SYNTHESIS OF VLC-PUFAS RELATING TO MACULAR DEGENERATIVE DISEASES: A SENIOR HONORS THESIS

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ABSTRACT

Very Long Chain Polyunsaturated Fatty Acids (VLC-PUFAs) are non-dietary fatty acids that are more than 24 carbons long and include more than one double bond. The specific compound we have focused on is 32:6 n-3: a 32-carbon molecule having 6 cis alkenes with the alkenes beginning at the omega 3 carbon position. These molecules are involved in the membrane structure of the retina and Stargardts disease while being anecdotally linked to age-related macular degeneration (AMD). Both diseases lead to degradation in vision. While Stargardts is an orphan disease, AMD is currently the leading cause of untreatable blindness in the United States. While we have completed a total synthesis of 32:6 n-3, optimization is required for scale-up. The final step of the synthesis proceeds in low yield and is labor intensive, promoting a need to develop a new solution such as a simpler purification process or the examination of new reactions.
ACKNOWLEDGEMENT

I would like to thank my mentor, Dr. Jon Rainier for allowing me to join his lab and for helping me to gain a better understanding of chemistry. He has been a very knowledgeable resource and a great teacher. I am incredibly thankful for the time that I have been able to spend in the Rainier lab and the skills that I have developed. I would also like to thank my graduate student mentor, Alex Wade, for allowing me to help him with his project. I also must acknowledge the other members of the Rainier lab for their willingness to answer questions and for the support that they have provided me. During my time in the lab, I have gained a better appreciation for chemistry, and it has led me to decide to pursue a graduate education in organic chemistry. A journey that I am very excited to begin. I am extremely grateful for my mom, Andrea Nebeker, and my grandpa, Dilworth Nebeker, who don’t have the best understanding of chemistry but who have never failed to support and encourage my love of science. I would not be where I am today without them. My love of chemistry began in high school with one enthusiastic teacher, Ivana Beeman. She fostered my interest in the subject, and she is the reason I decided to major in chemistry in college. I have loved my time at the University of Utah and am thankful for this opportunity to share my research, no matter how daunting I first viewed the task of writing this paper.
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INTRODUCTION

Very long chain polyunsaturated fatty acids (VLC-PUFAs) are rare and nondietary fatty acids that are found in low concentrations in humans in the brain, retinas, and testes. These fatty acids are found in the membranes as phospholipids. The phospholipid structure of VLC-PUFAs is shown below, along with the DHA derived VLC-PUFA 32:6n-3.

![Diagram 1: General Structure of VLC-PUFAs shown at left. Example VLC-PUFA found as a phosphatidylcholine that would be found in vivo shown at right.]

VLC-PUFAs are non-dietary, meaning that they rarely come from a dietary source however, they are synthesized in the retina by elongation of fatty acid precursors. These precursors include docosahexaenoic acid (DHA), arachidonic acid (AA), and eicosatetraenoic acid (EPA) and are elongated by the enzyme ELOVL4 (elongation of very long chain fatty acids -4).
The purpose for studying these compounds goes beyond their interesting structures. It has been proposed that they might be uniquely capable of improving eye health. VLC-PUFAs have been anecdotally linked to age-related macular degeneration (AMD), this disease is degenerative and leads to a loss of vision over time.\(^6\) Macular degeneration is the leading cause of untreatable blindness in the US (Seddon, 2001). There is more than one form of AMD and there are only therapies for some forms of the disease.\(^6\) It has been found that in patients suffering from AMD, concentrations of VLC-PUFA are lower in the retinas.\(^6\)

There is a lack of a supply of VLC-PUFAs, they are not commercially available and cannot be extracted from an environmental source.\(^5\) The scarcity of these compounds led to the need for a synthetic route. The target VLC-PUFA is 32:6 n-3: a 32-carbon molecule with six cis-alkenes that begin from the omega three carbon position, which is shown in Figure 1. When this project began, there were two other syntheses from Raman and Maharvi.\(^6\) The synthetic route determined by the Rainier group is shown below and can be modified to form other VLC-PUFAs, differing in the number of carbons and alkenes.

The VLC-PUFA biology studies have been led by Dr. Paul Bernstein at the Moran Eye Center, University of Utah. The synthesized product from Dr. Rainier’s lab have been delivered to the Bernstein group for whole cell, zebrafish, and mouse studies. Based on GC analysis of human donor eyes, it was found that the concentration of VLC-PUFAs in patients with age-related macular degeneration (AMD) was lower than patients without the disease.\(^1\) In mice that were fed the 32:6 n-3 VLC-PUFA we found that that they showed increases in the amount of retinal VLC-PUFA when compared to the controls.\(^2\) The mice with increased amounts of VLC-PUFA also showed an improvement in visual acuity.\(^2\) The goal of this project is to eventually understand the role of VLC-PUFAs in the retinas and possibly to develop a therapeutic that could be used for treatment of retinal degenerative diseases in humans.
The specific part of the project that I have been working on is the final step of the total synthesis, which is an oxidation from an aldehyde to a carboxylic acid. There are many complications that are associated with this step. Potential problems of concern during this last step included oxidation or isomerization of the alkenes.

Typically, an aldehyde can be oxidized to a carboxylic acid using chromium-based reagents such as chromic acid (Jones reagent, Fig.1).

![Jones reagent (H₂CrO₄)](image)

However, since the compounds that we are synthesizing are intended for animal feeding studies, the purity of the compound was important. Chromium is a highly toxic heavy metal and even trace amounts in the final compound were unacceptable, so we could not use reagents that could potentially incorporate them.

The current method that we have developed for the terminal oxidation step uses oxone in a solution of dimethylformamide (DMF). Oxone is a triple salt of potassium sulfate salts (KHSO₅, KHSO₄, K₂SO₄); the active oxidizing salt is potassium peroxymonosulfate (KHSO₅).

![Potassium peroxymonosulfate (KHSO₅)](image)

The issues that have arisen with this reaction were mainly due to the difficulty of removing DMF. This very polar solvent has a high boiling point making it challenging to concentrate the reaction mixture. Our compound was not only thermally sensitive and thus
decomposes when heated, but the oxone dissolves in DMF and residual oxone will continue to react with the compound, as well as being problematic for purification.

Oxone requires a highly polar solvent in order to dissolve, which is why DMF was chosen for the reaction. As part of my project, I performed solubility tests with oxone in different solvents. The goal of this experiment was to determine if a viable, lower boiling replacement for DMF existed.

Based on the results of the solubility tests, it was decided that methanol would be the best replacement solvent for DMF. We also predicted that using methanol would result in the formation of the methyl ester instead of the carboxylic acid. The ester can be easily saponified to the carboxylic acid or used as a therapeutic itself.

In addition to optimizing the oxone conditions we also examined other oxidants including N-hydroxyphthalimide, O₂ in acetonitrile (MeCN). Although this method was very interesting to us, it’s radical mechanism may cause undesirable effects with the alkenes during the oxidation.

![Figure 3: N-hydroxyphthalimide](image)

RESULTS AND DISCUSSION

The first step for the total synthesis was to protect 10-bromodecanol using a dihydropyran protecting group to protect the alcohol giving 1, in 57% yield (scheme 1). The second step converted docosahexaenoic acid to DHA aldehyde 2. This was carried out using a lithium aluminum hydride mediated reduction to reduce the carboxylic acid to an alcohol.
functional group; the alcohol was then reoxidized to the desired aldehyde by following a Swern oxidation. This procedure generated 2, DHA aldehyde, in a 57% yield over the 2 steps. A Grignard reaction combined 1, THP protected 10-bromodecanol, with 2, to form compound 3 in a 57% yield. To reduce the alcohol in 3, the material was converted into the corresponding mesylate and then immediately reduced via a second lithium aluminum hydride reduction to form 4, the THP protected VLC-PUFA, in 53% yield. The THP in 4 was removed next using acidic methanol to form the corresponding VLC-PUFA alcohol. The conversion of the VLC-PUFA alcohol into the desired VLC-PUFA involved two steps. The first converted the alcohol into the corresponding aldehyde 5, using a Swern oxidation. The final step of the synthesis was the aforementioned oxone oxidation, which resulted in 6, the final VLC-PUFA, in a 47% yield. For each step of the synthesis NMR spectroscopic data was collected and analyzed. The overall yield across the total synthesis was calculated to be 3.2%.

Scheme 1: Total synthesis of 32:6 n-3 VLC-PUFA
The goal of this project was to optimize the final oxidation reaction. Two different methods were proposed for the oxidation method. The first was an aerobic oxidation approach. The second was finding a new solvent system for the existing oxone reaction. Because the VLC aldehyde 5 was valuable test reactions were carried out with DHA aldehyde 2.

The aerobic oxidation method was attempted using Kang’s protocol. This used N-hydroxyphthalimide (Figure 3) as a catalyst (5% mol solution) for the reaction and O₂ as the oxidizing agent in a solution of acetonitrile. The reaction ran for 27 hours and gave a 31% yield of DHA.

\[
H\quad OH
\quad OH
\quad cat. \text{N-Hydroxyphthalimide, O}_2
\quad MeCN
\]

Scheme 2: Kang oxidation procedure

It was decided that this method would be difficult to reproduce on a larger scale. Based on these issues and the proposed radical mechanism, it was decided this method was not a viable replacement for the oxone reaction. As discussed above, the alkene region of the VLC-PUFA is sensitive to radicals and could easily become oxidized.
We next examined the effect of solvents on the oxone oxidation. Based on a paper from Webb and Ruszkay, it was proposed that using an aqueous solution of Ethylenediaminetetraacetic acid (EDTA) may help to better dissolve the oxone in less polar solvents. EDTA would bind to the potassium ions in oxone and make it more soluble in less polar solvents.

We initially examined the effect of EDTA in the oxone oxidation of DHA aldehyde 2 in DMF, isopropanol, and methanol. The solution of EDTA was at a concentration of $4 \times 10^{-3}$ M. Based on NMR spectroscopy, methanol worked closest to the DMF reaction and was easier to
work with as a solvent system. However, the aqueous EDTA solution was miscible in these solvents and formed interactions with the oxone. These interactions caused difficulty when removing all the oxone from the reaction mixture. The leftover oxone continued to react with the compound.

<table>
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<tr>
<th>Solvent</th>
<th>Amount of oxone dissolved (g/ 1 mL)</th>
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<tbody>
<tr>
<td>Methanol</td>
<td>0.0069</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>0.0007</td>
</tr>
<tr>
<td>DMF</td>
<td>0.0775</td>
</tr>
<tr>
<td>Toluene</td>
<td>0.0005</td>
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Table 1: Solubility of oxone in solvents

A set of control experiments was run to determine the solubility of oxone in different solvents. The solvents that we examined were methanol, isopropanol, DMF, and toluene and the results are shown in table 1. Based on these results, it was proposed to run reactions with a mixture of methanol and isopropanol and with methanol only, following the same oxone oxidation procedure as used in the total synthesis, only modifying the solvent.

Three test reactions were run with different mixtures of methanol and isopropanol, 30:70 (Iso:MeOH), 50:50 (MeOH: Iso), and 70:30 (Iso:MeOH). We observed similar yield for all three reactions (53%, 62%, 47%). Complicating these reactions was that the NMR results showed a mixture of methyl and isopropyl esters. To simplify the mixture of esters, it was determined that the reaction should be run in pure methanol, which was the solvent with the greatest oxone solubility.

The next issues that were focused on was getting the reaction to reach completion, use all the starting material, and reduce the formation of over oxidized biproducts. To better dissolve the
oxone in methanol, it was proposed to use tetrabutylammonium iodide as a phase transfer catalyst that would facilitate better dissolution of the oxone by replacing K$^+$ with Bu$_4$N$^+$. 

![Figure 6: Tetrabutylammonium Iodide structure](image)

The reactions with methanol as the solvent and a catalytic amount of tetrabutylammonium iodide were still being run using our the original conditions. The procedure used 1.1 equivalents of oxone, and the reaction was stirred at room temperature. Based on results from these reactions, it was seen that the tetrabutylammonium iodide helped the dissolution of oxone in solvent. However, the starting material was not being fully converted. It was decided to try reactions with different equivalents of oxone.

![Figure 7: oxone oxidation reaction](image)

In order to have a reference for the DHA ester, a sample of docosahexaenoic acid was esterified using a catalytic amount of p-toluenesulfonic acid. From this reaction, a clean NMR reference spectrum and a TLC sample were obtained. The reaction with 10 equivalents of oxone was stirred for 1.5 hours and the DHA aldehyde was over oxidized within that amount of time.

<table>
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<th>Oxone Equivalents</th>
<th>Temp (Celsius)</th>
<th>Purified % Yield</th>
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<tr>
<td>10</td>
<td>RT</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>-20</td>
<td>0</td>
</tr>
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To slow the formation of unwanted byproducts, we decided to run the reaction at 0, and in an acetone and dry ice bath at -20 °C. The reaction at -20 °C did not proceed. The reactions at 0 proceeded, however, it was difficult to tell if the temperature affected the formation of byproducts. The results are summarized in the table above. For the purposes of this paper a 0% yield means that nothing was collected after column chromatography purification, it does not necessarily mean that no reaction occurred.

One of the biggest challenges for this reaction was removing all the oxone and tetrabutylammonium iodide salts from the reaction. If all the salts were not removed, the crude product degraded during storage due to continued overoxidation by residual oxone. Our published procedure called for taking up the reaction mixture with a 1:20 mixture of ethyl acetate and hexanes and filtering off the oxone. In order to make sure all the oxone was removed and the product does not continue to be oxidized upon concentration. In general, the product was immediately purified using flash chromatography.

It was initially proposed to use 1M HCl to crash out oxone salts and to filter off the salts. The product was then extracted using ethyl acetate in a separatory funnel. The organic layer was collected and concentrated. After the product was concentrated and the resulting residue was
stored, we observed decomposition of the material. It was determined that not all the oxone was removed and the product had continued to oxidize.

To remove all the oxone salts, we proposed using a reducing agent that would react with the oxidizing salts. The reducing agents that were used were sodium bisulfite and sodium dithionite.

![Figure 9: Sodium Bisulfite and Sodium Dithionite structures (reducing agents)](image)

To precipitate the oxone salts, it was proposed to use a 1M aqueous solution of hydrochloric acid (HCl). After washing with 1 M HCl, the reaction mixture was filtered through a thin layer of celite, and the filtered solution was added to a separatory funnel where it was repeatedly washed with the reducing agents. The organic layer was collected and concentrated. While this solution worked better than other methods, the product, 9, had to be stored by freezing it in a solution of benzene and purified very soon after it was synthesized. The most accurate yield obtained for the reaction of oxone, tetrabutylammonium iodide, and methanol carried out at 0°C was 11%. This low yield was due to the difficulties mentioned previously with removing salts, the reported yield was after purification.

**CONCLUSION**

The goal for this project was to optimize the last step of the total synthesis by finding an alternative method for the oxidation. Progress was made with changing the solvent system. Once
alternative solvents were found, different variables were tested to improve the solubility of oxone and decrease the formation of byproducts. It was found that tetrabutylammonium iodide helped to increase the solubility of oxone in methanol. A pure NMR spectrum has not been obtained from these reactions due to degradation of material. More reactions will need to be run to finish optimizing this reaction and to fully understand the effect of temperature on the reaction.

EXPERIMENTALS

General
Glassware was either dried in an oven at 130 °C or flame dried under a N2 atmosphere prior to use. Unless described otherwise reactions were performed using common dry, inert atmosphere techniques. Solvents were purified according to the guidelines in Purification of Common Laboratory Chemicals (Perrin, Armarego, and Perrin: Oxford, 1966). Dichloromethane, triethylamine, and pyridine were distilled from CaH2. Reactions were monitored by TLC and visualized by a dual short wave/long wave UV lamp and stained with an ethanolic solution of potassium permanganate or p-anisaldehyde. Flash column chromatography was performed using F60 grade silica gel, 230-400 mesh. NMR spectra were recorded on Varian Unity-300, Varian VXR-500, Varian Inova-500, or Varian Inova-400 spectrometers. Chemical shifts for 1H NMR were reported as δ, parts per million (ppm), relative to the signal of tetramethysilane at 0 ppm or the CHCl3 signal at 7.26 ppm. The abbreviations s, d, t, q, dd, p, and m stand for the resonance multiplicity singlet, doublet, triplet, quartet, doublet of doublet, pentet, and multiplet, respectively. Concentration refers to removal of solvent under reduced pressure (house vacuum at ca. 20 mmHg).
(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenal (2) To a solution of docosahexaenoic acid (DHA) (1 gram, 3.04 mmol) in terahydrofuran (THF) (25 mL) at 0º C was added LiAlH₄ (0.57 grams, 15.2 mmol). The reaction mixture was stirred for 2 hours at 0º C before being quenched by a slow addition of saturated Na₂SO₄ (aq., 20 mL). The mixture was warmed to room temperature and stirred for 1 hour. The resulting solution was filtered and concentrated to yield a thick colorless oil, which was not purified further.

To a solution of oxalyl chloride (10.0 mL, 125.5 mmol) was added dimethyl sulfoxide (DMSO)(17.0 mL, 239.1 mmol) in dichloromethane (CH₂Cl₂)(200 mL) at -78º C. Compound 10 (DHA alcohol) was added to the resulting mixture after it had been stirred for 15 minutes. After the addition of compound 10, the reaction was stirred for 2 hours at 78º C and triethylamine (33.3 mL, 239.1 mmol) was added. The reaction mixture was warmed to room temperature after which it was quenched with water. The phases were separated, and the aqueous phase was extracted with dichloromethane (3 x 25 mL). The organic extracts were combined and concentrated. Flash chromatography (1:10 ethyl acetate: hexane) gave 2.016 grams (54%) of compound 2 (DHA aldehyde) as a pale-yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 9.76 (s, 1H), 5.81-5.24 (m, 12 H), 2.97-2.70 (m, 10H), 2.52-2.4 (m, 2H), 2.13-1.98 (m, 2H), 1.65-1.52 (m, 1H), 1.31-1.20 (m, 4H), 0.97 (t, J=6.4, 3H)
(12Z,15Z,18Z,21Z,24Z,27Z)-1-((tetrahydro-2H-pyran-2-yl)oxy)triaconta-12,15,18,21,24,27-hexaen-9-ol (3) To fresh magnesium powder (1.5 grams, 64 mmol) at room temperature was added 1 drop of dibromoethane and a solution of compound 1 (2.2 grams, 8.76 mmol) in THF (1.5 mL). The reaction mixture was stirred for 2 hours and then cooled to 0º C. To this reaction mixture was added a solution of compound 2 (DHA Aldehyde) (2 grams, 6.4 mmol) in THF (60 mL). The resulting mixture was immediately warmed to room temperature and then stirred for two additional hours. The reaction was quenched with saturated ammonium chloride (NH₄Cl)(aq., 30 mL). The aqueous phase was extracted with ethyl acetate (EtOAc) (3x20 mL), the extracts were combined and concentrated. Flash chromatography (a gradient of 1:10 ethyl acetate: hexanes to 1:4 ethyl acetate: hexanes) gave 1.98 (57%) grams of compound 3. ¹H NMR (500 MHz, CDCl₃) δ 5.87-5.25 (m, 12 H), 4.61-4.58 (m, 1H), 3.93-3.85 (m, 1H), 3.78-3.72 (ddd, J=9.4 Hz, 7.1 Hz, 7.1 Hz, 1H), 3.67-3.58 (m, 1H), 3.56-3.48 (m, 1H), 3.43-3.36 (m, 1H), 2.92-2.77 (m, 8H), 2.27-2.14 (m, 2H), 2.14-2.05 (m, 2H), 1.90-1.81 (m, 1H) 1.77-1.69 (m, 1H), 1.65-1.49 (m, 8H), 1.40-1.24 (m, 17 H), 1.00 (t, J=6.1 Hz, 3H)

2-((10-bromodecyl)oxy)tetrahydro-2H-pyran (1) To a solution of 10-bromo-1-decanol (5.4 grams, 21.5 mmol) in dichloromethane (100 mL) at room temperature was added 3,4-dihydro-2H-pyran (4.1 mL, 45.1 mmol) and a catalytic amount of p-toluene sulfonic acid monohydrate. The reaction mixture was stirred at room temperature for 16 hours and the concentrated. The concentrated material was purified by flash chromatography (1: 20 ethyl acetate: hexanes) giving 2.20 grams (44%) of compound 1 as a colorless liquid. ¹H NMR (500 MHz, CDCl₃) δ 4.58-4.54 (m, 1H), 3.90-3.82 (m, 1H), 3.76-3.69 (m, 1H), 3.53-3.46 (m, 1H), 3.42-3.34 (m, 3H), 1.88-1.78 (m, 3H), 1.75-1.66 (m, 1H), 1.63-1.46 (m, 7H), 1.46-1.37 (m, 3H), 1.37-1.23 (m, 12 H)

To a solution of compound 3 (1.0579 grams, 1.91 mmol) in dichloromethane (100 mL) at 0°C was added triethylamine (2.66 mL, 19 mmol) and Methanesulfonyl chloride (MsCl) (0.46 mL, 5.96 mmol). The reaction mixture was allowed to warm to room temperature and stirred for two hours. The reaction mixture was quenched with water (20 mL). The aqueous phase was extracted with dichloromethane (3x10 mL), the organic extracts were combined and concentrated. The compound was not purified and immediately taken into the next step.

To a mixture of mesylated material (1.2066 grams, 1.90 mmol) in THF (100 mL) at 0°C was added LiAlH₄ (0.87 grams, 22.9 mmol) very slowly. After the addition, the reaction mixture was warmed to room temperature and stirred for 8 hours. The reaction was quenched with saturated Na₂SO₄ (aq., 10 mL). After stirring for one hour the resulting mixture was filtered and concentrated. Flash chromatography (1:10 ethyl acetate: hexanes) gave 0.543 grams (53%) of compound 4 as a colorless oil. ¹H NMR (500 MHz, CDCl₃) δ 5.43-5.27 (m, 12H), 4.57 (t, J=7.8 Hz, 1H), 3.90-3.84 (ddd, J=11.7 Hz, 8.2 Hz, 3.1 Hz, 2.7 Hz, 1H), 3.75-3.69 (ddd, J=16.0 Hz, 6.8 Hz, 7.2 Hz, 1H), 3.52-3.46 (m, 1H), 3.40-3.34 (m, 1H), 2.89-2.75 (m, 10H), 2.10-2.00 (m, 4H), 1.87-1.79 (m, 1H), 1.75-1.67 (m, 1H), 1.62-1.48 (m, 10H), 1.38-1.21 (m, 30H), 0.97 (t, J=6.9 Hz, 3H)
(12Z,15Z,18Z,21Z,24Z,27Z)-triaconta-12,15,18,21,24,27-hexaenal (7) To a solution of compound 4 (0.9392 grams, 1.744 mmol) in a mixture of tetrahydrofuran (75 mL) and methanol (75 mL) at room temperature was added a catalytic amount of tolusulfonic acid (TsOH). The resulting mixture was stirred overnight, concentrated, and further used without purification.

To a solution of DMSO (2.9 mL, 41.34 mmol) in dichloromethane (100 mL) at -78°C was added oxalyl chloride (1.73 mL, 21.70 mmol). After the reaction had stirred for 0.5 hours, VLC alcohol (0.9392 grams, 2.06 mmol) was then added to the solution and the resulting mixture was stirred for 1 hour at -78°C. To this was added triethylamine (5.76 mL, 41.33 mmol) and the resulting reaction mixture was allowed to warm to room temperature, and the reaction was quenched with water (20 mL). The aqueous phase was extracted with ethyl acetate (3x20 mL), the organic extracts were combined concentrated. Flash chromatography (1:30 ethyl acetate: hexanes) gave 0.8812 grams (94%) of compound 5 as a light-yellow oil. 1H NMR (500 MHz, CDCl₃) 9.76 (s, 1H), 5.44-5.26 (m, 12H), 2.88-2.76 (m, 10H), 2.44-2.38 (m, 2H), 2.10-2.01 (m, 5H), 1.66-1.56 (m, 2H), 1.38-1.20 (m, 23 H), 0.96 (t, J=7.7, 3H)

(12Z,15Z,18Z,21Z,24Z,27Z)-triaconta-12,15,18,21,24,27-hexaenoic acid (8) To a solution of compound 5 (0.8812 grams, 1.94 mmol) in DMF (20 mL) at room temperature was added oxone (1 eq) for 1 hour. DMF was removed and the resulting compound 6 was yellow oil.
removed via repeated co-evaporation with hexanes. Concentration and flash chromatography (1:50 ethyl acetate: hexanes with 1% acetic acid) gave 0.4144 grams (47%) of compound 6 (VLC PUFA) as a colorless thick oil. $^1$H NMR (500 MHz, CDCl$_3$) δ 5.45-5.27 (m, 12H), 2.89-2.75 (m, 10H), 2.34 (t, J= 7.5 Hz, 2H), 2.11-2.01 (m, 4H), 1.66-1.59 (m, 2H), 1.38-1.24 (m, 24H), 0.97 (t, J= 7.7 Hz, 3H)

**methyl (4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoate (9)** To a solution of docosahexaenoic acid (1.0355 grams, 3.15 mmol) and methanol (50 mL) at room temperature was added a catalytic amount of p-toluenesulfonic acid. The reaction was brought to reflux and stirred for 16 hours. The reaction was quenched with aqueous sodium bicarbonate, the organic layer was separated and concentrated. The resulting product yielded 1.0034 grams of a light-yellow oil. $^1$HNMR (500 MHz, CDCl$_3$) δ 5.46-5.29 (m, 13H), 3.71-3.64 (m, 3H), 2.91-2.79 (m, 12 H), 2.45-2.34 (m, 5H), 2.13-2.03 (m, 3H), 1.63-1.52 (s, 4H), 0.99 (t, J= 7.60Hz, 3H)

**General Procedure for Aerobic Oxidation of Aldehydes to Carboxylic Acids**

A Schlenk tube was dried for 15 minutes and was filled with oxygen. To this tube was added a 5% mol solution of N-hydroxyphthalimide in dried MeCN and DHA Aldehyde (compound 2) at room temperature. The reaction was stirred for 27 hours. The reaction was stopped by filtering the solution through a pad of silica gel and washed with ethyl acetate (3x5 mL). The reaction was concentrated and purified by flash chromatography (1:10 ethyl acetate: hexanes with 1% acetic acid).
REFERENCES


