Supporting information

Mid-Chain Carboxylic Acids by Catalytic Refining of Microalgae Oil

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General Methods and materials

All catalyst precursors used were stored in a glovebox under a nitrogen atmosphere. All reactions were conducted under inert gas atmosphere (argon or nitrogen) using standard glovebox or Schlenk techniques. Dichloromethane was dried over CaH_2 and distilled under a nitrogen atmosphere. Methanol was dried over magnesium and distilled under a nitrogen atmosphere. All other solvents were standard analytical grade and used as received. CO was purchased from Air Liquide, cis/trans-2-butene (99 %) and n-Tetradecane from ABCR GmbH. Nonanedioic acid, hexanoic acid, octanedioic acid and nonanoic acid were purchased from Fluka. Methyl oleate (92.5 %) was kindly donated by DAKO AG. The compound was distilled under a nitrogen atmosphere prior to use. Methyl palmitate, methyl palmitoleate and methyl myristate were purchased from Nu-Check Prep, Inc. Methyl palmitoleate was purified by Kugelrohr distillation. EPA purchased from Carbosynth was distilled prior to use. Hoveyda-Grubbs 2nd generation was purchased from Carbosynth. 2-, 3- and 4-heptanone, 1,4-cyclohexadiene, decanoic acid, methyl laurate, decane, hexadecane, heptadecane and the metathesis catalysts, Grubbs 1st generation, Grubbs 2nd generation, Grubbs 3rd generation and Hoveyda-Grubbs 1st generation were purchased from Sigma-Aldrich. Catalyst precursors Umicore M73 SIPr, Umicore M2, Umicore M73 SIMes and Umicore M31 were kindly donated by Umicore. 1,3-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was kindly donated by Lipiod GmbH and Corden Pharma Switzerland LLC. Gas chromatography was performed on a Perkin-Elmer GC Clarus 500 equipped with an elite-5 column (Length = 30 m, Inner Diameter = 0.25 mm, Thickness = 25 µ) and a FID-detector via the following program: 3 min isothermal at 50°C, 20°C min^{-1} to 280°C, 280°C for 5 min with an injector temperature of 300°C and a detector temperature of 280°C. All free acids were esterified with methanol prior to GC analysis. GC-MS measurements were conducted on an Agilent GC7890A system equipped with an inert MSD 5975C triple-axis detector. GC-MS was performed with a HP-5ms column. NMR spectra were recorded on a Bruker Avance III 400 MHz. ^{1}H NMR spectra were referenced to residual protonated solvent ($CDCl_3$).

Algae cultivation and extraction

Phaedactylum tricornutum was cultivated in three 10 L flasks continuously in modified f/2 cultivation medium[1] aerated with sterile ambient air in a day/night rhythm of 16/8 hours at 20 °C and with a light intensity of 35 µmol x s^{-1} x m^{-2}.

30 L of algae culture were centrifuged for 10 min at 4000 g and 4°C using a Sorvall RC 6 Plus centrifuge equipped with a Sorvall SLA 3000 rotor. The obtained cell pellet was stored at -28°C. The oil was extracted via a modified method of Folch et al.[2] The cell pellet (48.6 g) was thawed and diluted with 30 ml ddH_2O. The mixture was put on ice and pre-treated by ultrasonication for 10 min with a pulse of 10 s and amplitude of 60%, using an ultrasound homogenizer D2200 from BANDELIN with a KE76 sonotrode. 190 ml Chloroform[3] and 90 ml methanol were then added to establish a ratio of $CHCl_3$:MeOH:ddH_2O = 8:4:3. The natural water content is supposed to be approximately 75%. The organic phase containing the lipids was collected and dried over $MgSO_4$. After removal of the solvent, 6.3 g algae oil was obtained.

General procedure for butenolysis

A Schlenk flask was sealed with a septum, evacuated and cooled in a cold bath (isopropanol/nitrogen) and 2-butene was condensed into a Schlenk flask. Via syringe, the fatty acid or ester or algae oil (with additional dichloromethane) and nonanoic acid as internal standard were added (ratio 2-butene per double bond = 10:1). In the experiment testing the effect of phosphocholine, 1 mol% of 1,3-dipalmitoyl-sn-glycero-3-phosphocholine was added. The flask was transferred to an ice/salt bath (-5 °C). In a separate Schlenk tube, the appropriate amount of Hoveyda-Grubbs 2nd generation catalyst was dissolved in dichloromethane and added via syringe to the pre-cooled Schlenk flask containing the butene. A catalyst loading of 0.1 mol% for the algae oil, methyl oleate and palmitoleate and for eicosapentenoic acid 0.1 mol% or 0.2 mol% per double bond was used. Samples were drawn with a pre-cooled, air tight syringe. The samples were quenched with ethyl vinyl ether and analysed by GC. For GC analysis the samples were trans-esterified first by treating the sample with methanol/dichloromethane and a catalytic amount of sulphuric acid followed by heating. The excess of 2-butene was distilled at ambient temperature under a slight vacuum and was reused without further purification.

General procedure for isomerizing methoxycarbonylation

Isomerizing alkoxycarbonylation reactions were performed in a 20 mL pressure reactor with a glass inlay and a magnetic stirrer heated in an aluminium block. The reactions were carried out under inert atmosphere. The catalyst precursor was weighed into a Schlenk tube, and MeOH as a solvent and the reaction mixture from butenolysis were added. This mixture was transferred via syringe into the

reactor. The reaction mixture was stirred at 90 °C and 20 bar CO for 7 days. The reaction mixture was diluted with CH_2Cl_2 and filtered to remove solids. Samples were taken directly from the reaction mixture and analysed by GC.

Butenolysis of model compounds

The butenolysis was performed at -5°C with a catalyst loading of 0.1 mol% and 10 equivalents of 2-butene. Butenolysis of methyl oleate produces methyl 9-undecenoate **E11:1** and 2-undecene **HC11:1** (Figure S 1). By applying a tenfold excess of 2-butene, self-metathesis of methyl oleate producing dimethyl 9-octadecenedioate **DE18:1** and 9-octadecene **HC18:1** can be supressed. GC analysis revealed that a conversion of 92 % of the starting material was reached after already 30 minutes. The selectivity for butenolysis products (methyl 9-undecenoate **E11:1** and 2-undecene **HC11:1**) was 96%. Apart from remaining methyl oleate **FA18:1** (4%) and the desired cross-metathesis products, only small amounts of self-metathesis products were found, 1% of dimethyl 9-octadecenedioate **DE18:1** and 1% 9-octadecene **HC18:1**.

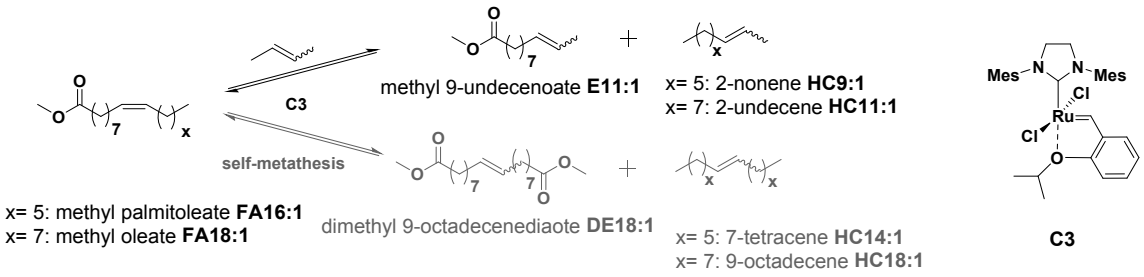


*Figure S1: Self- and cross-metathesis products of methyl oleate and methyl palmitoleate respectively with 2-butene and the used Hoveyda-Grubbs second generation catalyst (**C3**).*

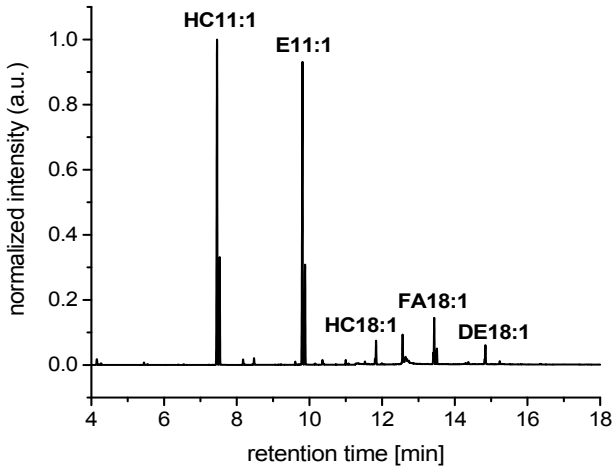


*Figure S2 GC trace of the reaction mixture of butenolysis of methyl oleate with labelling of the starting material (**FA18:1** methyl oleate), the butenolysis products (**E11:1** methyl 9-undecenoate, **HC11:1** 2-undecene) and self-metathesis products (**HC18:1** 9-octadecene, **DE18:1** dimethyl 9-octadecenedioate) are labelled.*

Methyl palmitoleate (**FA16:1**, Figure S1) was converted under the same condition as for methyl oleate. A conversion of 88 % and a selectivity toward butenolysis products (2-nonene **HC9:1** and methyl 9-undecenoate **E11:1**, Figure S1) was achieved with only 6% of self-metathesis products (dimethyl 9-octadecenedioate **DE18:1** and 7-tetradecene **HC14:1**).

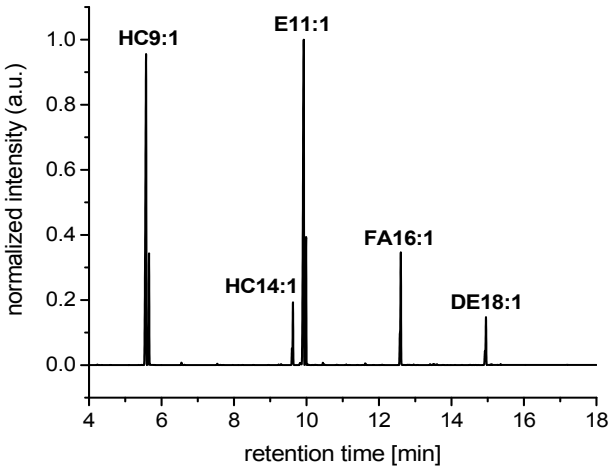
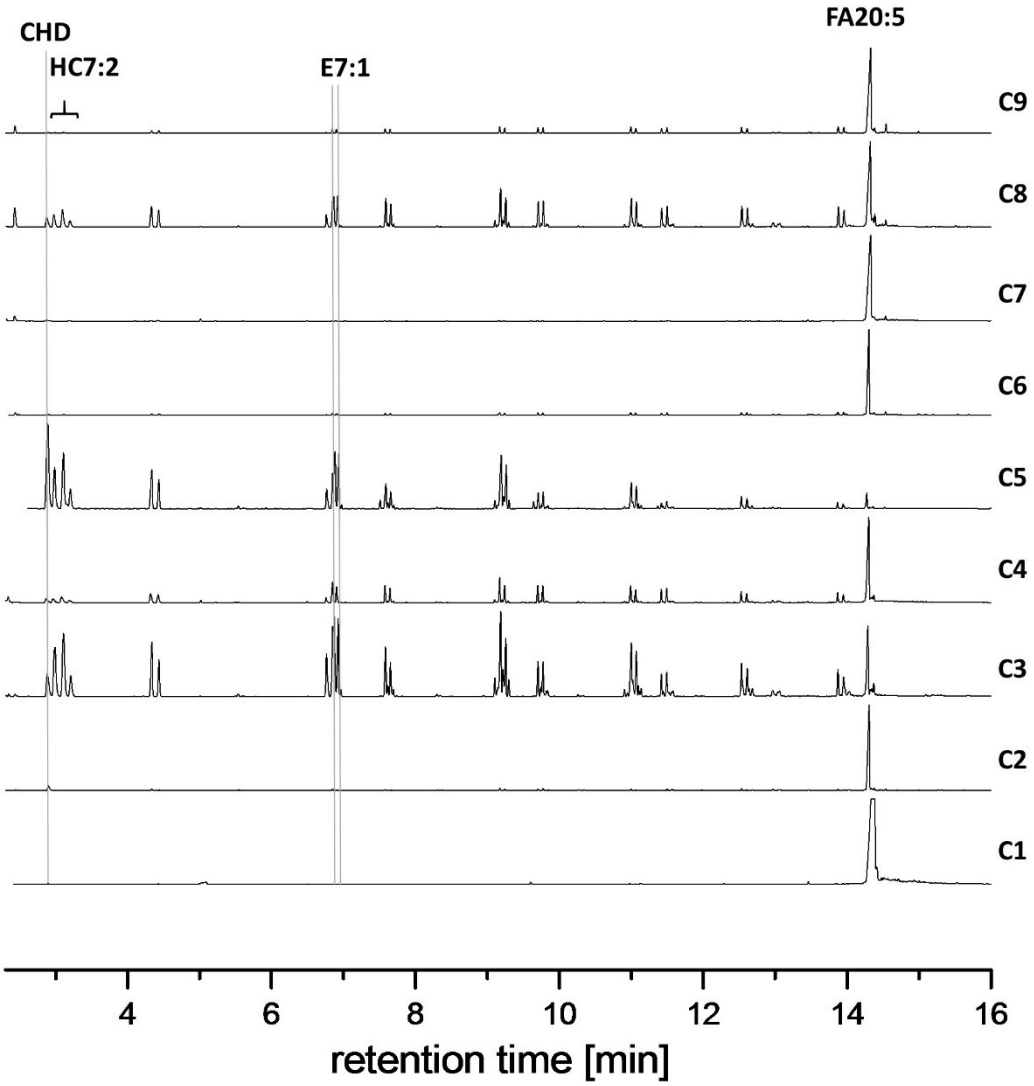


*Figure S3 GC trace of the reaction mixture of butenolysis of methyl palmitoleate, the starting material (**FA16:1** methyl palmitoleate), the butenolysis products (**E11:1** methyl 9-undecenoate, **HC9:1** 2-nonene) and self-metathesis products (**HC14:1** 7-tetracene, **DE18:1** dimethyl 9-octadecenedioate) are labelled.*



Butenolysis of EPA

*Figure S4: GC traces of catalyst screening of butenolysis of EPA with a catalyst loading of 0.1 mol% and 10 equivalents of 2-butene per double bond. (**C1**: Hoveyda-Grubbs 1st Generation; **C2**: Grubbs 1st Generation; **C3**: Hoveyda-Grubbs 2nd Generation, **C4**: Umicore M73 SIPr; **C5**: Grubbs 2nd Generation; **C6**: Grubbs 3rd Generation; **C7**: Umicore M2; **C8**: Umicore M73 SIMes; **C9**: Umicore M31, **CHD**: 1,4-cyclohexadiene, **HC7:2**: 2,5-heptadiene, **E7:1**: methyl 5-heptenoate). All non-labelled signals correspond to polyunsaturated products of incomplete butenolysis.*

The products of butenolysis of EPA with 0.1 mol% **C3** were identified by GC-MS. Additionally, the reaction mixture was hydrogenated to reduce the number of compounds and for further identification. This reaction mixture was also analysed by GC-MS and the GC-trace was compared to available genuine samples. Note that neither heptane nor octane, from 2,5-heptadiene and 2,5-octadiene were observed in the GC trace as the experimental procedure for hydrogenation reactions involves evacuation of the reaction vessels.

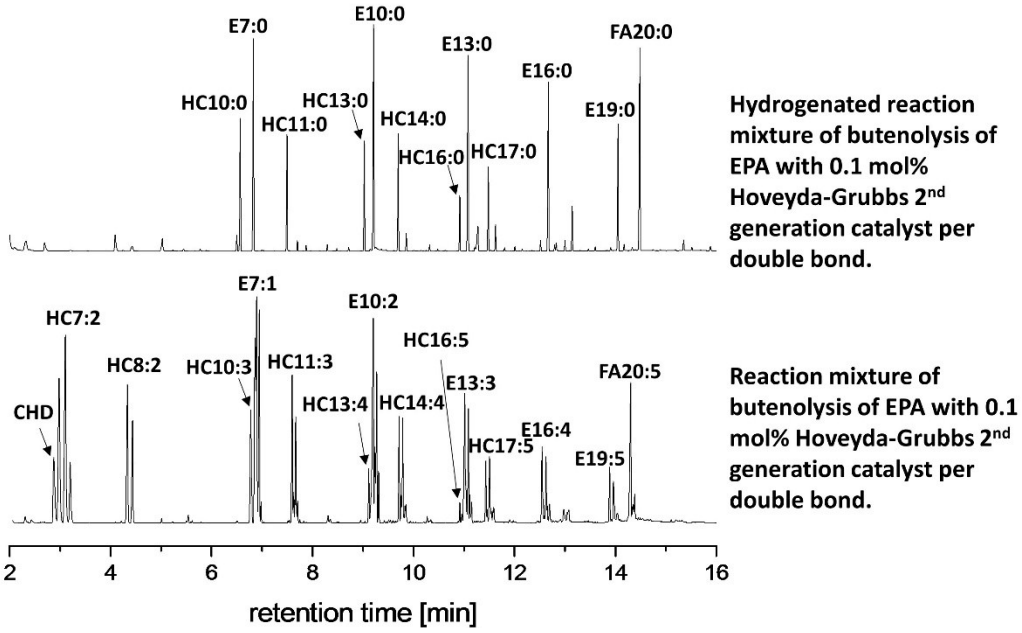


Figure S5 GC traces of butenolysis of EPA with 0.1 mol% Hoveyda-Grubbs 2nd generation catalyst (top) and the GC trace after hydrogenation (bottom). Products are labelled.

Compound	**Abbreviation**	**Calculated molecular weight [g/mol]**	**Mass found**
2,5-Heptadiene	HC7:2	96.17	96.1
2,5-Octadiene	HC8:2	110.20	110.1
2,5,8-Decatriene	HC10:3	136.24	136.1
Methyl 5-heptenoate	E7:1	142.20	142.1
2,5,8-Undecatriene	HC11:3	150.27	150.0
2,5,8,11-Tridecatetraene	HC13:4	176.30	176.1
Methyl 5,8-decadienoate	E10:2	182.26	182.1
2,5,8,11-Tetradecatetraene	HC14:4	190.33	157.1 (M-CH_3), 161.1 (M-CH_2CH_3), 147.1 (M-$CH_2CH_2CH_3$)
2,5,8,11,14-Hexadecapentaene	HC16:5	216.37	201.0 (M-CH_3), 187.0 (M-CH_2CH_3), 174.0((M-$CH_2CH_2CH_3$)
Methyl 5,8,11-tridecatrienoate	E13:3	222.33	222.1
2,5,8,11,14-Heptadecapentaene	HC17:5	230.4	215.1 (M-CH_3), 201.1 (M-CH_2CH_3), 187.0 (M-$CH_2CH_2CH_3$)
Methyl 5,8,11,14-hexadecatetraenoate	E16:4	262.39	262.1
Methyl 5,8,11,14,17-nonadecapentaenoate	E19:5	302.46	273.1 (M- CH_2CH_3), 247.0 (M-$CH_2CHCHCH_3$)
Decane	HC10:0	142.29	142.1
Methyl heptanoate	E7:0	144.21	144.0
Undecane	HC11:0	156.31	156.1
Tridecane	HC13:0	184.37	184.2
Methyl decanoate	E10:0	186.3	186.1
Tetradecane	HC14:0	198.39	198.1

Hexadecane	Hc16:0	226.45	226.1
Methyl tridecanoate	E13:0	228.38	228.2
Heptadecane	HC17:0	240.48	240.2
Methyl hexadecanoate	E16:0	270.46	270.1
Methyl heptadecanoate	E17:0	284.48	284.1
Methyl nonadecanoate	E19:0	312.54	212.2

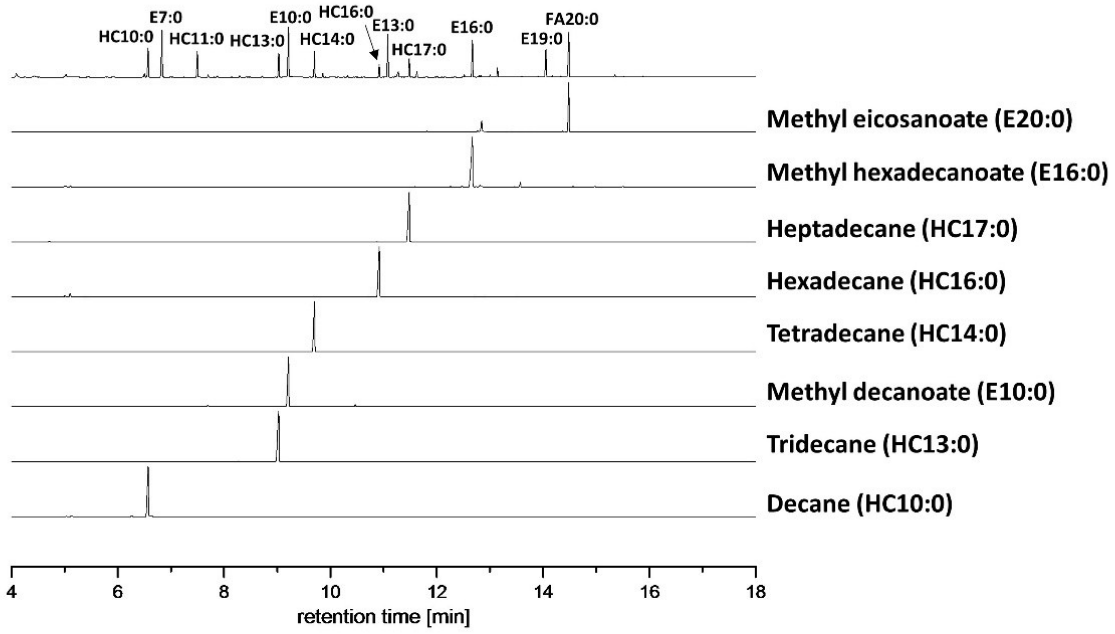


Figure S6 GC trace of the hydrogenated reaction mixture of butenolysis of EPA with 0.1 mol% Hoveyda-Grubbs 2nd generation catalyst (top) and GC traces of genuine samples for identification of the hydrogenated products.

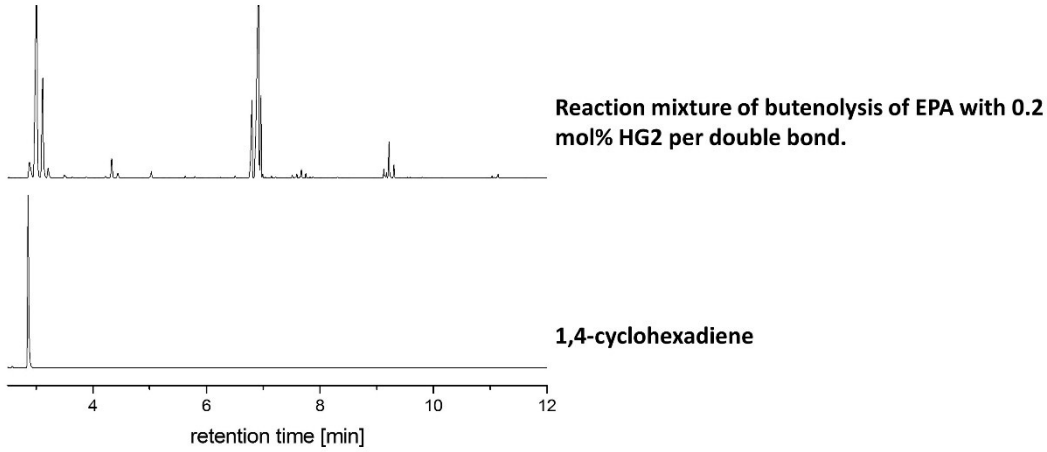


Figure S7 GC trace of butenolysis of EPA (top) and of a genuine sample of 1,4-cyclohexadiene (bottom).

Separation of Heptadiene

Heptadiene was isolated from the butenolysis reaction mixture of EPA by column chromatography with pentane as an eluent. A part of the 2,5-heptadiene isomerized to the conjugated 2,4-heptadiene. Pentane could not be removed completely due to the volatility of heptadiene.

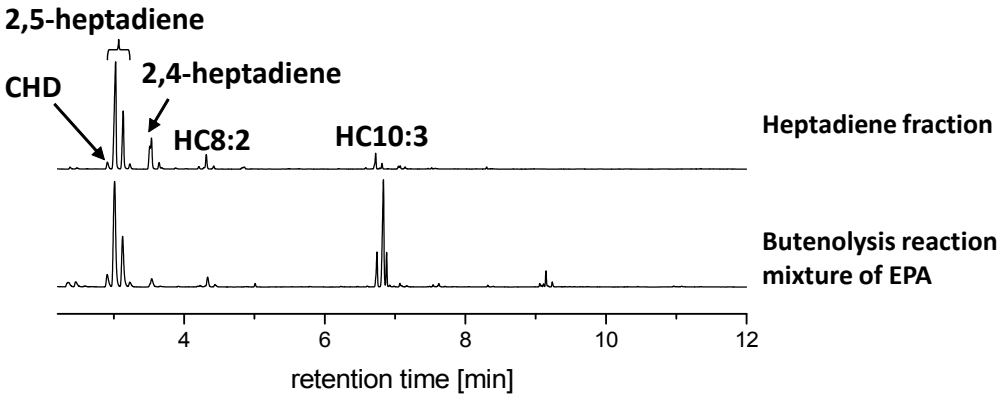


Figure S8 GC trace of the butenolysis reaction mixture of EPA (bottom) and of the separated heptadiene (top).

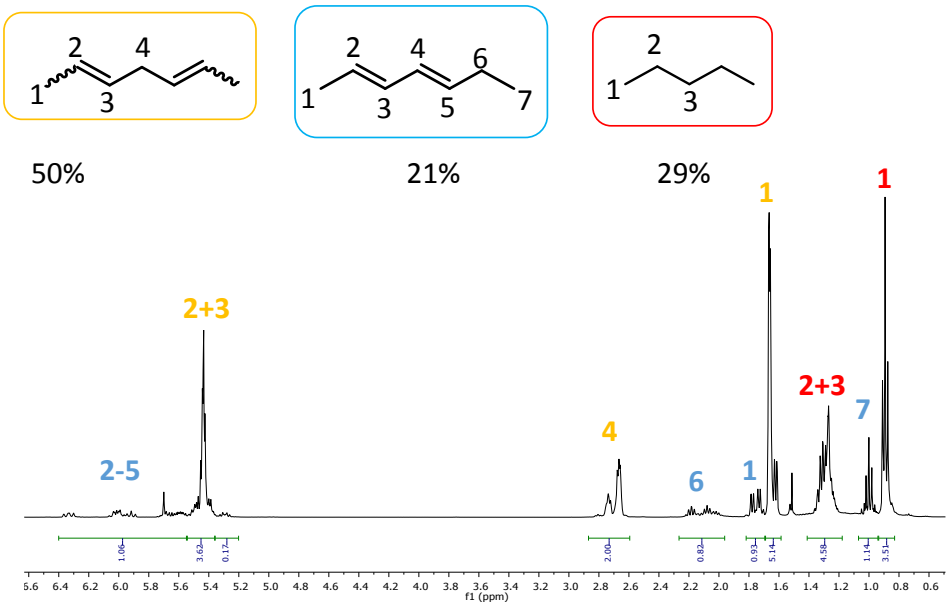


Figure S9 ^{1}H-NMR ($CDCl_3$, 400 MHz, 302 K) spectrum of the isolated heptadiene fraction.

Heptadiene was also separated by vacuum distillation and distillation under ambient pressure. Under ambient pressure more isomerization occurs.

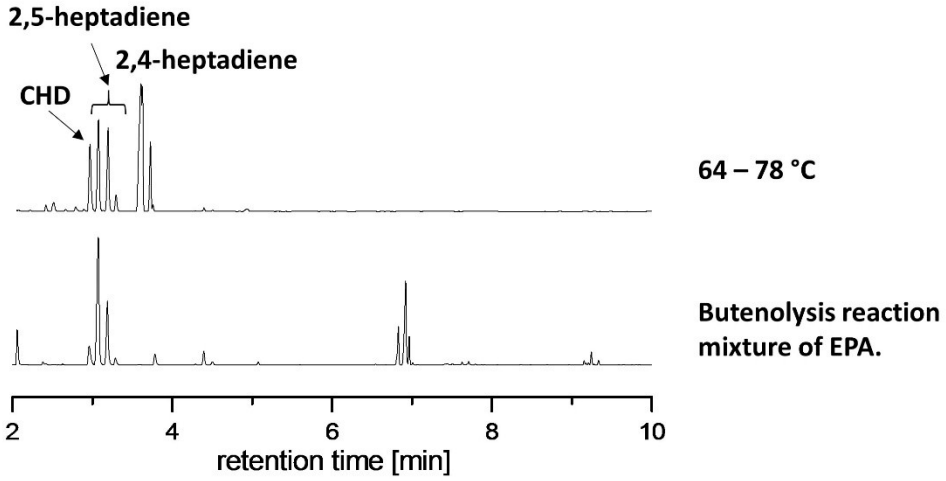


Figure S10 GC trace of the butenolysis reaction mixture of EPA (bottom) and GC traces of the distillate obtained by distillation at ambient pressure (top).

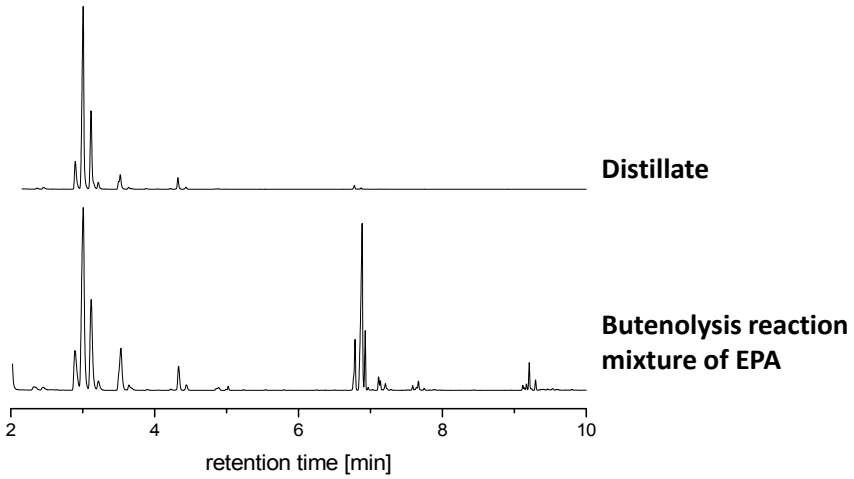


Figure S11 GC trace of the butenolysis reaction mixture of EPA (bottom) and of the distillate obtained by vacuum distillation (top).

Isomerizing Alkoxycarbonylation

Isomerizing Alkoxycarbonylation of the butenolysis reaction mixture of EPA and of isolated heptadiene

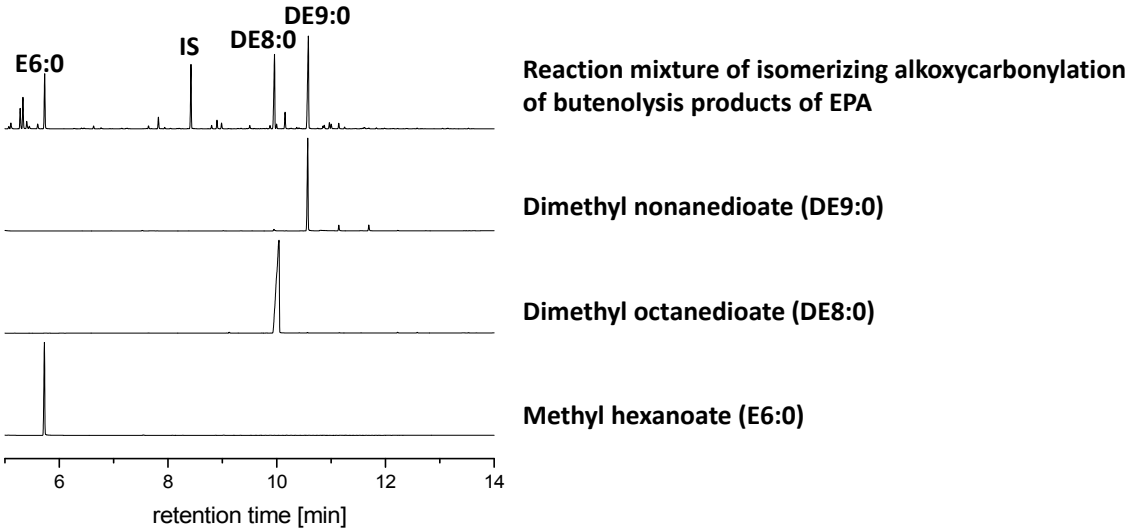


*Figure S12 GC trace of methoxycarbonylated butenolysis products of EPA with nonanoic acid as an internal standard (IS) and GC traces of genuine samples of methyl hexanoate **E6:0**, dimethyl octandioate **DE8:0** and dimethyl nonandioate **DE9:0**.*

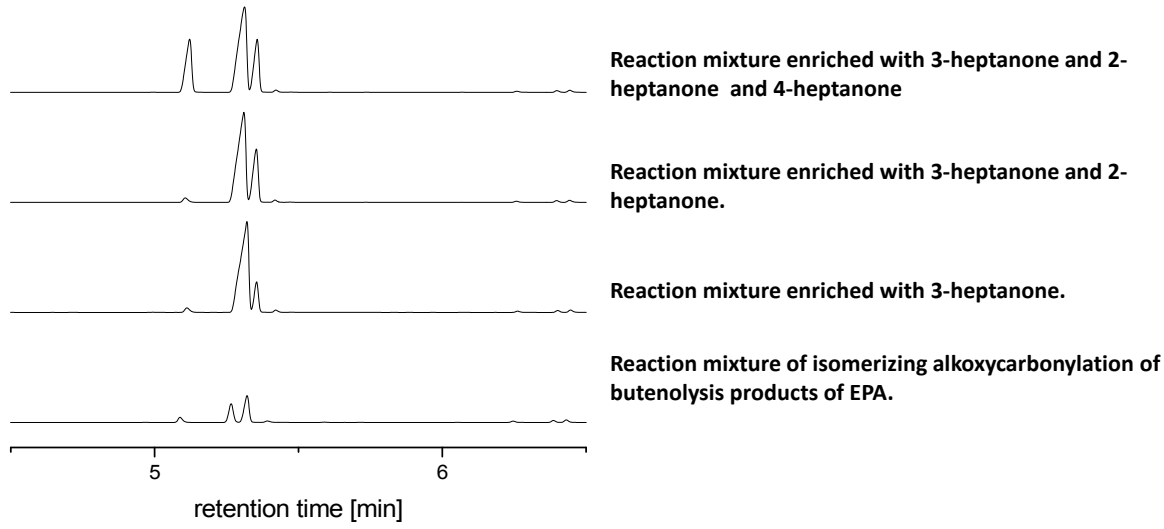


Figure S13 GC traces of methoxycarbonylated butenolysis products of EPA (bottom) and enrichment experiments with 2-, 3- and 4-heptanone.

The methoxy-ester side products (Figure S14) were identified by GC-MS:

Methyl methoxyoctanoate M= 188.27 g mol^{-1}; found 187.1 (M-H), 173.1 (M-CH_3), 159.0 (M-CH_2CH_3), 145.1 (M-$CH_2CH_2CH_3$), 127.1 (M-2x OCH_3), 87.1 ($\bullet CH_2CH_2COOCH_3$)

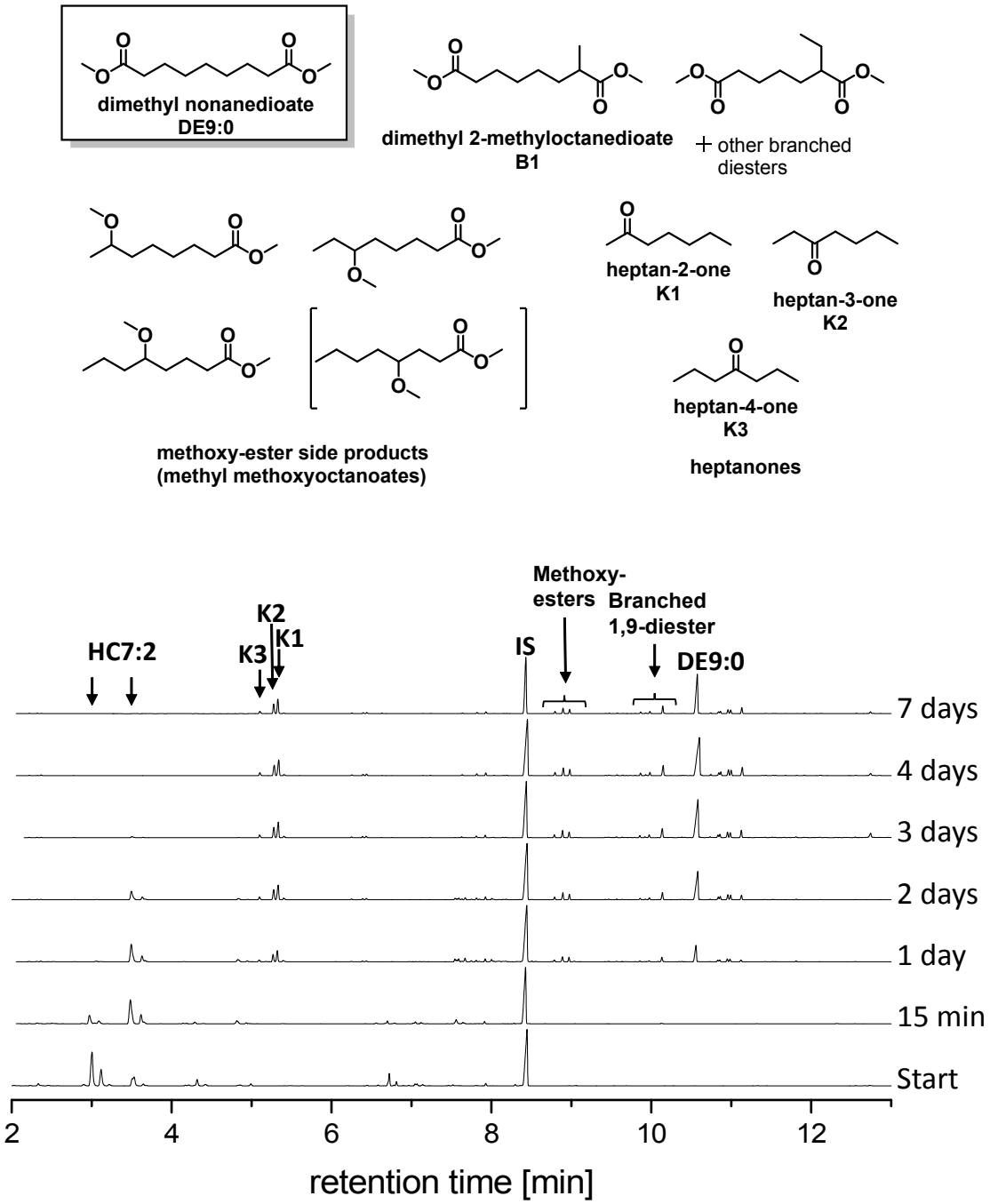


*Figure S14 GC traces of isomerizing alkoxycarbonylation of a mixture of 2,4- and 2,5-heptadiene (**HC7:2**) (0.8 mol% [Pd(dtbpx)(OTf)$_2$], 20 bar CO, 90 °C, nonanoic acid as an internal standard **IS**) and structures of the products.*

Isomerizing alkoxycarbonylation of the reaction mixture of butenolysis of crude algae oil

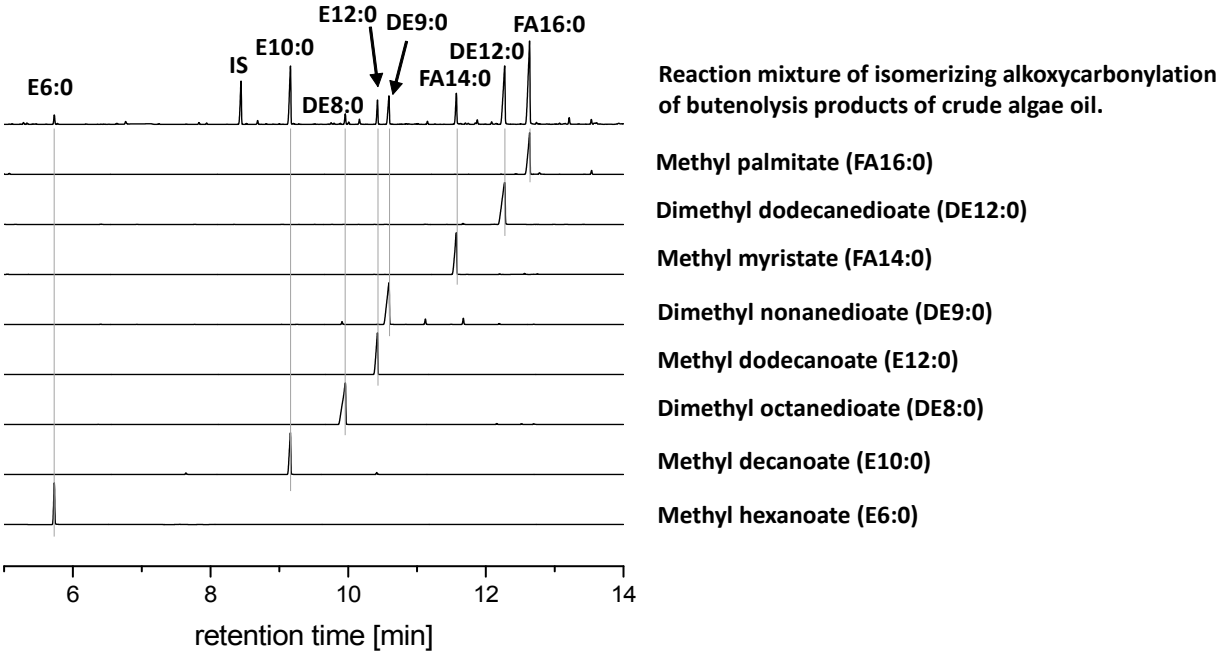


*Figure S15 GC trace of methoxycarbonylated butenolysis products of crude algae oil with nonanoic acid as an internal standard (IS) and GC traces of genuine samples of methyl hexanoate **E6:0**, methyl decanoate **E10:0**, dimethyl octandioate **DE8:0,** methyl dodecanoate **E12:0,** dimethyl nonandioate **DE9:0**, methyl myristale **FA14:0**, dimethyl dodecanedioate **DE12:0** and methyl palmitate **FA16:0**.*

Response Factors

All response factors were determined as an average of three measurements against nonanoic acid.

Name	Abbreviation	Response factor
Methyl myristate	FA14:0	1.35
Methyl palmitoleate	FA16:1	1.58
Methyl palmitate	FA16:0	1.49
Methyl oleate	FA18:1	1.88
Methyl eicosapentaenoate	FA20:5	1.15
Methyl nonanoate	E9:0	1
1,4-Cyclohexadiene	CHD	0.59
Methyl 5-heptenoate	E7:1	0.68*
2,5-Heptadiene	HC7:2	0.78
Methyl 5,8-decadienoate	E10:2	1.07*
2,5-Octadiene	HC8:2	0.96*
2,5,8-Decatriene	HC10:3	1.20*
Methyl 9-undecenoate	E11:1	1.10
2-Nonene	HC9:1	1.09*
2-Undecene	HC11:1	1.30*
Dimethyl octanedioate	DE8:0	0.74
Dimethyl nonanedioate	DE9:0	0.83
Methyl hexanoate	E6:0	0.59
Dimethyl dodecanedioate	DE12:0	1.12
Methyl decanoate	E10:0	1.07

Methyl dodecanoate	E12:0	1.38
Heptanone		0.74
Methoxyester		0.72*

*Derived from comparable compounds.

References

[1] R. R. L. Guillard and J. H. Ryther, *Can. J. Microbiol.*, 1962, **8**, 229-239.

[2] J. Folch, I. Ascoli, M. Lees, J. A. Meath and F. N. LeBaron, *J. Biol. Chem.*, 1951, **191**, 833-841.

[3] Chloroform is not a preferred solvent from the view point of environmental impact, and other solvents like supercritical CO_2 have also been reported for the extraction of microalgae. It was used here to stay compatible with earlier extraction data. Also concerning the focus of this article of critically assessing the compatibility of our procedure with real life microalgae oil, chloroform yields meaningful results in that is a good solvent that also includes possibly problematic impurities.