ORGANIC LETTERS

2006
Vol. 8, No. 5
927−930

Total Synthesis and Stereochemical Assignment of the Spiroisoxazoline Natural Product (+)-Calafianin[1]

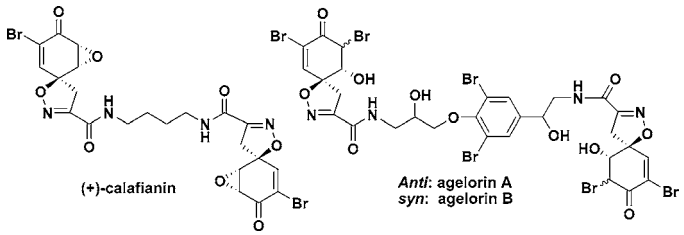
Sujata Bardhan, Daniel C. Schmitt, and John A. Porco, Jr.*

Department of Chemistry and Center for Chemical Methodology and Library Development, Boston University, 590 Commonwealth Avenue, Boston, Massachusetts 02215

porco@bu.edu

Received December 22, 2005

ABSTRACT



Synthesis of the spiroisoxazoline natural product (+)-calafianin is reported using asymmetric nucleophilic epoxidation and nitrile oxide cycloaddition as key steps. Synthesis and spectral analysis of all calafianin stereoisomers led to unambiguous assignment of relative and absolute stereochemistry.

The bromotyrosine-derived spiroisoxazolines are a structurally diverse class of physiologically active natural products. The family contains a number of structural types, including both monomeric and dimeric compounds derived from brominated tyrosine precursors (Figure 1).[2] A subset of molecules in this class (cf. aerothionin, **1**) possesses a brominated spiro-cyclohexadienyl isoxazoline core.[3] Noteably, an oxidized derivative, 11-hydroxyaerothionin, has been shown to inhibit *Mycobacterium tuberculosis*.[4] Additional members include (+)-calafianin (**3**),[5] bearing a bromo epoxy ketone moiety, and agelorins A and B (**4**, **5**),[6] which possess *anti*- and *syn*-bromohydrins, respectively. It is noteworthy that the agelorins were co-isolated with the natural product 11-*epi*-fistularin-3[7] (**2**), implying a biogenetic relationship

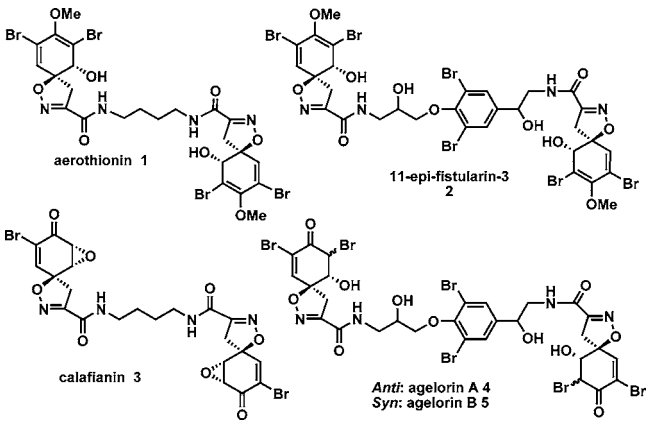


Figure 1. Dimeric spiroisoxazoline natural products.

(1) Presented in part at the 230th American Chemical Society National Meeting, Washington, D.C., Aug 28−Sep 1, 2005; ORGN abstract 027.

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10.1021/ol053115m CCC: $33.50
Published on Web 02/04/2006

between aerothionin- and agelorin-type natural products. Previous synthetic studies toward the spiroisoxazoline alkaloids include oxidative cyclization of phenolic oxime esters[8] as well as Diels−Alder cycloaddition of a chiral cyclopentadiene to produce enantiomerically pure cyclohexadienones.[9] A recent synthesis and structural revision of racemic calafianin[10] utilized acidic hydrolysis of an aerothionin- to an agelorin-type core structure.[11] In this paper, we report the total synthesis and stereochemical assignment of (+)-calafianin **3** and a derived agelorin-type structure featuring asymmetric nucleophilic epoxidation and nitrile oxide cycloaddition as key steps.

Our retrosynthesis is illustrated in Figure 2. We envisioned the preparation of agelorin core structure **6** from epoxy ketone **7** via regio- and stereoselective bromohydrin formation.[9] Compound **7** may be obtained from nitrile oxide cycloaddition[12] of vinyl epoxide **8** to afford the spiroisoxazoline core followed by acetal hydrolysis. We planned to utilize zirconium-catalyzed ester amide exchange methodology developed in our laboratory[13] to convert monomeric spiroisoxazolines to both monomeric and dimeric calafianin/agelorin structures.

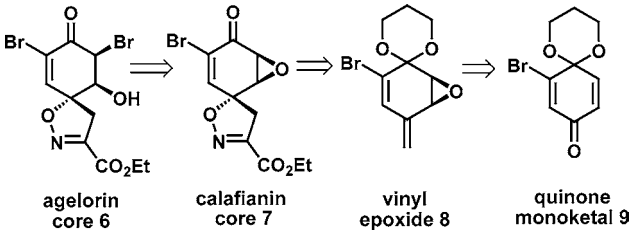
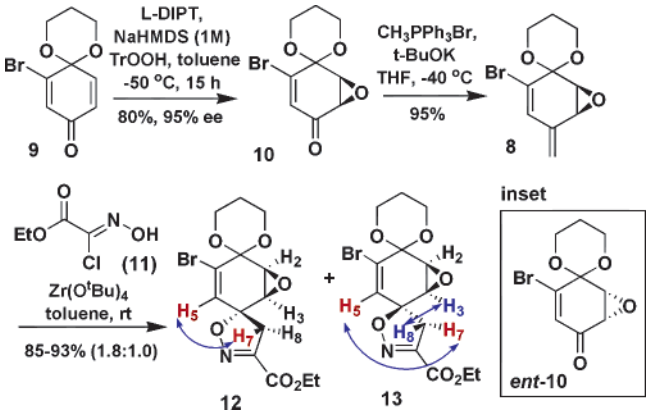


Figure 2. Retrosynthetic analysis.

The synthesis of (−)-calafianin core precursor **7** was initiated by enantioselective tartrate-mediated nucleophilic epoxidation[14] of the readily available quinone monoketal **9**[15] (Scheme 1). We have also prepared *ent*-**10**[16] in high yield

Scheme 1. Spiroisoxazoline Core Construction[a]



[a] Arrows indicate key NOE enhancements observed for **12** and **13**. See the Supporting Information for details.

and enantioselectivity (82%, 98% ee) using D-DIPT as chiral controller.[15] We next considered methylenation of epoxy ketone **10** to prepare the exocyclic vinyl epoxide **8**.[17] However, Wittig olefination proved to be challenging due to the lability of both starting material and vinyl epoxide product. Accordingly, methyltriphenylphosphonium halides were evaluated in conjunction with various bases, solvent, and concentrations at low temperature.[18] Use of lithium-derived bases (e.g., ^{n}BuLi, LiHMDS) led to considerable decomposition presumably due to epoxide chelation effects. An optimal condition was found for olefination employing KO^{t}Bu as base and methyltriphenylphosphonium bromide (THF, 0.03 M, −40 °C). This method was found to be practical for production of gram quantities of key intermediate **8**.

We next explored 1,3-dipolar cycloaddition of vinyl epoxide **8** for spiroisoxazoline construction.[19] Dipolar cycloaddition (Scheme 1) using a nitrile oxide generated in situ from ethyl chlorooximinoacetate **11** and diisopropylethylamine[20] led to significant formation of a furoxan dimer with low yields of the desired spiroisoxazolines **12** and **13**. Use of DBU[21] or inorganic bases such as $KHCO_3$[22] or

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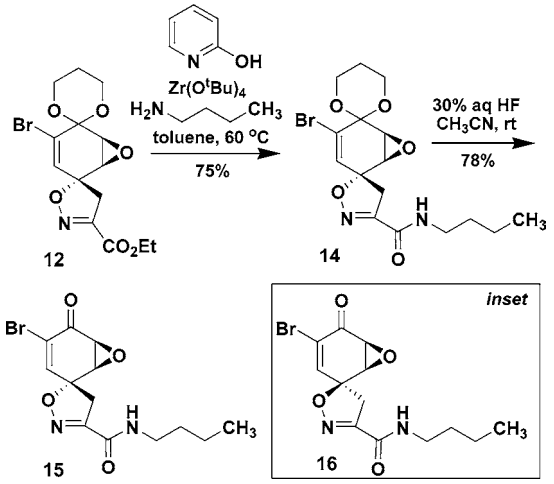
$NaHCO_3$ cleanly afforded spiroisoxazolines albeit in low yields and moderate selectivity. Recently, Kobayashi and coworkers have reported use of Zr(IV) alkoxides for highly stereoselective [3 + 2] cycloaddition of acylhydrazones and alkenes.[23] We thus considered use of group IV metal alkoxides for 1, 3-dipolar cycloaddition of nitrile oxides with alkenes. After considerable experimentation, we found use of $Zr(O^tBu)_4$ to be optimal, affording a moderate to high yield (85−93%) of **12**/**13** (dr = 1.8:1) and minimal formation of the furoxan dimer. Key NOE enhancements were observed between diagnostic protons (cf. Scheme 1) to enable assignment of the *syn* and *anti* spiroisoxazoline diastereomers. $Zr(O^tBu)_4$ apparently serves as a mild base which slowly deprotonates the ethyl chlorooximinoacetate and hence alleviates need for slow addition via syringe pump.

We next prepared the corresponding amides from both diastereomeric spiroisoxazolines obtained from 1, 3-dipolar cycloaddition. In light of literature precedent citing low yields[9c,10] (13−30%) for conventional diamidation of spiroisoxazoline carboxylic acids due to facile decarboxylation, we evaluated our recently developed catalytic ester−amide exchange methodology[13] in model amidations (Scheme 2).

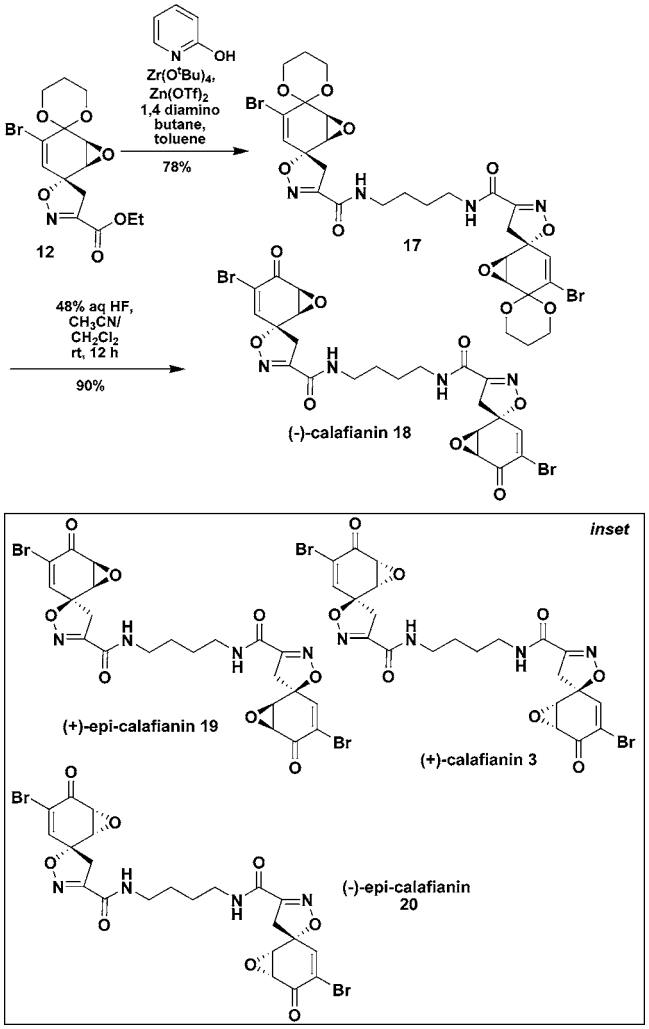
Scheme 2. Synthesis of Model, Monomeric Epoxyketone Spiro Isoxazolines



Use of nucleophilic activators such as HOBt and HOAt with 10 mol % $Zr(O^tBu)_4$ afforded low to moderate yields with difficult separation of the activator from the desired amide product. After evaluation of other activators, 2-hydroxypyridine (2-HYP)[24] afforded a 75% yield of **14** with facile separation of the activator. The protected monoamide **14** was efficiently deprotected using aqueous HF to afford amide **15** (Scheme 2). The corresponding diastereomer **16** was prepared using an analogous procedure from *syn*-cycloadduct **15**.

The synthesis of calafianin commenced with an extrapolation of the ester−amide exchange methodology. However, model amidation conditions resulted in low yields of the diamide product **17**. Further optimization involved screening of metal co-additives including $MgBr_2$,[25] $Sc(OTf)_3$,[26] and $La(OTf)_3$, along with other commercially available metal triflates. Gratifyingly, we found that addition of $Zn(OTf)_2$[26b] (20 mol %) in addition to Zr(IV)-2-HYP afforded increased yields of acetal-protected calafianin **17** (Scheme 3). However,

Scheme 3. Synthesis of (−)-Calafianin and Stereoisomers



late-stage acetal deprotection proved to be complicated by concomitant amide hydrolysis of the amide and subsequent epoxide ring opening. Exhaustive screening of acetal deprotection conditions (e.g., aq AcOH, 1 N HCl, TFA, CSA, PPTS,[27] K-10 clay,[28] $Bi(OTf)_3 \cdot 4H_2O$,[29] aqueous HF) on bis-

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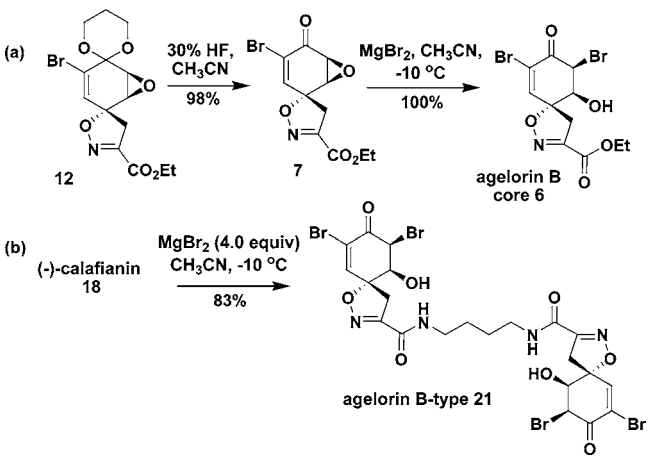
Scheme 4. Further Transformations to Agelorin B-Type Compounds



acetal **17** was conducted; most conditions generally led to product decomposition. Remarkably, we found that use of 48% aqueous HF in a binary solvent mixture of CH_3CN and CH_2Cl_2[30] effected clean hydrolysis of both cyclic ketals to afford calafianin **18** in high yield and purity with negligible hydrolysis and side reactions observed. Using the corresponding optimized synthetic route, all other stereoisomers including (+)- and (−)-*epi*-calafianin (Scheme 3, inset), derived from the *syn*-spiroisoxazoline diastereomer **13**, were synthesized and fully characterized. CD spectroscopy and optical rotation of all four stereoisomers led to unambiguous assignment of the absolute configuration for (+)-calafianin **3**.[16] Our studies thus confirm that **3** and aerothionin **1** have the same absolute configuration of the spiroisoxazoline core.

Further transformations of an epoxy ketone spiroisoxazoline core to agelorin B-type structures are shown in Scheme 4. Agelorins A and B[6] as well as related monomeric analogues[9a] have been shown to possess antitumor activity. Acetal 12 was hydrolyzed using aqueous HF to afford epoxy ketone **7**. Treatment of **7** with $MgBr_2$[31] at low temperature afforded *syn*-bromohydrin **6** as a single regioisomer (100%).[32] The latter compound was found to epimerize readily on SiO_2 to afford approximately a 1:1 mixture of *syn*- and *anti*-bromohydrin diastereomers. Direct ester-amidation of **6** proved difficult due to base sensitivity of the substrate leading to extensive decomposition. Accordingly, we performed bromohydrin formation directly on (−)-calafianin to afford the dimeric agelorin B-type structure **21** (Scheme 4b).

In conclusion, we have accomplished the asymmetric synthesis of the spiroisoxazoline natural product (+)-calafianin. Key transformations include asymmetric nucleophilic epoxidation, Zr(IV)-mediated nitrile oxide cycloaddition, and catalytic ester-amide exchange. Unambiguous stereochemical assignment of the natural product followed from the preparation of all possible stereoisomers and comparison of their circular dichroism (CD) spectra. Epoxy ketone core structures have also been efficiently converted to agelorin B-type structures using a stereoselective ring-opening process. Further studies toward the synthesis of additional spiroisoxazoline natural products are currently in progress and will be reported in due course.

Acknowledgment. Financial support from the American Cancer Society, Bristol-Myers-Squibb (Unrestricted Grant in Synthetic Organic Chemistry to J.A.P, Jr.), Novartis BioMedical Research Institute (Graduate Fellowship to S.B), Merck Research Laboratories, and the Boston University Undergraduate Research Opportunities Program (UROP) is gratefully acknowledged. We thank Prof. Carsten Christophersen for providing NMR and CD spectra of (+)-calafianin and Mr. Chris Singleton, Mr. Shun Su, and Dr. Gonzalo Hernandez (Boston University) for experimental assistance.

Supporting Information Available: Experimental procedures and characterization data for all new compounds and materials. This material is available free of charge via the Internet at http://pubs.acs.org.

OL053115M

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