CYCLOPROPANE FATTY ACID DERIVATIVES: BIOSYNTHESIS, FUNCTION AND TOTAL SYNTHESIS

Reported by Yuxin Zhao February 28, 2002

INTRODUCTION

Cyclopropane-containing natural products are very unique owing to their unusual bonding and inherent ring strain (27.5 kcal/mol).[1] This strained ring has been found in naturally occurring terpenes, steroids, amino acids, fatty acids, alkaloids, and nucleic acids.[2] For example, chrysanthemic acid derivatives, produced in plants via isoprenoid pathway are precursors to potent insecticides. Many cyclopropane-containing non-natural compounds also have important biological activities. An example is Cipro, a powerful anti-anthrax drug. Cyclopropane fatty acids (CFA) comprise an interesting family of primary and secondary metabolites (Figure 1). Most CFA derivatives contain only one three-membered ring, and occur as phospholipids **1** in bacterial membranes. Biosynthetically, they are derived from the corresponding unsaturated fatty acid (UFA) phospholipids. The formation of the cyclopropane ring is catalyzed by CFA synthase. The genetics and physiological role of CFA synthase are currently under active investigation.[3]

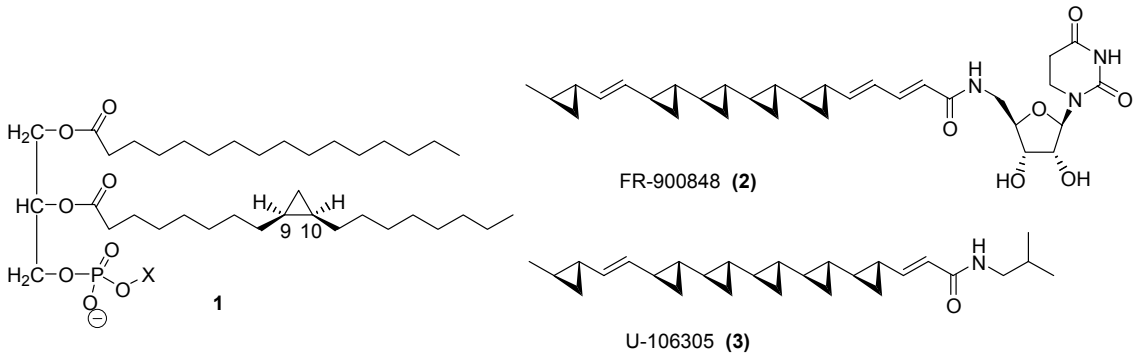


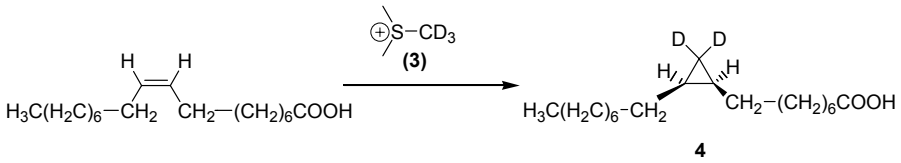
Figure 1. Cyclopropane Fatty Acid Derivatives

Two novel polycyclopropane fatty acid derivatives FR-900848 (**2**) and U-106305 (**3**) were recently isolated from fungal sources in 1990 and 1995.[4] While their biosynthetic origin and the properties of the related enzymes are still unclear, these poly CFA derivatives have attracted widespread synthetic interest owing to their novel structures. The enantioselective total syntheses of these two molecules were accomplished by Barret and Falck in 1996 using interative enantioselective Simmons-Smith reactions for the construction of the polycyclopropane system.[5] This abstract will focus on the biosynthesis and physiological function of mono CFA phospholipids as well as structures and asymmetric total synthesis of polyCFA derivatives.

BIOSYNTHESIS OF CYCLOPROPANE FATTY ACID (CFA)

The biosynthetic precursors of CFAs are unsaturated fatty acid (UFA) phospholipids, which in turn arise from acetyl CoA via the well-established polyketide pathway. The key step in CFA biosynthesis is the methylenation of the double bond in UFA. The source of the cyclopropane methylene carbon is the activated *S*-methyl group of *S*-adenosyl-L-methionine (SAM). A series of isotope labeling studies were carried out to elucidate the mechanism.[6] When *Lactobacillus plantarum* was cultured in the presence of SAM-methyl-d_3 (**3**), the CFA **4** isolated after hydrolysis contained deuterium located exclusively in the methylene group of the cyclopropane ring confirming that SAM is the methylene donor for the cyclopropanation process. Chiral methyl SAM incorporation experiments by Obrecht further revealed that the deprotonation of the cationic intermediate formed by the methylation of double bond proceeds in a stereospecific fashion[7] (Scheme 1).

Scheme 1. Deuterium Labeling Experiments on Mono CFA Biosynthesis [6, 7]



CYCLOPROPANE FATTY ACID SYNTHASE

The biosynthesis of CFA from UFA phospholipids is catalyzed by CFA synthases. Until recently very little was known about this enzyme owing to its high instability *in vitro*. Many attempts to purify CFA synthase using conventional procedures such as ion exchange and gel filtration failed. Cronan and coworkers found that the enzyme activity in crude extracts of *E. coli* was quite high, but that a dramatic loss of the activity occurred after ultracentrifugation. One possible explanation was that a stabilizing component in the crude extracts is lost upon ultracentrifugation. It is known that phospholipid vesicles formed during cell breakage are present in the crude extracts, and are removed by ultracentrifugation. Indeed, the first successful purification of the CFA synthase was accomplished by stabilizing the enzyme with phospholipid vesicles. This special purification procedure indicates that a specific protein-lipid interaction is required for the recognition and stabilization of the enzyme.[8]

It was found that when *E. coli* cells reach the stationary phase, 80% of the C_{16} UFA phospholipids are present as the corresponding CFA phospholipids. Since C_{16} UFA lipids are the major components of phospholipids on both leaflets of the membrane, CFA synthase seems to promote the cyclopropanation of double bonds of UFAs in both the inner and outer layers of cell membranes. Cronan and coworkers added trinitrobenzenesulfonic acid (TNBS), radiolabeled SAM, and acetate *in*

vitro to the standard mixture of CFA synthase and lipid vesicles. Under the conditions, TNBS reacts with the aminoethyl groups of outer membrane lipids but does not permeate into inner membrane. The reaction gave radioactive CFA both with and without the TNBS label, suggesting that CFA synthase can catalyze cyclopropanations on both sides of lipid bilayer.

Recently, the cloning of *E. coli cfa* gene was carried out in an effort to deduce the amino acid sequence of CFA synthase.[9] The enzyme was expressed using the T7 promoter/RNA polymerase system. The deduced amino acid sequence predicts a protein of 382 residues that lacks long hydrophobic segments. When compared with Genbank and Swiss Protein database, CFA synthase showed similarities to some other enzymes that also utilize SAM as cofactor. Two distinct motifs (GxGG and DxxxGxG) are present in both CFA synthase and over half of these known enzymes. Evaluation of the amino acid sequence revealed that the CFA synthase is a protein of only moderate hydrophobicity, which is consistent with its isolation from the cellular cytosol. The protein lacks any of the long hydrophobic regions usually found in membrane proteins. The detailed mechanism by which the enzyme gains access to the acyl chain double bonds on both leaflets of phospholipid vesicles remains unknown.

REGULATION OF THE CFA SYNTHASE GENE IN *E. COLI*

It is known that CFA phospholipids appear in the late exponential and early stationary phases of cell growth. In *E. coli*, a sharp increase of CFA synthase activity occurs in the early stationary phase and diminishes soon afterwards. Although many environmental changes such as pH and aeration degree can influence CFA formation,[10] genetics is believed to be the major factor in the regulation of CFA synthase activity.

Two functional promoters of the CFA synthase gene (*cfa*) have been identified. One promoter recognized by the σ^{70} subunit of RNA polymerase initiates the transcription of CFA synthase throughout all phases of cell growth. However, the other promoter recognized by the σ^{38} subunit of RNA polymerase becomes active only in the transition from the exponential phase to the stationary phase. These two factors suggest that the second promoter is essential for the rapid induction of CFA accumulation in the early stationary phase. The sigma factor σ^{38} is the product of the *rpoS* gene, which is responsible for the expression of a series of genes whose products accumulate in the stationary phase. Thus CFA formation is part of a complex regulation process influenced by an increased RpoS content in the early stationary phase.[11]

Another factor responsible for the regulation of CFA formation is the degradation of the CFA synthase. This could explain the sharp decline of CFA synthase after the induction in early stationary

phase. Pulse-chase labeling experiments of a biotin labeled CFA synthase showed a very short half-life of 5 min *in vivo*. Further results from tests with different strains of *E. coli* are consistent with the hypothesis that the diminished CFA synthase activity is mainly attributable to the instability of the enzyme itself. It was found that the degradation of this short-lived protein *in vivo* is dependent on the expression of the heat shock regulon. Preliminary research suggests that the corresponding energy-independent heat shock regulation protease is a novel enzyme.[12]

PHYSIOLOGICAL ROLE OF CYCLOPROPANE FATTY ACIDS

As discussed above, the majority of CFA phospholipids do not appear until the late exponential or early stationary phase of cell growth during which cyclopropanation of UFA phospholipids occurs. Thus this transformation can be regarded as a lipid modification during these specific cell-growing phases. Two hypotheses for the physiological role of CFA phospholipids have been proposed, including temporal energy storage and membrane fluidity modification. In both of these hypotheses, the formation of CFA phospholipids induces substantial physiological changes. On the other hand, Harley and coworkers proposed a contrary idea: rather than inducing dramatic physiological changes, CFA formation occurs to retain the physical properties of cell membrane during growth-phase transition. [13]

Since a relatively large amount of energy is consumed during CFA formation in bacterial membranes (each cyclopropanation costs three ATPs), early researchers hypothesized that the CFAs may act as the temporary energy storage sites. However, CFAs appear to be stable and less reactive than the corresponding UFA to oxidations by ozone or singlet oxygen.[14] This suggests that CFA is a stable membrane component rather than active energy source.

In another hypothesis, the function of CFAs is to modify membrane fluidity. It was proposed that the fluidity of bacteria membrane would change dramatically when UFA phospholipids are replaced by CFA counterparts owing to the loss of rigid double bond. However, NMR studies of acyl chain dynamics indicated that cyclopropane rings restrict the overall mobility and disorder of the acyl chain between the *cis* segment and polar head almost as effectively as a *cis* double bond.[15] Therefore, the overall membrane fluidity would not be influenced significantly upon the transformation from UFA to CFA phospholipids.

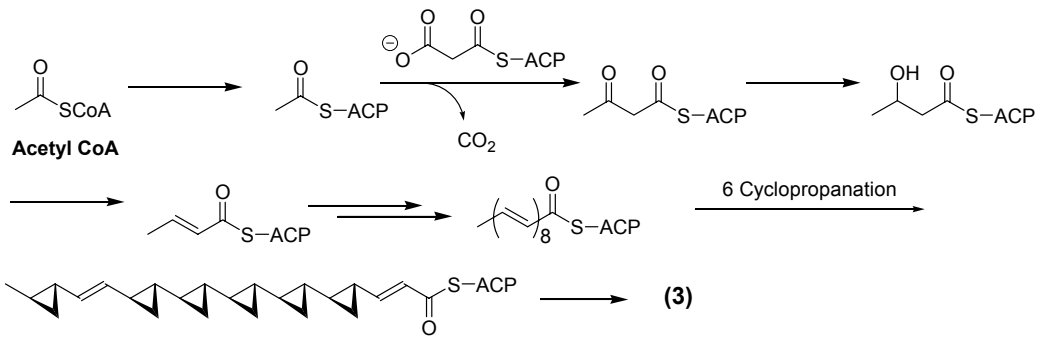
Harley and coworkers grew *E. coli* cells with various fatty acid compositions. The CFA-containing cells were found to survive better than UFA-containing cells upon treatment with hyperbaric oxygen.[16] Since both of the above hypotheses are inconsistent with experimental findings, Harley and coworkers proposed a different concept: the role of CFAs is to retain rather than alter the membrane properties during growth phase transition, which is beneficial for cell survival.

POLYCYCLOPROPANE FATTY ACID DERIVATIVES

Two polycyclopropane fatty acid derivatives, FR-900846 (**2**) and U-106305 (**3**) were isolated from *Streptoverticillium fervens* and *Streptomyces* in 1990 and 1995.[3] FR-900848 is a potent antibiotic against filamentous fungi, and U-106305 is an inhibitor of cholesteryl ester transfer protein (CETP). The structures and relative configurations of these two compounds were determined by chemical degradation with ozonolysis and NMR analysis. The absolute configurations were established by X-ray crystallographic analysis and total synthesis. [17]

Kou and coworkers studied the biosynthesis of U-106305 by ^{13}C labeling experiments.[3] When [1-^{13}C]acetate was fed to the culture, the corresponding ^{13}C enrichment was found at odd number positions of fatty acid backbone; correspondingly, ^{13}C enrichment was found at even number positions when [2-^{13}C]acetate was fed. Furthermore, ^{13}C enrichment in the cyclopropane methylene group was detected when ^{13}C labeled SAM was used as the cofactor. These results are consistent with a mechanism in which the fatty acid backbone is formed from acetyl CoA via polyketide pathway and SAM serves as the source of cyclopropane methylene carbon (Scheme 2). However, the intermediates, the timing of the cyclopropanation steps, and the origin of stereoselectivity are still unknown. In addition, the polycyclopropane fatty acid synthase (PCFA) has not yet been purified nor has the gene sequence of this enzyme (or enzymes) been determined.

Scheme 2. Biosynthesis of Poly CFA Derivative U-106305[3b]

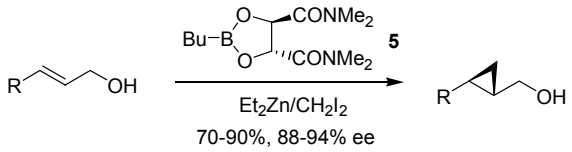


SYNTHESIS OF CYCLOPROPANE FATTY ACID DERIVATIVES

Total synthesis of poly CFAs includes two major tasks: the asymmetric cyclopropanations and fatty acid chain construction. Several methods are available for synthesis of cyclopropane rings from olefin precursors such as carbene addition with diazomethane/palladium acetate, olefin-diazo ester cyclopropanation, and the Simmons-Smith reaction. Among them, a modified enantioselective

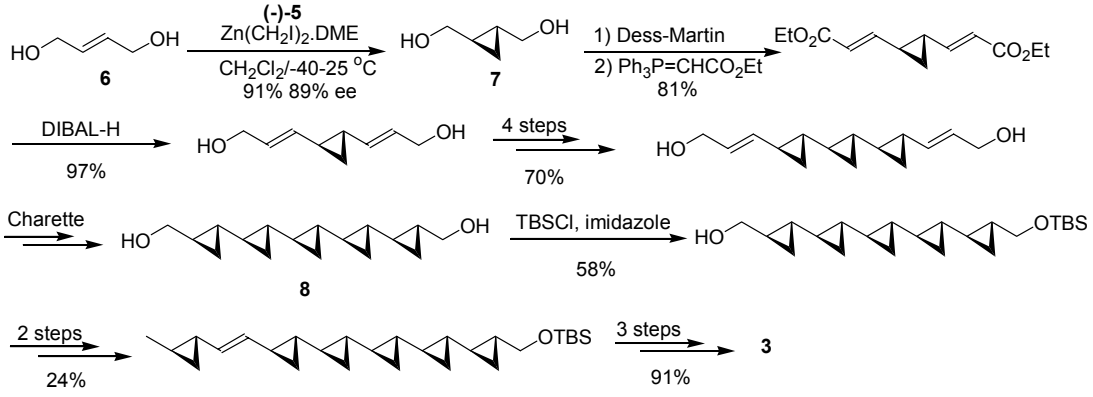
Simmons-Smith cyclopropanation procedure developed by Charette in 1994[18] was often employed recently in polycyclopropane fatty acid synthesis. In this reaction, the hydroxyl directed cyclopropanation of allylic alcohols is accomplished in high yield and excellent enantioselectivity with the chiral amphoteric bifunctional boronate ester **5**, diiodomethane, and diethyl zinc (Scheme 3).

Scheme 3. Charette Asymmetric Cyclopropanation[17]



Several different total syntheses of FR-900848 and U-106305 have been reported to date, all of which involved Charette cyclopropanation as the key step. The major differences were the strategy for fatty acid chain formation from individual cyclopropane precursors. In the first total synthesis of U-106305, Barret and coworkers employed asymmetric Simmons-Smith and Wittig reactions for the construction of the cyclopropane fatty acid side chain (Scheme 4). Enantioselective cyclopropanation of diol **6** with the reagent combination of Charette's dioxaborolane, CH_2I_2, and Et_2Zn gave **7** in 91% yield and good stereoselectivity (89% ee). Further carbon chain elongation by Wittig reactions and two subsequent asymmetric cyclopropanations afforded the C_2-symmetric pentacyclopropane diol **8**. Selective protection of one diol hydroxyl group followed by Wittig reaction was accomplished to attach the terminal cyclopropane ring. Reduction and formation of the isobutyl amide gave U-106305 in 5.5% overall yield over 15 steps.

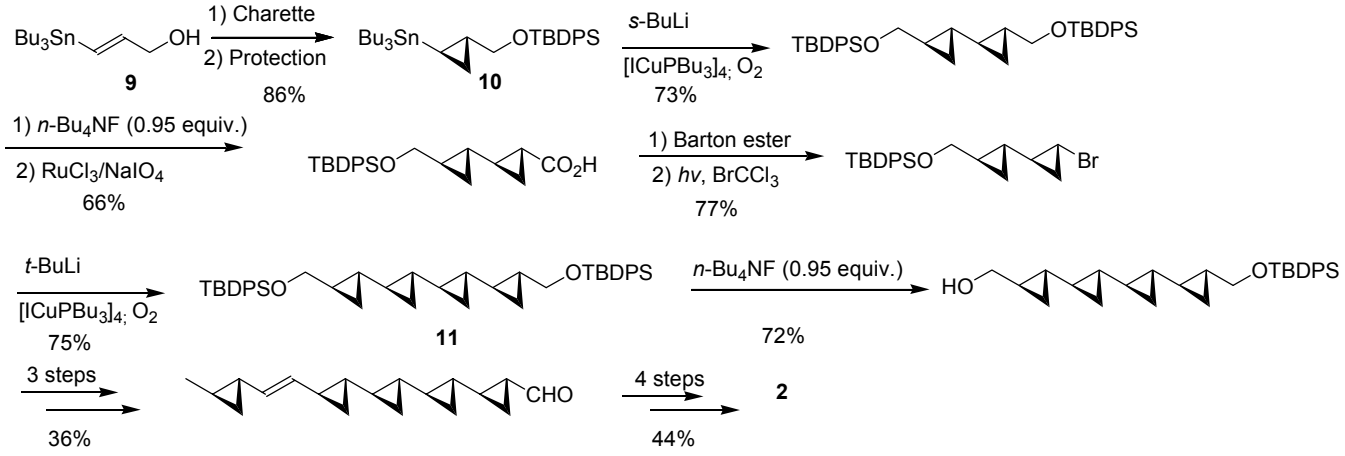
Scheme 4. Total Synthesis of U-106305 by Barret and Coworkers[5a]



In the total synthesis of FR-900848 (Scheme 5), Falck and coworkers took a different approach. A successive dimerization strategy was employed to construct the four contiguous cyclopropane rings

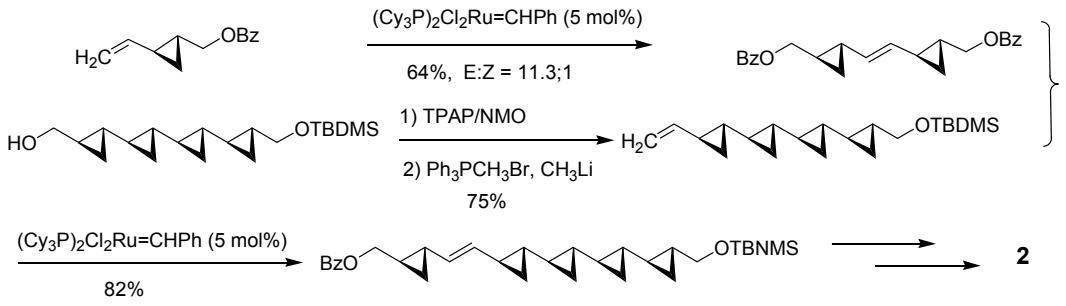
beginning with stannanyl alcohol **9**. The Charette procedure was again used for the asymmetric cyclopropanation of **9**. Two successive dimerizations of stannane **10** using *sec*-BuLi and $[ICuPBu_3]_4$ afforded the *trans*-tetracyclopropane compound **11** in 24% yield and excellent stereoselectivity. The synthesis of fatty acid chain was completed in another 9 steps including Mitsunobu condensation, Peterson type olefination, and Horner-Emmons homologation. The natural product FR-900848 was obtained by coupling of the corresponding carboxylic acid with dihydro-uridine.

Scheme 5. Total Synthesis of FR-900848 by Falck and Coworkers[5b]



Olefin cross-metathesis was also used in the synthesis of poly CFA compounds. Zercher and coworkers carried out the formal synthesis of FR-900848 using a combination of asymmetric Simmons-Smith reaction and Ru-catalyzed olefin metathesis (Scheme 6).[16] In this approach, two vinyl cyclopropane groups were connected via olefin metathesis using Grubbs' catalyst.

Scheme 6. Key Steps of FR-900848 Synthesis Using Olefin Cross-Metathesis



CONCLUSION

CFA derivatives show intriguing biological activities and they offer synthetic challenges. Mono CFA phospholipids play a very important physiological role in bacterial membranes. Although most of the genes associated with CFA biosynthesis have been identified and their functions are understood, much work still needs to be done to elucidate the biological functions of CFAs. Poly CFA derivatives mainly act as antibiotics or inhibitors of membrane enzymes. Besides their biological activity, the active research on the different approaches to synthesize these poly CFA derivatives will continue to be an attractive area for organic chemists.

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