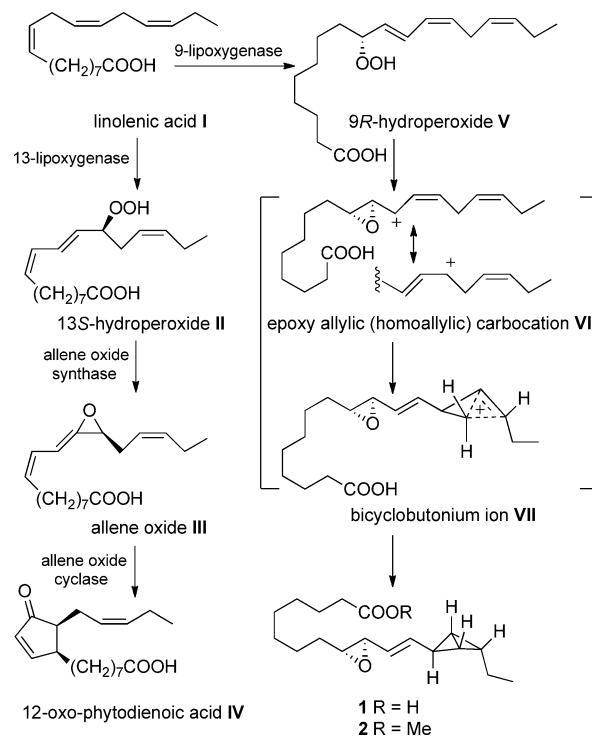


Synthesis of a Bicyclobutane Fatty Acid Identified from the Cyanobacterium *Anabaena* PCC 7120**

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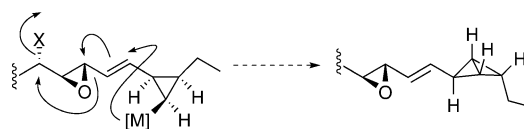
Fatty acids and their metabolites play critical roles in mammalian and nonmammalian cell biology as signaling molecules, components of membranes, and storage lipids.^[1] Enzymatic oxidation of polyunsaturated fatty acids leads to structurally diverse metabolites including unstable products such as allene oxides, divinyl ethers, and endoperoxides.^[2,3] For example, in plants 12-oxophytodienoic acid **IV** is produced by an initial oxidation of linolenic acid by 13-lipoxygenase, followed by a transformation to an unstable allene oxide **III** (Scheme 1).^[4] In 1988 Brash and co-workers showed that brief exposure of 13*S*-hydroperoxide **II** to a preparation of an allene oxide synthase followed by rapid organic extraction and treatment with diazomethane resulted in the isolation and characterization of the delicate allene oxide **III**.^[5] The advent of whole genome sequencing of microbes has further expanded the discovery of novel enzymatic transformations and characterization of unstable products.^[6,7] More recently, Brash and co-workers^[8] identified, by genomic analysis, a dual-function protein encoded in the cyanobacterium *Anabaena* PCC 7120; this protein consists of a lipoxygenase domain fused to a catalase-related domain. After in vitro reconstitution of this protein, linolenic acid (**I**) was examined as an enzyme substrate and found to yield bicyclobutane fatty acid **1**, the structure of which was confirmed by the characterization of methyl ester **2**. The novel two-step transformation was proposed to occur by lipoxygenase-mediated C9 oxidation (**I**→**V**, Scheme 1) followed by cyclization within the neighboring catalase-related domain to bicyclobutane **1** via intermediate bicyclobutonium ion **VII**. While ample precedent existed for the rearrangement of 9*R*-hydroperoxide **V** to an epoxy allylic carbocation,^[9] the formation of a bicyclobutonium ion **VII** leading to the bicyclo[1.1.0]butane ring system was unprecedented. Interestingly, cyclopropyl carbinyl cations have been implicated as intermediates in the biosynthesis of oxylipins.^[10] The



Scheme 1. Lipoxigenase/catalase-mediated modifications of linolenic acid in plants and cyanobacteria.

unique structure of bicyclobutane fatty acid **1** and its unknown bioactivity drew our attention, and lead to the total synthesis of bicyclobutane **2** described herein.

The high strain energy (66 Kcal) and acid lability of bicyclo[1.1.0]butanes makes them challenging structures for synthesis and isolation.^[11] Indeed, the parent bicyclobutane fatty acid (**1**) was not directly isolable, thus esterification with diazomethane in anhydrous solvent was required followed by careful purification of the methyl ester **2** at pH 8. When considering the synthesis of **2** we recognized that the installation of the delicate array of a vinyl epoxide conjugated to a strained bicyclobutane would be best achieved simultaneously, as illustrated in Scheme 2. The proposed reaction cascade^[12,13] starts with the generation of an all-*cis* metallated cyclopropane, followed by an S_N2' opening of the neighboring



Scheme 2. Proposed cascade reaction leading to bicyclobutane **2**.

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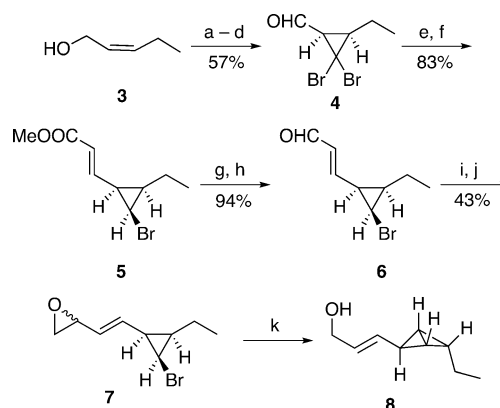


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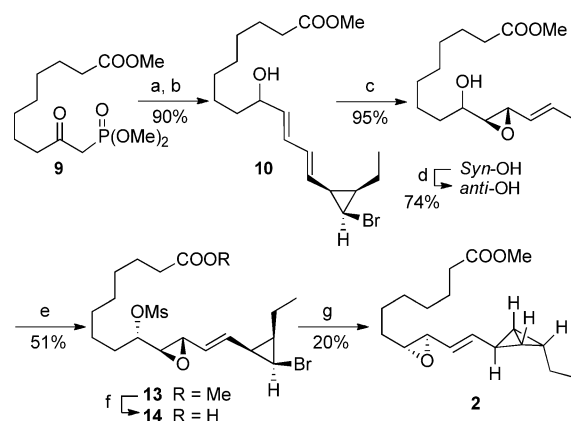
vinyl epoxide with release of an alkoxide appropriately oriented for displacement, to deliver the desired *trans* epoxide.

Precedent^[14,15] for the S_N2' opening of a vinyl epoxide by a cyclopropyl metal to form a bicyclobutane ring system lead us to initially examine the cyclization of vinyl epoxide **7** (Scheme 3). Preparation of **7** started with dibromocyclopropane addition to the TBS ether, which was derived from *cis*-2-pentenol (**3**), followed by desilylation and oxidation to afford aldehyde **4**. The Horner–Wadsworth–Emmons olefination of **4** and a subsequent stereoselective reduction of the *exo*-bromo group using triphenylstannane and triethylborane/oxygen^[16] as an initiator at low temperature afforded all-*cis* bromocyclopropane **5** in 83% yield.^[17] Conversion of ester **5** into aldehyde **6** was achieved by a standard two-step reduction/oxidation sequence. Aldehyde methylenation was accomplished using the protocol reported by Matteson and Sadhu,^[18] starting with the addition of (chloromethyl)lithium to **6**. The chlorohydrin products were treated with NaH in THF to give a 2:1 mixture of diastereomers (**7**); these diastereomers reacted convergently to give bicyclobutane **8** upon cyclization initiated by lithium–halogen exchange (*n*BuLi, diethyl ether, –78°C). Attempts to purify bicyclobutane **8** by chromatography led to decomposition, but ¹H and ¹³C NMR analysis of crude **8** was in full agreement with the assigned structure, as relevant NMR signals of fatty acid bicyclobutane **2** and bicyclobutane **8** coincided.^[8,19]

Having demonstrated the key carbanion-mediated cyclization we turned our attention to the synthesis of bicyclobutane fatty acid **2**, starting with the condensation of β-keto phosphonate **9**^[20] and aldehyde **6** (Scheme 4). A Luche^[21] reduction of the resulting keto ω-ester gave allylic alcohol **10**. Epoxidation of **10** using either metal-catalysis or oxidation using a peracid^[22] failed to afford the desired epoxy alcohol and led instead to presumed acid-promoted decomposition. Oxidation using dimethyldioxirane^[23] provided the desired epoxide as a 1:1 mixture of the *syn* (**11**) and the desired *anti* (**12**) epoxy alcohols.^[24] The *syn* isomer (**11**) was converted into the required *anti* diastereomer (**12**) using the Mitsunobu two-step alcohol inversion protocol.^[25] Mesylation of alcohol **12** provided sulfonate **13**, poised for the key cyclization cascade. Employing reaction conditions that were successful for the conversion of epoxide **7** into bicyclobutane **8** (*n*BuLi then CuI·2LiCl) resulted in only decomposition of **13**. Addition of mesylate **13** to a solution of *n*BuLi and subsequent warming from –78 to –20°C lead to bicyclobutane formation, as determined by ¹H NMR analysis, but the epoxide formation did not occur, and there was an accompanying loss of the methyl ester group. However, treatment of **13** with 4 equivalents of *tert*-butyllithium in THF at –78°C did lead to the desired reaction cascade, unfortunately the cyclization was accompanied by conversion of the terminal methyl ester into the corresponding *tert*-butyl ketone. Altering the number of equivalents of *tert*-butyllithium did not provide a satisfactory solution, but by using the corresponding carboxylic acid **14** the formation of the *tert*-butyl ketone was avoided and the desired methyl ester was obtained after treatment of the crude product with diazomethane.^[26] Attempts to isolate the parent fatty acid **1** failed, as observed in the original isolation



Scheme 3. Synthesis of bicyclobutane **8**. a) TBSCl, Et₃N, CH₂Cl₂, RT, 94%; b) CHBr₃, BnEt₃NCl, 50% NaOH (aq), RT, 80%; c) TBAF, THF, RT, 95%; d) SO₃·Pyr, DMSO, *i*Pr₂EtN, CH₂Cl₂, –20°C, 79%; e) (EtO)₂P(O)CH₂CO₂CH₃, NaH, THF –78°C, 87%; f) Et₃B, Ph₃SnH, PhMe, –78°C, 95%; g) DIBAL-H, CH₂Cl₂, –78°C, 95%; h) MnO₂, CH₂Cl₂, RT, 99%; i) ICH₂Cl, *n*BuLi, THF, –78°C, 51%; j) NaH, THF, –78°C, 84%; k) i. *n*BuLi, THF, –78°C, ii. [CuI·2(LiCl)], THF, –78 to –20°C. DIBAL-H = diisobutylaluminum hydride, DMSO = dimethylsulfoxide, Pyr = pyridine, TBAF = tetra-*n*-butylammonium fluoride, TBS = *tert*-butyldimethylsilyl, THF = tetrahydrofuran.



Scheme 4. Synthesis of bicyclobutane **2**. a) KHMDS, THF, –78°C → RT, then **6**, 92%; b) NaBH₄, CeCl₃, MeOH, RT, 98%; c) DMDO, CH₂Cl₂, 0°C, 95%; d) PPh₃, DIAD, *p*-nitrobenzoic acid, RT then K₂CO₃, MeOH, 74%; e) MsCl, Et₃N, DMAP, CH₂Cl₂, 0°C to RT, 56%; f) LiOH (aq), THF, 60°C; g) *t*BuLi, THF, –78 to –20°C, then CH₂N₂, 20%. DIAD = diisopropyl azodicarboxylate, DMAP = 4-dimethylaminopyridine, DMDO = dimethyl dioxirane, HMDS = hexamethyldisilazide, Ms = methanesulfonyl.

work.^[8] A solution of methyl ester **2** in [D₆]DMSO was estimated to have a half life of 3 days.

In conclusion we have developed a 13-step synthesis of bicyclobutane fatty acid methyl ester (±)-**2**. Other related structurally novel and/or unstable lipids such as thromboxane A₂ and pentacycloanammoxic acid (ladderane) have important biological functions.^[1,27] Future work will be directed toward the preparation of the unstable parent fatty acid **1** and studies aimed at defining the biological properties of this unusual oxylipin natural product.

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