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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-

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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.

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

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]

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 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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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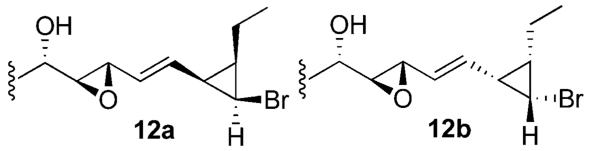

26. The yield of the crude product **2** was near quantitative (see the Supporting Information for a ^{1}H NMR spectrum of the crude product **2**). Extensive decomposition occurs upon purification leading to a yield of 20%.
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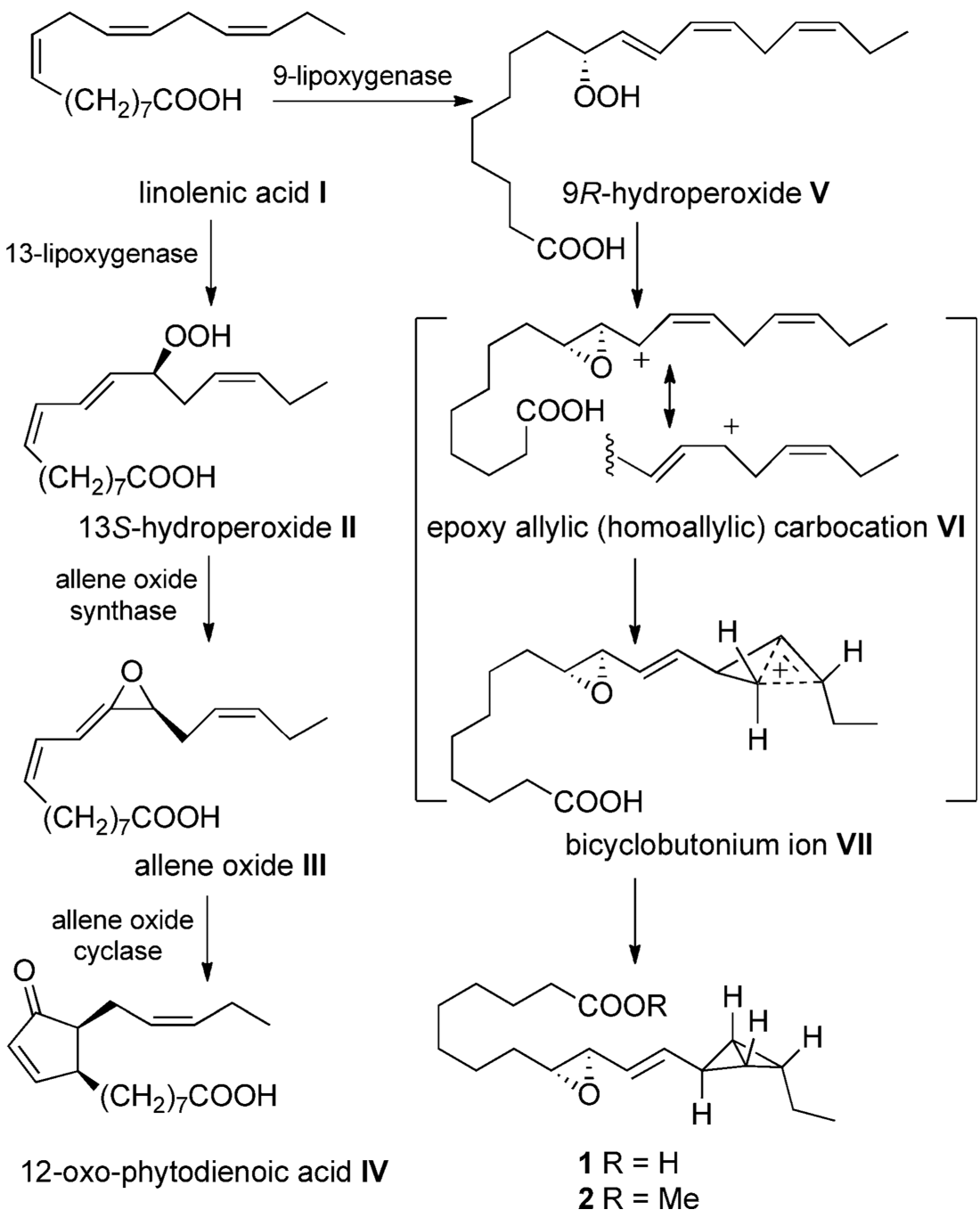
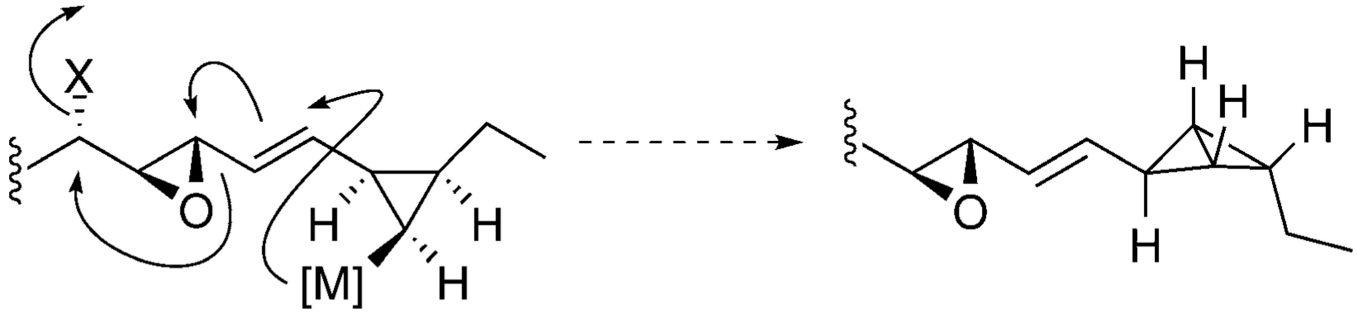
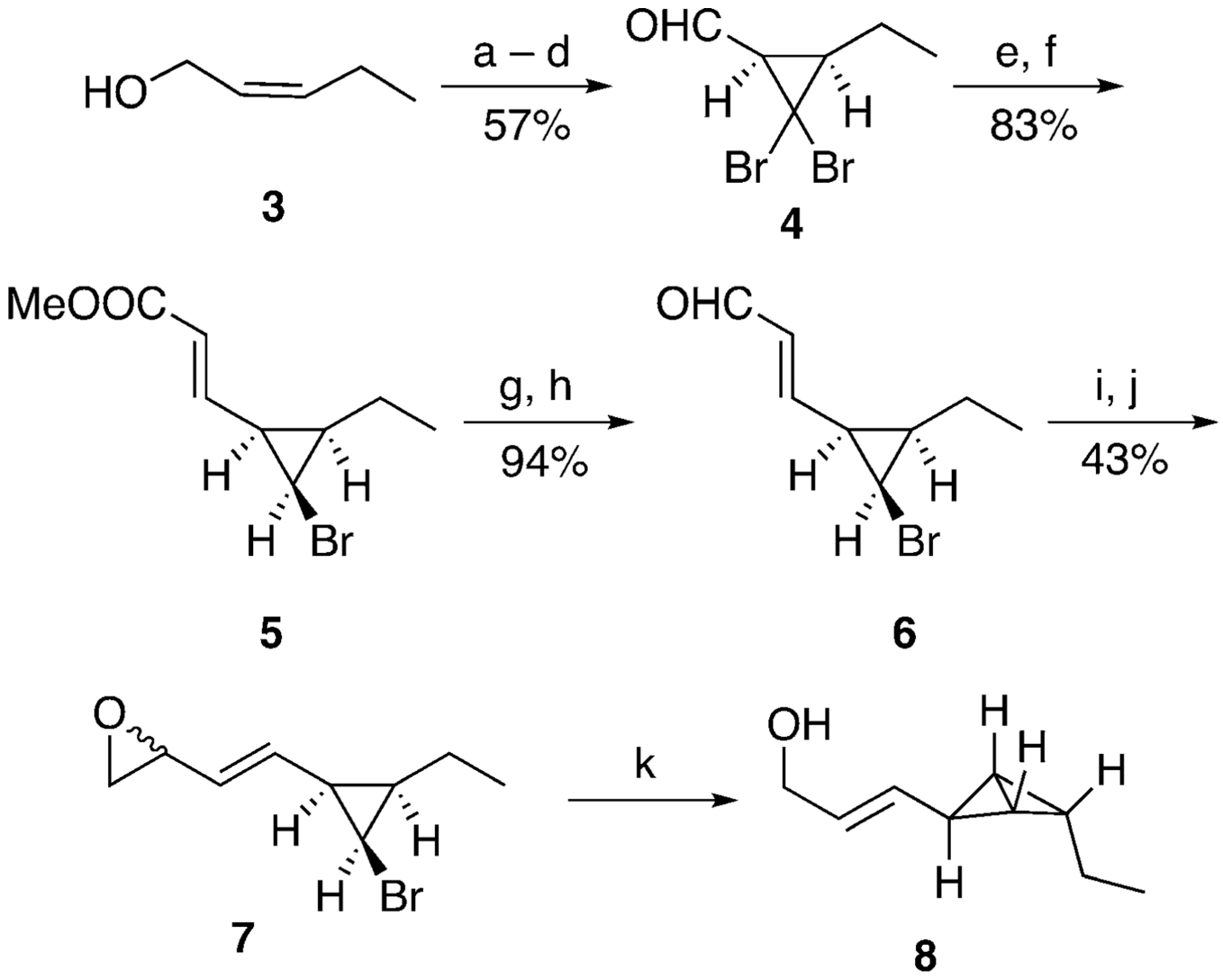


Scheme 1.
Lipoxygenase/catalase-mediated modifications of linolenic acid in plants and cyanobacteria.

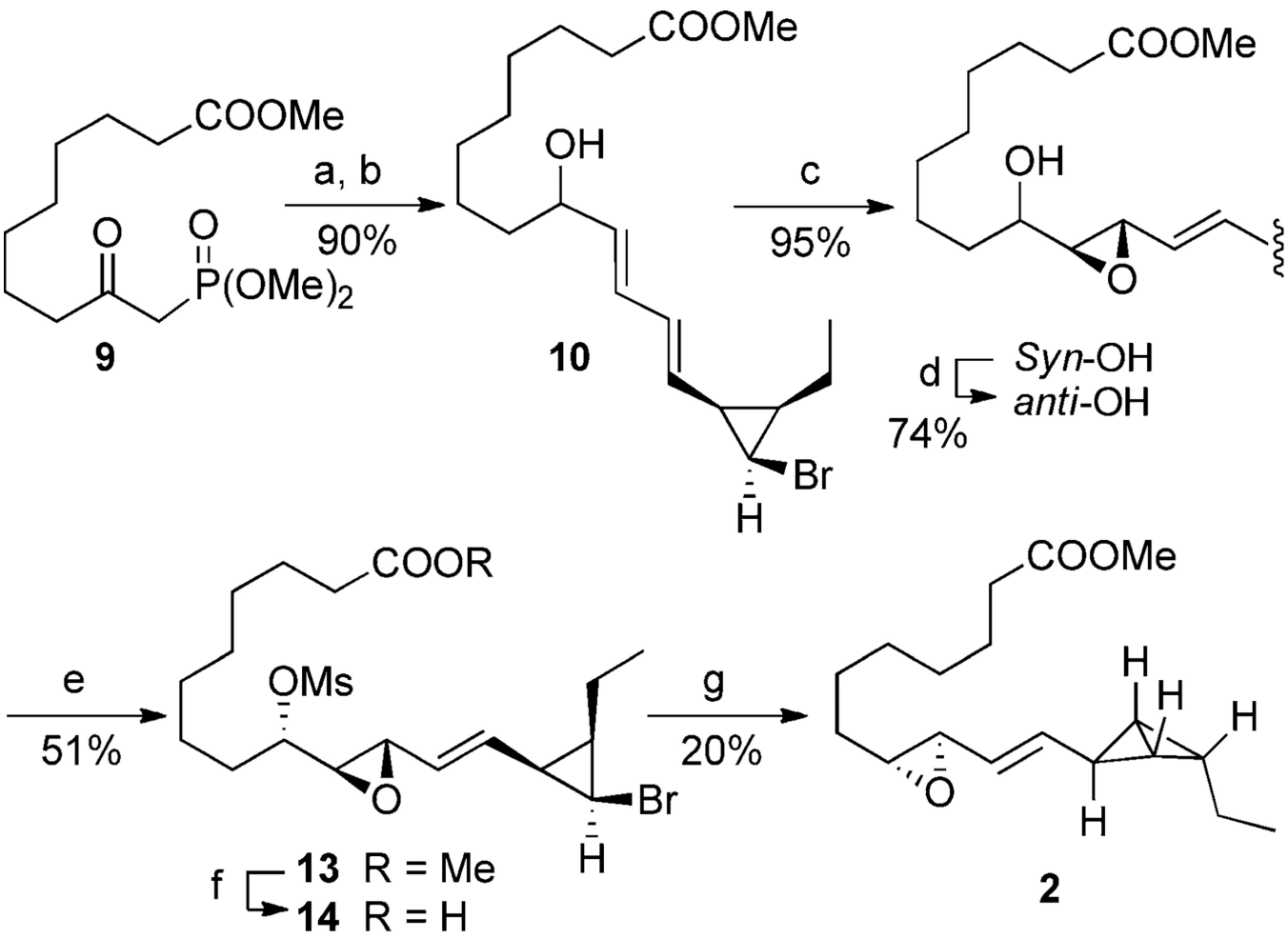


Scheme 2.
Proposed cascade reaction leading to bicyclobutane **2**.



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.



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.