Synthetic Polyester from Algae Oil**

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Dedicated to Professor Gerhart von Graevenitz on the occasion of his 70th birthday

Abstract: *Current efforts to technically use microalgae focus on the generation of fuels with a molecular structure identical to crude oil based products. Here we suggest a different approach for the utilization of algae by translating the unique molecular structures of algae oil fatty acids into higher value chemical intermediates and materials. A crude extract from a microalga, the diatom Phaeodactylum tricornutum, was obtained as a multicomponent mixture containing amongst others unsaturated fatty acid (16:1, 18:1, and 20:5) phospho choline triglycerides. Exposure of this crude algae oil to CO and methanol with the known catalyst precursor [{1,2 (tBu$_2$PCH$_2$)$_2$C$_6$H$_4$}Pd(OTf)](OTf) resulted in isomerization/ methoxycarbonylation of the unsaturated fatty acids into a mixture of linear 1,17 and 1,19 diesters in high purity (>99%). Polycondensation with a mixture of the correspond ing diols yielded a novel mixed polyester 17/19.17/19 with an advantageously high melting and crystallization temperature.*

The generation of energy, transportation fuels and chemicals likewise today still strongly relies on fossil feedstocks. However, the availability of fossil deposits is limited on the long term, increasing environmental risks are associated with the recovery of oil and gas from non conventional sources, and the net release of carbon dioxide upon combustion can disadvantageously affect the global climate. Renewable resources like biomass from land plants can contribute to resolve these pressing problems, however, this approach raises urgent issues such as consumption of land and irrigation water, a competition with food production, inefficient yields per time and area, and the associated logistics of harvesting and collection.[1]

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Considering this background, algae oils are currently receiving much awareness. Microalgae can be cultivated in brackish or seawater on non arable land that is unapt for food production. Furthermore, no costly nutrients like glucose or peptones are required necessarily.[2] Rather, sunlight and CO$_2$ may serve as an energy source and raw material, respectively. The relatively high division rates of individual algal strains may result in a duplication of the biomass within 24 h.[3] At the same time, the amount of fatty acids in microalgae can be substantial with around 20 50% of the dry weight.[3,4]

While these advantages of algae oils are often and increasingly highlighted,[5 7] the application of algae oils so far is mostly restricted to the extraction of the naturally occurring ingredients and their utilization as biofuels, pig ments, vitamins or for human and animal nutrition. A prominent example are polyunsaturated ω 3 fatty acids used as food additives. On the other hand, efforts to use algae oils industrially comprise deoxygenation, pyrolysis, or even gasification to C$_1$ building blocks and their subsequent Fischer Tropsch conversion to higher alkanes. All these studies aim at using algae oil to generate hydrocarbons that ideally cannot be differentiated from the corresponding fractions, like jet fuel, based on crude oil.[8,9] However, the production of algae is associated with an energy input which currently still has a significant impact on the overall balance of fuel generation. This supports the general perception that generation of more valuable products than fuels for combus tion is worthwhile. Interestingly, algae do not only produce a large amount of lipids, they are also a unique resource for "unusual" classes of lipids and fatty acids differing from those available from higher animals and plant organisms.[10] This opens up unique opportunities for the production of chem icals from these oils, which may have a broader variety of structures and which can also be tailored specifically for sophisticated applications. Accordingly, we aim at translating the unique feedstock structure of algae oil into otherwise inaccessible products with useful properties rather than replicating crude oil based structures. Here, in a pioneering approach (Scheme 1), we demonstrate the conversion of algae oil into difunctional molecules, which are novel mono mers for the preparation of polyesters.
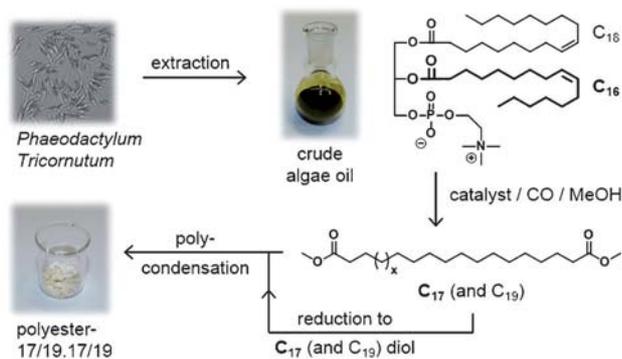
We chose the diatom *Phaeodactylum tricornutum* as a source for lipids, because published data suggested this strain to be very effective in the production of the desired unsaturated fatty acids.[11,12] This species belongs to the group of diatoms, ubiquitously present in aquatic and unicellular microalgae, which are major players in the global carbon cycle with an estimated share of up to 20% of total global carbon

Scheme 1. Approach to α,ω dicarboxylic acid intermediates and poly ester materials from algae oil.

Figure 2. Light microscopical images of *Phaeodactylum tricornutum* before (left) and after (right) treatment with ultrasound and acidic hydrolysis. Insert in left image shows a single cell, the dark area being a chloroplast (inset scale bar 10 μm). The arrows indicate lipid droplets in the intact cells (left) and released lipids after breaking the cells (right).

fixation.[13,14] Furthermore the genomic information of this alga is available[15] and genetic manipulation is feasible.[16]

In contrast to traditional plant oils,[17 19] algae do not contain fatty acids primarily in the form of triacylglycerides, but rather as diacylglycerides substituted with polar substitu ents such as galactosyl or phosphate groups on the third hydroxy moiety of glycerol (Figure 1).[20] This feature is important, as direct extraction of fatty acids with hydrocarbon solvents is hampered. Note, that also triacylglycerides can be present, especially when the algae are in the stationary growth phase for a longer period.
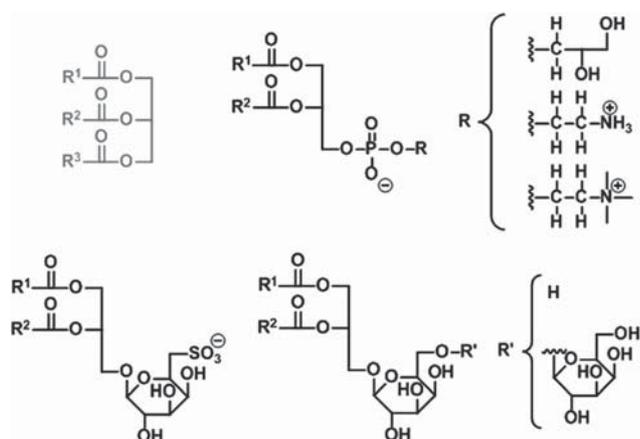

Figure 1. Fatty acids as they appear in plants (gray, top left) and algae (black).

Phaeodactylum tricornutum starts accumulating visible lipid droplets in the late stationary phase (Figure 2, left). In order to extract the fatty acids, different techniques were tested, namely direct extraction with hydrocarbon solvent, additional acidic hydrolysis to cleave ester functionalities, and ultrasonication, homogenization, and microwave treatment to destroy the cells. To release the lipid droplets from the cells (Figure 2, left), hydrolysis and mechanical disintegration were found to be crucial (Figure 2, right), and disintegration by ultrasound was most favorable. Subsequent lipid extraction from a 30 L algae culture being in the stationary phase for
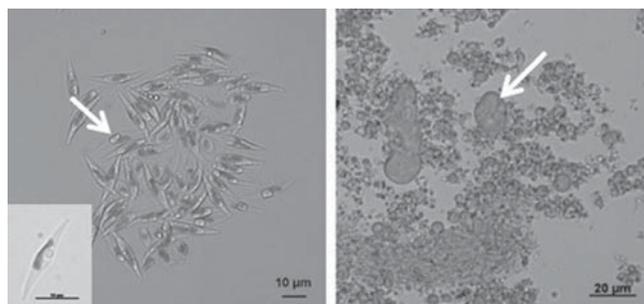
about six weeks yielded 5.8 g of algae oil. These results compare favorably with optimized yields reported from established methods for lipid extraction.[21,22]

Fatty acids in the extracted algae oil were identified by gas chromatography (GC) by comparison of retention times and enrichment with genuine samples of the respective fatty acids (Figure 3). 48.2 % of monounsaturated fatty acids (37.0 %
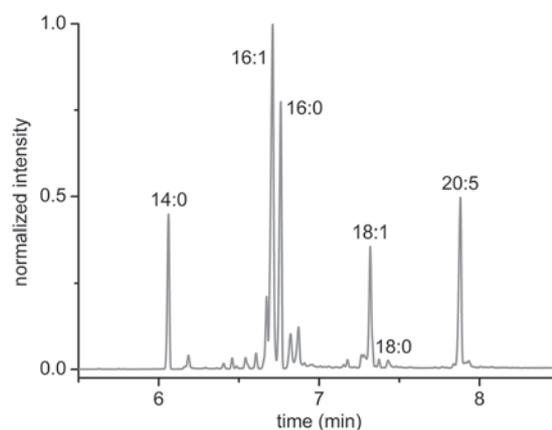

Figure 3. GC trace of crude algae oil extracted from *Phaeodactylum tricornutum* (after transesterification to the methyl esters for analysis).

palmitoleic acid (16:1)[23] and 11.2 % oleic acid (18:1)), 16.2 % of the multiple unsaturated fatty acid eicosapentaenoic acid (20:5), and 35.0 % of saturated fatty acids (23.4 % palmitic acid (16:0) and 11.6 % myristic acid (14:0)) were identified. However, not only fatty acids are present in the algae oil, which was already suggested by its green color and evidenced by gas chromatography (Figure 3) and ^{1}H NMR spectroscopy (Figure S6, Supporting Information, SI). Possible ingredients, both ubiquitously present in algae, may be carotenoids or chlorophylls.

A synthesis of polyesters via polycondensation requires further conversion to difunctional molecules as monomers. This requires catalysts compatible with the various compo nents of the crude algae oil. For example, the phosphate moieties may block coordination sites detrimentally, partic

ularly for electrophilic cationic active species. Tetraalkyl ammonium salts may alkylate phosphine ligands. The activity of (neutral) ruthenium alkylidene metathesis catalysts was reported to be decreased tremendously by phosphates, for example.[24]

Against this background, the extracted algae oil (5.8 g) was exposed to 20 bar CO pressure at 90 °C in methanol as a reagent and solvent in a pressure reactor in the presence of [(dtbpx)Pd(OTf)](OTf) (970 µmol; dtbpx = 1,2 bis((di *tert* butylphosphino)methyl)benzene), a known catalyst precursor for isomerizing methoxycarbonylation.[25 27] GC analysis of the crude reaction mixture (Figure 4, top trace) clearly
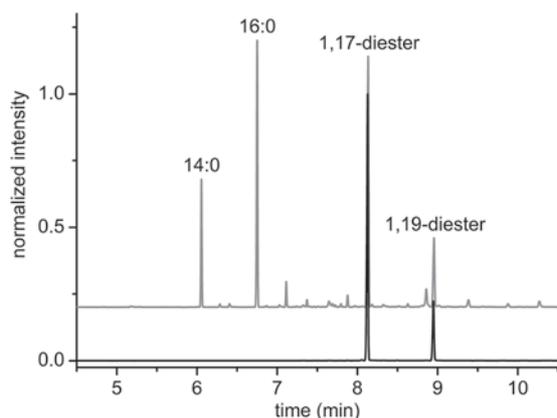

Figure 4. GC trace of the crude reaction mixture obtained from the isomerizing methoxycarbonylation of algae oil (top, gray), and after recrystallization from methanol and heptane (bottom, black).

evidences that the monounsaturated fatty acids (16:1 and 18:1) are completely converted to the desired linear 1,17 and 1,19 diester, respectively. These diesters were assigned unam biguously by enrichment with genuine samples prepared independently by methoxycarbonylation of pentadeca 1,14 diene and methyl oleate, respectively (SI). The signal of eicosapentaenoic acid also disappeared during the isomeriz ing methoxycarbonylation of the algae oil. Apparently, under these conditions eicosapentaenoic acid was not transformed straightforwardly to the 1,21 diester as evidenced by hydro genation of the reaction mixture and enrichment with a genuine sample (for preparation of this 1,21 diester see the SI). Possible additional reaction pathways may be multi ple methoxycarbonylation or hydromethoxylation.[28] Workup yielded 2.1 g of a mixture of 82 % 1,17 and 18 % 1,19 diester in polycondensation grade purity (> 99 %, Figure 4, bottom trace). A part of this mixture was reduced to the respective diols using LiAlH₄. After recrystallization from chloroform, a mixture of 85 % 1,17 and 15 % 1,19 diol was obtained in polycondensation grade purity (> 99 %). The ratio between the diols with different chain lengths was slightly different from the ratio of its respective diesters, presumably due to different solubility of the diols in the recrystallization. However, this is not a problem for the following polyconden sation reaction (see below).

The remarkable insensitivity of the catalyst towards the multi component mixture represented by crude algae oil was

further underlined by methoxycarbonylation of pure 1,2 dioleoyl *sn* glycero 3 phosphocholine as a substrate (sub strate double bonds/Pd = 125:1). A smooth conversion to dimethyl nonadecane 1,19 dioate occurred (for details see SI).

To achieve a substantial molecular weight by means of classical step growth polycondensation reactions, a very accu rate control of the stoichiometric ratio of functional groups is necessary.[29] To this end, the appropriate amount of the diol and the diester components, generated from the algae oil, were calculated from the peak area percentage obtained from the GC analyses (SI). Polycondensation catalyzed by titanium alkoxides afforded a mixed linear long chain polyester 17/19.17/19[30] with a number average molecular weight of $M_n = 4.0 \times 10^4 \, \mathrm{g\,mol^{-1}}$ as determined by both ¹H NMR spectroscop ic analysis of the end groups and high temperature size exclusion chromatography (SEC). The obtained material showed a melting point of 99 °C ($T_c = 75$ °C, $\Delta H_m = 136 \, \mathrm{J\,g^{-1}}$, Figure 5).
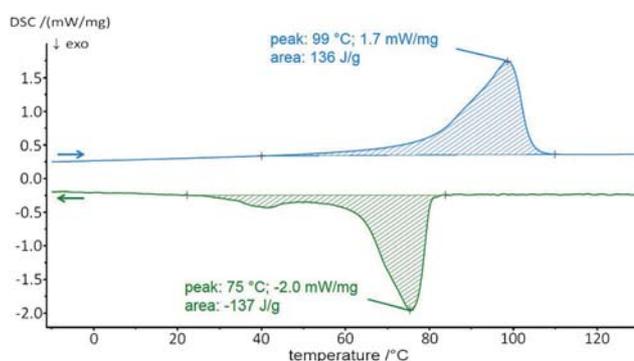

Figure 5. DSC trace of the algae derived polyester.

Other than known long chain polyesters, the material prepared here contains a mixture of repeat units with different numbers of carbon atoms (C_{17} and C_{19}). Such a less regular spacing of the ester groups could hinder the formation of sheets of ester groups and result in reduced order and melting points.[31 33] However, no such adverse effect was observed and the melting points are in line with neat model polyesters X.X prepared from the genuine samples for gas chromatographic identification (X = 17: $T_m = 99$ °C; X = 21: $T_m = 104$ °C and X = 25: $T_m = 108$ °C, see SI for details) and reported data for other polyesters X.X (X = 18: $T_m = 95$ °C[34] and X = 19: $T_m = 103$ °C[26]). These thermal properties com pare to typical thermoplastics, like low density polyethylene (LDPE).

In summary, a utilization of algae oils for the generation of higher value chemicals and polymer materials is an attractive alternative to biofuel production. This requires appropriate chemical conversions and catalysts compatible with algae oil. In an exemplary approach (Scheme 1) we have synthesized polyesters from crude lipids of the diatom *Phaeodactylum tricornutum*. Our findings support the feasibility of the generation of functional chemical intermediates by modern catalytic methods from algae oil, as exemplified by the

preparation of dimethyl heptadecane 1,17 dioate and hepta decane 1,17 diol. These molecules are based on a C_{16} building block (palmitoleic acid) which is a major component of algae oils but not of traditional plant oils. α,ω Difunctional com pounds can be obtained in high purity, even enabling step growth polycondensation to a high molecular weight polyes ter. This novel polyester possesses an advantageously high melting point as a result of the crystallizable linear segments originating from the algae oil lipids.

Notably, the transition metal catalyzed methoxycarbony lation is possible on the crude algae extract. Neither the five fold unsaturated eicosapentaenoic acid nor other non fatty acid components of the algae (amongst others phosphocho lines, see Figure 1) shut down catalysis. While an optimization of the work up procedures for larger scale synthesis was not a focus of this work, it can be noted that already at this stage a complicated chromatographic workup of the catalysis products is not necessary. Instead, a recrystallization was sufficient, which can be readily scaled.

A detailed investigation on different algae strains including genetic modification for the production of fatty acids, alternative extraction methods, and the conversion by other synthetic methods are part of our ongoing research in this topic.

Experimental Section

Detailed procedures and characterization of monomers and polymers (including NMR spectra, GC , SEC and DSC traces, and WAXS) are given in the Supporting Information.

Growth of algae: *Phaeodactylum tricornutum* UTEX 646 was obtained from the Culture Collection of Algae at the University of Texas in Austin and grown in 10 L flasks in a modified f/2 medium[35,36] (pH 7) with artificial half concentrated sea salts (16.6 g L^{-1}, tropic marine) and 0.09 μM MnCl$_2$ at a light intensity of 35 μmol s^{-1} m^{-2} in a day/night rhythm of 16/8 h at 20 °C. Ambient air was bubbled through the flasks. The algae were cultivated for 7 8 weeks, being in the stationary phase for at least six weeks. Centrifugation of 30 L algae culture yielded 45.3 g of fresh weight algae, after freeze drying 12.8 g of dry weight algae were obtained. Work up by ultrasonication, acidic hydrolysis, and solvent extraction yielded 5.8 g of crude algae oil, which was directly subjected to isomerizing methoxycarbonylation.

Isomerizing methoxycarbonylation: Crude algae oil (5.8 g) and the catalyst precursor [(dtbpx)Pd(OTf)](OTf) (0.97 mmol), or 1,2 dioleoyl *sn* glycero 3 phosphocholine (450 mg, 0.57 mmol) and the catalyst precursor (0.009 mmol), respectively, were dissolved in methanol. In a pressure reactor, the solution was pressurized with 20 bar CO and heated under stirring to 90 °C for seven days. The resulting suspension was diluted with methylene chloride and filtered to remove solids. The solvents were evaporated, and the crude product mixture was analyzed by GC. Recrystallization yielded 2.1 g and 320 mg of the desired linear diesters, respectively.

Polymerization of algae oil monomers: Stoichiometric amounts of the algae oil based linear diester and diol mixtures were mixed in a mechanically stirred Schlenk tube. Titanium(IV) tetrabutoxide was added as catalyst and the mixture was heated stepwise to 200 °C under reduced pressure to yield the algae oil based polyester.

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