Supporting Information

From a biosynthetic pathway toward a biocatalytic process and chemocatalytic modifications: Three-step enzymatic cascade to the plant metabolite \(cis-(+)-12\)-OPDA and metathesis-derived products

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Experimental Information

**Sequences and plasmid maps**

*pET28a-AtOAC2_WTΔchl(C)His*

**Figure 1**: Allene Oxide Cyclase (AOC2) from Arabidopsis thaliana in pET28(a)+, plasmid map, without chloroplast target sequence.

Allene Oxide Cyclase (AOC2) from *Arabidopsis thaliana* wild-type sequence, without chloroplast target sequence$^{[1]}$.

ATGGGCAAGCAGCATCATCATCATCATCACAGCAGCGGCTGGTGCCGCAGGCAGG
CATATGCTTTGGTTCTCAAAATCTTGGTATCTCATCTAAGCGTTCAGATT
TCTCCTATCCATACAGTTTACATGCAAGAAGACCTCAGTCTCGAGTCTCAGG
AAAACGGGAATATCGAAAACCCTAGACCAAGCAAAGTTCAAGAACTGAGTGTGTACG
ATCAACGAATTAGATGACAGGCAGCCTTTCAAAAGACGAAGTTCAAGAATGTGTTTCAAGG
GGTCTCGGAGATCTCGTACCAGTACGAATCTACAAAGAAGAAGACTCAGGCTACAGAAGACG
Allene Oxide Cyclase (AOC2) from Arabidopsis thaliana in pQE30 wild-type sequence, without chloroplast target sequence[1]

ATGAGAGGATCGCATCACCATCACCATCACCAGGATCCATGCTTGGTTCCTCTAAATCCTTCCAAAATCTTGGTATCTCATCTAACGGTTCCAGATTTCTCCTATCCATCAGTTTCTAAGTTAACTATACCAACTAA-

Amino acid sequence

MGSSHHHHHHSSGLVPRGSMLGSSKFQNLGISSNSGDFSYSSFTAKKNTLASRALSQNGNIENPRPSKVQSLSVEINELDRSPKILKNAFLMFLGLDVLVPFTNLKLYTGDLLKRVQITAGLCVVEHPVEKKGERFEATYSFYGDFYGLSVOQPYLYEDSLAITGGAGIFEGAYQVQLQQLVYPTKLYTFYLFGLANDLPHELGTVPVPSKIEPAEAKEPSGVISNYTN

pQE30-AtAOC2 _WTΔchl(p(N)His

pQE30_AT-AOC2_WTΔchl(p(N)His

4187 bp

m7 T1 Terminator

m8 T1 Terminator

His-Tag

T5-Promoter

lac-Operator

lac-Operator

4000
CAAGAAGAACCTCAGCTTCTCGAGGCTCTCTCCCAAAACGGGAATATCGAAAACCCTAGACCAAGCAAAGTTCAAGAACTGAGTGTGACGAAATCTACAGAATTAGATCGACACAGCCCAAGATTCTTAAAAACGCATTCAGCTTAATGTTCGGTCTCGGAGATCTCGTACCATTCACAAACAACTCTACACAGGCGATCTCAAGAAACGCGTGGGCATCACGGCAGGTCTCTGGCGTCGTCATCGAACACGTCCCAGAGAAGAAAGGTGAAAGATTCGAAGCTACTTATAGC
TTCTACTTCCGAGAATATGCCGACTTTGGCCAGTCGGTCTACAGAGAGCTGTTCTCGGATCTCCCTTTAAAGGTTGGCTAATGATTGGCGTTGGAGCTCAGGACACGCAACCGGGAACACCGGTACCGCCGTCTAAGGACATAGAGCCGCAGCGGGAAAGCTAAGGCCTAGCCGAGTTTATAAGTAACTATACCACG
Amino acid sequence
MRGSHHHHHHGSLMGSSKSFQNLGISSNGSDFSYSSFTAKKNLTASRALSGNQEBPAPPSKINDLPLELTGTPVPPSKDIEPAPEAKALEPSGVISNYTN-
Allene Oxide Cyclase (AOC2) from *Arabidopsis thaliana* in pUC18, Nucleotide-sequence, without chloroplast target sequence

ATGAGAGGATCGCATCACCATACCACATCCGGATCCCTTGGTTCCTCTCTAAATCCTTCCA
AAATCTTGGTATCTCATCTAAGGGATCTAGTTCTCTTCTCTTACCATCAAGTTTCACTGCCAA
GAAGAACCCTACTGCTTCTCGAGCTCTCTCCCAAAACGGGAATATCGAAAACCCTAGAC
CAAGCAAAGTTCAAGAACTGAGTGTGTACGAAATCAGAATTACAGTGACAGCATGGCC
AAATGCTTAAAAACGCATTCCAGTTAATGGGATCTGGTCTCAGAGATCTCGTACCATTACA
AACAAACTCTCACAGAGCCGATCTCAAGAAACCCGCTGGGCATCACGGCAGGTCTCTGCG
TCGTCATCGAAGACCGCGATCTCAAGAAACCGCGTGGGCATCACGGCAGGTCTCTGCG
TCTCGAGGAAGACTATGGCGCACTTTGCCTGAAGGACCACTTGCACCTTACAGAAGGATTC
GTTCCTGCCATCACGTGTTGCTCTTACGAGAATTTCAGGCTACGACAGGCTCAAG
CTCTACAGCTTGTGATCCGAACAAAACGGCACTTTTGCTACACTTTTAACCTTAAAGGTTGCT
AATGATTTCGCTTGGAGTACCGGAGACCCGAGGAACAGCCTCGGACTACAGAGGCTCAAG
CGGCCGCGGAAGCTAAGGCAAGTGCAGCCTAGCGGAGTTATAAGTAACTATACCAACTA
A

Amino acid sequence

MRGSHHHHHHHGLGSSKSFSQNLGISSNGDSFYSSFTAKKNLTASRALSONGNINPRPS
KVQELSVYEINELDRHSPKILKNAFLSMFGLDVPTNKLYTGDLKRRVGTAGLCSVIEHVP
Allene Oxide Synthase (AOS) from *Arabidopsis thaliana* in pET28(a). Nucleotide-sequence, without chloroplast target sequence and solubility-sequence GCAAAAAAACATCATCA
ATAGAAGAACCTCTCATACATTTATGTCTACCACCGGCGTTAGTCAAATCTGATTAC
CAGAGACTCTACGAGTTTTTCTTAGAATCCGCCGGTGAGATTCTCGTTGAAGCCGATAA
ATTGGGTATCTCACGAGAAGAAGCTACTCACAATCTTCTCTTCGCCACGTGCTTCAACAC
GTGGGGTGGGATGAAGAT TTTGTTTCCGAATATGGTTAAACGTATCGGGCGGGCGGGT
CATCAAGTTTCAAAACCAGATTAGCGAGGTAGGAGATCTGTGATTAAATCCAACCGGCGG
AGAACTCAGATGGGAGCAGCATTAGGAAATAGGAGTTAACCAATACAGTGTTTACGAT
GTCTCCGTGTAGAACCCAGGTACGGCTCAATACGGTAGAGCGAAGAGATCTGTGAT
TATCGAAGAGCCAGCAGCAGCGGCTTTAAAGTCAAAAGCAGGTTAAATGCTTTACCGGT
AACCATTTCGAGCACGAGATCCGAAGATTTTTGATCAGGGCGGATGAGTTTGTGCGGCGA
GAGATTCTCGAGAAGAAGAAGAAGGCTACCTTTTAGGCATGTGTTGTGGTCGAATGGA
CCGAGAGCCAGAGACTCCCGACCGGTTGAGAAATAACATGCGCCGTAAGGATTGTTTCTGT
TTTGGTGCGAGGTAGTGGTGGTGTGAGATTTGAGATTCTCCGCGATATGATTCTCGTTATTG
AGGGGTACGTCGCGGTAGGAAGCTCCGCTTAAATACGTTGTAAGGAAAGCTAGC
TTTGTCCGAAGCTTGGCGGCGGCGGCACTCGGGGACCACCACCACCACCTAA

Amino acid sequence

MAKKTSS ASGSETPDLTVATRTGSKDLPIRNIPNGYGLPIVGIPKDRTWDYFYDQGAEFFKS
RIRKYNSTVVRVNMPGPAGIAENPQVVALLGDGKSFPLVDKVEKDLFTGTYMPSTELTG
GYRILSYLDPSEPKHEKKNLFLLLKSSRNNRFPEFOATYSELFSDLEKELSLSKADSFSGS
SDGTAEFNLARAFGYTNPADTKLKAADAPGLTIKWLFNLHPSSILPRVIEEPIHTFSLPPA
LVKSDYQRLYEFFLESAGEILVEADKLGISREEATHNLLFATCFNTGGMKILPFNVMKRIGR
AGHQLVHRNLEAEEIRSVIKSNGGELTMGAIEKMLTKSVVYECRLREFFVPVTAYGFRKAKDLVIE
ESHDAAFKVKAGEMLGYQPLATRDPKIFRADEOFVFPERFVGEGEKLLRHVLWSNPGPETE
TPTGNKQCAKDFVVLVARLVFVIEIFRYSDFDEVGTSPLGSSNFSSLKASFVDKLAA
LGHHHHHH.

For all sequences his-tags are shown in yellow, the gene itself in grey and solubility sequence in magenta.
Microbiological and biochemical procedures

Production of competent cells

From the preculture, 250 μL were isolated at transferred into a 250 mL sterile flask with 25 mL LB-Medium. When the culture reached an OD of 0.35-0.4, the cells were harvested and incubated on ice. The cells were transferred to sterile vessels and centrifuged at 5000 xg and 4 °C for 15 minutes. The cell pellet was suspended in 5 mL of calcium chloride solution (100 mM), the suspension was incubated on ice for 20 minutes. Subsequently, the suspension was centrifuged at 5000 xg and 4 °C for 10 minutes, the supernatant was discarded. The cells were dissolved in 500 μl of a solution of calcium chloride (100 mM) with 15% glycerol solution. The resuspension was aliquoted (100 μL) and stored at -80 °C.

Transformation of competent cells with plasmid-DNA

After digestion 10 μL plasmid DNA was added to chemical competent cells (50 μL) and incubated for 30 minutes on ice. The cells were heated at 42°C for 90 seconds and incubated again for five minutes on ice. Afterwards 1 mL of LB media was added. The mixture was heated for three hours at 37°C and 800 rpm. Subsequently, the cells were cultured on LB agar plates with suitable antibiotic and incubated overnight at 37°C.

Production of whole cell catalyst

For the production of the whole-cell catalyst, first one plasmid was transformed into E.coli BL21(DE3). Subsequently, competent cells were prepared from these cells. Afterwards, the other plasmid is transformed into the same E.coli BL21(DE3).

Heterologous expression of AtAOS and AtAOC

TB medium (400 mL) or AI medium (400 mL) with kanamycin (50 μg/mL) and ampicillin (50 μg/mL) were inoculated with 1% (v/v) overnight culture. The cultures were grown at 37 °C and 180 rpm. When the EsLeuDH-DM culture reached an OD_{600nm} of 0.6, cell cultures were induced with 400 μL of IPTG (1M). For expression the temperature was reduced to a lower temperature.
Cell Disruption

The cells were suspended in sodium phosphate buffer (50 mM, pH 7) (25% cell suspension). The cells were digested under ultrasound sonification (3x 5 minutes, 5x 10 cycles, 10-20% power) on ice. Afterwards the suspension was centrifuged (15,000 rpm, 30 minutes).

Biochemical methods

SDS-PAGE Analysis

The overexpression of the enzymes was verified via SDS-PAGE. Therefore, the cells suspension was disrupted by ultrasound sonification on ice. Before the first centrifugation-step, a sample was taken (insoluble fraction). For the isolation of the inclusion bodies, the supernatant after the first centrifugation-step (4000 xg, 30 minutes, 4°C) was used and centrifuged again (20000 x g, 30 minutes, 4°C), the pellet contains the inclusion bodies. The 10 µL of the diluted crude extract (1 mg protein/mL) were transferred into the collection gel and analyzed via SDS-PAGE (15% separation gel).

Figure 1: SDS-PAGE pET28a_AtAOS. Stained with Coomassie Brilliant Blue R-250, lane 1: marker (Thermo Scientific PageRuler® Prestained Protein Ladder); all genes were expressed in E. coli BL21(DE3) at 25 °C, lane 2: insoluble fraction AtAOS; lane 3: inclusion bodies AtAOS.
Figure 2: SDS-PAGE BL21(DE3) of AtAOS-WTΔchlp and AtAOC2-WT in different vectors. Stained with Coomassie Brilliant Blue R-250, lane 1: crude extract of pET28-AtAOC2-WT with chloroplast target sequence, lane 2: insoluble fraction pET28-AtAOC2-WT with chloroplast target sequence, lane 3: crude extract of pET21-AtAOC2-WT without chloroplast target sequence, lane 4: insoluble fraction of pET21-AtAOC2-WT without chloroplast target sequence, lane 5: crude extract of pET28-AtAOC2-WT without chloroplast target sequence, lane 6: insoluble fraction of pET28-AtAOC2-WT without chloroplast target sequence, lane 7: marker (Thermo Scientific PageRuler® Prestained Protein Ladder), lane 8: crude extract of pET28-AtAOS-WT without chloroplast target sequence, lane 9: insoluble fraction of pET28-AtAOSWT without chloroplast target sequence, lane 10: crude extract of pACYCT2-AtAOS-WT without chloroplast target sequence, lane 11: insoluble fraction of pACYCT2-AtAOSWT without chloroplast target sequence.
Figure 3: SDS-PAGE BL21(DE3) of AtAOS-WTΔchip and AtAOC2-WT in pET28a, expressed at different temperatures. Stained with Coomassie Brilliant Blue R-250. lane 1: crude extract of pET28-AtAOC2-WT without chloroplast target sequence, expressed at 25 °C, lane 2: insoluble fraction pET28-AtAOC2-WT without chloroplast target sequence expressed at 25 °C, lane 3: crude extract of pET28-AtAOC2-WT without chloroplast target sequence, expressed at 30 °C, lane 4: insoluble fraction pET28-AtAOC2-WT without chloroplast target sequence expressed at 30 °C, lane 5: crude extract of pET28-AtAOS-WT without chloroplast target sequence, expressed at 37 °C, lane 6: insoluble fraction pET28-AtAOS-WT without chloroplast target sequence expressed at 37 °C, lane 7: marker (Thermo Scientific PageRuler® Unstained Protein Ladder), lane 8: crude extract of pET28-AtAOS-WT without chloroplast target sequence, expressed at 25 °C, lane 9: insoluble fraction pET28-AtAOS-WT without chloroplast target sequence expressed at 25 °C, lane 10: crude extract of pET28-AtAOS-WT without chloroplast target sequence, expressed at 30 °C, lane 11: insoluble fraction pET28-AtAOS-WT without chloroplast target sequence expressed at 30 °C, lane 12: crude extract of pET28-AtAOC2-WT without chloroplast target sequence, expressed at 37 °C, lane 13: insoluble fraction pET28-AtAOC2-WT without chloroplast target sequence expressed at 37 °C.
Figure 4: SDS-PAGE BL21(DE3)CodonPlus RIL of AtAOS-WTΔchl and AtAOS2-WTin pET28a at 25 °C. Stained with Coomassie Brilliant Blue R-250, lane 1: crude extract of pET28-AtAOC2-WT without chloroplast target sequence, lane 2: insoluble fraction pET28-AtAOC2-WT with chloroplast target sequence, lane 3: marker (Thermo Scientific PageRuler® Unstained Protein Ladder), lane 4: crude extract of pET28-AtAOS-WT without chloroplast target sequence, lane 5: insoluble fraction pET28-AtAOS-WT with chloroplast target sequence.
Figure 5: SDS-PAGE of expressed enzymes in BL21(DE3) at 25 °C. Stained with Coomassie Brilliant Blue R-250, lane 1: marker (Thermo Scientific PageRuler® Unstained Protein Ladder, lane 2: insoluble fraction WCC consisting of pET28a-AtAOS and pQE30-AtAOC2 lane 3: crude extract of WCC consisting of pET28a-AtAOS and pQE30-AtAOC2, lane 4: insoluble fraction of pQE30-AtAOC2, lane 5: crude extract of pQE30-AtAOC2, lane 6: insoluble fraction of pET28a-AtAOS, lane 7: crude extract of pET28a-AtAOS.
Activity Assay

First, an absorbance spectrum was recorded from a stock solution of 1 μL / mL purified 13-HPOT (2) in sodium phosphate buffer (50 mM, pH 7).

![Absorbance spectrum for 13-HPOT.](image)

Afterwards, 13-HPOT (2) was dissolved in sodium phosphate buffer (50 mM, pH 7, 1 mL) at different concentrations and the absorption was measured.

| 13-HPOT (2)/(mg/L) | Absorption Ø | Extinction factor /L/(mol cm) |
|--------------------|--------------|-------------------------------|
| 1.                  |              |                               |
| 8.8                | 0.81         | 28572                         |
| 5.9                | 0.49         | 25887                         |
| 4.1                | 0.28         | 21432                         |
| 2.                  |              |                               |
| 8.5                | 0.69         | 25281                         |
| 11.3               | 0.94         | 25660                          |

Ø: 25383 L/mol cm
The extinction factor was calculated via the *Lambert-Beer* equation. The enzyme activity is defined as \( \mu \text{mol} \ \text{min}^{-1} \):

\[
U = \frac{\Delta E \cdot V_t}{t \cdot V_p \cdot d \cdot \varepsilon \cdot F}
\]  

(1)

\( \Delta \text{Abs} \) is the change in absorption, \( V_t \) is the total volume of the sample, \( t \) is the time and \( V_p \) is the total volume of the enzyme sample. \( d \) is the thickness of the cuvette and \( F \) is the dilution factor. \( \varepsilon \) is the extinction coefficient. In the assay for the determination of the activity of the 13-LOX, the formation of 13-HPOT (2) was examined at 237 nm within 60 seconds. Therefore, the extinction factor of 13-HPOT (2) was estimated \( \varepsilon = 25383 \ \text{L} \ \text{mol}^{-1} \ \text{cm}^{-1} \). The temperature was changed from 25 to 40°C in 5°C steps. The composition of the assay is shown in the table below:

**Table 2**: Composition of activity assay for 13-LOX.

| Compound | volume/µL | Final |
|----------|-----------|-------|
| Buffer (NH₄Cl, pH 9; 20, 50, 100 mM) (NH₄Cl, 100 mM, pH 7, 8, 9), (NaPi 100 mM), (TRIS·HCl 100 mM), (NH₄Cl, 100 mM, 0/5/10% ethanol) | 890 |  
| \( \alpha \)-Linolenic acid (1) (1 mg/25 mL (Buffer)) | 100 | 0.014 µmol |
| 13-LOX (3 mg/ 25 mL(Buffer)) | 20 | 0.0012 mg |
**Activity assay 13-LOX**

![Activity assay 13-LOX](image)

**Figure 4**: Activity of 13-LOX at different conditions.

**Table 3**: Activity of 13-LOX at different conditions.

| Buffer    | pH | c/mM | T/°C | EtOH/% | Abs/min | Abs/min | Abs/min | Ø(Abs/min) | U/mg |
|-----------|----|------|------|--------|---------|---------|---------|------------|------|
| NH₄Cl     | 7  | 100  | 25   | 0      | 0.00    | 0.00    | 0.00    | 0.00       | 0.00 |
| NH₄Cl     | 8  | 100  | 25   | 0      | 0.02    | 0.02    | 0.02    | 0.02       | 2.47 |
| NH₄Cl     | 9  | 100  | 25   | 0      | 0.05    | 0.06    | 0.06    | 0.05       | 5.84 |
| NH₄Cl     | 9  | 20   | 25   | 0      | 0.02    | 0.02    | 0.02    | 0.02       | 1.80 |
| NH₄Cl     | 9  | 50   | 25   | 0      | 0.04    | 0.04    | 0.03    | 0.04       | 3.82 |
| NH₄Cl     | 9  | 100  | 25   | 0      | 0.05    | 0.06    | 0.06    | 0.05       | 5.84 |
| NaPi      | 9  | 100  | 25   | 0      | 0.06    | 0.04    | 0.36    | 0.05       | 4.52 |
| TRIS-HCl  | 9  | 100  | 25   | 0      | 0.02    | 0.02    | 0.02    | 0.02       | 2.23 |
| NH₄Cl     | 9  | 100  | 20   | 0      | 0.03    | 0.03    | 0.03    | 0.03       | 3.06 |
| NH₄Cl     | 9  | 100  | 30   | 0      | 0.06    | 0.06    | 0.06    | 0.06       | 6.70 |
| NH₄Cl     | 9  | 100  | 35   | 0      | 0.62    | 0.67    | 0.65    | 0.65       | 6.69 |
| NH₄Cl     | 9  | 100  | 40   | 0      | 0.04    | 0.05    | 0.42    | 0.04       | 4.54 |
| NH₄Cl     | 9  | 100  | 35   | 5      | 0.06    | 0.07    | 0.66    | 0.06       | 6.72 |
| NH₄Cl     | 9  | 100  | 35   | 10     | 0.02    | 0.02    | 0.01    | 0.02       | 1.46 |
**Activity assay 13-LOX and derivatives at different pH**

**Figure 5:** Activity of 13-LOX and the corresponding esters at different conditions.

**Table 4:** Activity of 13-LOX and the corresponding esters at different conditions.

| Substrate              | pH | Abs/min | Abs/min | Abs/min | Ø(Abs/min) | Abs/min | U/mg |
|------------------------|----|---------|---------|---------|------------|---------|------|
| α-LA (1)               | 7  | 0.00    | 0.00    | 0.00    | 0.00       | 0.00    | 0.19 | 0.20 |
| α-LA (1)               | 8  | 0.00    | 0.01    | 0.00    | 0.00       | 0.00    | 0.43 | 0.45 |
| α-LA (1)               | 9  | 0.06    | 0.10    | 0.08    | 7.63       | 8.03    |      |      |
| α-methyl-LA (5)        | 7  | 0.00    | 0.00    | 0.00    | 0.00       | 0.21    | 0.22 |      |
| α-methyl-LA (5)        | 8  | 0.00    | 0.00    | 0.00    | 0.00       | 0.22    | 0.23 |      |
| α-methyl-LA (5)        | 9  | 0.00    | 0.01    | 0.00    | 0.00       | 0.33    | 0.35 |      |
| α-ethyl-LA (6)         | 7  | 0.01    | 0.00    | 0.00    | 0.00       | 0.46    | 0.48 |      |
| α-ethyl-LA (6)         | 8  | 0.01    | 0.01    | 0.01    | 0.01       | 0.53    | 0.56 |      |
| α-ethyl-LA (6)         | 9  | 0.00    | 0.01    | 0.00    | 0.00       | 0.46    | 0.48 |      |
Activity assay AtAOS

The purification of the AOS by means of Ni\textsuperscript{2+}-NTA column chromatography was not possible; therefore, the inclusion bodies were isolated in order to test them for activity. For this purpose, some \textit{E. coli} BL21(DE3) cells containing AtAOS were disrupted by the means of ultrasound sonification. For the isolation of the inclusion bodies, the supernatant after the first centrifugation-step (4000 xg, 30 minutes, 4 °C) was used and centrifuged again (20000 xg, 30 minutes, 4 °C), the pellet contains the inclusion bodies. The pellet of the last centrifugation step was resuspended in sodium phosphate buffer (50 mM, pH 7). The protein concentration was determined via Bradford-assay (0.31 mg/mL).

Table 5: Composition of activity assay for AtAOS.

| Compound                                                                 | volume/μL |
|--------------------------------------------------------------------------|-----------|
| Buffer (NH\textsubscript{4}Cl, pH 9; 20, 50, 100 mM) (NH\textsubscript{4}Cl, 100 mM, pH 7, 8, 9), (NaPi 100 mM), (TRIS-HCl 100 mM), (NH\textsubscript{4}Cl, 100 mM, 0/5/10% ethanol) | 890       |
| 13-HPOT (2) (0.13 mg/25 mL (Buffer))                                      | 100       |
| AtAOS (0.31 mg/ 1 mL(Buffer))                                            | 10        |

Figure 6: Activity of AtAOS at different conditions
Table 6: Activity of AtAOS at different conditions

| Buffer | pH | c/mM | T/°C | EtOH/% | Abs/min | Abs/min | Abs/min | Ø(Abs/min) | U/mg |
|--------|----|------|------|--------|---------|---------|---------|------------|------|
| NH₄Cl  | 9  | 20   | 25   | 0      | 0.17    | 0.18    | 0.19    | 0.18       | 5.62 |
| NH₄Cl  | 9  | 50   | 25   | 0      | 0.17    | 0.15    | 0.14    | 0.15       | 4.75 |
| NH₄Cl  | 9  | 100  | 25   | 0      | 0.11    | 0.13    | 0.18    | 0.14       | 4.32 |
| TRIS-HCl| 9  | 100  | 25   | 0      | 0.21    | 0.24    | 0.27    | 0.24       | 7.52 |
| NaPi   | 9  | 100  | 25   | 0      | 0.23    | 0.24    | 0.27    | 0.24       | 7.61 |
| NaPi   | 9  | 100  | 25   | 0      | 0.21    | 0.18    | 0.16    | 0.18       | 5.64 |
| NaPi   | 9  | 100  | 20   | 0      | 0.13    | 0.12    | 0.15    | 0.13       | 4.12 |
| NaPi   | 9  | 100  | 30   | 0      | 0.13    | 0.15    | 0.10    | 0.13       | 3.91 |
| NaPi   | 9  | 100  | 35   | 0      | 0.09    | 0.07    | 0.13    | 0.10       | 3.01 |
| NaPi   | 9  | 100  | 40   | 0      | 0.15    | 0.19    | 0.12    | 0.15       | 4.77 |
| NaPi   | 9  | 100  | 25   | 5      | 0.12    | 0.16    | 0.14    | 0.14       | 4.35 |
| NaPi   | 9  | 100  | 25   | 10     | 0.17    | 0.18    | 0.19    | 0.18       | 5.62 |
Chemical syntheses of linolenic acid esters

*Synthesis of linolenic acid methyl ester (5)*

\[
\text{Linolenic acid methyl ester (5)}
\]

\[
\text{C}_{19}\text{H}_{32}\text{O}_2 \\
292.46 \text{ g/mol}
\]

\(\alpha\)-Linolenic acid (1) (55 mg, 0.19 mmol) was dissolved in methanol (15 mL), toluene (10 mL), HCl (8% in methanol, 4°C, 3 mL) with molecular sieve (3 Å, 110 mg). The reaction was stirred for 1 h. The solvent was removed *in vacuo*. Afterwards, the crude product was extracted with water (10 mL) and *n*-hexane (10 mL). Magnesium sulfate was added to remove residual water. The solvent was removed *in vacuo*.

**Yield:** 57 mg, 0.12 mmol, 98%.

**TLC:** \((R_f) = 0.81\) (Cyclohexane/EtOAc/AcOH, 4.5:0.5:0.1 v/v)

\(^1\text{H-NMR}\) (500 MHz, CDCl\(_3\)) \(\delta/\text{ppm} = 5.45 – 5.28 \, (m, 6H), 3.67 \, (s, 3H), 2.81 \, (ddd, \, ^3\text{J} = 9.3, \, ^2\text{J} = 4.7, \, 2.3 \, \text{Hz}, \, 4H), \, 2.31 \, (t, \, ^3\text{J} = 7.6 \, \text{Hz}, \, 2H), \, 2.12 – 2.01 \, (m, \, 4H), \, 1.62 \, (t, \, ^3\text{J} = 7.3 \, \text{Hz}, \, 2H), \, 1.38 - 1.24 \, (m, \, 8H), \, 0.98 \, (t, \, ^3\text{J} = 7.5 \, \text{Hz}, \, 3H).

The spectral data are in accordance with those given in literature.\(^2\)

*Synthesis of linolenic acid ethyl ester (6)*

\[
\text{Linolenic acid ethyl ester (6)}
\]

\[
\text{C}_{20}\text{H}_{34}\text{O}_2 \\
306.49 \text{ g/mol}
\]

Linolenic acid (1, 100 mg, 0.40 mmol) were dissolved in ethanol (130 mL), toluene (20 mL), HCl (8% in ethanol, 4°C, 3 mL) with molecular sieve (4 Å, 110 mg). The reaction was stirred under reflux for 24 h. The solvent was removed *in vacuo*. Afterwards, the crude product was extracted with water (10 mL) and *n*-hexane (10 mL). Magnesium sulfate was added to remove residual water. The solvent was removed *in vacuo*. 

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Yield: 58 mg (0.2 mmol), 58%

TLC: (Rf) = 0.86 (Cyclohexane/EtOAc/AcOH, 4.5:0.5:0.1, v/v)

\(^1\)H-NMR (500 MHz, CDCl\(_3\)) δ/ppm = 5.37 – 5.18 (m, 6H), 4.12 (q, \(^3\)J = 7.1, 2H), 2.81 (t, \(^3\)J = 6.3 Hz, 4H), 2.29 (t, \(^3\)J = 7.6 Hz, 2H), 2.11 – 2.01 (m, 4H), 1.62 (q, \(^3\)J = 7.7 Hz, 2H), 1.31 – 1.19 (m, 8H), 1.26 (t, \(^3\)J = 7.1 Hz, 3H), 0.98 (t, \(^3\)J = 7.5 Hz, 3H).

\(^{13}\)C-NMR (126 MHz, CDCl\(_3\)) δ/ppm = 174.05, 132.10, 130.42, 128.42, 128.40, 127.86, 127.25, 60.31, 34.54, 29.72, 29.32, 29.26, 29.25, 27.35, 25.77, 25.68, 25.12, 20.70, 14.43, 14.41.

EI-MS [m/z]: 329.30 [M+Na]⁺

HRMS (ESI) calculated for [M+Na]⁺ 329.2451, found 329.2446

FT-IR/cm\(^{-1}\): 3009 (CH- ν), 2926 (CH-ν), 1735 (C=O-ν)
α-Linolenic acid (1) was diluted in ethanol (1 mL). The solution was dissolved in ammonium chloride buffer (100 mM, pH 9). Lipoxygenase from Glycine max was dissolved in ammonium chloride buffer (100 mM, pH 9). The reaction was performed under oxygen saturation at room temperature. Reaction was controlled via TLC (cyclohexane/EtOAc/AcOH 6:2:0.1 v/v). Afterwards the reaction mixture was acidified to pH 2 with hydrochloric acid (2 M) and extracted twice with DCM (1:1, v/v). Magnesium sulfate was added to remove residual water. The solvent was removed via rotary evaporator. The reaction compositions (which consists of either pure product or a mixture of substrate and product, but no side product) are shown in detail in the following table:

Table 7: Composition and results for the reaction towards 13-HPOT.

| Experiment | NH₄Cl/mL | α-LA/µL | 13-LOX/mg | O₂ | t/min | Conv./%a) |
|------------|---------|---------|-----------|----|-------|-----------|
| A          | 25      | 12 (0.04 mmol) | 0.35     | -  | 180   | 15%       |
| B          | 250     | 120 (0.43 mol)  | 3.12     | +  | 60    | 20%       |
| C          | 250     | 120 (0.43 mol)  | 3.16     | +  | 30    | 32%       |
| D          | 250     | 120 (0.43 mol)  | 3.00     | ++ | 30    | 56%       |
| E          | 250     | 120 (0.43 mol)  | 3.00     | ++ | 20    | 76%       |

a) Reaction in an open flask; b) Oxygen (balloon filled with oxygen) added during reaction, c) Oxygen (balloon filled with oxygen) added before and during the reaction, d) Determined by the means of ¹H-NMR, based on the consumption of substrate, no side products were observed

¹H-NMR (500 MHz, CDCl₃) δ/ ppm: 6.58 (ddt, 3J = 15.3, 11.1, 1.0 Hz, 1H), 5.99 (t, 3J = 11.0 Hz, 1H); 5.58 (dd, 3J = 8.0 Hz 4J = 15.2, 1H), 5.53 – 5.47 (m, 2H), 5.33 (ddt, 3J = 7.3 Hz, 4J = 10.7, 1.7 Hz, 1H), 4.43 (dt, 3J = 8.2, 6.6 Hz, 1H), 2.50 – 2.44 (m, 1H), 2.34 (t, 3J = 7.4 Hz, 2H), 2.32 – 2.28 (m, 1H), 2.18 (ddt, 3J = 7.4 Hz, 4J = 1.5, 15.0 Hz, 2H), 2.05 (pd, 3J = 7.4 Hz, 4J = 1.5 Hz, 2H), 1.63 (p, 3J = 7.3 Hz, 3H), 1.25 (td, 3J = 7.1, 4.6 Hz), 0.96 (t, 3J = 7.5 Hz, 3H).

¹³C-NMR (126 MHz, CDCl₃) δ/ ppm: 178.46, 134.53, 134.26, 130.42, 130.36, 127.68, 123.22, 86.36, 33.89, 30.76, 29.42, 29.00, 28.94, 27.80, 24.77, 20.85, 14.25.

EI-MS [m/z]: 333.26 [M+Na]+ 349.17 [M+K]+

HRMS (ESI) calculated for [M+Na]+ 333.2036, found 333.2043
**FT-IR/cm**⁻¹: 3500-3000 (OH- ν), 2924 (CH- ν), 2853 (CH- ν), 1704 (C=O- ν), 1456-1409 (CH₃/CH₂- δ), 1243 (CH₃- δ), 1200 ((E)-CH₂-progression bands), 948 (OH- δ).
Ammonium chloride buffer (100 mM, pH 9) was saturated with oxygen. *E.coli* BL21(DE3)CodonPlusRIL containing AOS and AOC2 were suspended in the buffer. The cell and buffer mixture was saturated with oxygen for five minutes. Lipoxygenase from *Glycine max* was dissolved in ammonium chloride buffer and dissolved in reaction mixture and saturated with oxygen for five minutes. α-Linolenic acid was dissolved in ethanol and afterwards added to the reaction mixture. The reaction was performed under oxygen saturation at room temperature. Reaction was controlled via TLC (cyclohexane/EtOAc/AcOH 6:2:0.1 v/v). After 1 h reaction time, the reaction mixture was centrifuged (10.000x g, 15 min, 4 °C). The supernatant was acidified with 2 M hydrochloric acid and extracted with DCM. Magnesium sulfate was added to remove residual water. The solvent was removed in vacuo, and the diastereomeric ratio of 12-OPDA (determined by comparison with literature $^1$H-NMR data$^{[4]}$ for the cis- and trans-diastereomers of 12-OPDA) as well as the ratio between α-ketol and cis-(+)-12-OPDA were determined from the resulting crude product by means of $^1$H-NMR spectroscopy. In all experiments the diastereomeric ratio of the cis-OPDA diastereomer to the trans-12-OPDA diastereomer was in the range of 90:10 (cis:trans) to 95:5 (cis:trans). The optical rotation measurements were done on a polarimeter “Model 341” from Perkin Elmer. The measurements were performed in a cuvette with a layer thickness of 1 cm at room temperature and at a wavelength of 589 nm (sodium D-line). The substances were measured as solutions with concentrations of ~1 g/100 mL in chloroform. The crude product was purified via automated column chromatography (MeCN/H$_2$O/AcOH), leading to cis-(+)-12-OPDA as an isolated product.
Optimization of the synthesis of cis-\((+)-12\)-OPDA (4)

Ammonium chloride buffer (100 mL, 100 mM, pH 9 or 500 mL, 100 mM, pH 9 for experiment 4) was saturated with oxygen. The enzymes were added as whole cells in *E. coli* BL21(DE3)CodonPlusRIL, the compositions can be taken from Table 8. AOS and AOC2 in one whole cell catalyst were used with 300 mg or 1.5 g for experiment 4. AOS and AOC2 in separate whole cells were used with 150 mg of each type of cells. The whole cells were suspended in the buffer. The cell and buffer mixture were saturated with oxygen for five minutes. Subsequently, lipoxygenase from *Glycine max* (3 mg, 40 U or 15 mg, 200 U for experiment 4), which was dissolved in ammonium chloride buffer (1 mL, 100 mM, pH 9), was added to this mixture and saturated with oxygen for five minutes. Then, the reaction was started by addition of \(\alpha\)-linolenic acid (1, 100 mg, 0.36 mmol or 500 mg, 1.80 mmol for experiment 4), which was dissolved in ethanol (5 mL or 25 mL for experiment 4), to this mixture. The reaction was performed under oxygen saturation at room temperature. The reaction was controlled via TLC (cyclohexane/EtOAc/AcOH 6:2:0.1 v/v). After 1 h reaction time, the reaction mixture was centrifuged (10,000x g, 15 min, 4 °C). The supernatant was acidified with 2 M hydrochloric acid (4 mL or 20 mL for experiment 4) and extracted with DCM (3x 50 mL or 3x 250 mL for experiment 4). Magnesium sulfate was added to remove residual water. The solvent was removed *in vacuo*, and the diastereomeric ratio of 12-OPDA (determined by comparison with literature \(^1\)H-NMR data\(^4\) for the cis- and trans-diastereomers of 12-OPDA) as well as the ratio between \(\alpha\)-ketol and \(cis\)-(+)\(-12\)-OPDA were determined from the resulting crude product by means of \(^1\)H-NMR spectroscopy.

Table 8: Composition and results for the reaction towards \(cis\)-(+)\(-12\)-OPDA.

| Experiment | AOS             | AOC2              | Ratio 12-OPDA:\(\alpha\)-Ketol |
|------------|----------------|-------------------|--------------------------------|
| 1          | pET28a-AtAOS   | pET28a-AtAOC2     | 69:31                          |
| 2          | pET28a-AtAOS   | ...               | 23 (racemic\(^b\) 12-OPDA):77  |
| 3          | pET28a-AtAOS   | pUC18-AtAOC2      | 69:31                          |
| 4          | pET28a-AtAOS   | pQE30-AtAOC2      | 90:10                          |

\(^a\) In all experiments the diastereomeric ratio of the \(cis\)-OPDA diastereomer to the \(trans\)-12-OPDA diastereomer was found to be in the range of 90:10 (\(cis\):trans) to 95:5 (\(cis\):trans). \(^b\) For the 12-OPDA sample of this experiment, the measured optical rotation was as follows: \(\alpha\)\(^D\)\(^b\): 0° (c 30, CHCl\(_3\)).
The crude product of experiment 4 was purified via automated column chromatography (MeCN/H₂O/AcOH), leading to cis-(-)-12-OPDA (4) as an isolated product.

**Yield of 4 (experiment 4):** 31% (150 mg, 0.53 mmol)

**Diastereomeric ratio of 4 (experiment 4):** d.r.(cis:trans)=90:10

**1H-NMR** (500 MHz, CDCl₃) δ/ppm = 7.73 (dd, 3J = 6.0, 4J = 2.7 Hz, 1H), 7.60 (d, 3J = 5.7 Hz, 1H), 6.18 (dd, 3J = 5.9, 4J = 1.7 Hz, 1H), 6.12 (d, 3J = 5.9 Hz, 1H), 5.46 – 5.32 (m, 2H), 2.97 (ddt, 3J = 5.9, 10.8, 7.6, 4J = 3.6 Hz, 1H), 2.50 (dt, 3J = 15.3, 5.4 Hz, 1H), 2.47 – 2.41 (m, 1H), 2.35 (t, 3J = 7.5 Hz, 2H), 2.17 – 2.10 (m, 1H), 2.06 (d, 3J = 7.5 Hz, 2H), 1.72 (td, 3J = 11.1, 5.0 Hz, 1H), 1.63 (p, 3J = 7.2 Hz, 3H), 1.32 (q, 3J = 7.1, 5.9 Hz, 8H), 1.15 (dtd, 3J = 14.3, 9.6, 4J = 4.5 Hz, 1H), 0.97 (t, 3J = 7.5 Hz, 3H).

The spectral data are in accordance with those given in literature[3,4] and according to the comparison of the 1H-NMR spectroscopy data of the cis-diastereomer and the trans-diastereomer, which are reported in literature,[4] a diastereomeric ratio (cis:trans) of 90:10 was found for this experiment.
Ammonium chloride buffer (900 mL, 100 mM, pH 9) was saturated with oxygen. The enzymes were added as whole cells in *E.coli* BL21(DE3)CodonPlusRIL. AOS and AOC2 (2.7 g) were used in one whole cell catalyst. The whole cells were dissolved in the buffer. The cell and buffer mixture were saturated with oxygen for five minutes. Lipoxygenase from *Glycine max* (27 mg, 360 U) was dissolved in ammonium chloride buffer (1 mL, 100 mM, pH 9), afterwards dissolved in reaction mixture and saturated with oxygen for five minutes. α-Linolenic acid (1, 900 mg, 3.23 mmol) was dissolved in ethanol (45 mL) and afterwards added to the reaction
mixture. The reaction was performed under oxygen saturation at room temperature. Reaction was controlled via TLC (cyclohexane/EtOAc/AcOH 6:2:0.1 v/v). After 1 h reaction time the reaction solution was divided into three parts. Each part was centrifuged (10,000x g, 15 min, 4 °C). The supernatant was acidified with 2 M hydrochloric acid and extracted with DCM (3x 50 mL or 3x 250 mL for experiment 4). Magnesium sulfate was added to remove residual water. The solvent was removed in vacuo. For the ultrasound approach, the cell pellet was dissolved in DCM (15 mL) and disrupted under ultrasound sonification (1x 5 minutes, 5x 10 cycles, 10-20% power), afterwards the mixture was centrifuged (10,000 xg, 5 min). The procedure was done for two times. The supernatants were combined and the solvent was removed in vacuo. The crude product was purified via automated column chromatography (MeCN/H2O/AcOH).

Yield for product 4 obtained from the ultrasound method: 61% (176 mg, 0.64 mmol).

Optical rotation for product 4 obtained from the ultrasound method:

\[ \alpha_0^{20} : +104^\circ \text{ (c 10, CHCl}_3) \]

[Literature: \( \alpha_0^{20} : +104.0^\circ \text{ (c 9.5, CHCl}_3) \)]

Diastereomeric ratio of 4 obtained from the ultrasound method: d.r.(cis:trans)=94:6

\( ^1\text{H-NMR for product 4 obtained from the ultrasound method} \) (500 MHz, CDCl3) δ/ppm = 7.73 (dd, \( ^3\text{J} = 6.0, \quad ^4\text{J} = 2.7 \text{ Hz, 1H} \)), 7.60 (d, \( ^3\text{J} = 5.7 \text{ Hz, 1H} \)), 6.18 (dd, \( ^3\text{J} = 5.9, \quad ^4\text{J} = 1.7 \text{ Hz, 1H} \)), 6.12 (d, \( ^3\text{J} = 5.9 \text{ Hz, 1H} \)), 5.46 – 5.32 (m, 2H), 2.97 (ddt, \( ^3\text{J} = 5.9, 10.8, 7.6, \quad ^4\text{J} = 3.6 \text{ Hz, 1H} \)), 2.50 (dt, \( ^3\text{J} = 15.3, 5.4 \text{ Hz, 1H} \)), 2.47 – 2.41 (m, 1H), 2.35 (t, \( ^3\text{J} = 7.5 \text{ Hz, 2H} \)), 2.17 – 2.10 (m, 1H), 2.06 (d, \( ^3\text{J} = 7.5 \text{ Hz, 2H} \)), 1.72 (td, \( ^3\text{J} = 11.1, 5.0 \text{ Hz, 1H} \)), 1.63 (p, \( ^3\text{J} = 7.2 \text{ Hz, 3H} \)), 1.32 (q, \( ^3\text{J} = 7.1, 5.9 \text{ Hz, 8H} \)), 1.15 (ddt, J = 14.3, 9.6, \( ^4\text{J} = 4.5 \text{ Hz, 1H} \)), 0.97 (t, \( ^3\text{J} = 7.5 \text{ Hz, 3H} \)).

The spectral data are in accordance with those given in literature and according to the comparison of the \( ^1\text{H-NMR spectroscopy data of the} \) cis-diastereomer and the trans-diastereomer, which are reported in literature, a diastereomeric ratio (cis:trans) of 94:6 was found.
**Esterification cis-(-)+12-OPDA**

At first, cis-(-)+12-OPDA (4, 126 mg, 0.43 mmol) was dissolved in 5 mL EtOH, 770 mg molecular sieves (0.4 nm) and CAL-B (97 mg) are added. The reaction was stirred for 16 h at room temperature. The reaction was performed under oxygen saturation at room temperature. The molecular sieve is then filtered off and washed with H2O (2x 5 mL) and extracted with n-hexane (2x 5 mL). The solvent was removed *in vacuo*.

**Yield:** 127 mg (0.39 mmol), 92%

**$^1$H-NMR** (500 MHz, CDCl$_3$): $\delta$ (ppm) = 7.73 (dd, $^3$J= 6.0, $^4$J= 2.7 Hz, 1H), 7.59 (d, $^3$J= 5.7 Hz, 1H) 6.17 (dd, $^3$J= 5.9, $^4$J= 1.7 Hz, 1H, H3), 6.11 (d, $^3$J= 5.9 Hz, 1H), 5.46 – 5.32 (m, 2H), 4.11 (q, $^3$J= 7.1 Hz, 2H), 2.97 (tt, $^3$J= 8.4, $^4$J= 3.7 Hz, 1H), 2.49 (dd, $^3$J= 5.5, $^4$J= 15.3 Hz, 1H), 2.46 – 2.41 (m, 1H), 2.28 (t, $^3$J= 7.5 Hz, 3H), 2.16 – 2.07 (m, 1H), 2.07 – 2.03 (m, 2H), 1.76 – 1.68 (m, 1H), 1.60 (q, $^3$J= 7.1 Hz, 3H), 1.30 (s, 8H), 1.24 (t, $^3$J= 7.1 Hz, 3H), 1.14 (dtd, $^3$J= 18.6, 10.0, 4.6 Hz, 1H) 0.96 (t, $^3$J= 7.5 Hz, 3H).

**$^{13}$C-NMR** (126 MHz, CDCl$_3$): $\delta$ (ppm) = 210.84, 173.83, 167.11, 133.90, 132.98, 132.4, 126.98, 125.08, 60.20, 49.86, 44.29, 34.32, 30.78, 29.61, 29.42, 27.60, 24.91, 23.79, 20.80, 20.60, 14.27.

**MS (ESI):** m/z = 343.27 [M+Na]$^+$

HRMS (ESI) calculated for [M+Na]$^+$ 343.2244, found 343.2241

**IR (neat)/cm$^{-1}$:** 2926 (s, v, -CH$_2$), 2921 (m, v, -CH$_3$), 1730 (s, v, -C=O), 1707 (m, v, -C=C).

**General procedure (GP1) Metathesis reactions**

The reaction was done with cis-(-)+12-OPDA-ethylester (7, 1 eq.) and olefin (3 eq.) in degassed dichloromethane (10 mL) with *Hoveyda-Grubbs* catalyst™ 2nd generation (C1, 10 mol%). for 17.5 h. Afterwards the solvent was removed, and the crude product purified via thin layer chromatography (cyclohexane/EtOAc v/v 3:1).
Synthesis of Ethyl 8-((1S,5S)-5-((Z)-5-hydroxyhex-2-en-1-yl)-4-oxocyclopent-2-en-1-yl)octanoate (8)

The reaction was done with cis-(-)-12-OPDA-ethylester (7, 23.0 mg, 0.07 mmol) and 4 penten-2-ol (21.5 µL, 0.21 mmol, 3 eq.) in degassed dichloromethane (10 mL) with Hoveyda-Grubbs catalyst™ 2nd generation (C1, 4.49 mg, 0.006 mmol, 10 mol%) for 17.5 hours. Afterwards the solvent was removed, and the crude product purified via thin layer chromatography (cyclohexane/EtOAc v/v: 3:1).

Yield: 6.1 mg (17 µmol), 24%

$^1$H-NMR (500 MHz, CDCl$_3$): δ (ppm)= 7.71 (ddt, $^3$J = 5.8, $^4$J = 11.8, 3.2 Hz, 1H), 7.59 (d, $^4$J = 2.8 Hz, 1H), 6.19 – 6.14 (m, 1H), 6.13 – 6.09 (m, 1H), 5.61 (dq, $^4$J = 13.2, $^3$J = 6.4 Hz, 1H), 5.47 (ddt, $^3$J=7.5 Hz, $^4$J= 29.7, 15.0, 1H), 4.12 (q, $^3$J = 7.1 Hz, 2H), 3.83 (dt, $^3$J = 12.6, $^4$J = 6.4 Hz, 1H), 2.98 (m, 1H), 2.45 (dt, $^3$J=6.5, $^4$J = 16.4 Hz, 1H), 2.29 (t, $^3$J = 7.5 Hz, 2H), 2.26 – 2.18 (m, 1H), 2.06 (ddd, $^3$J=6.7, $^4$J = 16.0 Hz, 1H), 1.76 - 1.68 (m, 1H), 1.62 (t, $^3$J = 7.3 Hz, 3H), 1.32 (m, 12H), 1.26 (t, $^3$J= 7.1 Hz, 3H), 1.20 (dd, $^3$J= 6.3, $^4$J = 3.4 Hz, 3H).

$^{13}$C-NMR (126 MHz, CDCl$_3$): δ (ppm)= 204.0, 174.47, 134.78, 133.07, 131.59, 128.19, 126.56, 125.98, 67.33, 51.59, 42.69, 42.66, 34.24, 32.74, 29.49, 29.20, 29.07, 25.07, 22.77.

MS (ESI): m/z = 375.27 [M+Na]$^+$

HRMS (ESI) calculated for [M+H]$^+$ 351.25299, found 351.25366

IR (neat)/cm$^{-1}$: 3439 (s, ν, OH) 2925 (s, ν, -CH$_2$), 2921 (m, ν, -CH$_3$), 1732 (s, ν, -C=O), 1702 (m, ν, -C=C) 1347 (w, δ, -CH$_2$).

Synthesis of Ethyl 8-((1S,5S)-4-oxo-5-((Z)-6-oxohept-2-en-1-yl) cyclopent-2-en-1-yl)octanoate (9)
The reaction was done with cis-(+)-12-OPDA-ethylester (7, 24.0 mg, 0.07 mmol) and hexen-5-one (22.0 µL, 0.22 mmol, 3 eq.) in degassed dichloromethane (10 mL) with Hoveyda-Grubbs Catalyst™ 2nd generation (C1, 4.69 mg, 0.006 mmol, 10 mol%) for 17.5 hours. Afterwards the solvent was removed, and the crude product purified via thin layer chromatography. 3:1).

**Yield:** 8.0 mg (22 µmol), 30%

1H-NMR (500 MHz, CDCl3): δ (ppm) = 7.72 (dd, 3J = 6.0, 4J = 2.9 Hz, 1H), 6.20 – 6.13 (m, 1H), 5.40 – 5.30 (m, 2H), 4.12 (q, 3J = 7.1 Hz, 2H), 2.97 (m, 1H), 2.50 (m, 1H), 2.46 (dt, 4J = 14.3, 3J = 7.1 Hz, 1H), 2.32 (t, 3J = 6.4 Hz, 2H), 2.28 (t, 3J = 7.4 Hz, 2H), 2.14 (s, 1H), 2.12 (s, 3H), 2.03 (dq, 4J = 24.9, 3J = 7.9 Hz, 2H), 1.61 (m, 4H), 1.31 (s, 8H), 1.25 (t, 3J = 7.1 Hz, 3H)

13C-NMR (126 MHz, CDCl3): δ (ppm) = 210.62, 208.49, 173.96, 167.19, 132.92, 132.57, 131.46, 129.60, 129.18, 127.66, 60.34, 51.50, 49.42, 46.80, 44.43, 43.47, 34.46, 30.11, 26.80, 25.05, 21.71, 14.41.

**MS (ESI):** m/z = 385.30 [M+Na]+

HRMS (ESI) calculated for [M+H]+ 363.25299, found 363.25271

IR (neat)/cm⁻¹: 2925 (s, v, CH₂), 2854 (m, v, CH₂), 1715 (vs, v, C=O-Ester), 1586 (w, v, C=C).

**Synthesis of Ethyl 8-(((1S,5S)-5-((Z)-6-cyanohex-2-en-1-yl)-4-oxocyclopent-2-en-1-yl)octanoate (12)**

The reaction was done with cis-(+)-12-OPDA-ethylester (7, 24.0 mg, 0.06 mmol) and hex-5-enenitril (21.7 µL, 0.19 mmol, 3 eq.) in degassed dichloromethane (10 mL) with Hoveyda-Grubbs Catalyst™ 2nd generation (C1, 3.80 mg, 0.006 mmol, 10 mol%) for 17.5 hours.
Afterwards the solvent was removed, and the crude product purified via thin layer chromatography (cyclohexane/EtOAc v/v 3:1).

**Yield:** 9.0 mg (25 µmol), 40%

**1H-NMR** (500 MHz, CDCl₃): δ (ppm) = 7.72 (dd, 3 J = 5.9, 4 J = 2.7 Hz, 1H), 6.16 (d, 3 J = 5.9 Hz, 1H), 5.47 – 5.38 (m, 2H), 4.11 (q, 3 J = 7.2 Hz, 2H), 3.61 (m, 2H), 2.98 (m, 1H), 2.60 (q, 3 J = 8.4 Hz, 2H), 2.49 (m, 1H), 2.43 (m, 1H), 2.33 (t, 3 J = 7.0 Hz, 2H), 2.28 (t, 3 J = 7.4 Hz, 2H), 2.18 (q, 3 J = 6.6 Hz, 1H), 1.72 (dq, 4 J = 15.1, 3 J = 7.3 Hz, 2H), 1.61 (t, 3 J = 7.0 Hz, 2H) 1.33 (s, 8H), 1.25 (t, 3 J = 7.2 Hz, 3H).

**13C-NMR** (126 MHz, CDCl₃): δ (ppm) = 210.86, 173.85, 167.15, 167.12, 132.43, 127.15, 126.97, 67.21, 60.20, 53.42, 49.45, 34.33, 30.76, 29.60, 29.55, 29.09, 28.66, 27.57, 25.68, 24.91, 14.26.

**MS (ESI):** m/z = 382.30 [M+Na]+

HRMS (ESI) calculated for [M+Na]+ 382.23526, found 382.2348

**IR (neat)/cm⁻¹:** 2925 (s, ν, CH₂), 2854 (m, ν, CH₂), 2250 (w, ν, C≡N), 1732 (s, ν, C=O-ester), 1702 (vs, ν, C=O-carbonyl), 1586 (w, ν, C=C)

**Synthesis of Ethyl 8-((1S,5S)-5-((Z)-6-bromohex-2-en-1-yl)-4-oxocyclopent-2-en-1-yl)octanoate (10)**

The reaction was done with cis-(+)-12-OPDA-ethylester (7, 19.4 mg, 0.06 mmol or 30.0 mg, 0.09 mmol) and 5-bromopentene (21.5 µL, 0.18 mmol, 3 eq. or 41.0 µL, 0.34 mmol, 4 eq.) in degassed dichloromethane (10 mL) with Hoveyda-Grubbs Catalyst™ 2nd generation (C1, 3.79 mg, 0.006 mmol, 10 mol% or C1, 13.3 mg, 0.016 mmol, 18 mol%) for 17.5 hours. Afterwards the solvent was removed, and the crude product purified via thin layer chromatography (cyclohexane/EtOAc v/v 3:1).

**Yield:** 10 mg (24 µmol), 40% or 23 mg (56 µmol), 61%
\textbf{\textsuperscript{1}H-NMR} (500 MHz, CDCl\textsubscript{3}): $\delta$ (ppm)= 7.72 (dd, $^3J$= 5.8, $^4J$= 2.7 Hz, 1H), 6.16 (d, $^3J$= 5.8 Hz, 1H), 5.61 – 5.36 (m, 2H), 4.11 (q, $^3J$= 7.2 Hz, 2H), 3.38 (dt, $^4J$= 15.1, $^3J$= 6.6 Hz, 2H), 3.01 – 2.94 (m, 1H), 2.60 (m, 2H), 2.50 (m, 1H), 2.47 – 2.36 (m, 1H), 2.28 (t, $^3J$= 7.5 Hz, 2H), 2.20 – 2.15 (m, 1H), 1.90 (m, 2H), 1.77 – 1.68 (m, 1H), 1.67 – 1.58 (m, 2H), 1.31 (s, 8H), 1.25 (t, $^3J$= 7.1 Hz, 4H).

\textbf{\textsuperscript{13}C-NMR} (126 MHz, CDCl\textsubscript{3}): $\delta$ (ppm)= 210.73, 173.96, 167.18, 132.61, 130.18, 129.59, 60.34, 49.43, 44.46, 34.47, 33.34, 32.42, 31.04, 30.97, 29.75, 29.30, 29.21, 28.88, 27.73, 25.05, 14.42.

**MS (ESI):** m/z = 435.229 m/z [M+Na]$^+$, 437.186 m/z [M+Na]$^+$.  
HRMS (ESI) calculated for [M+Na]$^+$ 435.1505, found 435.1506

**IR (neat)/cm\textsuperscript{-1}:** 2925 (s, v, CH\textsubscript{2}), 2854 (m, v, CH\textsubscript{2}), 1732 (s, v, C=O-Ester), 1702 (vs, v, C=O-carbonyl), 1586 (w, v, C=C), 600 (w, v, C-Br).
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