Natural Products

DOI: 10.1002/ange.201104366

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

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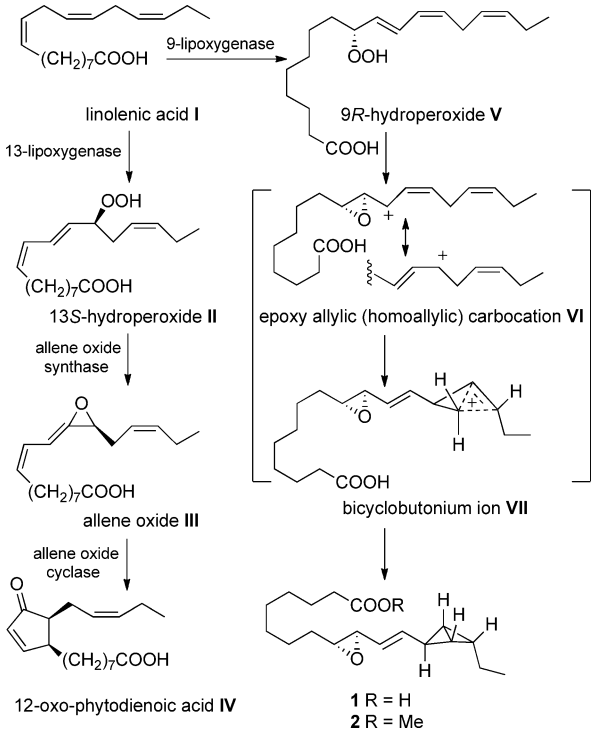
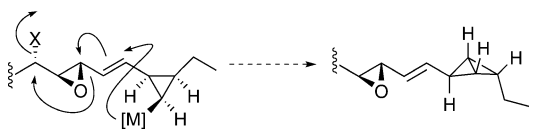
Fatty acids and their metabolites play critical roles in mammalian and nonmammalian cell biology as signaling molecules, components of membranes, and storage lipids.[1] Enzymatic oxidation of polyunsaturated fatty acids leads to structurally diverse metabolites including unstable products such as allene 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 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.



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

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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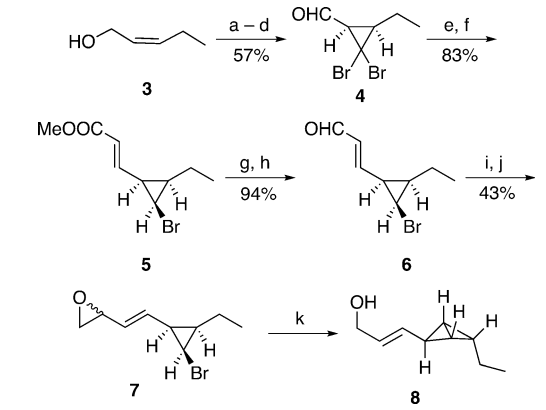
[**] We thank the National Institutes of Health (5RO1CA059515) for support of this research. S.M.D. acknowledges the support of the Vanderbilt Chemical Biology Interface (CBI) training program (T32 GM065086). We gratefully acknowledge Prof. Alan Brash for valuable discussion.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201104366.

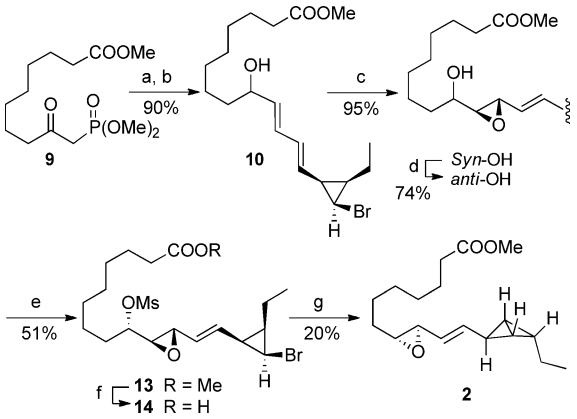
vinyl epoxide with release of an alkoxide appropriately oriented for displacement, to deliver the desired *trans* epoxide.

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 dibromocarbene 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 ^{1}H and ^{13}C 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]



Scheme 3. Synthesis of bicyclobutane **8**. a) TBSCl, Et_3N, CH_2Cl_2, RT, 94%; b) $CHBr_3$, $BnEt_3NCl$, 50% NaOH (aq), RT, 80%; c) TBAF, THF, RT, 95%; d) SO_3·Pyr, DMSO, iPr_2EtN, CH_2Cl_2, −20 °C, 79%; e) $(EtO)_2P(O)CH_2CO_2CH_3$, NaH, THF −78 °C, 87%; f) Et_3B, Ph_3SnH, PhMe, −78 °C, 95%; g) DIBAl-H, CH_2Cl_2, −78 °C, 95%; h) MnO_2, CH_2Cl_2, RT, 99%; i) ICH_2Cl, *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 = diisobutylaluminium hydride, DMSO = dimethylsulfoxide, Pyr = pyridine, TBAF = tetra-*n*-butylammonium fluoride, TBS = *tert*-butyldimethylsilyl, THF = tetrahydrofuran.

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·2 LiCl) 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 ^{1}H 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 4. Synthesis of bicyclobutane **2**. a) KHMDS, THF, −78 °C→RT, then **6**, 92%; b) $NaBH_4$, $CeCl_3$, MeOH, RT, 98%; c) DMDO, CH_2Cl_2, 0 °C, 95%; d) PPh_3, DIAD, *p*-nitrobenzoic acid, RT then K_2CO_3, MeOH, 74%; e) MsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to RT, 56%; f) LiOH (aq), THF, 60 °C; g) *t*BuLi, THF, −78 to −20 °C, then CH_2N_2, 20%. DIAD = diisopropyl azodicarboxylate, DMAP = 4-dimethylaminopyridine, DMDO = dimethyl dioxirane, HMDS = hexamethyldisilazide, Ms = methanesulfonyl.

work.[8] A solution of methyl ester **2** in [D_6]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 A_2 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.

Received: June 23, 2011
Published online: September 7, 2011

Keywords: cascade reaction · cyclization · lipids · natural products · strained molecules

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[24] For simplicity only one *syn* (**11**) and one *anti* (**12**) diastereomer is represented in Scheme 4. Upon cyclization (via mesylate **13**), both *anti* diastereomers (**12a** and **12b**) react convergently to give **2** with formation of the achiral [1.1.0]bicyclobutane ring. When starting from the assigned *syn* diasteromers (**11**) only decomposition was observed under the same reaction conditions. This observation supports the assigned relative stereochemistry.
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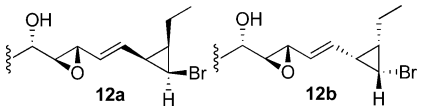

[26] The yield of the crude product **2** was near quantitative (see the Supporting Information for a ^{1}H NMR spectrum of the crude product **2**). Extensive decomposition occurs upon purification leading to a yield of 20 %.
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