.3	506.0	-	-
19	3.00	15.2	500.3	0	1 (Triton® CG-110)
20	3.01	15.9	500.6	0	1 (TEAB)
21	3.05	15.6	501.7	0	1 (TPGS-750[3])
22	3.10	15.2	500.6	1	1 (TEAB)
23	3.08	15.3	502.7	2.5	-
24	6.00	30.3	1005.5	2.5	-

a WCC produced in high-cell density fermentation
b cell wet weight

Table S. 9: Results of the approaches 11 - 17. The reaction time was 30 min.

Approach	α-LA (1) / %	13-HPOT (2) / %	12-OPDA (4) / %	α-ketol (5) / %	*cis-trans* of 4
11	0	0	100	0	>99:1
12	0	0	97.5	2.5	>99:1
13	0	0	98.5	1.5	>99:1
14	0	0	96.4	3.6	>99:1
15	0	0	98.5	1.5	>99:1
16	0	0	100	0	>99:1
17[a]	4 (1)	0	96 (99)	0	>99:1

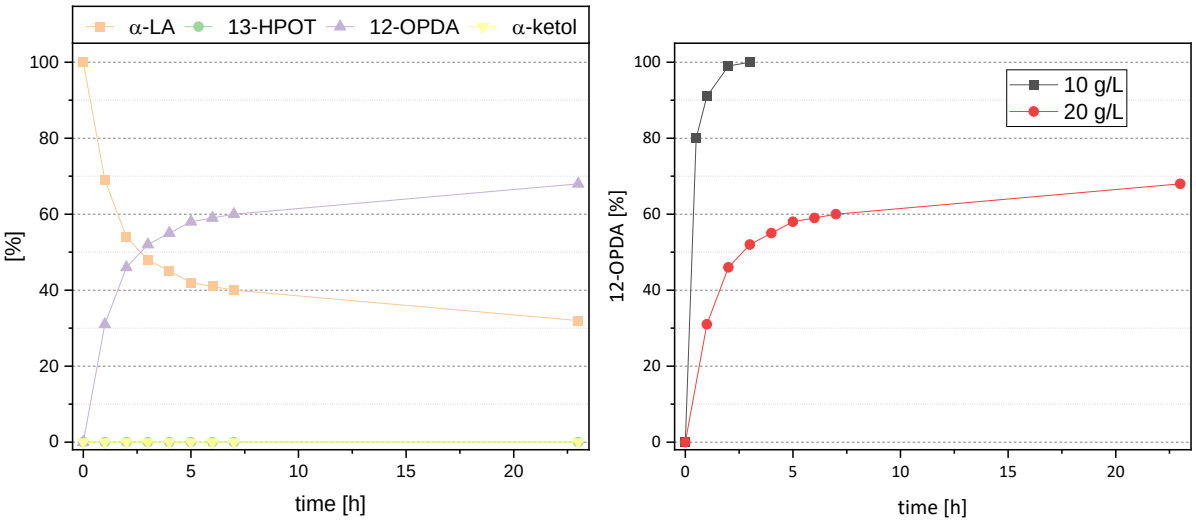
a WCC produced in high-cell density fermentation; in brackets: results of 1 h reaction time

Table S. 10: Comparison of the approaches 18 - 23.

Results: α-LA (1) - 13-HPOT (2) - 12-OPDA (4) - α-ketol (5)						
Time / min	**Buffer (18)**	**Triton (19)**	**TEAB (20)**	**TPGS (21)**	**TEAB + Ethanol (22)**	**Ethanol (23)**
0	100 - 0 - 0 - 0	100 - 0 - 0 - 0	100 - 0 - 0 - 0	100 - 0 - 0 - 0	100 - 0 - 0 - 0	100 - 0 - 0 - 0
30	56 - 0 - 44 - 0	72 - 0 - 28 - 0	59 - 0 - 41 - 0	54 - 0 - 45 - 1	25 - 0 - 75 - 0	20 - 0 - 80 - 0
60	29 - 0 - 71 - 0	56 - 0 - 44 - 0	25 - 0 - 75 - 0	36 - 0 - 63 - 1	11 - 0 - 89 - 0	9 - 0 - 91 - 0
120	-	45 - 0 - 55 - 0	11 - 0 - 89 - 0	24 - 0 - 74 - 2	8 - 0 - 92 - 0	1 - 0 - 99 - 0
180	3 - 0 - 97 - 0	42 - 0 - 57 - 1	8 - 0 - 92 - 0	21 - 0 - 77 - 2	8 - 0 - 92 - 0	0 - 0 - >99 - 0
240	3 - 0 - 97 - 0	40 - 0 - 59 - 1	6 - 0 - 94 - 0	18 - 0 - 80 - 2	8 - 0 - 92 - 0	-
300	2 - 0 - 98 - 0	39 - 0 - 60 - 1	5 - 0 - 95 - 0	18 - 0 - 80 - 2	4 - 0 - 96 - 0[a]	-

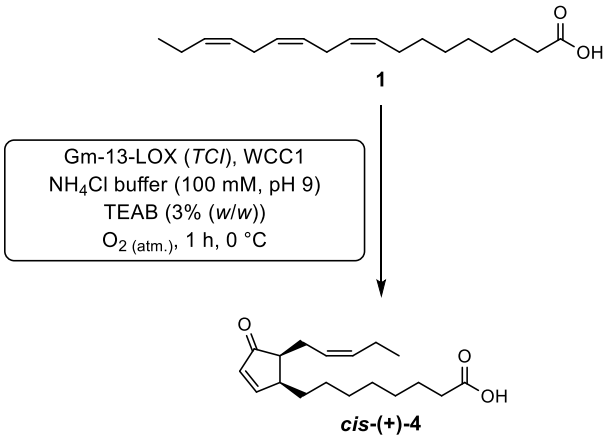
a Result after extraction. Reaction control showed: 8 - 0 - 92 - 0.

Table S. 11: Comparison of the approaches 23 and 24. Left) Every component of the approach 24 is shown; right) comparison between the approaches 23 and 24.



Results: *α*-LA (1) - 13-HPOT (2) - 12-OPDA (4) - *α*-ketol (5)		
Time / h	**10 g/L (23)**	**20 g/L (24)**
0	100 - 0 - 0 - 0	100 - 0 - 0 - 0
0.5	20 - 0 - 80 - 0	-
1	9 - 0 - 91 - 0	69 - 0 - 31 - 0
2	1 - 0 - 99 - 0	54 - 0 - 46 - 0
3	0 - 0 - >99 - 0	48 - 0 - 52 - 0
4	-	45 - 0 - 55 - 0
5	-	42 - 0 - 58 - 0
6	-	41 - 0 - 59 - 0
7	-	40 - 0 - 60 - 0
23	-	32 - 0 - 68 - 0

2.1.3 Optimization of the work-up



Scheme S. 3: Reaction scheme of the biocatalytic synthesis of *cis*-(+)-12-OPDA (***cis*-(+)-4**) starting from *α*-linolenic acid (**1**) by using commercially available Gm-13-LOX (*TCI*, L0059) and a whole-cell catalyst.

NH_4Cl buffer (39.5 mL, 100 mM, pH 9) was added to a three-necked-flask (100 mL). The buffer was cooled down with an ice bath while stirring with overhead stirrer (500 rpm). Gm-13-LOX (*TCI*, L0059, 7.5 mg) was dissolved in the cold buffer and added to the flask. The WCC1 (1.57 g (cell wet weight)) was resuspended in residual buffer (10 mL) and added to the flask. The additives were added. The tetraethylammonium bromide (TEAB, 0.5 mL, 15 mg/mL stock solution) and *α*-linolenic acid (*α*-LA, **1**, 252.0 mg, 0.91 mmol) were moved to the flask last. The suspension was stirred (overhead, 500 rpm) under oxygen atmosphere (needle size: inlet = 0.9 x 50 mm; outlet = 0.5 x 40 mm). The reaction progress was controlled *via* ^{1}H-NMR: The sample (1 mL) was taken, acidified with HCl (2 M, 40 µL) and extracted with $CDCl_3$ (850 µL). The mixture was vortexed and centrifuged (10000x g, 5 min). The organic layer was separated and directly used for NMR. After full conversion, the reaction mixture was moved to separation funnel and diluted with water, saturated KCl or NaCl solution (25 mL) and acidified with HCl (2 M, 2 mL). Afterwards, the suspension was extracted with EtOAc (1 x 50 mL). The aqueous layer was separated, and the mixed layer was centrifuged (10000x g, 10 min). The organic layer was dried over $MgSO_4$. The solvent was removed in vacuum.

Table S. 12: Shown are all amounts which were used fort he approaches 25 - 27.

Approach	WCC[b] / g	13-LOX / mg	α-LA (1) / mg	TEAB[a] / mL	Extraction additive	Extracted amount
25	1.57	7.5	252.0	0.5	dH_2O	160.9 mg
26	1.54	7.5	253.4	0.5	sat. KCl	187.1 mg
27	1.65	8.5	255.4	0.5	sat. NaCl	253.5 mg

a stock solution: 15 mg/mL; TEAB = tetraethylammonium bromide
b cell wet weight

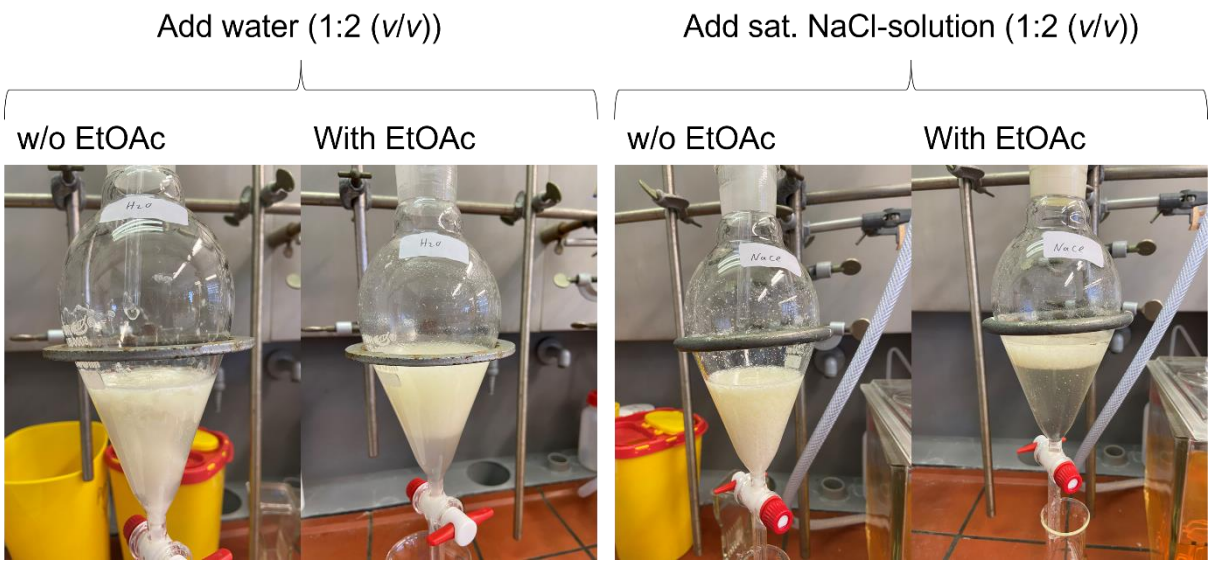


Figure S. 5: NaCl has a massive impact on the phase separation during the work up. The influence on nebulizing the cell debris is visible as well.

The most effective extraction was performed with NaCl as additive. The purity (56%) of the gained 12-OPDA (**4**) was determined with an internal standard (1,3,5-trimethoxybenzene). The diastereomeric excess was excellent (>99% de). The remove the impurities, we ran a reversed-phase automated column chromatography with the *Biotage IsoleraOne*-System. We used a C18 column (Biotage® Sfär C18 D Duo 100 Å 30 µm 12 g) and used acetonitrile and water with 0.1% acetic acid as cosolvent. The used sequence is shown in Table S. 13.

Table S. 13: Gradient for automated column chromatography. Acetonitrile (MeCN) and water (H_2O) were used as solvents with acetic acid (AcOH; 0.1%) as cosolvent. The used volume is shown in column volume (CV). The pump speed was 12 mL/min.

Gradient	**MeCN + AcOH (0.1%) / %**	**H_2O + AcOH (0.1%) / %**	**Length / CV**
Equilibration	50	50	3
1	50	50	1
2	50 - 100	50 - 0	10
3	100	0	2

To be sure, that all impurities are removed, we just combine the fractions 7 and 8. In fraction 6 were impurities (Figure S. 6). The solvent was removed with a rotary evaporator and in high vacuum over 3 h. The recovery of the column chromatography was 64% and can be increased by optimization of the column chromatography. The use of a lyophile resulted in high isomerization of the product ***cis*-(+)-4** (70% *de*) and should be avoided. In the end we obtained the *cis*-(+)-12-OPDA (***cis*-(+)-4**) as a colorless oil with a purity of >99% and an overall yield of 35% (91.0 mg, 0.31 mmol). The diastereomeric excess was very high (96% de) The product purity was determined with an internal standard (1,3,5-trimethoxybenzene) and visualized with ^{1}H-NMR (Figure S. 7)

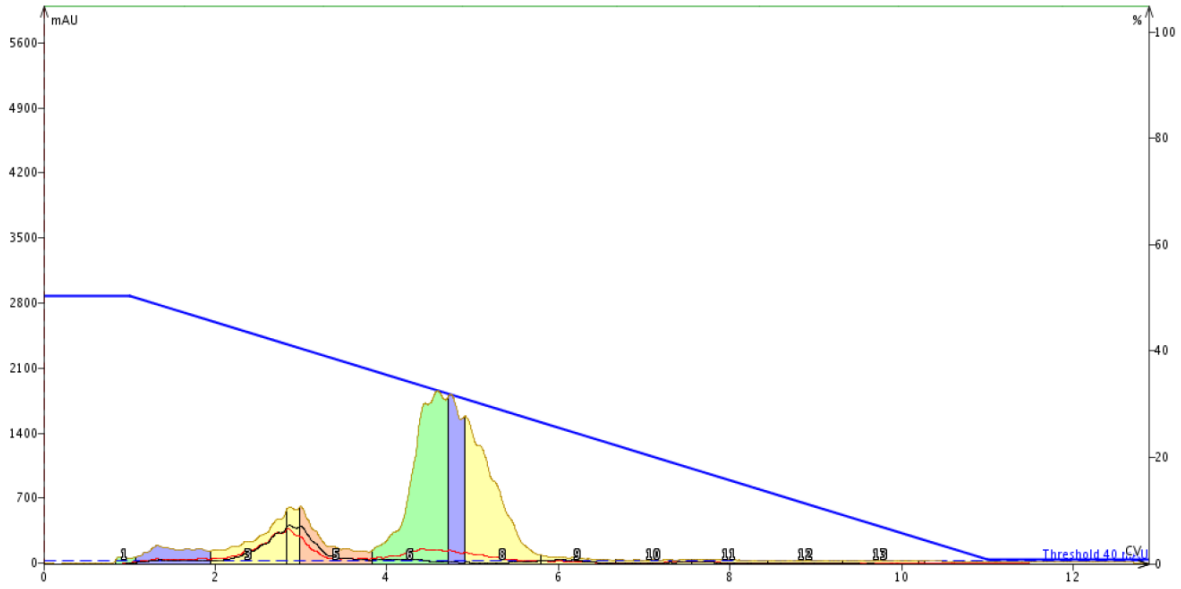


Figure S. 6: Chromatogram of the purification *via* reversed-phase automated column chromatography. Red line = 254 nm; black line = 280 nm; brown line = λ all; blue line = gradient of water.

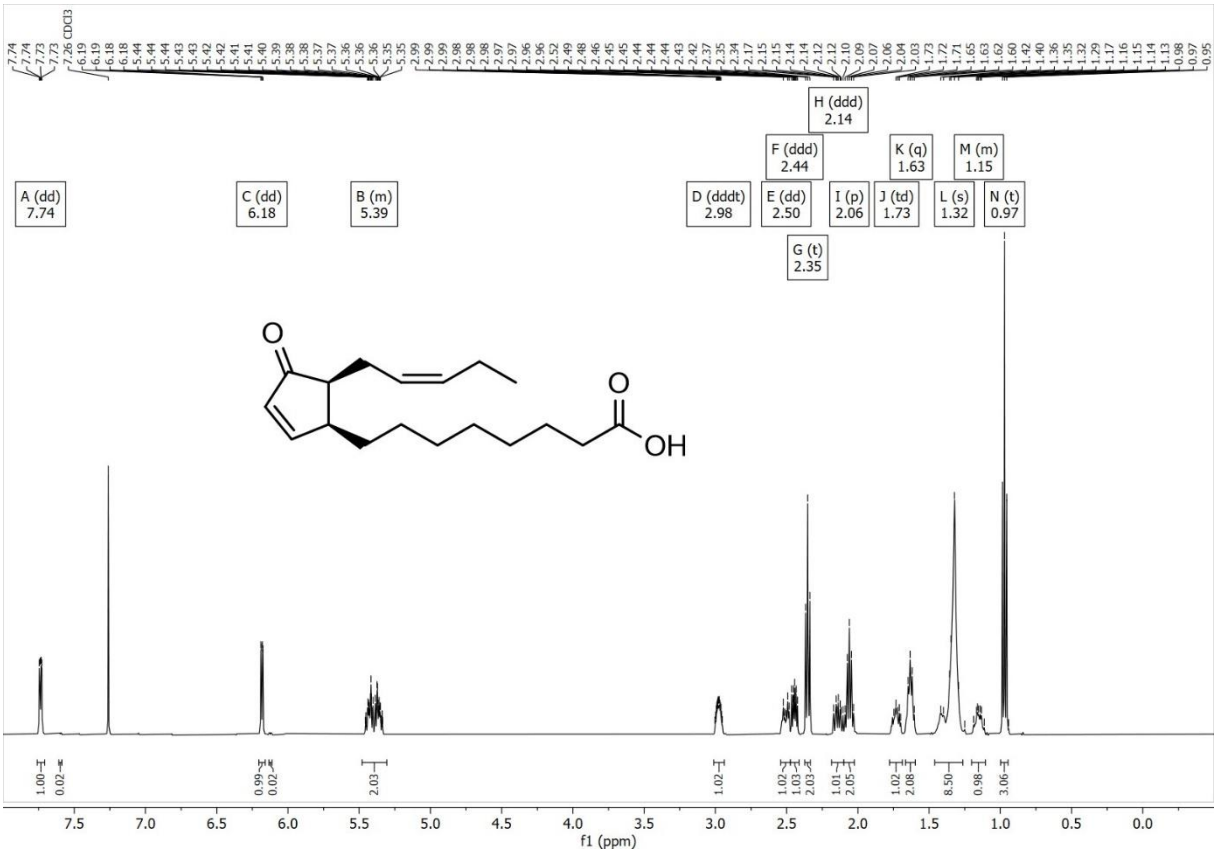
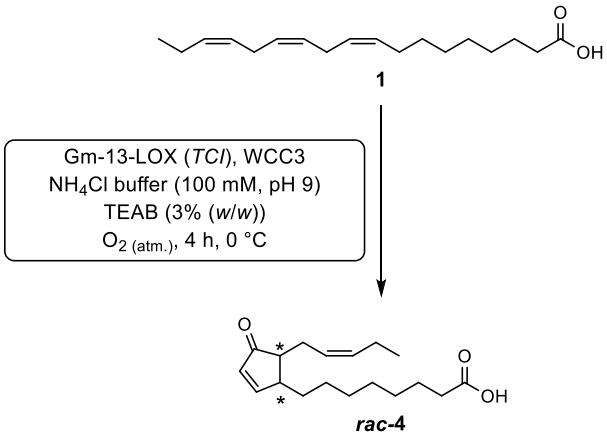


Figure S. 7: ^{1}H-NMR spectra of *cis*-(+)-12-OPDA (***cis*-(+)-4**).

^{1}H-NMR (500 MHz, $CDCl_3$) δ / ppm = 7.74 (dd, ^{3}J = 5.8 Hz, ^{4}J = 2.8 Hz, 1H), 6.18 (dd, ^{3}J = 5.8 Hz, ^{4}J = 1.8 Hz, 1H), 5.47 – 5.32 (m, 2H), 2.98 (dddt, ^{3}J = 13.0, 6.7, 4.7 Hz, ^{4}J = 2.4 Hz, 1H), 2.50 (dd, ^{3}J = 15.4, 5.6 Hz, 1H), 2.44 (ddd, ^{3}J = 9.6, 6.2, 4.6 Hz, 1H), 2.35 (t, ^{3}J = 7.5 Hz, 2H), 2.14 (ddd, ^{3}J = 15.9, 9.9, 7.6 Hz, 1H), 2.06 (p, ^{3}J = 7.4 Hz, 2H), 1.73 (td, ^{3}J = 11.1, 4.9 Hz, 1H), 1.63 (q, ^{3}J = 7.3 Hz, 2H), 1.32 (m, 8H), 1.19 – 1.10 (m, 1H), 0.97 (t, ^{3}J = 7.5 Hz, 3H).

2.1.4 Synthesis of racemic 12-OPDA



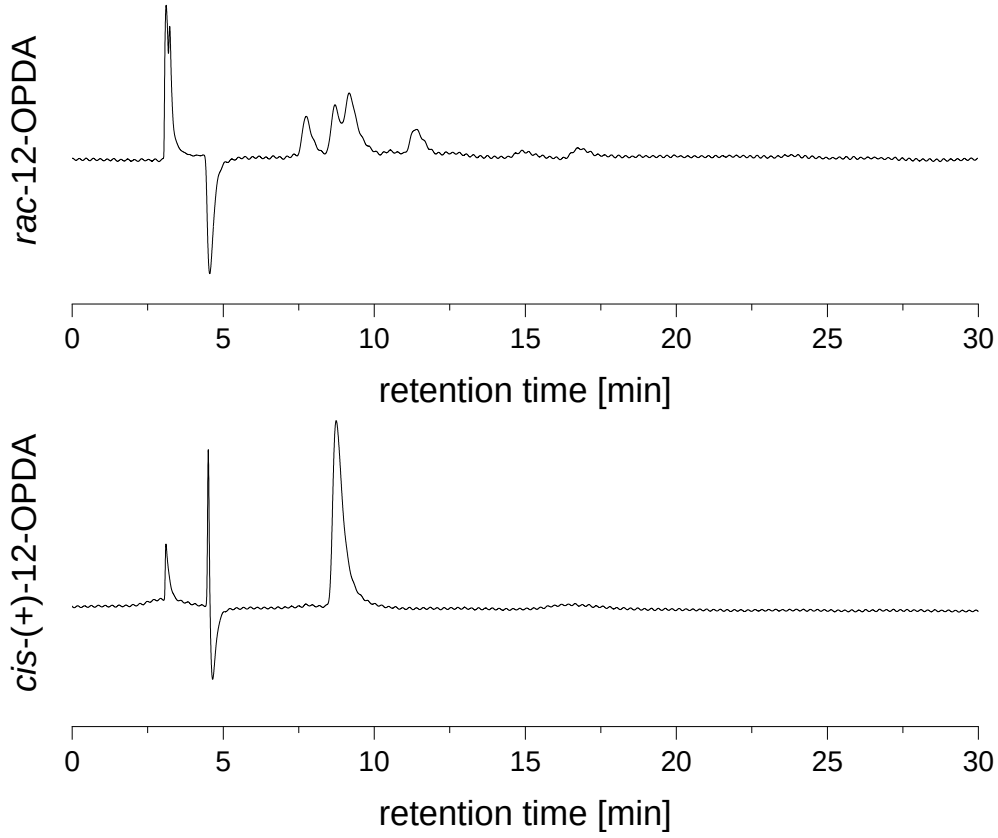
Scheme S. 4: Reaction scheme of the biocatalytic synthesis of *rac*-12-OPDA (***rac*-4**) starting from *α*-linolenic acid (**1**) by using commercially available Gm-13-LOX (*TCI*, L0059) and a whole-cell catalyst.

NH_4Cl buffer (38 mL, 100 mM, pH 9) was added to a three-necked-flask (100 mL). The buffer was cooled down with an ice bath while stirring (500 rpm). Gm-13-LOX (*TCI*, L0059, 9.1 mg) was dissolved in the cold buffer and added to the flask. The WCC3 (1.57 g (cell wet weight)) was resuspended in residual buffer (10 mL) and added to the flask. The additives were added. The tetraethylammoniumbromide (0.5 mL) were used as stock-solution (15 mg/mL). *α*-linolenic acid (*α*-LA, **1**, 255.7 mg, 0.92 mmol) was dissolved in ethanol (2 x 0.5 mL) and moved to the flask last. The suspension was stirred (KPG, 500 rpm) at 0 °C under oxygen atmosphere (needle size: inlet = 0.9 x 50 mm; outlet = 0.5 x 40 mm). The reaction progress was controlled *via* ^{1}H-NMR: The sample (1 mL) was taken, acidified with HCl (2 M, 40 µL) and extracted with $CDCl_3$ (850 µL). The mixture was vortexed and centrifuged (10000x g, 5 min). The organic layer was separated and directly used for NMR. After full conversion, the reaction mixture was moved to separation funnel and diluted with saturated NaCl solution (25 mL) and acidified with HCl (2 M, 2 mL). Afterwards, the suspension was extracted with EtOAc (1 x 50 mL). The aqueous layer was separated, and the mixed layer was centrifuged (10.000x g, 10 min). The organic layer was dried over $MgSO_4$. The solvent was removed in vacuum. The crude product (155.6 mg) was obtained as yellow oil and was purified *via* automated column chromatography with *Biotage Isolera One* (C18; MeCN, H_2O, AcOH (0.1%)). The purified racemate (7.7 mg, 0.03 mmol, 3%) was analyzed by ^{1}H-NMR spectroscopy and NP-HPLC. However, only the *cis*-enantiomers were obtained. Thus, we stored the half under argon at -20 °C and the other half at room temperature. After half a year, the sample at room temperature was fully isomerized to the *trans*-isomer. Both were combined and measured in the NP-HPLC.

3 Analysis

3.1 HPLC

For determination of the diastereomeric excess, we used NP-HPLC from *Knauer* with universal valve drive ASM 2.2L, detector DAD 2.1L, pumps P 6.1L, autosampler AS 6.1L, column oven CT 2.1, degassing unit degasi GPC was purchased from *Biotech*. As solvent mixture we used pentane (90%), ethanol (10%) and a small amount of acetic acid (0.1%) for protonation of 12-OPDA (**4**). The flowrate was set to 1 mL/min. As stationary phase, we used the Chiralpak® AD-H column from *Daicel Chiral Technologies*.



Compound	Peak 1	Peak 2	Peak 3	Peak 4
rac-12-OPDA	7.78 (*trans*-(+))	8.74 (*cis*-(+))	9.18 (*cis*-(-))	11.46 (*trans*-(-))
cis-(+)-12-OPDA	-	8.71	-	-

Figure S. 8: Spectra of HPLC analysis of *rac*- and *cis*-(+)-12-OPDA (**4**).

3.2 NMR

3.2.1 Determination of conversions

The determination of conversions was done completely by ^{1}H-NMR spectroscopy. Therefore, we compared the integrals *I* of characteristic signals of all possible substrates, intermediates, and products (Table S. 14). All spectra were set up in $CDCl_3$ and measured with 16-32 scans.

Table S. 14: Compounds from 12-OPDA synthesis and the characteristic signals in ^{1}H-NMR.

Compounds	δ / ppm	# protons
α-linolenic acid (α-LA; **1**)	2.80	4
13-HPOT (**2**)	4.42	1
12-OPDA (**4**)	7.74 (*cis*) + 7.60 (*trans*)	1
α-ketol (**5**)	4.30	1

However, we choose the signals according to literature[1] and calculate the proportion of compound X with the equation the following equation. The compound X is one of the compounds from Table S. 14.

$$Proportion\ of\ compound\ X = \frac{\frac{I(compound\ X)}{\#protons_{compound\ X}}}{\left(\frac{I(\alpha - LA)}{4}\right) + I(13 - HPOT) + I(12 - OPDA) + I(\alpha - ketol)}$$

For determination of diastereomeric excess *de* of 12-OPDA (**4**) we used the equation:

$$de = \frac{I(cis - (+)) - I(cis - (-)) - I(trans - (+)) - I(trans - (-))}{I(cis - (+)) + I(cis - (-)) + I(trans - (+)) + I(trans - (-))}$$

With the analysis *via* HPLC, we could observe, that the (-)-enantiomers were not formed at all. Thus, we set the integrals to "0" and removed them from the equation:

$$de = \frac{I(cis - (+)) - I(trans - (+))}{I(cis - (+)) + I(trans - (+))}$$

Both isomers, *cis* and *trans,* are also possible to differentiate *via* ^{1}H-NMR spectroscopy (Table S. 14). Thus, we calculated the diastereomeric excess with the *cis-trans* ratio.

4 Literature

[1] J. Löwe, K.-J. Dietz, H. Gröger, *Adv. Sci.* **2020**, *7*, 1902973.

[2] N. Montua, N. Sewald, *ChemBioChem* **2023**, *24*, e202300478.

[3] B. H. Lipshutz, S. Ghorai, A. R. Abela, R. Moser, T. Nishikata, C. Duplais, A. Krasovskiy, R. D. Gaston, R. C. Gadwood, *J. Org. Chem.* **2011**, *76*, 4379.