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GREEN CHEMISTRY

Supporting Information

Flexible polyurethanes, renewable fuels, and flavorings from a microalgae oil waste stream

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MATERIALS AND METHODS

Materials.

Nannochloropsis salina was obtained from the National Center for Marine Algae and Microbiota, Maine, USA. Chemical reagents were purchased from Fisher Chemical, Macron Fine Chemicals, Sigma Aldrich, Acros, Fluka, Alfa Aesar Chemicals or TCI-America. All chemicals were regent grade and used without further purification. Analytical grade solvents such as acetone, hexane, and methanol were purchased from Fisher Chemical and used as received. Deuterated NMR solvents such as chloroform-d, DMSO-d6 were purchased from Cambridge Isotope Laboratories.

Equipment.

Gas chromatography mass spectrometry (GC-MS) was run on an Agilent 7890A GC system connected to a 5975C VL MSD quadrupole MS (EI). Samples were separated on a 60m DB23 Agilent GCMS column using helium as carrier gas and a gradient of 110 ^{o}C to 200 ^{o}C at 15 ^{o}Cmin^{-1}, followed by 20 minutes at 200 ^{o}C. ^{1}H NMR spectra was recorded on a JOEL ECA 500 or a Varian VX500 spectrometer equipped with an Xsens Cold probe. ATR-FTIR was performed on a Perkin Elmer Spectrum RXI equipped with a ZnSe 1mm ATR cell. 18 scans were taken at a 1 cm^{-1} resolution. Gel permeation chromatography (GPC) was performed on Agilent GPC/SEC system, the samples were run in chloroform at 45 ^{o}C using a refractive index detector and analyzed against polystyrene standard. Fluorescence spectra were recorded at room temperature on a Thermo Scientific Varioskan LUX at the excitation wavelength of 350 nm. Differential Scanning Calorimetry (DSC) was measured on Perkin-Elmer in a temperature range of -50 to 600 ^{o}C under Ar at flow rate of 20 mL min^{-1} with a heating rate of 10 K min^{-1}. All data are referred to the second heating cycle. Ozone is produced from the Triogen LAB2B Ozone generator. Irradiation of photochemical reaction was carried out using 12V 5630 LED Strip Light purchased from IEKOV. The wavelength of Blue LEDs light was measured by the Compact CCD Spectrometer.

Step 1: Purification of fatty acids from biomass.

Nannochloropsis salina [1] was chosen as a strain for outdoor growth of microalgae biomass due to its robust growth and ability to accumulate high concentrations of polyunsaturated fatty acids [2]. The procedure for algae culturing and harvesting of biomass has been described in our previous publication[3]. The harvested algae paste was collected and dried by centrifugation and storing at -20^{o}C and the Triacylglycerides (TAGs) were extracted from using hexane and isopropanol using a liquid-liquid extraction technique [4]. After a process of TAG hydrolysis, omega-3 fatty acids were isolated using fractional distillation[5], providing a mixture of saturated and monounsaturated fatty acids methyl esters (FAMEs). Acidic hydrolysis of these FAMEs provided a raw oil containing a mixture of saturated and monounsaturated fatty acids partially contaminated with photosynthetic pigments other small molecules.

To remove non-fatty acid contaminants, saponification was carried out on 127 g of FAME mixture with 400 mL aqueous KOH 3N. The collected soft soap was washed with acetone several times obtain the purified soap. The fatty acids were recovered by acidification with 6N aqueous HCl (Figure S1). Yield: 98 g, 78%

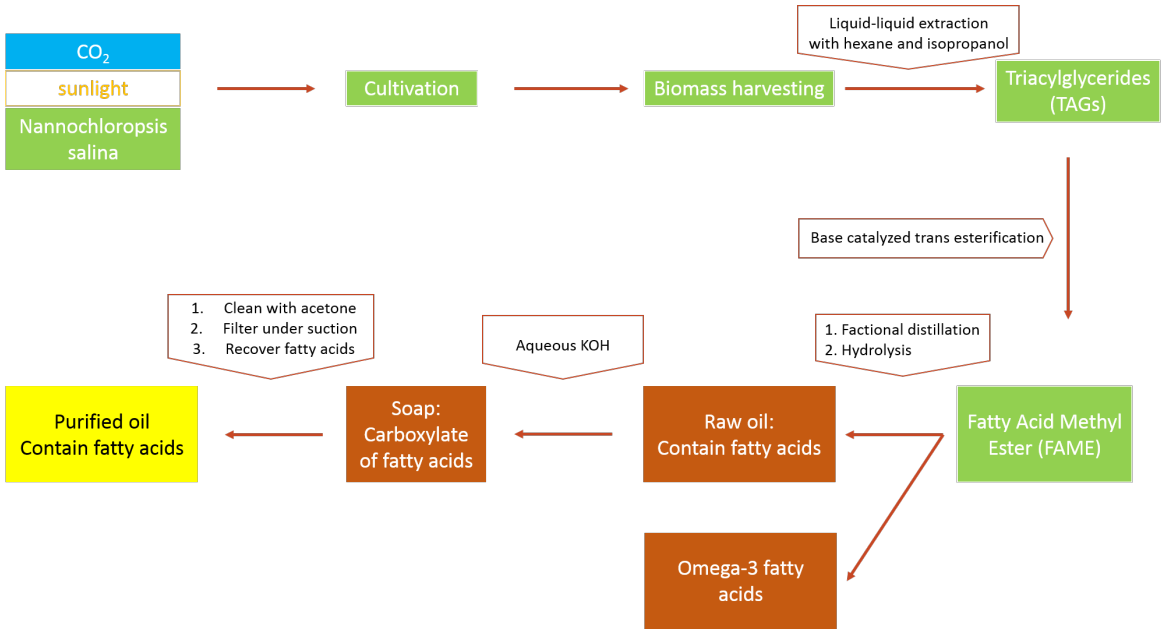


Figure S1. Fatty acids purification process

Step 2: Isolation mono-unsaturated fatty acid C16-1.

Palmitoleic acid was isolated from a mixture of FFAs using a urea complexation method under optimal conditions (Figure S2)[6, 7]. The free fatty acids (100g) were mixed with 123 g urea in 770 mL methanol, then heated at 70 °C until the mixture became a homogeneous solution. The resulting mixture was slowly cooled to room temperature for 30 minutes before storing overnight at 4 °C for crystal formation. The crystals were separated from liquid by filtration under vacuum. Methanol was removed from the filtrate with a rotary evaporation, which was then washed with warm water (70 °C) and extracted with an equal volume of hexane. The hexane layer containing mono-unsaturated fatty acid was dried with anhydrous sodium sulfate before solvent removal by rotary evaporation to obtain pure palmitoleic acid (49.8 g, 85 % yield). The signal at δ 0.71-1.11 corresponds to the terminal alkyl methyl, while the four peaks between δ 1.15 - 2.46 are assigned to the methylene groups nearest to the double bond and carboxyl group. Figure S10 shows the ^{1}H NMR spectra of the isolated palmitoleic acid C16-1 and palmitic acid C16-0. The palmitoleic acid C16-1 (Figure S10a) is identified by the high intensity signal at δ 5.24-5.50 belong to double bond. In contrast, there is a very low intensity signal at δ 5.22-5.44 in the ^{1}H NMR spectrum of palmitic acid C16-0 (Figure S10b), indicative of the presence of small amount of C16-1. These findings agreed with GC-MS data (Figure S9).

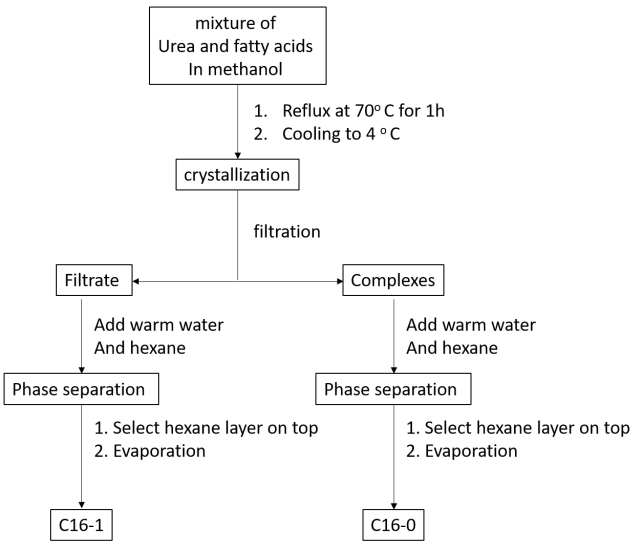
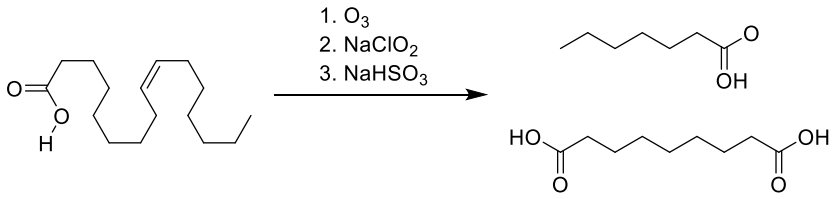


Figure S2. Urea complexation procedure

Step 3: Oxidative cleavage of C16-1.

Azelaic acid was synthesized by oxidative cleavage of mono-unsaturated fatty acid C16-1 with ozone as shown in Scheme 1. The procedure is similar to the process of synthesis of azelaic acid from oleic acid[8-11], except that a quench reaction was accomplished by sodium chlorite ($NaClO_2$)[12]. C16-1 (20 g, 0.07 mol, 1 equiv) was dissolved in a mixture of 150 mL acetonitrile and 15 mL H_2O. The solution was cooled to 0 °C in ice bath and treated with ozone until the reaction complete, as confirmed by TLC. Once the ozonolysis completed, a 157 mL aqueous solution of 2M sodium chlorite (35.54g, 0.31 mol, 4 equiv) was added dropwise into the cold reaction with the temperature controlled at 0 °C. The reaction mixture turns yellow upon sodium chlorite addition. After standing overnight at room temperature, the mixture was reduced by slow addition of aqueous 2M sodium bisulfite (166 mL, 34.6g, 0.33 mol, 4 equiv) under controlled temperature of 0 °C. Once completed, the solution turned colorless and clarified, and the mixture was stirred for 10 minutes. Ethyl acetate (100 mL) added, and the two layers were separated. The organic phase, with azelaic acid and heptanoic acid, was dried by rotary evaporator to obtain a white paste product, which was diluted with hexane and extracted with hot water. Upon cooling the aqueous phase, azelaic acid (AA) formed as white crystals, which were filtered, washed several times with cold water, and dried (12.4 g, 84 % yield.) The hexane layer containing heptanoic acid was dried over Na_2SO_4, filtered, and concentrated as an oily liquid (8.5 g, 83 % yield.) The product was analyzed by GC-MS (Figure S11) and ^1H & ^{13}C NMR (Figures S12a and S13a). Due to the relatively high boiling point of azelaic acid (286 °C), analysis was performed by converting azelaic acid into the dimethyl ester and analyzing by GC-MS (Figure S11). The dimethyl azelate was identified by matching mass spectral data to the NIST library database. As shown in Figure S11, the retention time of the dimethyl azelate appeared at 13.16 minutes. The mass spectra of this observed peak was characterized with a cluster of fragmentation patterns and ions, which match well with the mass spectra reference library of dimethyl azelate. As shown in Figure S12a, the signals at δ 1.0 – 1.8 ppm was assigned to methyl and methylene groups of azelaic acid, a triplet peak at δ 2.2 ppm correspond to C-$H_{aliphatic}$ protons near the carboxylic group, and a broad signal around δ 12 is from carboxylic acid proton. The two peaks between δ 2.4 and 3.3 ppm belong to DMSO and water, respectively. Analyzed data of azelaic acid: ^1H NMR (500 MHz, DMSO-d_6) δ = 11.95 (s, 1H), 2.15 (t, *J*=7.4, 2H), 1.44 (p, *J*=6.9, 2H), 1.21 (d, *J*=6.3, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ = 175.10, 34.16, 28.95, 24.97.HR-ESI-MS calcd. for azelaic acid – $C_9H_{16}O_4$ $[M-H]^-$: 187.22, found 187.25

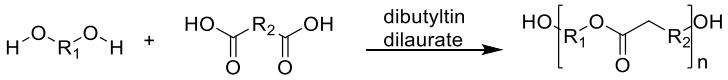
The heptanoic acid product was also produced at over 85%, as analyzed by ^{1}H & ^{13}C NMR (Figures S12b and S13b). Analyzed data of heptanoic acid: ^{1}H NMR (500 MHz, DMSO-d_6) δ = 2.13 (t, *J*=7.4, 1H), 1.50 – 1.38 (m, 1H), 1.26 – 1.17 (m, 4H), 0.81 (t, *J*=7.0, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ = 175.17, 34.18, 31.48, 29.53, 28.72, 24.97, 22.49, 14.38. HR-ESI-MS calcd. for heptanoic acid – $C_7H_{14}O_2$ $[M-H]^-$: 129.18, found 129.23



Scheme S1. Ozonolysis of palimitoleic acid

Step 4: Polycondensation of ethylene glycol and azelaic acid for polyester polyol synthesis.

The reaction procedure for polyol synthesis was adapted from a literature report[13]. To a 100mL 3-neck flask, 17.8 g of azaleic acid and 8.7 g of ethylene glycol were combined. One neck was fitted with a gas inlet to allow dry nitrogen to flow through at a fixed flow rate to around 80 mL/min. The central neck was fitted with a thermometer for temperature verification. The right neck was attached to a Dean-Stark apparatus to collect the water released by the esterification reaction. The apparatus was heated to 140 °C using a heating mantle with stirring to facilitate melting of the azelaic acid. At this point, 10 µL of dibutyltin dilaurate was added and the temperature gradually increased to 200 °C over the course of an hour. The reaction was allowed to proceed for a further 14 hours, until all of the monomers were consumed.



Scheme S2. Synthesis of polyester polyols

The polyols were characterized by OH number and acid number according to ASTM E1899 and D664, respectively using a Mettler Toledo G20S auto-titrator using a non-aqueous electrode. For the OH number titrations, four replicates of between 0.1 and 0.3 grams of polyol were reacted with p-toluene sulfonyl isocyanate (TSI) to form the carbamate, which was subsequently titrated with a standardized solution of 0.1M tetrabutylammonium hydroxide in acetonitrile. The acid number titrations were performed in duplicate. 1 g of sample was diluted in a 50:49:1 solution of toluene, isopropanol, and water, then titrated with a standardized solution of 0.1M KOH in isopropanol.

Step 5: Polyurethane polymerization with methylenediphenyl diisocyanate (MDI)

A stainless steel mold with three 1" cube slots was used to fabricate the foam samples. The mold was heated in an oven to 50 °C to ensure that the exothermic urethane reaction is sustained. Mold release (Stoner S236) was applied by lightly spraying on to the mold sidewalls, to ensure ease of demold. Polyols were heated to 50 °C to liquefy and reduce viscosity. All other components were used at room temperature. Polyol, catalyst, surfactant, water and isocyanate components were weighed into a cup and mixed with a DAC 600.1 Flacktek speed mixer at 2000 rpm for 17 seconds. The cube mold was placed on a balance. Each cube was hand poured from the cup into the mold to ensure consistent mass across cubes. The mold was then sealed and cured in an oven for 1 hour at 50 °C, and then cooled to room temperature before demolding the cubes.
This study characterized four physical properties of the polyester polyurethane material: density, hardness, hysteresis, and peak force. The mass of each foam cube was measured on an analytical balance with an accuracy of plus or minus 0.01 grams. Density was determined by dividing the mass by the mold volume for

each 1 inch cube. Hardness was measured by pressing a digital shore A durometer, made by FstDgte, into the center of each cube according to ASTM method D2240. The reported hardness is an average of the durometer measurements from all six faces of each cube.

Hysteresis and peak force were calculated using an AFG 2500N compression tester by MecMesin, with a MultiTest - dV sample stage. The test method was a compression of 50% of the original height of each cube, at a speed of 100 mm per minute. This instrument output a curve displaying each data point of force versus height of displacement. Energy loss was calculated as the integral under the curve for the compression, minus the integral under the curve for decompression. Percent hysteresis was calculated dividing the energy loss by the energy in. The peak force was measured on the 10th cycle of compression, in order to illustrate load-bearing capacity of the material.

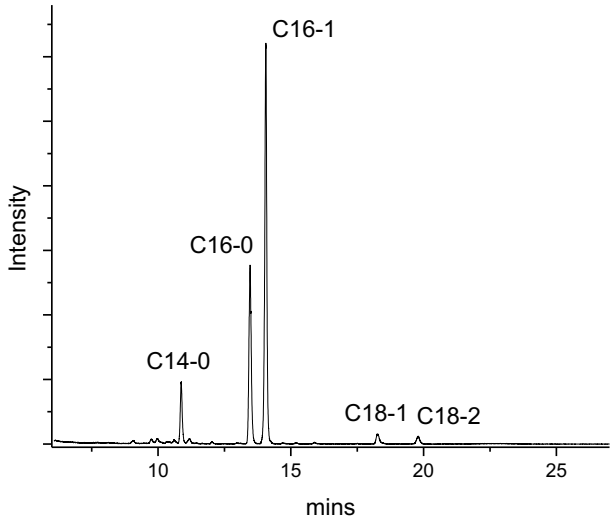


Figure S3. GC-MS of fatty acid methyl ester (FAME) from raw oil

Table S1: Fatty acid components and contents in samples analyzed by GC-MS

Samples / Fatty acids contents	Initial fatty acids Percentage of fatty acid methyl ester	Isolated palmitoleic acid C16-1 Percentage of fatty acid methyl ester	Isolated palmitic acid C16-0 Percentage of fatty acid methyl ester
C14 - 0	8.3	3.4	4.9
C16 - 0	28.9	8	85.9
C16 - 1	58.7	86	8.8
C18 - 1	2.3	1.8	0.4
C18 - 2	1.8	0.8	0

Note: C14 – 0: “14” is the number of carbon atoms in the fatty acids molecule, while “0” is the number of carbon-carbon double bonds.

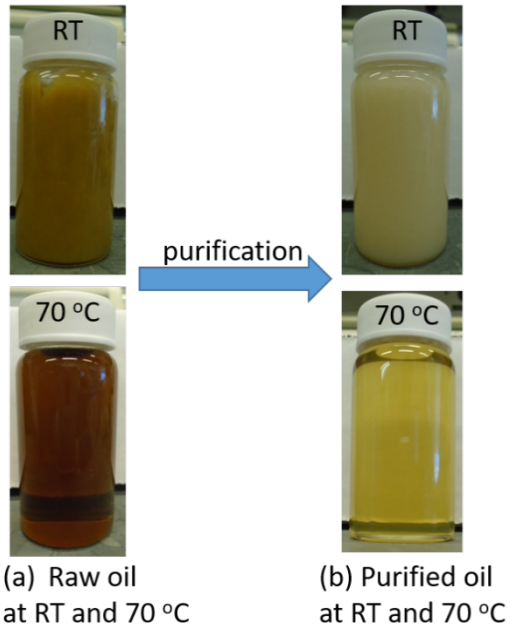


Figure S4. Images of the raw oil (a) and purified oil (b)

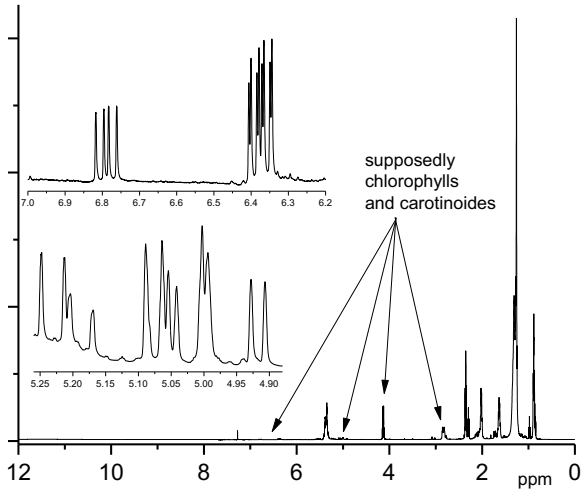


Figure S5. ^{1}H NMR spectrum of raw oil

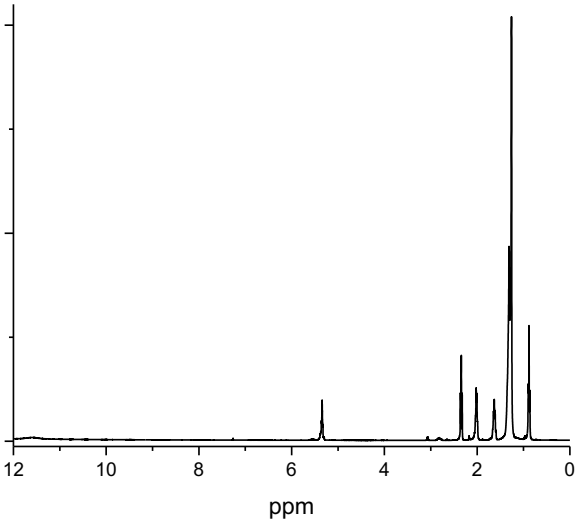


Figure S6. ^{1}H NMR spectrum of purified oil

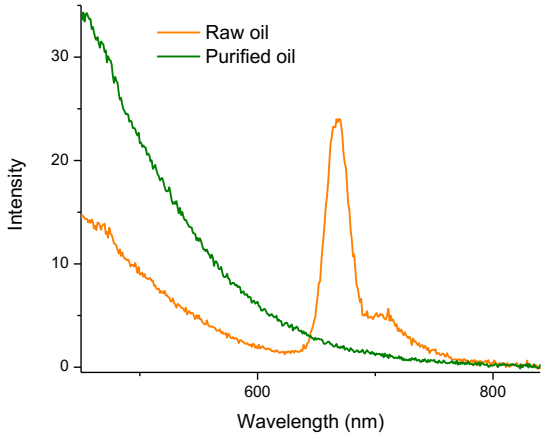


Figure S7. Fluorescence comparison of raw oil and purified oil

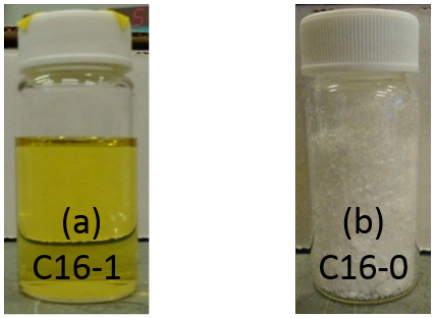


Figure S8: Images of palmitoleic acid (C16-1) and palmitic acid (C16-0)

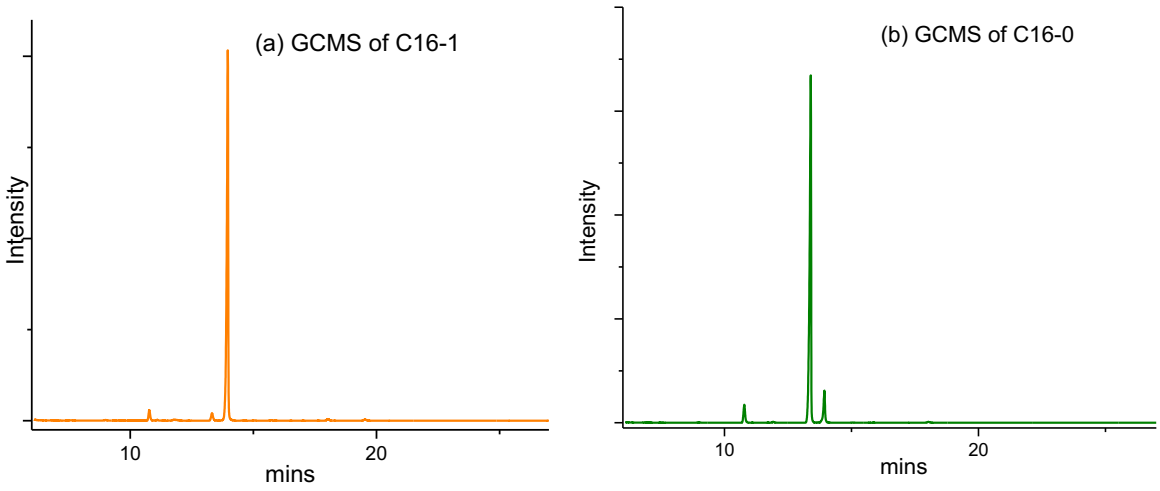


Figure S9: GC-MS of (a) palmitoleic acid C16-1 and (b) palmitic acid C16-0

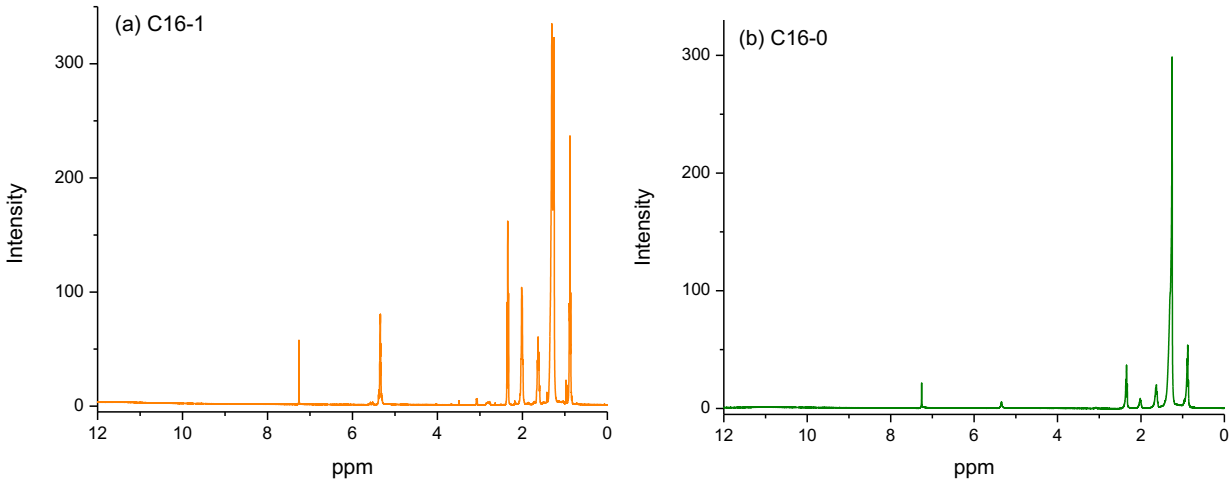


Figure S10: ^{1}H NMR of (a) palmitoleic acid C16-1 and (b) palmitic acid C16-0

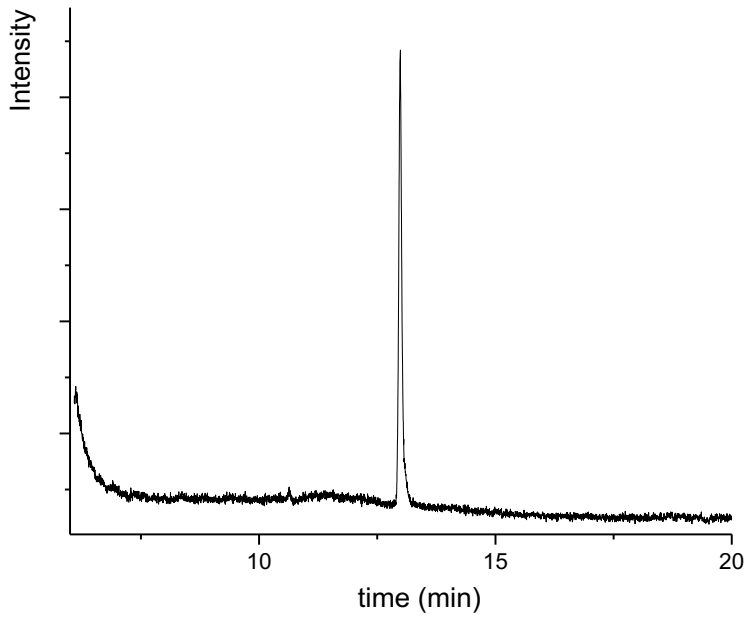


Figure S11: GC-MS of dimethyl azelate originate from synthesized azelaic acid

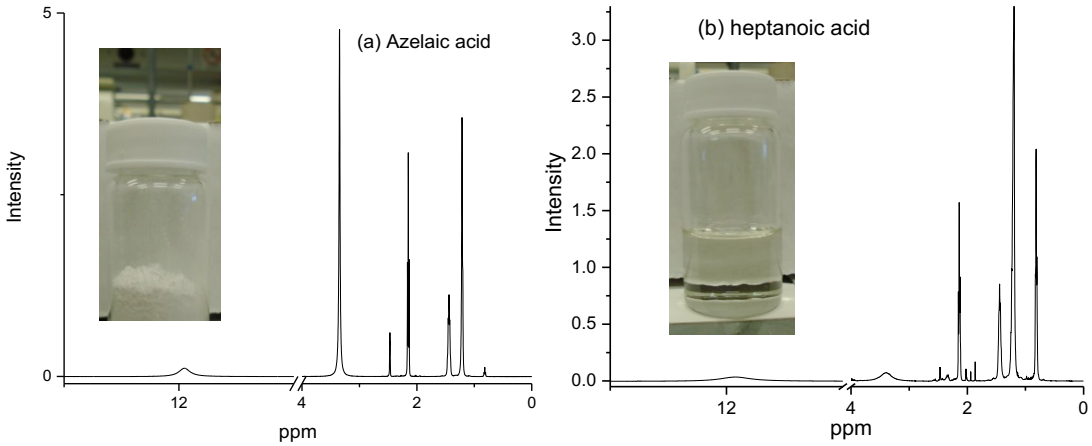


Figure S12: ^{1}H NMR of synthesized azelaic acid (a) and heptanoic acid (b)

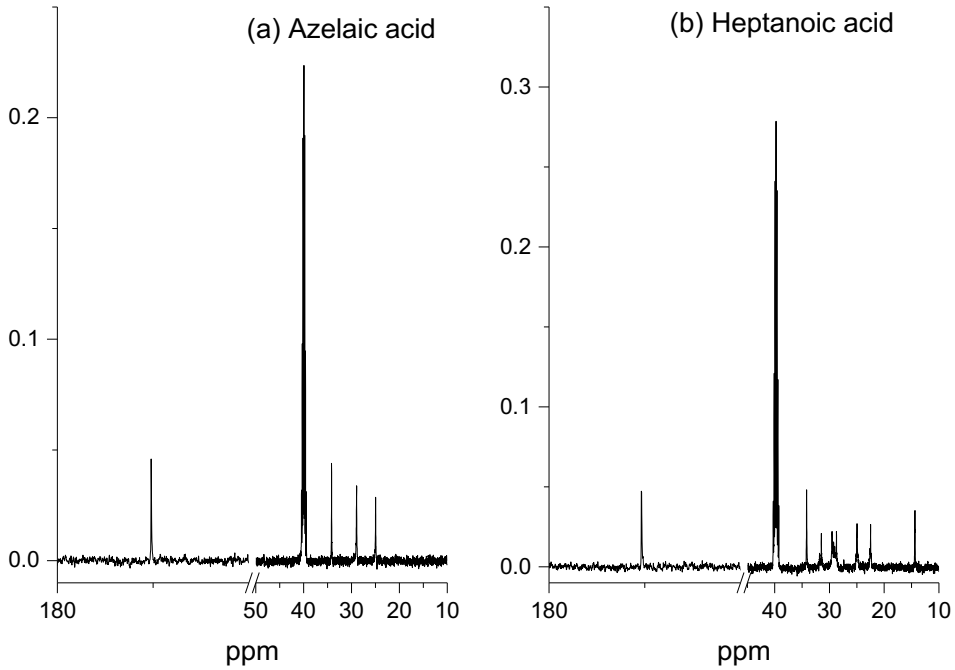


Figure S13: ^{13}C NMR of synthesized azelaic acid (a) and heptanoic acid (b)

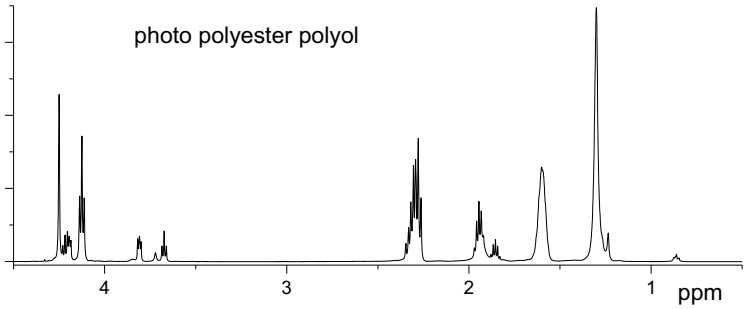


Figure S14. ^{1}H NMR of polyester polyol

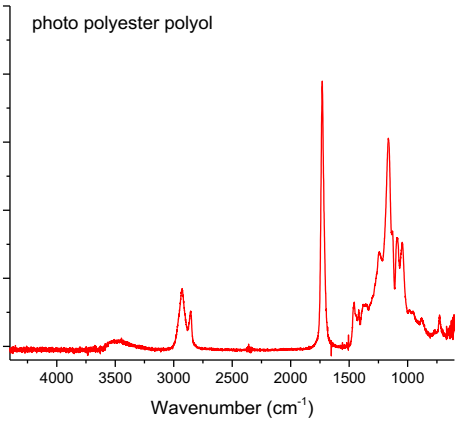


Figure S15. FT–IR of polyester polyol

Table S2: Properties of polyester polyol

Polyol properties	Photosynthetic azelaic polyol
OH number, mg KOH/g	109 ± 2
Acid number, mg KOH/g	0.28 ± 0.02
GPC analysis – M_w , $gmol^{-1}$ (weight average molecular weight)	10600
GPC analysis – M_n , $gmol^{-1}$ (number average molecular weight)	4000
GPC analysis – polydispersity index (PDI)	2.6

Table S3: Table for the cube foam PU formulation

Component	Parts per hundred	Weight (g)
Photosynthetic polyol	100	14.482
Momentive L1507	1.74	0.258
Niax A1	0.1	0.015
TEDA	0.2	0.03
Fomrez UL-29	0.03	0.006
Water	1	0.148
MDI	89.9	13.02

Hydrodecarboxylation of heptanoic acid via Organic Photoredox catalysis to produce hexane:

Hexane was prepare according to the followed procedure [14]: To a 50 mL round bottom flask was added 63 mg of diphenyl disulfide ($(PhS)_2$) , 37 mg of N,N-diisopropylethylamine (i-Pr_2NET), 33 mg of 9-Mesityl-10-phenyl acridinium tetrafluoroborate (Mes-Acr-Ph), and 190 mg of heptanoic acid, followed by 3.9 mL 2,2,2-trifluoroethanol and 1 mL of ethyl acetate. The mixture was allowed to react at ambient temperature under irradiation for 48 h. The reaction mixture was passed through a plug of silica into a vial containing internal standard (methyl nonadecanoate) before GC-MS analysis (49mg, 40% yield).

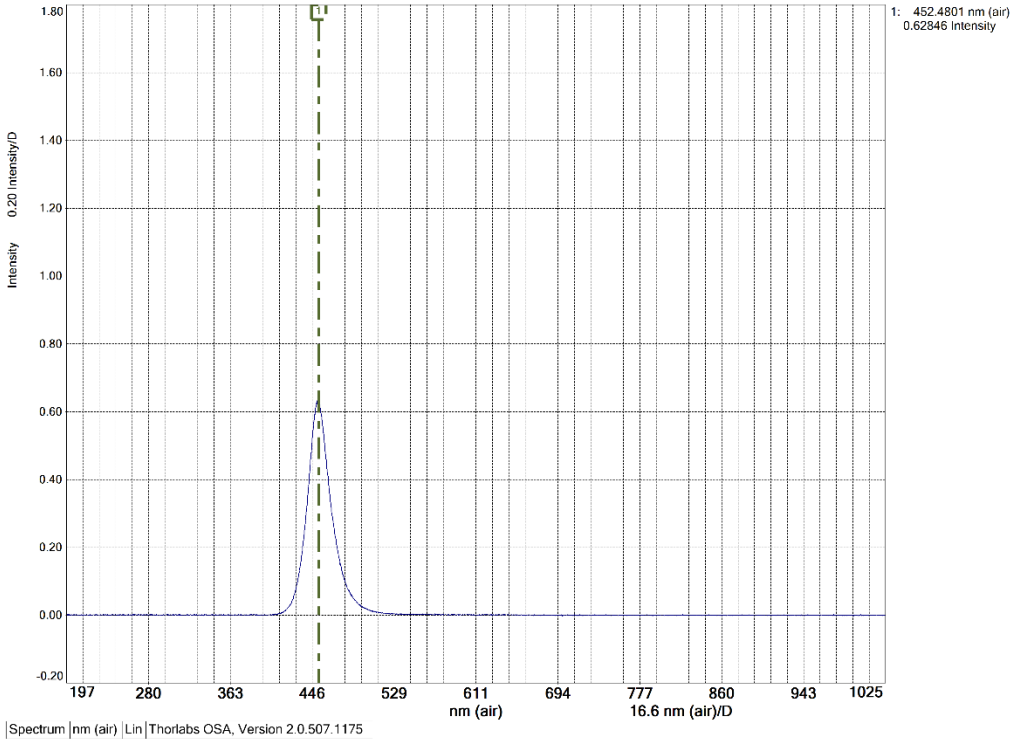
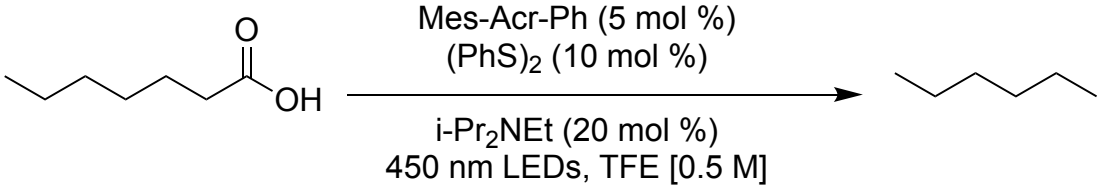


Figure S16: The wavelength of Blue LEDs detected by the Compact CCD Spectrometer



Scheme S3. Hydrodecarboxylation of heptanoic acid.

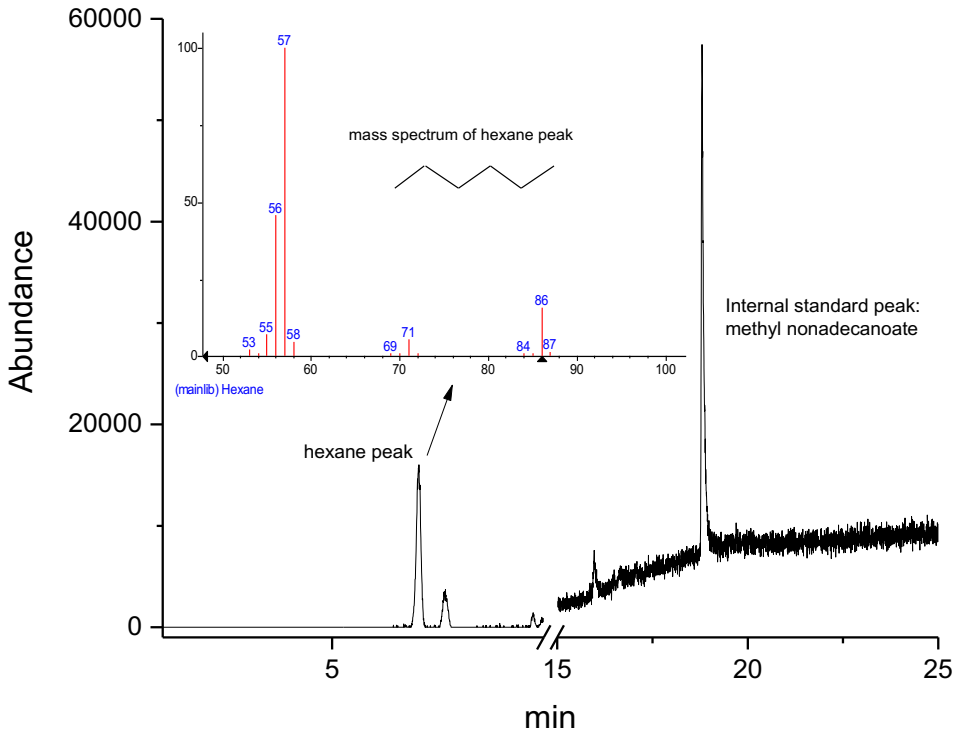
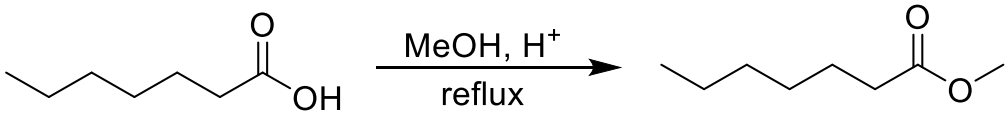


Figure S17. GC-MS chromatogram of obtained hexane and internal standard

Synthesis of methyl heptanoate via esterification

Adding 20 g (0.15 mol) of heptanoic acid followed by 130 mL Methanolic HCl 1M to a 250 mL round bottom flask. The reaction was refluxed for 2h at 85°C. Once the reaction complete and it was cooled to room temperature. The product was extracted with hexane then washed with 5% aqueous sodium carbonate and saturated aqueous sodium chloride. After drying over sodium sulfate, hexane was removed by rotary evaporator to obtain methyl heptanoate as colorless oil with grape smell (19.8 g, 90 % yield.) Methyl heptanoate: ^{1}H NMR (500 MHz, Chloroform-*d*) δ = 3.64 (s, 3H), 2.31 – 2.25 (m, 2H), 1.59 (t, *J*=7.3, 2H), 1.34 – 1.21 (m, 6H), 0.90 – 0.81 (m, 3H).



Scheme S4. Synthesis of methyl heptanoate via esterification

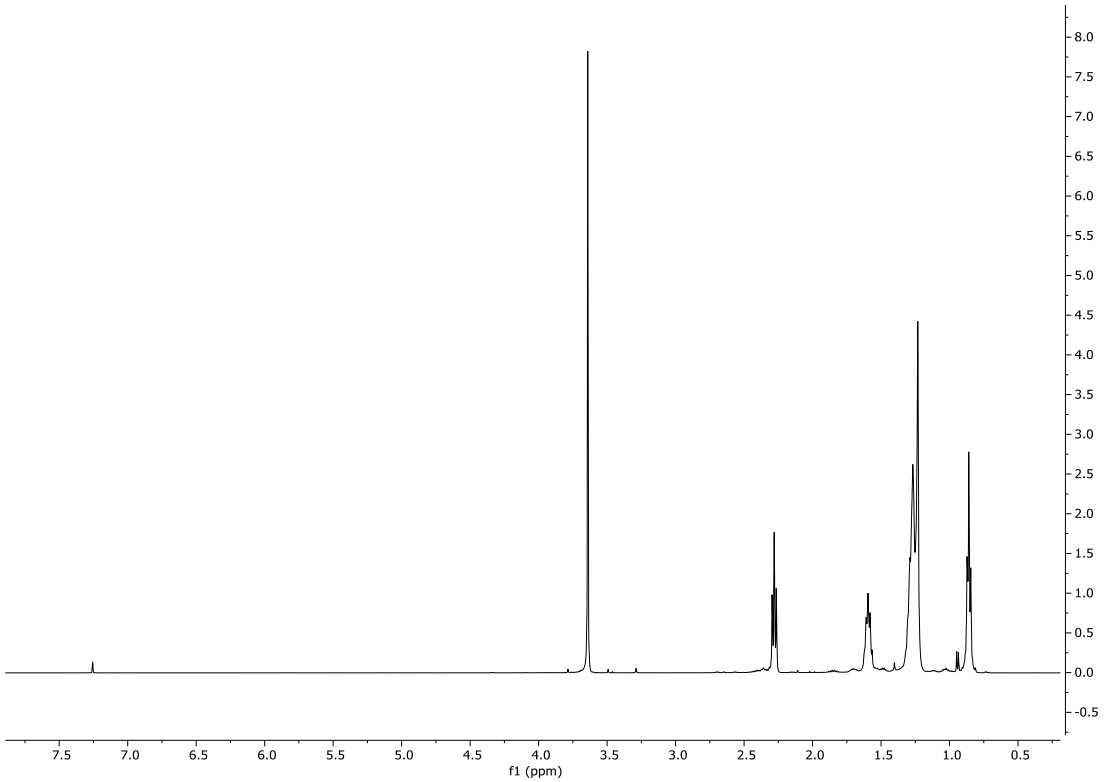


Figure S18. ^{1}H NMR of methyl heptanoate

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