UNIVERSITY OF CALIFORNIA SAN DIEGO

Synthesis of New Polyunsaturated Fatty Acid Ester of Hydroxyl Fatty Acid (FAHFA): Stearoyl Acid Ester of Leukotriene B4 (LTB4)

A thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Chemistry

by

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Professor Julia Megan Stauber
Professor Emmanuel Theodorakis

2022

The thesis of Zhichen Ma is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

University of California San Diego

2022

DEDICATION

I want to dedicate my thesis to my parents who have been supporting and encouraging me throughout my life. I also want to dedicate my thesis to my present and previous lab colleagues: Dr.Srihari Konduri, Joshua Schweer and Naga Purnima who have helped me to go through the research process.

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I would also like to acknowledge Dr. Meric Erikci Ertunc, Peter Gray and Alan Saghatelian for providing test for my synthesized new 5-SAHEA and 12-SAHEA. With their help, this research set first step on discovering this new family of FAHFAs.

ABSTRACT OF THE THESIS

Synthesis of New Polyunsaturated Fatty Acid Ester of Hydroxyl Fatty Acid (FAHFA): Stearoyl Acid Ester of Leukotriene B4 (LTB4)

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Master of Science in Chemistry

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Professor Dionicio Siegel, Chair

Fatty acid ester of hydroxyl fatty acid (FAHFA) is a molecule family of lipid ester. In recent years, some of them have showed function on anti-inflammation, anti-diabetes. Among them, a unique kind of polyunsaturated FAHFA-13-HODE was discovered and found it reversed the original harmful effect as a

mediator in metabolism from its hydroxyl fatty acid part. Inspired by this phenomenon, Leukotriene B_4 as a mediator in cell leading to many harmful effects was chosen as the hydroxyl fatty acid part of new FAHFAs and these FAHFAs were expected to have beneficial biological function. In this research, in order to test this hypothesis, polyunsaturated FAHFAs 5-stearoyl acid ester of LTB_4 and 12-stearoyl acid ester of LTB_4 were synthesized, which consisted of hydroxyl fatty acid (HFA)-racemic leukotriene B_4 analogies and fatty acid (FA)-stearoyl acid

INTRODUCTION

1.1 Background of FAHFAs

Fatty acid ester of hydroxy fatty acid (FAHFA) is a family of lipid compounds. Its feature is an estolide consist of fatty acyl group (FA) and hydroxyl fatty acid backbone (HFA).[5] In Figure 1.1, there are examples for FA and HFA that make up FAHFAs.

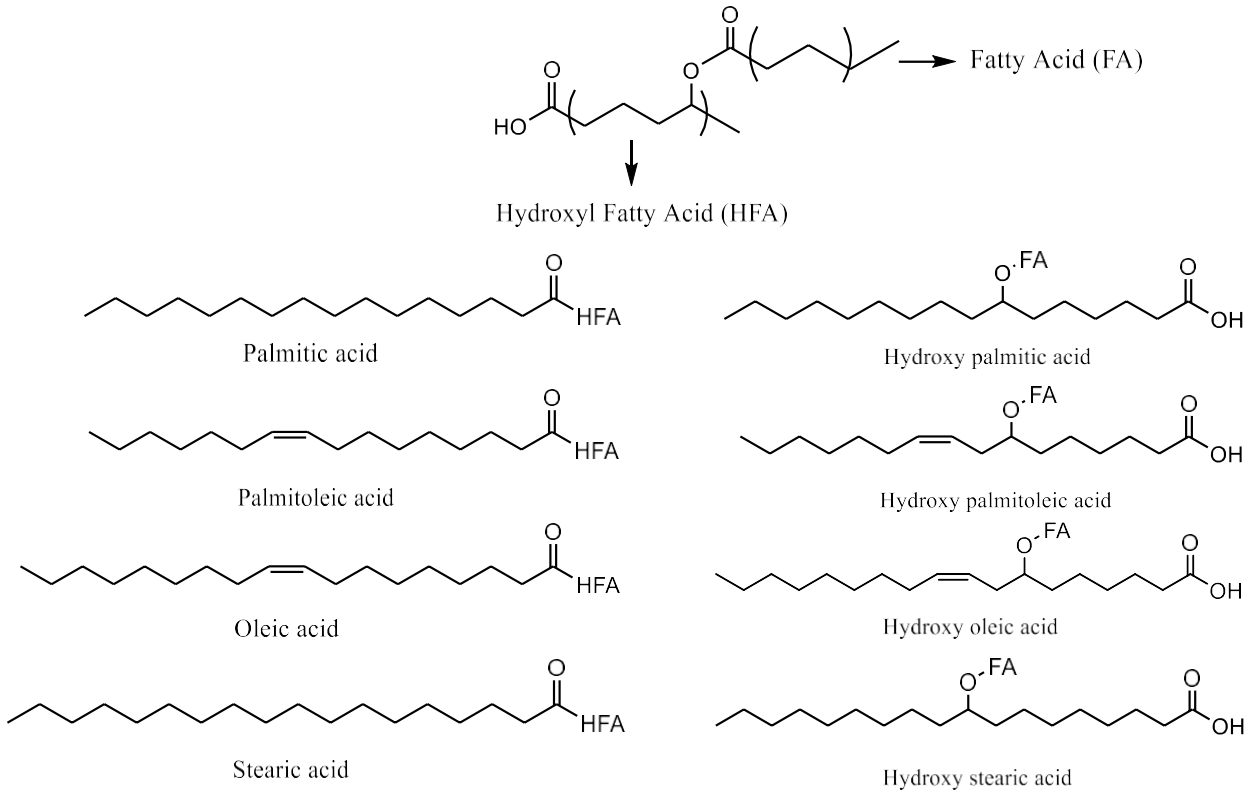


Figure 1.1 Illustration of FAHFA's structure and example molecules for FA and HFA. The figure shows the general structure of one FAHFA molecule, which contains fatty acid (FA) and hydroxyl fatty acid (HFA). There are four examples of FAs: palmitic acid, palmitoleic acid, oleic acid and stearic acid. As well as corresponding HFA.

1.2 Discovered Biological Effects of FAHFAs

In 2014, Yore et al. discovered FAHFAs molecules through lipidomic analysis of adipose tissue and found one isomer type from this family-PAHSA (Plamitic-acid-hydroxy-stearic-acid) reducing adipose tissue inflammation and improving insulin-stimulated glucose uptake.[1] After that, more discoveries were found on PAHSAs' beneficial biological effects in different organs and tissues[6,7,8].

Besides saturated fatty acid, in recent years. Unsaturated FAHFAs were also isolated and synthesised with proved function on anti-inflammatory and pro-resolving effect[2] (13-DHAHLA), suppressing secretion of cytokines and expression of pro-inflammatory genes[9] (13-LAHLA). These outcomes of unsaturated FAHFAs suggest them have promising properties and more polyunsaturated FAHFAs need to be discovered and synthesized to test their effects.

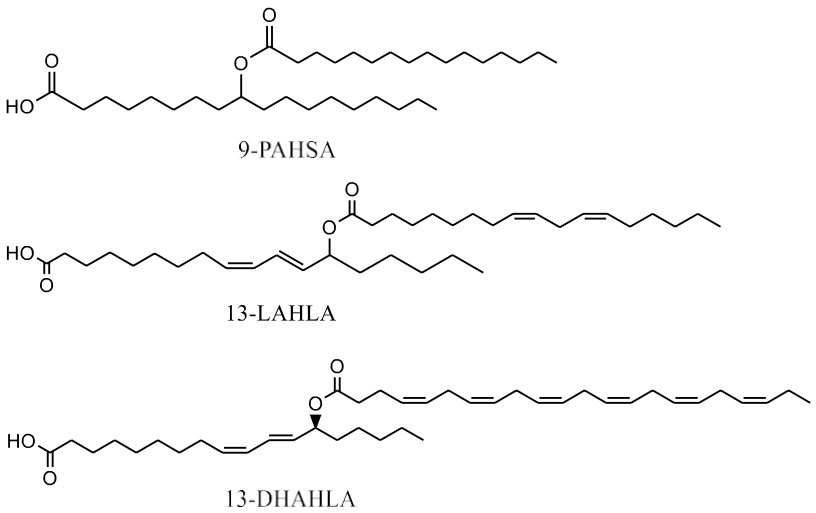


Figure 1.2 Structures of example FAHFAs with reported beneficial biological effects. Three FAHFAs with discovered beneficial biological effects. 9-PAHSA is a saturated FAHFA. 13-LAHLA and 13-DHAHLA are polyunsaturated FAHFAs.

1.3 Special 13-HODE and New Hypothesis

In 2016, among identified omega-3 polyunsaturated fatty acid derived FAHFAs, one docosahexaenoic acid (DHA) esterified to (9*Z*, 11*E*)-13-hydroxyoctadeca-9,11-dienoic acid (13-HODE) derivatives was found. This compound displayed anti-inflammatory and pro-resolving effects.[2] 13-HODE is the stable oxidation products of linoleic acid through increased oxidative stress and the increased level of it contributed to atherosclerosis progression, myocardial infraction or stroke.[3] Surprisingly, from esterification on 13-HODE, the 13-(S)-DHAHLA completely silenced its HFA's part original function and contributed to beneficial biological effect. This special phenomenon inspired the creation of new kind of FAHFAs using other symptom mediator lipid molecules as HFA part.

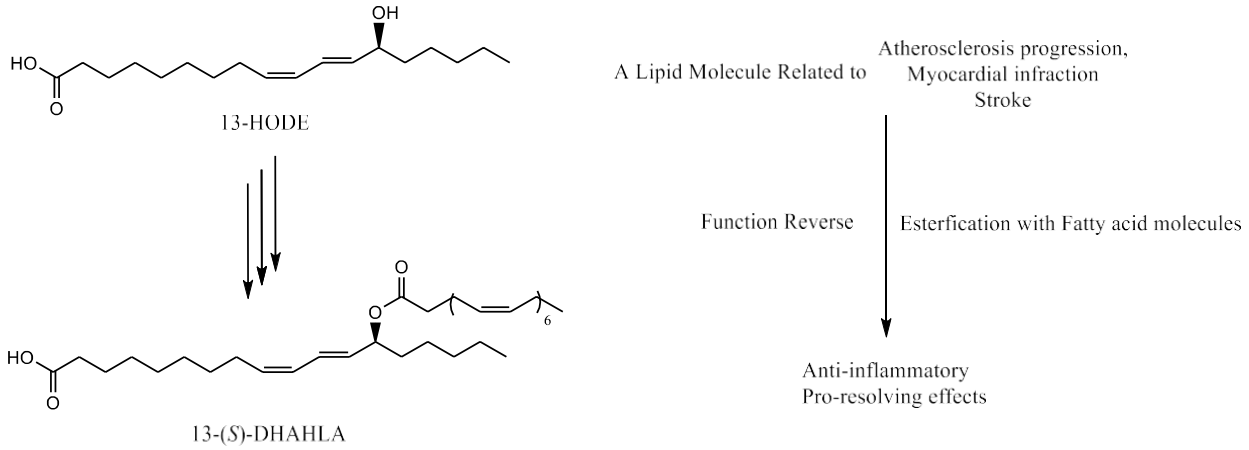
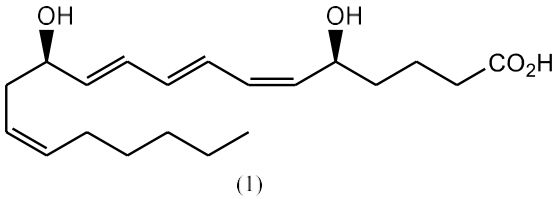


Figure 1.3 13-HODE and its special phenomenon. The phenomenon appears on 13-(S)-DHAHLA: 13-HODE as a molecule related to several syndromes was esterified and showed anti-inflammatory and pro-resolving effects.

1.4 Background of Leukotriene B_4 and Design of New FAHFAs

Leukotriene B_4 ((5*S*,6*Z*,8*E*,10*E*,12*R*,14*Z*)-5,12-Dihydroxy-6,8,10,15-icosatetraenoic acid) (LTB_4) is a molecule in lipid family as an ecosatetraenoic acid.[10]



(1)

Figure 1.4.1 Structure of Leukotriene B_4 (LTB_4) The structure of leukotriene B_4 molecule and it is described as (5S,6Z,8E,10E, 12R,14Z)-5,12-Dihydroxy-6,8,10,15-icosatetraenoic acid.

In studies, this molecule was found mediating its biological effects through two G protein-coupled receptors, BLT1 and BLT2. BLT1 appears to mediate the major activities of LTB4 on leukocytes leading to inflammation[11], whereas BLT2 involved in various aspects of cancer progression.[12] This molecule also prompts insulin resistance by acting on macrophages[13]. Due to its functions and structure as a lipid molecule, LTB_4 is believed as a good candidate for the HFA part of new FAHFAs. In this research, this hypothesis will be tested and it planned to synthesize a new kind of LTB_4 based FAHFAs. They are stearoyl acid esterified LTB_4 as 5-stearoyl acid ester LTB_4 and 12-stearoyl acid ester LTB_4. In order to do

test hypothesis and determine the synthesis strategies, a racemic mixture of LTB_4 were be used on the target molecules.

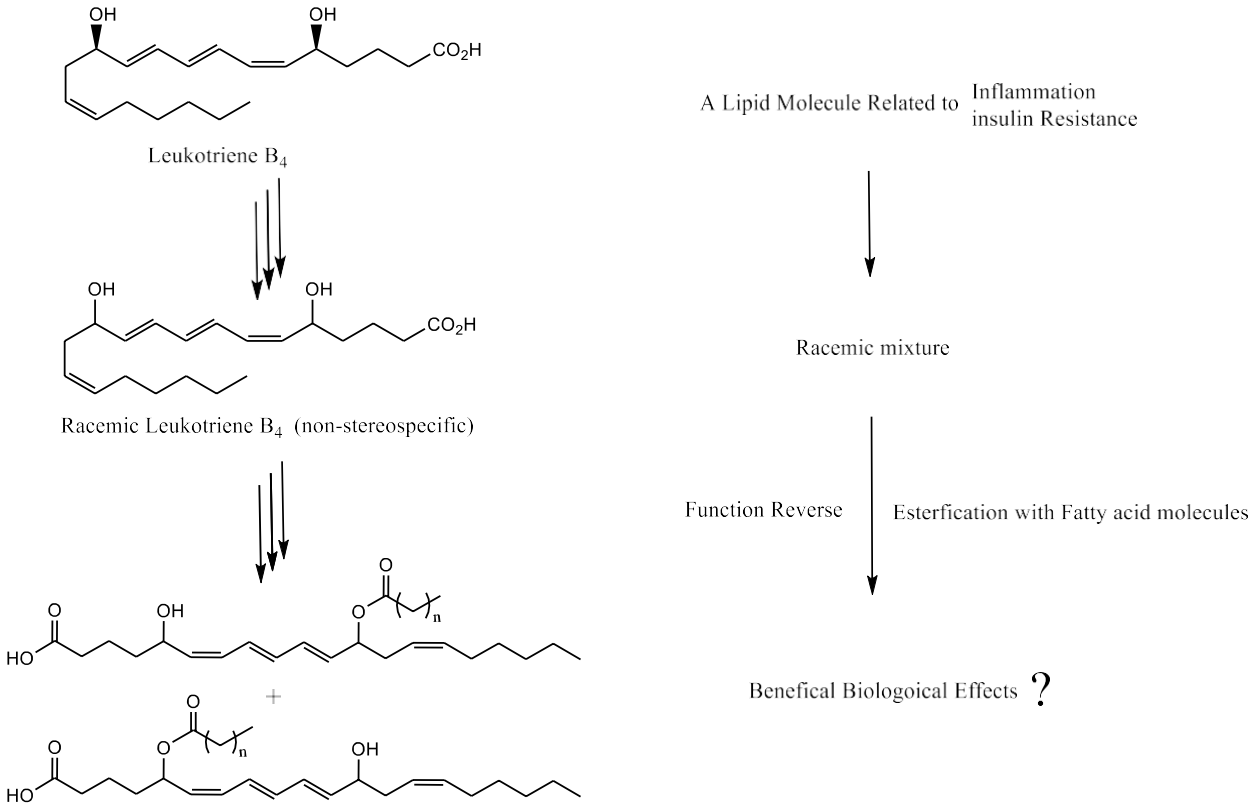


Figure 1.4.2 Plan of synthesis LTB_4 based FAHFA and its hypothesis. The hypothesis of new FAHFAs based on LTB_4: The biological function of LTB_4 is expected to be reversed through esterification with fatty acids. And it is expected to have beneficial biological effects.

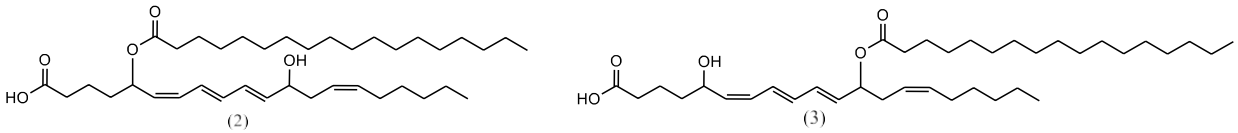


Figure 1.4.3 Structure of target molecules 5-stearoyl acid ester LTB_4 (2) and 12-stearoyl acid ester LTB_4. (3). New FAHFAs was designed to esterify racemic LTB_4 with stearic acid: 5-stearoyl acid ester LTB_4 and 12-stearoyl acid ester LTB_4.

1.5 Pervious Approaches to Synthesis LTB_4 Backbone

In previous synthesis of LTB_4, all the synthesis strategies were creating two hydroxyl groups on two separate parts and connecting them. For connecting two parts, one major approach was connecting

through alkene bond synthesis, such as Horner-Wasdworth-Emmons reaction and Wittig reaction[14,15,16,17] The other approach was using cross-coupling to make the conjugated structure (double –double bond formation or double- triple bond formation)[18,19,20,21]. Comparing with two approaches on reaction condition and precursors' preparation, in this research, the cross-coupling will be applied with milder reaction condition and easier prepared reagents.

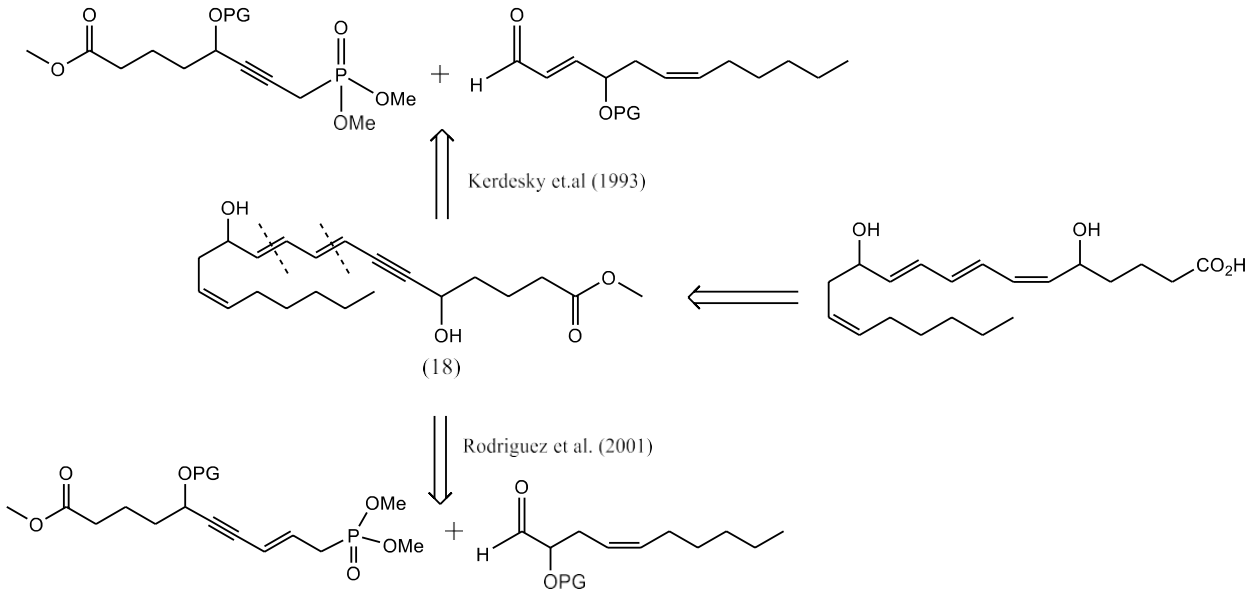


Figure 1.5.1 Key Synthesis approach of LTB_4 through Horner-Wasdworth-Emmons reaction
Previous synthesis route to form the backbone of LTB_4. Two examples applied Horner-Wasdworth-Emmons reaction to form the olefin bonds and connect two separated hydroxyl groups. (PG=Protecting Group)

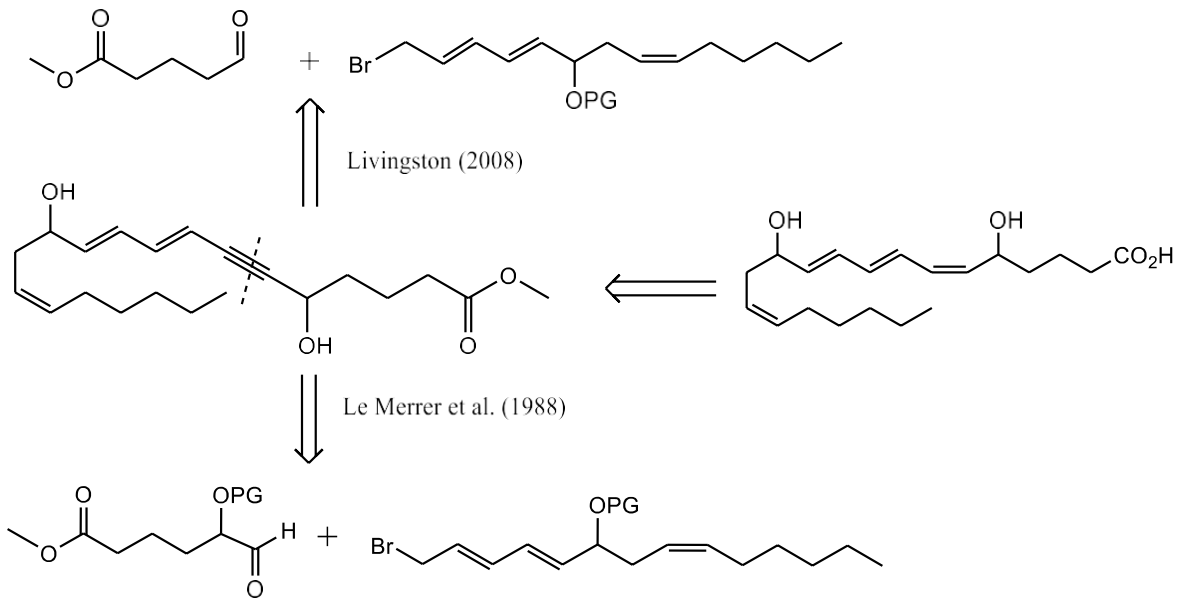


Figure 1.5.2 Key Synthesis approach of LTB_4 through Wittig reaction. Previous synthesis route to form the backbone of LTB4. In the synthesis applied by Livingston et al, Wittig reaction was done followed by strong base catalyzed elimination reaction to form terminal alkyne. This terminal alkyne was added to aldehyde. In Le Merrer et al.'s work, Wittig reaction was used to form the olefin bonds and connect two separated hydroxyl groups. (PG=Protecting Group)

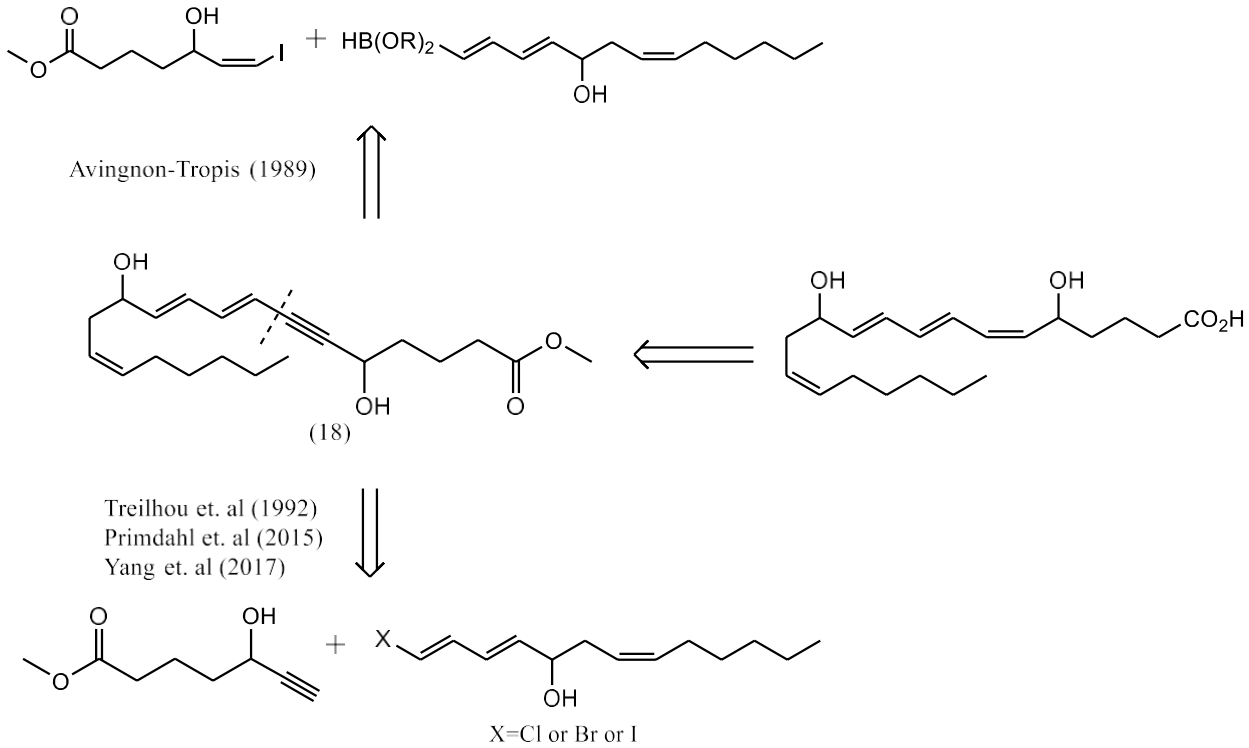


Figure 1.5.3 Key Synthesis approach of LTB_4 through cross-coupling reaction. Cross-coupling methods were also used to form the backbone. In the work of Avingnon-Tropis et al. Suzuki coupling was

used to connect diene and vinyl iodine from alkynyl iodine. Also, Sonogashira couplings were used to connect two hydroxyl parts.

1.6 Strategies of Synthesis FAHFAs

For synthesis the ester bond on FAHFAs, two strategies were applied: first, the HFA ester group was formed before the acylation with FA chain.[22, 23] Second, the HFA ester group was formed through oxidation after the acylation on FA chain[24,25]. In this research, the stearoyl acid ester chain on LTB_4 was synthesized before the carbonyl acid group was formed through hydrolysis of the methyl ester groups. This was due to the facts that there was unstable conjugated structure on LTB_4. Also, methyl ester group is formed in LTB_4 synthesis which had reported strategies for saponification.[9,23]

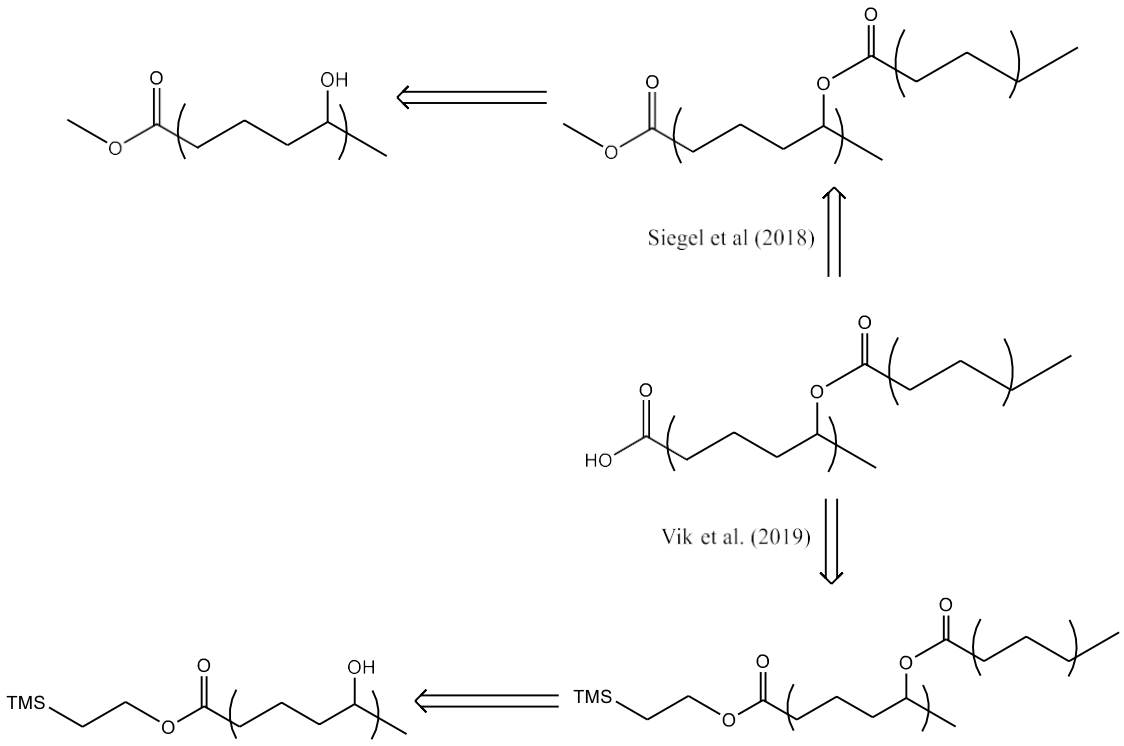


Figure 1.6.1 General Strategies for synthesis FAHFAs (HFA ester group was formed before the acylation with FA chain.) In two works above, the carbonyl groups were formed as ester before the esterification with FA groups and acid group was afforded through de-esterification at end of synthesises.

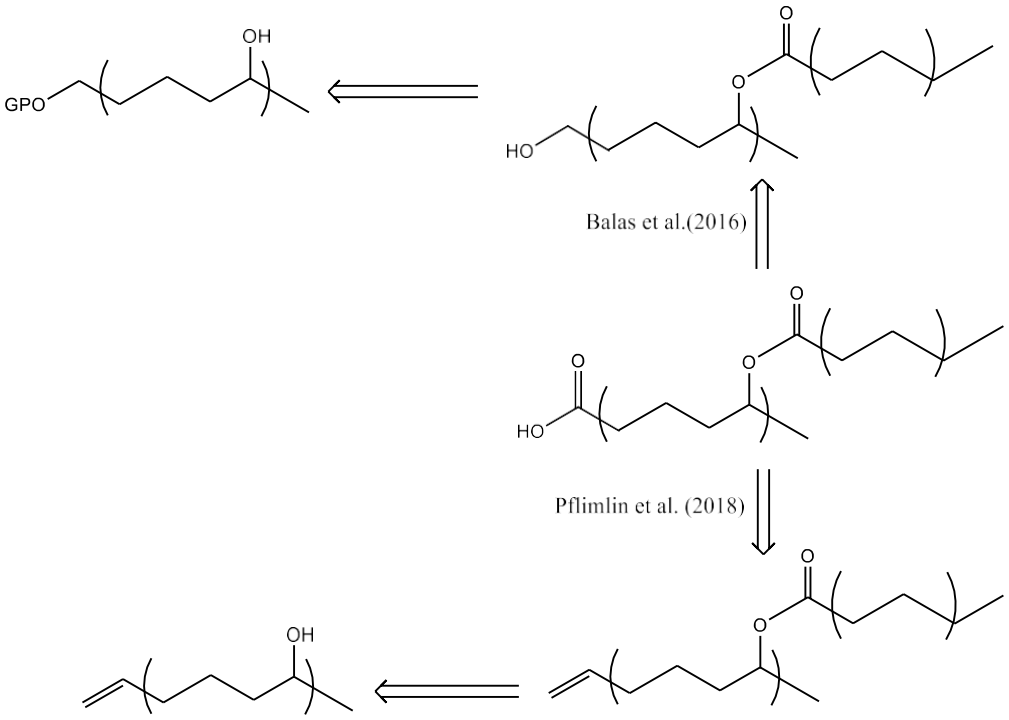


Figure 1.6.2 General Strategies for synthesis FAHFAs (HFA ester group was formed through oxidation after the acylation on FA chain.). In two examples above, FA formed the ester bond first. The acid group on FAHFA was formed though later stage oxidation.

1.7 Key Disconnection

The retrosynthesis began with breaking the ester bond between racemic LTB_4 and stearoyl side chain. This esterification process can be realized by reacting hydroxyl groups on LTB_4 with stearoyl chloride as commercially available product. For the LTB4 analogous part, the key strategic is to connect (6) and (7) through Sonogashira reaction and *cis*-specific reduction by active Zn. (7) was obtained from desilylation, coupling and reduction from trimethylsilylate (TMS) enynic alcohol (16). Since racemic TMS enynic alcohols were the target, direct addition of *n*-butyl lithium deprotonated trimetylsilyacetylene towards aldehyde was applied. Similarly, the same addition strategies was used to form (6). After the oxidation of the alcohol group (12) to aldehyde (13), alkynyl addition to aldehyde and desilylation, (6) was formed. This strategy separated LTB_4 from two parts each containing one single hydroxyl group which was the potential chiral center, which makes future synthesis of stereo-pure reagent possible. The esterification strategies applied above can also apply to other FA derivatives for future studies.

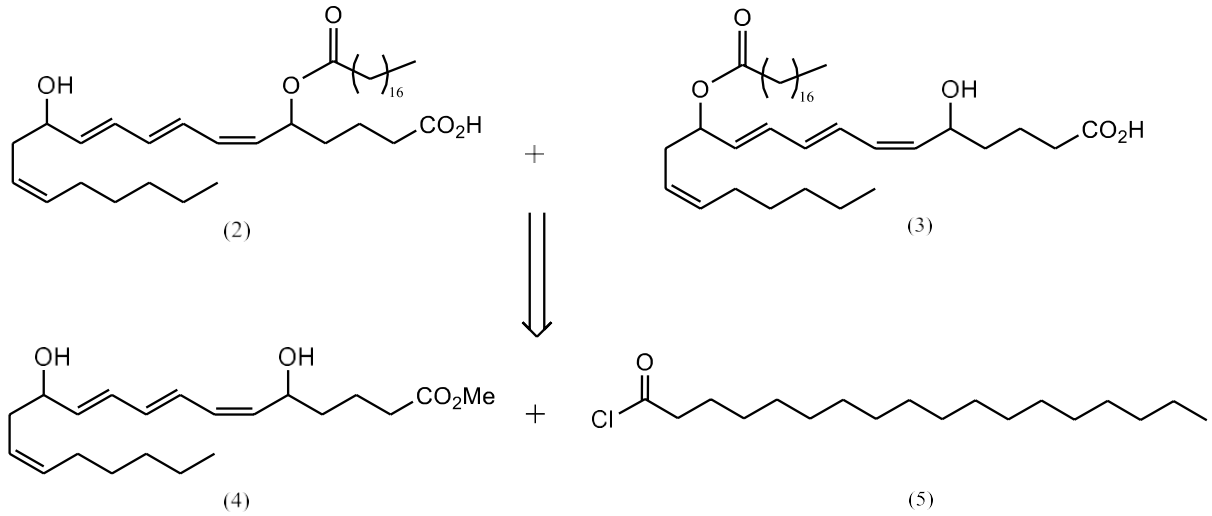


Figure 1.7.1 Key Disconnections of 5-stearoyl acid ester LTB$_4$ (2) and 12-stearoyl acid ester LTB$_4$ (3) (Part 1) In this part, the key connection was through esterification between HFA (racemic methyl ester LTB$_4$) and FA (stearoyl chloride)

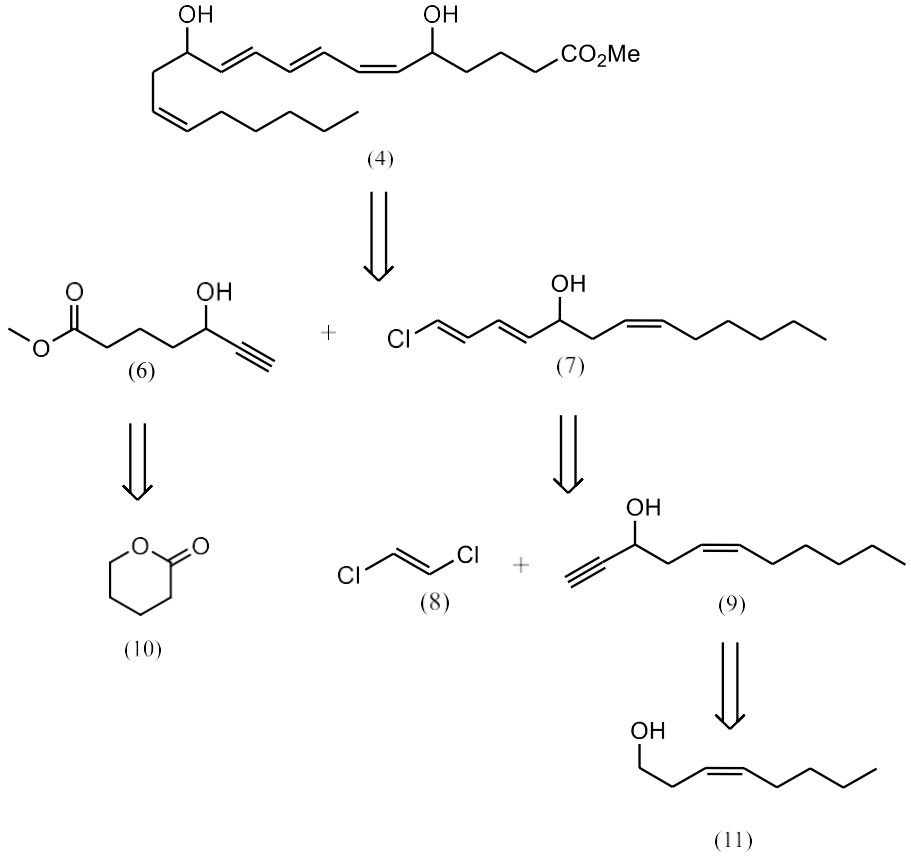


Figure 1.7.2 **Key Disconnections of 5-stearoyl acid ester LTB$_4$ (2) and 12-stearoyl acid ester LTB$_4$ (3) (Part 2)** The key connection in this part was cross-coupling between terminal alkyne and chloride diene. The alkyne was from cyclolactone 10; chloride diene was from olefin alcohol 11.

1.8 Conclusion

This thesis presents the synthesis of 5-stearoyl acid ester of LTB_4 and 12- stearoyl acid ester of LTB_4, which were synthesized in the first time with an overall yield of 3.5% and 2.7% respectively. These two molecules were expected to process beneficial biological effects, which reversed LTB_4 original effects. Through current synthesis route, a foundation was built for future synthesis to the analogies of 5-stearoyl acid ester of LTB_4 and 12- stearoyl acid ester of LTB_4. The long term plan is to test their functions and expand library of derivatives.

RESULT AND DISCUSSION

2.1 Efforts towards Methyl 5-hydroxyhept-6-ynoate (6)

2.1.1 Synthesis of Formyl Ester (13).

The preparation of (6) commenced by base-induced methanolysis of lactone (10) to give hydroxyl ester (12) in 96%.[26] The oxidation of (12) to formyl ester (13) was achieved using Swern oxidation in 54% yield[27].

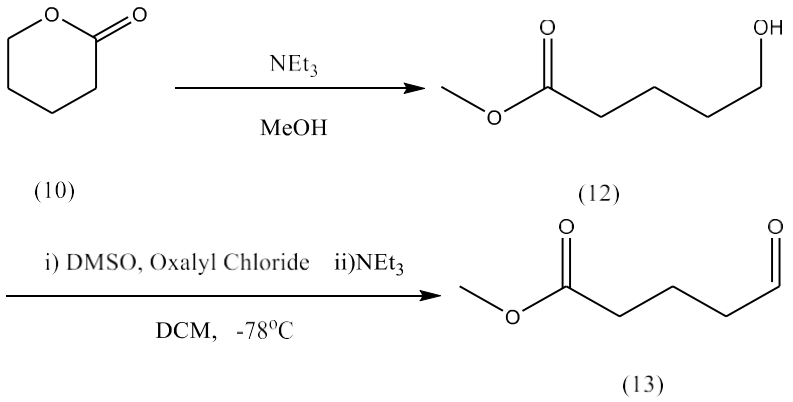


Figure 2.1.1 Synthesis of formyl ester 13. Methyl ester alcohol was formed through base catalyzed ring opening. The aldehyde group on 3 was formed through oxidation on the hydroxyl group on 12.

2.1.2 Synthesis of Racemic Methyl 5-hydroxyhept-6-ynoate (6)

Due to need for synthesis of racemic target product (6), the direct addition to the aldehyde group using deprotonated trimethylsilyacetylene was applied using *n*-butyl lithium as base.

In this step, the temperature of reaction was found to have the key impact on yield. It was found that after the addition of (13), if the temperature was returned to room temperature and reacted for 2 hours, from the ^{1}H NMR spectrum of separated (14) after silica gel chromatography, there were unwanted side-reaction products. With same Rf value as (14), it made these impurity impossible to remove. After consulting literature[28], the whole reaction process was performed under -78 ^{o}C for 2 hours. The TMS alkynol ester (14) was generated as the only addition product in 62% yield.

After desiylation using tetra-n-butylammonium fluoride (TBAF), (14) was converted into alkynol ester (6) with 89% yield.

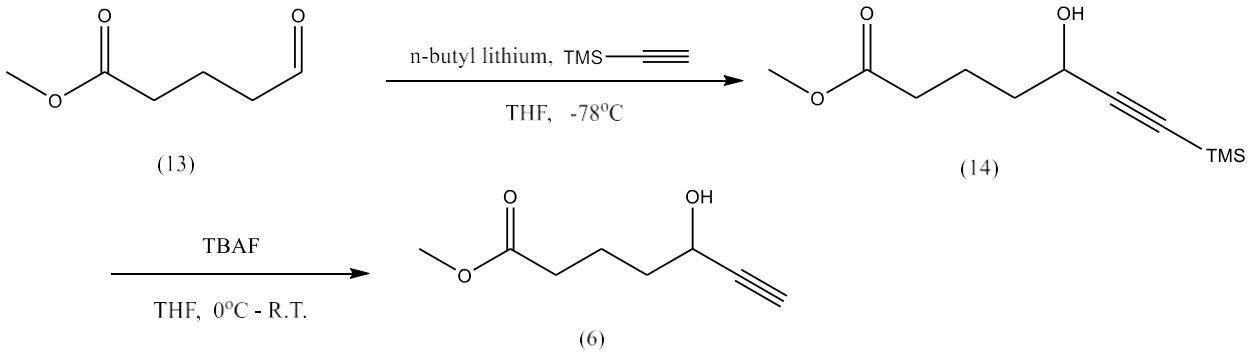


Figure 2.1.2 Synthesis of racemic Methyl 5-hydroxyhept-6-ynoate 6. The racemic hydroxynoate 6 was formed through TMS acetylene addition to the aldehyde group on 13 by n-butyl lithium and TBAF desilylation.

2.2 Efforts towards (1*E*,3*E*,7*Z*)-1-Chlorotrideca-1,3,7-trien-5-ol (7)

2.2.1 Problematic Product (15) and Specified Separation Methods.

In the synthesis of (7), oxidation of olefinic alcohol (11) to aldehyde (15) was achieved using Dess-Martin periodinane (DMP). In the process of isolation of (15), normal methods (method 1 and method 2) led to the isomerization on the olefin group creating inseparable impurities. After screening of literatures, two methods (Method 3 and Method 4) was applied[21,29]. Method#4 provided (15) with relative higher yield of 96.1% and clean product.

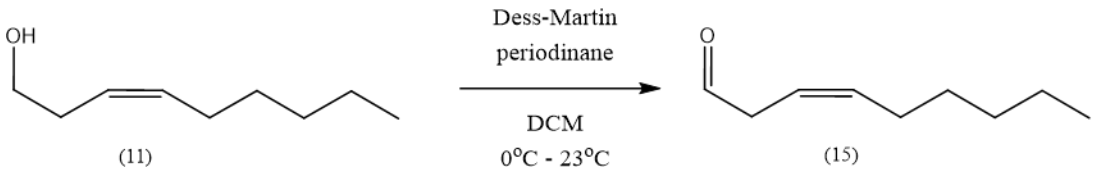


Figure 2.2.1 Synthesis of aldehyde (15) Oxidation of olefinic alcohol by DMP.

Table 2.2.1 Method applied in separation of 15. Four trials on different methods to quench and separate product 15. The method #4 provided most effective strategies with 96.1% yield.

Method #	Method Description	Isomerization	Yield
1	Remove solvent *in vacuo*, purified by silica gel.	Yes	N/A
2	Quenched by saturated sodium thiosulfate solution and washed by saturated sodium bicarbonate solution	Yes	N/A
3	Dilute by diethyl, cool to -30°C, filter through Celite, Distilled under vacuum at 80°C	No	41.0%
4	Dilute with pentane, cool to -15°C, filter through rinsed silica gel (diethyl ether: pentane 1:4), concentrate under reduced pressure.	No	96.1%

2.2.2 Continue Synthesis to Racemic Chlorotriene Alcohol (7).

Using n-butyl lithium to deprotonate trimetylsilyacetylene, the addition reaction on aldehyde (15) led to the formation of racemic TMS enynic alcohol (16). After desiylation by TBAF, enynic alcohol (9) was afford.

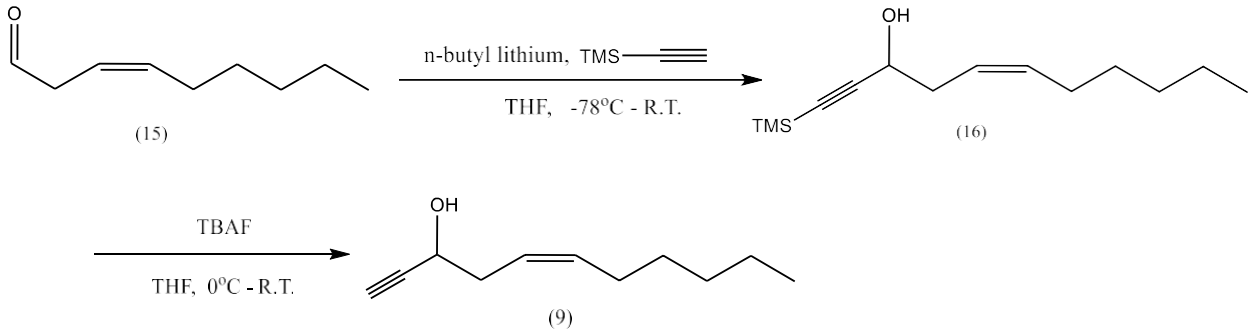


Figure 2.2.2.1 Synthesis of racemic enynic alcohol 9. After TMS acetylene addition mediated by n-butyl lithium and TBAF desilylation, racemic enynic alcohol 9 was formed.

The next step was Sonogashira coupling with (E)-1,2-dichloroenthene. By applying the protocol of Yang et al[21], the compound (17) was formed with only 35% yield and there was recrystallized $Pd(PPh_3)_4$ solid co-eluted with product. The purification for this solid further decrease the yield of (17). The optimal condition was tested inspired by Linstrumelle et al's method, with 0.02 equivalent of $Pd(PPh_3)_4$, chloroenynic alcohol (17) was afforded in 71% conversion rate[30].

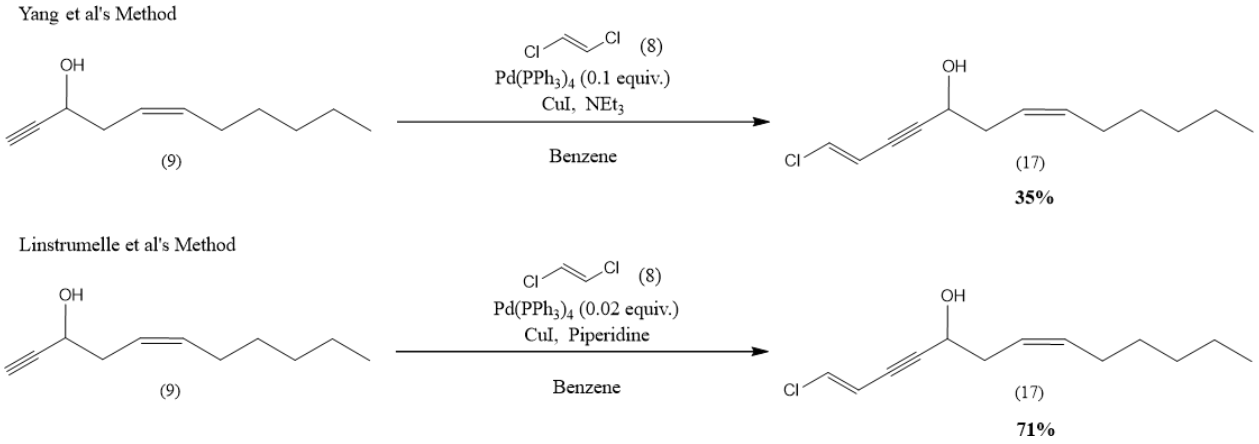


Figure 2.2.2.2 conditions for synthesis 17. Two different conditions on Sonogashira reaction. By replacing NEt_3 to piperidine, less catalyst loading provided higher yield.

The reduction of (17) applying $LiAlH_4$ resulted in the formation of *trans* double bond in chlorotriene alcohol (7) with 67% yield.[30]

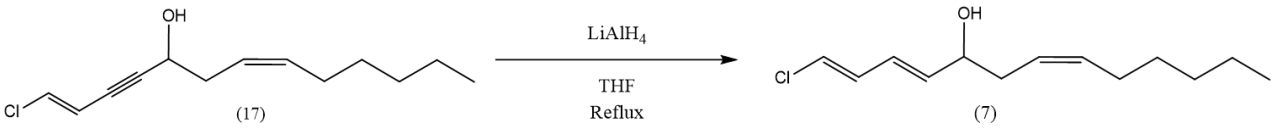


Figure 2.2.2.3 Synthesis of chlorotriene alcohol 7. The diene chloride was formed through reduction using $LiAlH_4$.

2.3 Formation of LTB_4 backbone

2.3.1 Sonogashira Cross-coupling

With both (6) and (7) in hand, the key step in forming LTB_4 backbone was to use Sonogashira coupling again to synthesis dienyne (18). By applying two protocols from literatures using $Pd(PPh_3)_4$ as catalyst, the yield was below 40%[21,30]. After consulting Linstrumelle's modification on Sorogashira reaction, bis(benzonitrile)palladium dichloride ($Pd(PhCN)_2Cl_2$) was used as catalyst and the conversion rate rose to 57%[31].

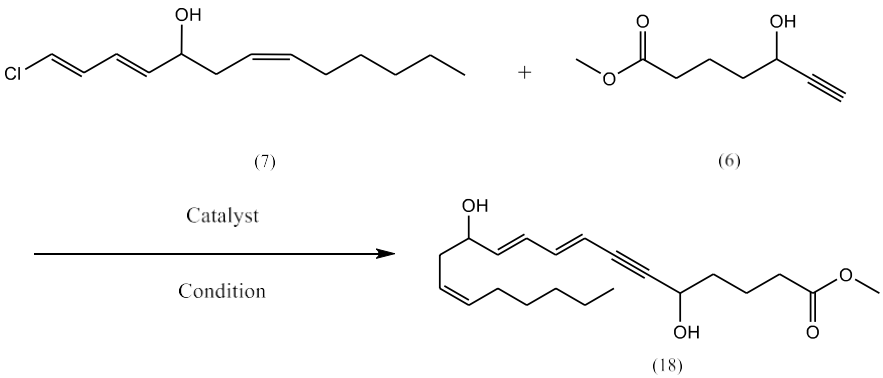


Figure 2.3.1 Synthesis of dienyne 18 by Sonogashira coupling. The key backbone formation was expected through Sonogashira coupling between 7 and 6.

Table 2.3.1 Conditions for Sonogashira Coupling and their yield. The screens on different Sonogashira reaction condition. By applying the conditions of Method#4, the yield was the highest.

Method #	Catalysts	Condition	Yield
1	$Pd(PPh_3)_4$ (0.1 equiv.), CuI (0.2 equiv.), NEt_3 (10.0 equiv.)	Benzene, 23°C, Overnight	11%
2	$Pd(PPh_3)_4$ (0.05 equiv.), CuI (0.1 equiv.), *n*-butylamine (10.0 equiv.)	Benzene, 23°C, Overnight	33%
3	$Pd(PPh_3)_4$ (0.2 equiv.), CuI (0.4 equiv.), Piperidine (10.0 equiv.)	Benzene, 23°C, Overnight	29%
4	$Pd(PhCN)_2Cl_2$ (0.05 equiv.) CuI (0.1 equiv.) Piperidine (20.0 equiv.)	Benzene, 23°C, Overnight	57%

2.3.2 *cis*-Reduction

In order to form the desired *Z,E,E*-triene (4), several *cis*-reduction methods were tested. The semihydrogenation using Brown' s P-2 Ni catalyst in the presence of ethylene diamine was applied on compound (18).[23] after 20 minutes, no desired product was found and the starting material was not retrieved. It was expected that (18) decomposed and this condition was harsh.

From literatures, Boland's Z-specific hydrogenation was applied with mild condition and exclusive cis-product formation.[33] Two conditions were first tested, but method #2 only provide product with 13% yield and method #3 retrieved starting material.[30,33] By screening the literatures, the improved version of Boland reduction was found with adding trimethylsilyl chloride (TMSCl) to further activate Zn.[34] This method (method #4) furnished desired product with 37.5% yield and less reaction time.

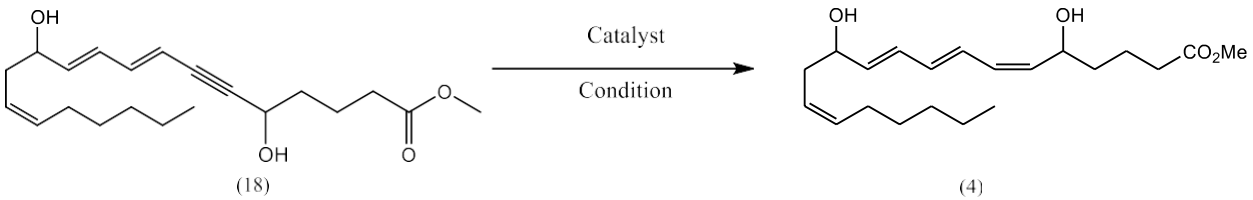


Figure 2.3.2 Cis-reduction of 18 to 4. The key step in forming the LTB4 backbone was to form the cis-alkene group through reduction.

Table 2.3.2 Reduction for 4. Screens of cis-reduction condition were listed. By using the condition in Method#4, the yield was 37.5%.

Method#	Catalyst	Condition	Reaction Time	Yield
1	$Ni(OAc)_2$, $NaBH_4$, Ethylene diamine	EtOH, H_2 atomasphere.	20 min	N/A
2	Active Zn (Zn/Cu/Ag)	Methonal:H_2O = 1:1	5 h	13%
3	Active Zn (Zn/Cu/Ag)	Methonal:H_2O = 9:7	Overnight	0%
4	Active Zn (Zn/Cu/Ag), TMSCl (10.0 equiv)	Methonal:H_2O = 1:1	4 h	37.5%

2.4 Synthesis of 5-stearoyl acid ester LTB_4 and 12-stearoyl acid ester LTB_4.

2.4.1 Esterification

With the main part racemic LTB4 being synthesized, the next step was the esterification. There was reported esterification of hydroxyl fatty acids with fatty acid carbonyl chloride under pyridine-induced condition.[35] However, comparing with other FAHFAs' hydroxyl fatty acid chain, LTB_4 has two hydroxyl groups and esterification will be expected to happen at either position or both. Two mono-esterified products were expected to have same Rf value, which made them hard to separate in silica gel chromatography.

In the trial reaction, compound 4 was reacted with commercially available stearoyl chloride (5) using pyridine in DCM. In this reaction, in order to surpass unwanted di-esterfication, 0.8 equivalent of (5) was used. It was delight to find that two ester products (19) and (20) from esterification on two different hydroxyl groups showed obvious difference on Rf value (ΔRf=0.15 in Hexane:EtoAc=6:4). Through eluting with Hexane/EtoAc from 9:1 to 7:3, two methyl ester FAHFA (19) and (20) was separated with overall 18.8%.

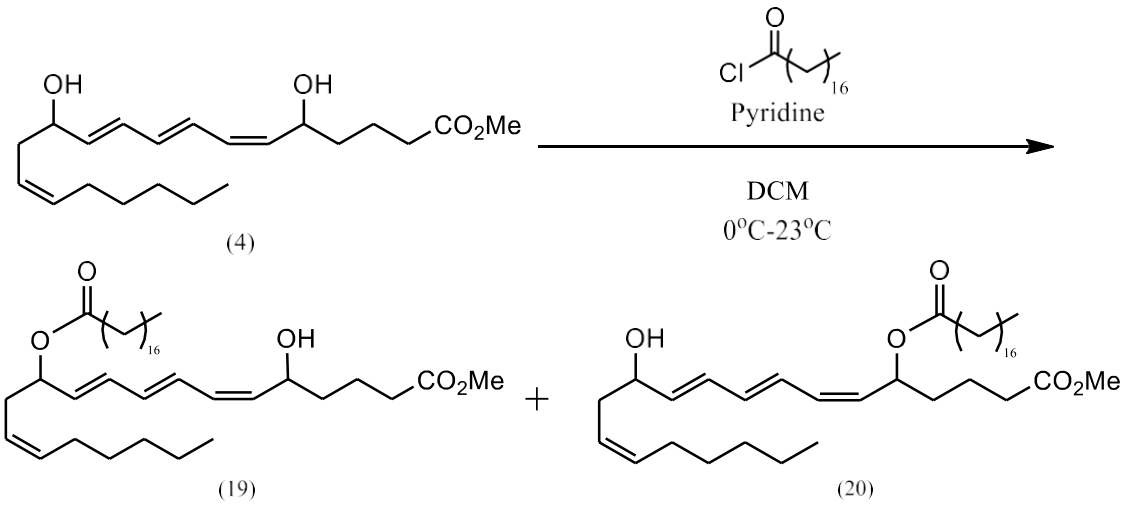


Figure 2.4.1 Esterification of Compound 4. Compounds 19 and 20 were formed directly through pyridine-catalyzed esterification between 4 and stearoyl chloride.

2.4.2 Synthesis of Carbonyl Acid Groups on 5-stearoyl acid ester LTB_4 and 12-stearoyl acid ester LTB_4

2.4.2.1 Researches on Transesterification Approaches

In order to synthesis the carbonyl acid group on final products, one strategy was to change the methyl ester group to other esters with more-easily being removed groups (TMS ethyl ester or trifluoroethyl ester).

For transesterification to trifluoroethyl ester, Otera's catalyst was applied by reacting with trifluoroethanol (TFE) .[36] However, all trials on (6) and (18) failed to provide desired products. The only isolated product was the intramolecular cyclized lactone.

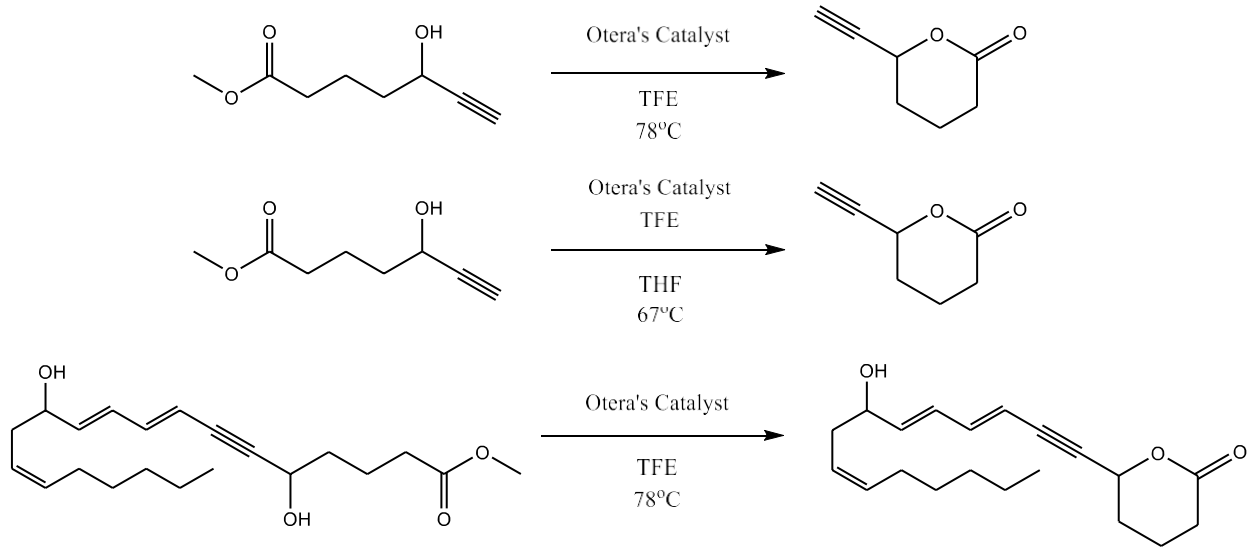


Figure 2.4.2.1.1 Transesterification using Otera's Catalyst. Trials on transesterification to change methyl groups into trifluoride ethyl groups. All experiment provided only one cyclolactone product through intramolecular esterification.

In the studies for synthesis the TMS ethyl ester $Ti(i\text{-}Pro)_4$ was used as catalyst, (6) was transferred to its TMS ethyl ester.[37] After the Sonogashira coupling, the TMS ethyl ester of (18) was formed. But, when it was treated under the cis-reduction condition, neither desired product nor starting material were found.

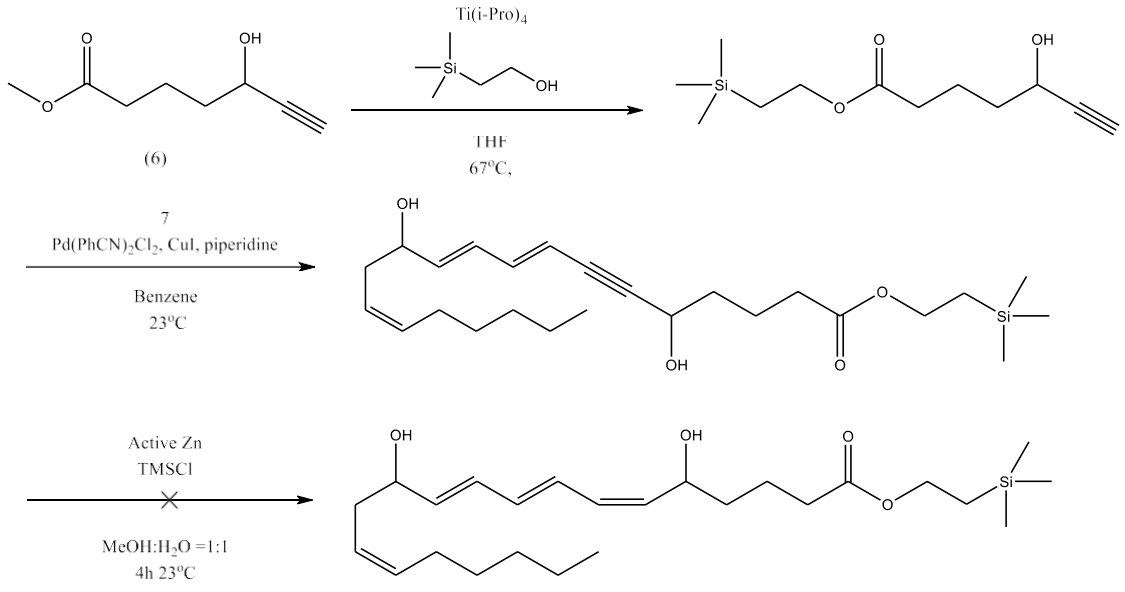


Figure 2.4.2.1.2 Transesterification to TMS ethyl ester and attempted synthesis on corresponding triene. Trial to form TMS ethyl esterified 4 form 6. The expected product was not formed.

2.4.2.2 Research on Saponification Conditions

For synthesis of product (19) and (20), 5-stearoyl acid ester LTB_4 and 12-stearoyl acid ester LTB_4 were also expected to achieve through saponification at their methyl ester group.[9,23] Compound (20) was used for trials. Applying previously reported method used in saponification of methyl ester FAHFAs, there were two side-products (1.1) and (4) formed without the trace of (2).

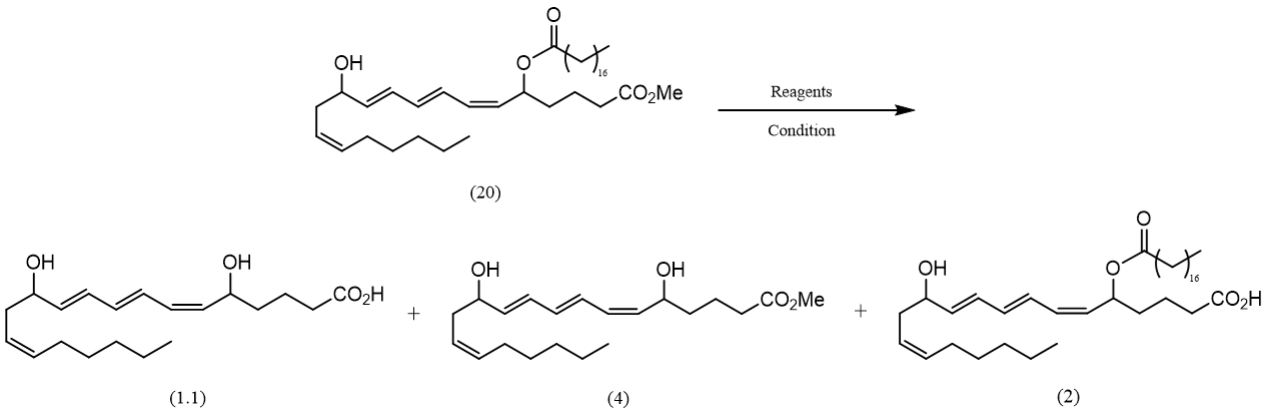


Figure 2.4.2.2.1 Products from saponification. There are three products from saponification of 20. The compound 2 is the desired product.

In order to find the conditions for synthesis desired product (2), screening of conditions was performed. After screening for reaction conditions, the method using LiOH H_2O in 3:1:1 THF: MeOH: H_2O mixture system was the most effective.[38] This method gave final products (2) and (3) with yield of 37% and 29% respectively.

Table 2.4.2.2 Screen of saponification Conditions. Screen for the saponification conditions. By applying the condition in Method#7, desired product 2 was formed.

Method #	Reagent	Solvent system	Temperature	Time	(1.1)	(4)	(2)
1	LiOH (2.0 eq.)	THF	23°C	Overnight	Major	No	No
2	LiOH (2.0 eq.)	THF:H_2O =1:1	23°C	Overnight	Major	No	No
3	LiOH (2.0 eq.)	THF:H_2O =1:1	23°C	4.5 h	No	Major	No
4	LiOH (2.0 eq.)	THF:H_2O =1:1	0 °C	7 h	No	Major	No
5	$KOSi(Me)_3$ (1.2 eq.)	THF	23 °C	6h	No	No	No
6	LiOH H_2O (4.0 eq.)	THF:MeOH: H_2O = 3:1:1	0°C to 23 °C	1h	No	Major	No
7	LiOH H_2O (2.0 eq.)	THF:MeOH: H_2O = 3:1:1	0°C to 23 °C	40 min	No	Minor to trace amount	Major

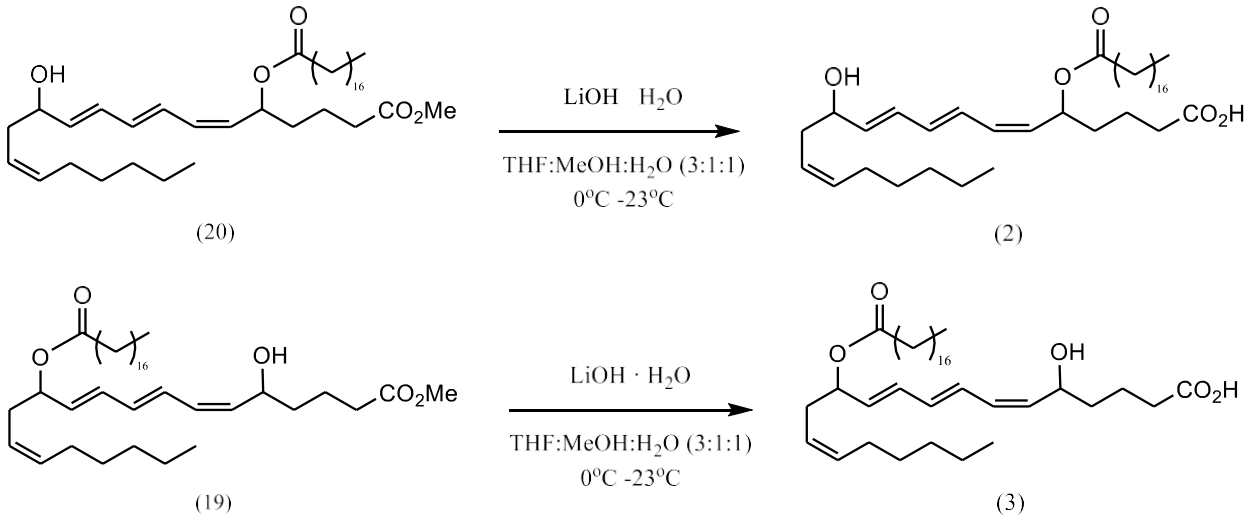


Figure 2.4.2.2.2 Saponification to synthesis 5-stearoyl acid ester LTB_4 (2) and 12-stearoyl acid ester LTB_4 (3). The formation of final compound 2 and 3 through saponification of 19 and 20.

2.5 Conclusion

In conclusion, I have synthesized 5-stearoyl acid ester LTB_4 and 12-stearoyl acid ester LTB_4 based on proposed synthesis strategy. The alkynol alcohol and chloridiene alcohol were synthesized from commercially available reagents. Sonogashira coupling to connect two parts and *cis*-reduction on alkyne group with optimal conditions were the key steps in scheme. Next, direct esterification with stearoyl chloride provided two methyl FAHFAs. Lastly, the desired 5-stearoyl acid ester LTB_4 and 12-stearoyl acid ester LTB_4 was obtained through saponification using lithium hydroxide in overall yield of 2.7% for 3 and 3.5% for 2.

Experimental

General Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Varian 600 MHz (^{1}H at 600 MHz, ^{13}C at 150 MHz, ^{13}C HSQC and HMBC at 150 MHz). All spectra were taken in $CDCl_3$ with shifts reported in parts per million (ppm) referenced to protium or carbon of the solvent (7.26 or 77.16, respectively).They are reported as; position, multiplicity and integration. Standard abbreviations are used throughout (s-singlet, d-doublet, dd-doublet of doublets, dt-doublet of triplets, dq-doublet of quartets, ddd-doublet of doublet of doublets, dtd-doublet of triplet of doublets, dtt-doublet of triplet of triplets, t-triplet, td-triplet of doublets, tt-triplet of triplets, tdd-triplet of doublet of doublets, q-quartet, qd-quartet of doublet, p-pentet, m-multiplet). Mass spectra including Electrospray ionization (ESI) and Atmospheric pressure chemical ionization (APCI) was LRMS, acquired on a Thermo LCQdeca mass spectrometer and reported as m/z for the molecular ion $[M+Na]^+$, $[M+H]^+$, $[M-H2O+H]^+$and $[M-H]^-$. Accurate mass measurement are correct to ±0.01

Organic solutions were concentrated by rotary evaporator at 30 millibar with the water bath heated to not more than 50 °C. Tetrahydrofuran (THF), dichloromethane (DCM), benzene and diethyl ether were purified with a Pure-Solve MD5 Solvent Purification System (Innovative Technology). All other commercially available reagents were purified as necessary following standard. Thin-layer chromatography (TLC) was performed using 0.2 mm commercial silica gel plates (silica gel 60, F254, EMD Chemicals) and visualized with a UV lamp (short and long wave) and/or aqueous potassium permanganate ($KMnO_4$) stain.

Methyl 5-hydroxypentanoate (12)

Dissolve commercially available lactone (9) (1000 mg, 9.9 mmol, 1.0 equiv.) in MeOH (10 ml), add trimethylamine (NEt_3) (0.47 ml, 3.7 mmol, 0.34 equiv.) into solution. After stirring at 23°C for 18 hours, the solution was filtered through a short silica gel plug and washed by MeOH (20 ml). The collected solution was concentrated under vacuum and can be used without further purification. Compound 11 (1269.6 mg, 9.6 mmol) was synthesised as clear oil compound with yield of 96.1%.

^{1}H NMR (600 MHz, $CDCl_3$) δ 3.60 (s, 3H), 3.56 (t, *J* = 6.5 Hz, 2H), 2.29 (t, *J* = 7.5 Hz, 2H), 1.64 (p, *J* = 7.4 Hz, 2H), 1.52 (dq, *J* = 9.9, 6.5 Hz, 2H).

^{13}C NMR (150 MHz, $CDCl_3$**)** δ 174.30, 61.98, 51.58, 33.67, 31.96, 20.53

LRMS (ESI) calc. for $C_6H_{13}O_3$ $[M+H]^+$: 133.08, obs, 133.00

Methyl 5-oxopentanoate (13)

To a flame-dried flask, liquid oxalyl chloride (1524.2 mg, 12.0 mmol, 1.25 equiv.) was added into dry DCM (60 ml). Then, dimethyl sulfoxide (1916.3 mg, 24.5 mmol, 2.5 equiv.) in dry DCM (12 ml) was added into the solution dropwise at -78°C under argon. After stirring at same temperature for 30 minutes, 11's dry DCM solution (12 ml) was added into system dropwise and stirred for 30 minutes at same temperature. Dry NEt_3 (3888.3 mg, 39.2 mmol, 4.0 equiv.) was added dropwise at -78°C before transferring the flask into ice bath. After all starting material 11 being oxidized monitored by Thin-lay-

chromatography (TLC) (Hexane:EtoAc = 1:1), water (20 ml) was added to quench reaction. DCM (10 ml x3) was used to extract the aqueous phase three times. All organic parts was then washed by brine (20 ml), dried using Na_2SO_4 and concentrated under vacuum. The crude product was purified using flash chromatography on silica gel (Hexane:EtoAc = 1:1) to afford compound 12 (678.3 mg, 5.2 mmol) as yellow oil with 54.2% yield. R_f=0.58 (Hexane:Ethyl Acetate(EtoAc)=1:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.72 (t, J = 1.4 Hz, 1H), 3.62 (s, 3H), 2.48 (td, *J* = 7.2, 1.3 Hz, 2H), 2.32 (t, *J* = 7.3 Hz, 2H), 1.70 – 1.51 (m, 2H).

^{13}C NMR (150 MHz, $CDCl_3$**)** δ 201.46, 173.30, 51.57, 42.85, 32.85, 17.26.

LRMS (ESI) calc. for $C_6H_{10}O_3$ $[M+H]^+$: 131.06, obs, 131.05

Methyl 5-hydroxy-7-(trimethylsilyl)hept-6-ynoate (14)

In a flame-dried flask, after its air was removed and filled with argon, commercial product trimethylsilyacetylene (563.1 mg, 5.7 mmol, 1.1 equiv) was added into dry THF (60 ml) and whole solution was cooled to -78^{o}C before adding n-butyl lithium (367.3 mg, 5.7 mmol, 1.1 equiv. 1.6M in Hexane) dropwise. After stirring under argon at same temperature for 1 hour, compound 12 (678.3 mg, 5.2 mmol, 1.0 equiv.) in dry THF (20 ml) was added dropwise into it. The system was stirred at -78^{o}C under argon for 2 hours before returned to 23^{o}C and quenched by saturated NH_4Cl aqueous solution (50 mL). The aqueous phase was extracted by ether (20 ml X3) and the combined organic layers were washed by brine (20 ml) and dried by Na_2SO_4. After concentrated in *vacuo* , the crude product was purified using silica gel chromatography (Hexane:EtoAc = 9:1) to give yellow oil-like product (742.9 mg, 3.3 mmol) with yield of 62.4%. R_f=0.45 (Hexane:EtoAc=10:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.36 (t, J = 6.3 Hz, 1H), 3.66 (s, 3H), 2.36 (t, J = 7.2 Hz, 2H), 1.85 – 1.63 (m, 4H), 0.15 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 174.06, 106.50, 89.71, 62.44, 51.70, 36.99, 33.66, 20.66, 0.03.

LRMS (APCI) calc. for $C_{11}H_{20}O_3Si$ $[M+H]^+$: 228.12, obs. 228.96

Methyl 5-hydroxyhept-6-ynoate (6)

After drying a flask using flame, tetra-n-butylammonium fluoride (TBAF) (935.6 mg, 3.6 mmol, 1.1 equiv) (1 M in THF) was added into dry THF (10 ml) and cooled to 0^{o}C using ice bath. After being stirred for 5 minutes, compound 13 (742.9 mg, 3.5 mmol, 1.0 equiv.) in dry THF (3 ml) was added into solution dropwise. The reaction system was stirred at 23^{o}C under argon for 20 minutes. When all compound was reacted monitored by TLC (Hexane:EtoAc = 7:3) , the saturated NH_4Cl aqueous solution (10 ml) was used to quench solution. Then ether (5 ml X 3) was used to extract aqueous phase. The collected organic phase was washed by brine (10 ml) and dried by Na_2SO_4. The concentrated residues was passed through a silica column using hexane-EtoAc (8:2) as eluent to give yellow oil-like product 6 (455.6 mg, 2.9 mmol) in 89.6 % yield. R_f=0.59 (Hexane:EtoAc=55:45)

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.34 (td, *J* = 6.3, 2.2 Hz, 1H), 3.63 (s, 3H), 2.43 (d, *J* = 2.2 Hz, 1H), 2.33 (t, *J* = 6.3 Hz, 2H), 1.83 – 1.64 (m, 4H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 174.08, 84.74, 73.01, 61.62, 51.68, 36.82, 33.56, 20.49.

LRMS (APCI) calc. for $C_8H_{12}O_3$ $[M+H]^+$: 157.08, obs. 156.95

(*Z*)-Non-3-enal (15)

In a flame-dried flask, Commercially available 10 (1000 mg, 7.03 mmol, 1.0 equiv.) was dissolved in dry DCM (70 ml) under argon atmosphere and cooled to 0°C by ice bath. After stirring for 5 minutes, solid Dess-Martin periodinane (DMP) (3870 mg, 9.13 mmol, 1.3 equiv.) was added in quickly. The solution was returned to 23 °C. After stirring for 1 hours, the solution was cooled to -15°C for 20 minutes and pentane (70 ml) was added to dilute solution. The suspension was filtered through a pad of silica gel pre-rinsed with diethyl ether/pentane (1:4). The silica pad was washed by diethyl ether/pentane (1:4) system (200ml). Combined solution was cooled to -50°C for 30 minutes. Then solvent was removed under vacuum. This afforded 15 (947.5 mg, 6.76 mmol) as clear oil with yield of 96.1%

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.63 (t, *J* = 2.0 Hz, 1H), 5.68 (dtt, *J* = 10.6, 7.3, 1.6 Hz, 1H), 5.51 (dtt, *J* = 10.7, 7.3, 1.7 Hz, 1H), 3.16 (dt, *J* = 7.3, 1.9 Hz, 2H), 2.04 – 1.98 (m, 2H), 1.39 – 1.21 (m, 6H), 0.86 (td, *J* = 7.1, 3.6 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 200.02, 135.62, 118.02, 42.63, 31.51, 29.06, 27.65, 22.60, 14.08.

LRMS (APCI) calc. for $C_9H_{16}O$ $[M+H]^+$: 141.12, obs, 141.18.

(*Z*)-1-(Trimethylsilyl)undec-5-en-1-yn-3-ol (16)

Just after compound 14 was formed, in a flame-dried flask, liquid trimethylsilyacetylene (1225.9 mg, 12.5 mmol, 1.5 equiv) was dissolved in dry THF (35 ml) under argon atmosphere. The solution was chilled to -78°C before n-butyl lithium (799.5 mg, 12.5 mmol, 1.5 equiv.) (1.6 M in hexane) was added dropwise and was stirred under argon for 1 hour at same temperature. Compound 14 (1166.8 mg, 8.3 mmol, 1.0 equiv.)'s THF solution (5ml) was added dropwise into the system before the whole reaction was returned to 23°C. After reacting for 2 hours, saturated NH_4Cl aqueous solution (20 ml) was added to quench reaction. The aqueous phase was extracted by ether (10 ml x3), washed by brine (30 ml) and dried by Na_2SO_4. After solvent was removed under reduced pressure, the crude product was purified by silica gel chromatography using hexane:EtoAc 10:1 as eluent to afford product 15 (1107.1 mg, 4.6 mmol, 55.8 %) as yellow oil-like liquid. R_f=0.53 (Hexane:EtoAc=10:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.60 (dtd, *J* = 10.7, 7.3, 1.6 Hz, 1H), 5.45 (dtt, *J* = 10.8, 7.4, 1.6 Hz, 1H), 4.37 (t, *J* = 6.3 Hz, 1H), 2.46 (tt, *J* = 11.0, 4.3 Hz, 2H), 2.09 – 2.02 (m, 2H), 1.39 – 1.23 (m, 6H), 0.88 (td, *J* = 7.1, 2.7 Hz, 3H), 0.16 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 134.43, 123.39, 106.45, 89.55, 62.53, 35.74, 31.64, 29.45, 27.62, 22.68, 14.18, -0.02

LRMS (APCI) calc. for $C_{14}H_{25}Si$ $[M-H_2O+H]^+$: 221.17, obs, 221.25.

(*Z*)-Undec-5-en-1-yn-3-ol (9)

TBAF (621.2 mg, 2.4 mmol, 1.1 equiv) (1 M in THF) was added into dry THF (20 ml) under argon atmosphere in a flame-dried flask. After solution was cooled to 0°C by ice bath, compound 15 (515 mg, 2.2 mmol, 1.0 equiv) in THF (5 ml) was added dropwise into TBAF solution. The reaction was then returned to 23°C and reacted for 20 minutes. To the solution, saturated aqueous NH_4Cl solution (10 ml)

was used to quench the reaction. The aqueous parts was extracted by diethyl ether (10 ml x 3), washed by brine (10 ml) and dried by Na_2SO_4. The crude product was concentrated under reduced pressure and purified to give compound 8 (327.8 mg, 1.97 mmol, 91.3%) as yellow oil-like liquid through silica chromatography using hexane;EtoAc 9:1. R_f=0.39 (Hexane:EtoAc=9:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.61 (dtt, *J* = 10.6, 7.2, 1.6 Hz, 1H), 5.46 (dtt, *J* = 10.8, 7.3, 1.7 Hz, 1H), 4.38 (td, *J* = 6.3, 2.2 Hz, 1H), 2.52 – 2.46 (m, 2H), 2.45 (d, *J* = 2.2 Hz, 1H), 2.09 – 2.03 (m, 2H), 1.38 – 1.22 (m, 6H), 0.87 (t, J = 7.0 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 134.64, 123.06, 84.64, 73.04, 61.89, 35.57, 31.60, 29.37, 27.57, 22.65, 14.15.

LRMS (APCI) calc. for $C_{11}H_{17}$ $[M-H_2O+H]^+$: 149.13, obs, 149.24

(1*E*,7*Z*)-1-Chlorotrideca-1,7-dien-3-yn-5-ol (17)

In a flame-dried Schlenk tube, air was removed and tube was charged with argon. Solid $Pd(PPh_3)_4$ (28.9 mg, 0.025 mmol, 0.02 equiv.), solid CuI (23.8 mg, 0.12 mmol, 0.1 equiv.) were added in one portion, followed by piperidine liquid (252.8 mg, 2.5 mmol, 2 equiv) and commercially available liquid 7 (1211.0 mg, 12.5 mmol, 10 equiv.). Dry benzene (5ml) was injected into mixture. The tube was put into -78^{o}C acetone solution to Freeze-Pump-Thaw three times. After degassing, compound 8 (207.7 mg, 1.2 mmol, 1.0 equiv.) was dissolved in argon-bubbled dry benzene solvent (2ml) and added into mixture in one portion at 23^{o}C. The reaction was stirred at same temperature overnight under argon. Saturated NH_4Cl aqueous solution (10 ml) was used to quench solution and the aqueous phase was extracted by diethyl ether three times (5 ml x3). The combined organic part was dried by Na_2SO_4. Then the solvent was remove in vacuo to afford the crude product which was purified using silica gel chromatography with hexane: EtoAc 9:1. Compound 16 (200.9 mg, 0.89 mmol) was synthesized with as yellow oil-like liquid with 71.1% yield. R_f=0.38 (Hexane:EtoAc=9:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 6.53 (d, J = 13.7 Hz, 1H), 5.94 (dd, J = 13.6, 2.0 Hz, 1H), 5.62 (dq, J = 14.8, 7.3 Hz, 1H), 5.44 (dd, J = 12.3, 6.1 Hz, 1H), 4.53 – 4.43 (m, 1H), 2.55 – 2.42 (m, 2H), 2.12 – 1.97 (m, 2H), 1.39 – 1.22 (m, 6H), 0.88 (t, J = 6.9 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 134.82, 130.96, 123.00, 113.31, 92.52, 80.04, 62.47, 35.60, 31.62, 29.39, 27.61, 22.68, 14.15.

LRMS (APCI) calc. for $C_{13}H_{17}Cl$ $[M-H_2O+H]^+$: 209.10, obs, 209.30

(1*E*,3*E*,7*Z*)-1-Chlorotrideca-1,3,7-trien-5-ol (7)

$LiAlH_4$ solution (67.4 mg, 1.78 mmol, 2.0 equiv.) (4.0 M in ether) was injected dropwise into dry THF (5 ml) in a flame-dried flask under argon at 0^{o}C (ice bath). After stirring for 5 minutes, compound 16 (200.7 mg, 0.88 mmol, 1.0 equiv) in THF (5 ml) was added into it dropwise at same temperature. The reaction system was connected to a reflux tube and heated to reflux at 68^{o}C under argon. After two hours, the solution was returned to 23^{o}C before adding water (0.072 ml), 15% NaOH aqueous solution (0.072 ml) and water (0.24 ml). The quenched mixture was stirred at 23^{o}C overnight. Celite was used to filter the diethyl ether (10 ml) diluted solution and it was rinsed by diethyl ether twice (5 ml x2). The combined organic parts was concentrated under reduced pressure and purified by silica chromatography

(Hexane:EtoAc = 7:3) to afford yellow oil-like liquid (137.9 mg, 0.60 mmol, 67.9 %). R_f=0.56 (Hexane:EtoAc=9:1)

^{1}H NMR (600 MHz, $CDCl_3$) δ 6.41 (dd, *J* = 13.2, 10.9 Hz, 1H), 6.21 – 6.11 (m, 2H), 5.71 (dd, *J* = 15.4, 6.0 Hz, 1H), 5.57 – 5.49 (m, 1H), 5.37 – 5.29 (m, 1H), 4.15 (q, *J* = 6.3 Hz, 1H), 2.33 – 2.24 (m, 2H), 2.03 – 1.97 (m, 2H), 1.36 – 1.20 (m, 6H), 0.86 (t, *J* = 7.0 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 136.62, 133.95, 133.02, 126.05, 124.01, 120.92, 71.57, 35.33, 31.57, 30.96, 29.32, 27.47, 22.61, 14.01.

LRMS (APCI) calc. for $C_{13}H_{20}Cl$ $[M-H_2O+H]^+$: 211.12 obs, 211.11

(8*E*,10*E*,14*Z*)-Methyl 5,12-dihydroxyicosa-8,10,14-trien-6-ynoate (18)

In a flame-dried Schlenk tube, air was removed and tube was charged with argon. Solid $Pd(PhCN)_2Cl_2$ (12.7 mg, 0.033mmol, 0.05 equiv.), solid CuI (12.6 mg, 0.066 mmol, 0.1 equiv.) were added in one portion, followed by piperidine liquid (1124.12 mg, 13.20 mmol, 20.0 equiv). Compound 17 (151.0 mg, 0.66 mmol, 1.0 equiv.) was dissolved in dry benzene (1 ml) and injected into the mixture in one portion. The tube was put into -78 ^{o}C acetone solution to Freeze-Pump-Thaw three times. Compound 6 (154.6 mg, 0.99 mmol, 1.5 equiv.) was dissolved in argon-bubbled benzene (1ml) and added into system dropwise. The whole reaction was stirred under argon overnight at 23^{o}C. Diethyl ether (5 ml) was used to dilute solution and saturated NH_4Cl aqueous solution (5 ml) was added to quench it. After extracting the aqueous phase three times using diethyl ether (2 ml x3), combined organic parts was dried using Na_2SO_4 and concentrated in vacuo. The crude product was passed through a column loaded with silica gel using hexane:EtoAc 8:2 to afford yellow oil-like product. (137 mg, 0.38 mmol, 56.9%) R_f=0.31 (Hexane:EtoAc=6:4)

^{1}H NMR (600 MHz, $CDCl_3$) δ 6.56 (dd, *J* = 15.6, 10.9 Hz, 1H), 6.28 (dd, *J* = 15.3, 10.8 Hz, 1H), 5.82 (dd, *J* = 15.3, 6.0 Hz, 1H), 5.64 – 5.54 (m, 2H), 5.35 (dtt, *J* = 11.0, 7.6, 1.7 Hz, 1H), 4.52 (td, *J* = 6.2, 1.9 Hz, 1H), 4.22 (q, *J* = 6.2 Hz, 1H), 3.67 (s, 3H), 2.38 (t, *J* = 7.1 Hz, 2H), 2.32 (q, *J* = 6.8 Hz, 2H), 2.03 (m, *J* = 5.6 Hz, 2H), 1.84 – 1.71 (m, 3H), 1.37 – 1.22 (m, 7H), 0.88 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 174.16, 141.42, 138.38, 133.77, 129.23, 124.00, 110.72, 92.48, 83.97, 71.68, 62.34, 51.66, 37.00, 35.20, 33.58, 31.52, 29.27, 27.42, 22.57, 20.63, 14.08.

LRMS (ESI) calc. for $C_{21}H_{32}O_4Na$ $[M+Na]^+$: 371.22, obs, 371.35.

(6*Z*,8*E*,10*E*,14*Z*)-Methyl 5,12-dihydroxyicosa6,8,10,14-tetraenoate (4)

Zn powder (3.5 g) was charged into a flask in one portion, then water (20 ml) was added into and the powder was stirred for 15 minutes under argon at 23^{o}C. $Cu(OAc)_2.H_2O$ (0.35 g) was dissolved in water (10 ml) and injected in the solution. After stirring for 15 minutes at 23^{o}C, $AgNO_3$ (0.35 ml) in water (3 ml) was added into mixture and the system was stirred for 30 minutes at same temperature. The reaction mixture was filtered and washed in sequence of water (15 ml x2), methanol (15 ml x 2), acetone (15 ml x2) and diethyl ether (15 ml x2). The freshly washed metal power was ready to be used.

In a flask, compound 18 (137 mg, 0.38 mmol, 1.0 equiv.) was added in and the flask was degassed, recharged with argon. The freshly made Zn/Cu/Ag powder (3.08 g) was added into flask in one portion

quickly. Next, H_2O:MeOH 1:1 (46 ml) was added into the flask and it was covered by aluminum foil to keep it in dark. Then, trimethylsilyl chloride liquid (406.08 mg, 3.8 mmol, 10.0 equiv.) was added into mixture dropwise. The reaction was done under argon atmosphere with vigorously stirring (1600 rpm). After 4 hours, reaction was stopped, and diluted with diethyl ether (30 ml) and filtered using diethyl ether rinsed Celite. The combined solution system gained after washing the Celite twice with diethyl ether (20 ml x2) was first put under vacuum to remove ether and methanol. The remaining residue was redissolve in diethyl ether (30 ml) to extract the aqueous phase. After extracting the aqueous phase for three times with diethyl ether (10 ml x3), organic part was washed by brine (20 ml) and dried by Na_2SO_4. Silica gel chromatography was used to purify concentrated crude product and product 4 (51.7 mg, 0.16 mmol) was collected in yield of 37.5% as clear oil-liked liquid. R_f=0.32 (Hexane;EtoAc=6:4)

^{1}H NMR (600 MHz, $CDCl_3$) δ 6.46 (dd, *J* = 14.6, 11.4 Hz, 1H), 6.31 – 6.14 (m, 2H), 6.05 (t, *J* = 11.2 Hz, 1H), 5.75 (ddd, *J* = 15.0, 6.4, 2.5 Hz, 1H), 5.58 – 5.50 (m, 1H), 5.43 – 5.31 (m, 2H), 4.56 (dt, *J* = 9.1, 6.2 Hz, 1H), 4.18 (p, *J* = 5.9 Hz, 1H), 3.64 (s, 3H), 2.37 – 2.24 (m, 4H), 2.02 (td, J = 8.3, 4.0 Hz, 2H), 1.76 – 1.57 (m, 2H), 1.53 – 1.43 (m, 1H), 1.39 – 1.19 (m, 5H), 0.86 (t, *J* = 6.9 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 174.13, 136.93, 134.09, 133.83, 130.18, 127.52, 124.21, 71.91, 67.59, 51.65, 36.82, 35.40, 33.90, 31.59, 29.36, 27.50, 22.64, 20.85, 14.14.

LRMS (ESI) calc. for $C_{21}H_{34}O_4Na$ $[M+Na]^+$: 373.24, obs, 373.37.

General procedures for (19) and (20)

In a flame-dried flask, compound 4 (95 mg, 0.27 mmol, 1.0 equiv.) was dissolved in dry DCM (3 ml) and solution was put into ice bath (0^{o}C) under argon. Pyridine as liquid (107.20 mg, 1.36 mmol, 5.0 equiv.) was injected into solution dropwise. Next, the mixture was stirred for 15 minutes at 0^{o}C. Commercially available stearoyl chloride solid 5 (65.68 mg, 0.22 mmol, 0.8 equiv.) was dissolved in dry DCM and added into mixture immediately. The reaction system was allowed to stir at 23^{o}C overnight under argon atmosphere. To quench the reaction, water (5 ml) was added and solution was stirred for 10 minutes. After DCM layer was collected, aqueous layer was extracted by DCM three times (3 ml x3) and the collected organic layer was wash 1 M HCl aqueous solution (1ml), saturated sodium bicarbonate aqueous solution (1ml), brine (5 ml) and dried by Na_2SO_4. The crude product residue was purified using silica chromatography after solvent was removed under reduced pressure. The column was washed using hexane:EtoAc from 9:1 to 7:3. This gave both product 19 (18.4 mg, 0.030 mmol) and 20 (13.1 mg, 0.021 mmol) as white solid with 18.8 % as combined yield.

(6*Z*,8*E*,10*E*,14*Z*)-1-methoxy-1-oxoecosa-6,8,10,14-tetraen-12-yl stearonoate (12-stearoyl acid ester LTB_4 methyl ester) (19)

R_f= 0.88 (Hexane:EtoAc=6:4)

^{1}H NMR (600 MHz, $CDCl_3$) δ 6.49 (dd, *J* = 14.7, 11.4 Hz, 1H), 6.33 – 6.16 (m, 2H), 6.06 (t, *J* = 11.2 Hz, 1H), 5.66 (td, *J* = 15.0, 7.6 Hz, 1H), 5.49 (dt, *J* = 14.0, 7.4 Hz, 1H), 5.42 (t, *J* = 9.8 Hz, 1H), 5.30 (dq, *J* = 13.2, 7.2 Hz, 2H), 4.58 (q, *J* = 6.5 Hz, 1H), 3.66 (s, 3H), 2.42 (dt, *J* = 14.3, 7.0 Hz, 1H), 2.35 (p, *J* = 6.6, 5.8 Hz, 4H), 2.28 (q, *J* = 6.5, 5.5 Hz, 3H), 2.01 (q, *J* = 7.3 Hz, 2H), 1.78 – 1.55 (m, 8H), 1.50 (dq, *J* = 11.9, 5.6 Hz, 1H), 1.37 – 1.22 (m, 33H), 0.88 (td, *J* = 6.9, 4.2 Hz, 6H).

^{13}C NMR-HSQC (150 MHz, $CDCl_3$) δ 174.18, 173.25, 134.15, 133.87, 133.38, 132.45, 132.27, 130.19, 128.28, 123.53, 73.76, 67.73, 51.70, 36.85, 36.62, 34.74, 34.02, 33.93, 32.54, 29.84, 29.80, 29.75, 29.73,

29.63, 29.58, 29.50, 29.42, 29.39, 29.34, 29.29, 29.21, 27.52, 25.14, 24.85, 22.83, 22.72, 20.87, 14.26, 14.21.

LRMS (ESI) calc. for $C_{39}H_{68}O_5Na$ $[M+Na]^+$: 639.50, obs. 639.53

(6*Z*,8*E*,10*E*,14*Z*)-1-methoxy-1-oxoecosa-6,8,10,14-tetraen-5-yl stearonoate (5-stearoyl acid ester LTB_4 methyl ester) (20)

R_f=0.73 (Hexane:EtoAc=6:4)

^{1}H NMR (599 MHz, Chloroform-d) δ 6.58 (dd, *J* = 14.8, 11.4 Hz, 1H), 6.34 (dd, *J* = 15.2, 10.8 Hz, 1H), 6.24 (dd, *J* = 14.7, 10.7 Hz, 1H), 6.11 (t, *J* = 11.2 Hz, 1H), 5.78 (dd, *J* = 15.1, 6.3 Hz, 1H), 5.67 (dt, *J* = 9.3, 6.4 Hz, 1H), 5.61 – 5.54 (m, 1H), 5.37 (dtt, *J* = 11.0, 7.6, 1.7 Hz, 1H), 5.32 (t, *J* = 10.0 Hz, 1H), 4.22 (q, *J* = 6.3 Hz, 1H), 3.67 (s, 3H), 2.34 (tdd, *J* = 11.2, 7.1, 3.2 Hz, 4H), 2.27 (t, *J* = 7.6 Hz, 2H), 2.05 (qd, *J* = 7.3, 1.6 Hz, 2H), 1.76 – 1.53 (m, 6H), 1.38 – 1.20 (m, 34H), 0.88 (td, *J* = 6.9, 4.1 Hz, 6H).
^{13}C NMR-HSQC (150 MHz, $CDCl_3$) δ 173.83, 173.37, 136.99, 134.67, 133.93, 131.74, 130.58, 129.03, 127.75, 124.18, 72.07, 69.75, 51.69, 35.38, 34.67, 34.27, 34.20, 33.76, 32.06, 31.64, 29.83, 29.79, 29.78, 29.60, 29.57, 29.50, 29.41, 29.40, 29.38, 29.25, 29.20, 27.54, 25.10, 24.82, 22.82, 22.69, 20.67, 14.24, 14.18.

LRMS (ESI) calc. for $C_{39}H_{68}O_5Na$ $[M+Na]^+$: 639.50, obs. 639.49

(6*Z*,8*E*,10*E*,14*Z*)-12-((stearoyl)oxy)ecosa-6,8,10,14-tetraenoic acid (12-stearoyl acid ester LTB_4) (3)

Compound 19 (18.4 mg, 0.030 mmol, 1.0 equiv.) was dissolved in THF (0.6 ml). Next, MeOH (0.2 ml) and water (0.2 ml) were added in sequence. The mixture was move into ice bath and stirred for 5 minutes. Solid LiOH H_2O (2.50 mg, 0.060 mmol, 2.0 equiv.) was weight and added in one portion. The reaction was returned to 23°C and charged with argon. After most compound 19 was reacted monitored by TLC (DCM:MeOH=95:5), the reaction was titrated by saturated $Na_2H_2PO_4$ aqueous solution to pH=5. Then, water was added (1 ml), followed by adding diethyl ether (2 ml). The aqueous phase was extracted by diethyl ether three times (1 ml x3) and the organic parts were washed by brine (3 ml) and dried by Na_2SO_4. After crude product was concentrated under reduced pressure, a silica gel column was used to purify product using DCM/MeOH system (99:1 -95:5). This afforded white solid compound 3 (5.3 mg, 0.0088 mmol) with 29.5% yield. R_f=0.32 (DCM: Methanol=95:5)

^{1}H NMR (600 MHz, $CDCl_3$) δ 6.50 (dd, *J* = 14.7, 11.5 Hz, 1H), 6.33 – 6.25 (m, 1H), 6.19 (m, 1H), 6.07 (t, *J* = 11.2 Hz, 1H), 5.67 (dt, *J* = 15.2, 6.4 Hz, 1H), 5.49 (dt, *J* = 14.2, 7.4 Hz, 1H), 5.43 (t, *J* = 9.8 Hz, 1H), 5.31 (m, 2H), 4.64 – 4.58 (m, 1H), 2.46 – 2.32 (m, 4H), 2.29 (dd, *J* = 9.7, 5.4 Hz, 2H), 2.01 (q, *J* = 7.4 Hz, 2H), 1.81 – 1.50 (m, 6H), 1.38 – 1.17 (m, 36H), 0.88 (td, *J* = 6.9, 3.9 Hz, 6H).

^{13}C NMR-HSQC&HMBC (150 MHz, $CDCl_3$) δ 178.43, 173.32, 134.19, 133.95, 133.45, 132.34, 132.30, 130.36, 128.40, 123.56, 73.85, 67.84, 36.82, 34.82, 34.78, 33.92, 33.35, 32.08, 31.66, 29.86, 29.81, 29.77, 29.75, 29.64, 29.52, 29.47, 29.44, 29.35, 29.30, 29.22, 27.55, 25.16, 24.87, 22.85, 22.73, 20.69, 14.28, 14.22.

LRMS (ESI) calc. for $C_{38}H_{65}O_5$ [M-H]-:601.49, obs. 601.46

(6*Z*,8*E*,10*E*,14*Z*)-5-((stearoyl)oxy)ecosa-6,8,10,14-tetraenoic acid (5-stearoyl acid ester LTB_4) (2)

Compound 20 (13.1 mg, 0.021 mmol, 1.0 equiv.) was hydrolyzed using same method as compound 3. This afforded white solid compound 2 (4.8 mg, 0.0080 mmol) with 37.5% yield. R_f=0.27 (DCM: Methanol=95:5)

^{1}H NMR (600 MHz, $CDCl_3$) δ 6.58 (dd, *J* = 14.8, 11.4 Hz, 1H), 6.34 (dd, *J* = 15.2, 10.8 Hz, 1H), 6.24 (dd, *J* = 14.7, 10.8 Hz, 1H), 6.12 (t, *J* = 11.2 Hz, 1H), 5.79 (dd, *J* = 15.1, 6.2 Hz, 1H), 5.68 (dt, *J* = 9.3, 6.4 Hz, 1H), 5.62 – 5.54 (m, 1H), 5.41 – 5.29 (m, 2H), 4.22 (q, *J* = 6.5 Hz, 1H), 2.39 – 2.31 (m, 4H), 2.27 (t, *J* = 7.6 Hz, 2H), 2.08 – 2.02 (m, 2H), 1.75 (dtd, *J* = 14.8, 6.7, 3.5 Hz, 1H), 1.69 – 1.50 (m, 7H), 1.39 – 1.19 (m, 34H), 0.88 (td, *J* = 6.9, 3.9 Hz, 6H).

^{13}C NMR-HSQC&HMBC (150 MHz, $CDCl_3$) δ 178.47, 173.51, 137.17, 134.71, 134.16, 131.81, 130.64, 129.19, 127.81, 124.28, 124.28, 72.18, 69.84, 34.88, 34.36, 34.03, 33.99, 32.08, 29.86, 29.81, 29.79, 29.77, 29.74, 29.62, 29.59, 29.51, 29.47, 29.42, 29.39, 29.35, 29.31, 29.28, 29.22, 27.69, 24.85, 22.85, 14.27, 14.20.

LRMS (ESI) calc. for $C_{38}H_{65}O_5$ $[M-H]^-$:601.49, obs. 601.48

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