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New Sources of Chemical Diversity Inspired by Biosynthesis: Rational Design of a Potent Epothilone Analogue

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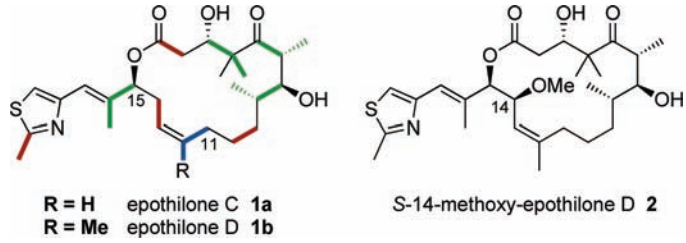
ABSTRACT



A concise total synthesis of (*S*)-14-methoxyepothilone D has been accomplished. (*S*)-14-Methoxyepothilone D represents a conceptually novel example of polyketide analogue design based on an alternative biogenetic pattern of extender units. The significant biological activity observed for this compound provides a foundation to support studies designed to prepare derivatives of this type through fermentation of genetically engineered organisms expressing the epothilone PKS gene cluster.

Polyketide natural products are typically produced by microorganisms to create an environmental advantage for the producing organism through antibiotic and antifungal activity. Their structures evolve during the ancestral history of the producing organism through modification in their PKS gene clusters and the development or sequestration of genes encoding post-PKS processing enzymes.[1] The polyketide backbone is generated through the sequential incorporation of two-carbon extender units derived most commonly from malonyl-CoA or methylmalonyl-CoA. Advances in the genetic engineering of the gene clusters responsible for the production of biological active polyketides represents a valuable new source of chemical diversity essential to identification of the next generation of chemotherapeutic agents.[2] Unfortunately, the biological implications of even minor structural modifications are typically difficult to predict. Our unique approach to this classic structure−activity relationship (SAR) problem was based on the initial appreciation that the main role for the functionality that adorns a polyketide is to control the overall conformation. Through extensive computer-based conformational analysis coupled with solution NMR studies on the naturally occurring epothilones as well as designed analogues, we were able to develop a conformational-activity relationship profile which was used to propose a binding conformation for the class.[3−6] With this information in hand, it is possible to design

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biologically active analogues of the epothilones, such as epothilones C and D, Figure 1, which may be accessible through genetic engineering.

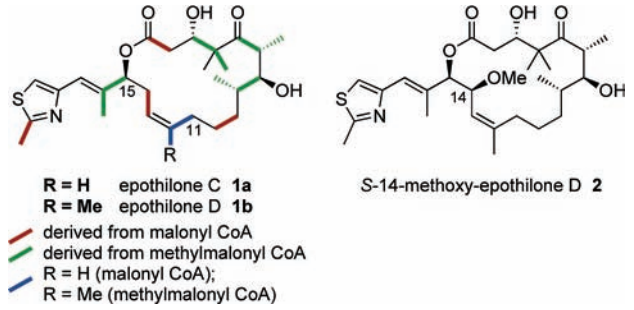
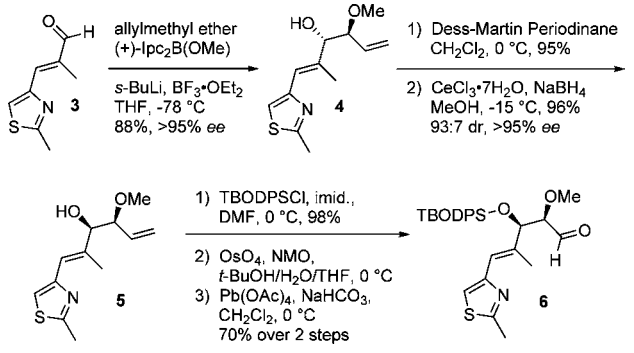


Figure 1. Structures and biogenetic pattern for epothilones C and D; the structure of (*S*)-14-methoxyepothilone D.

Herein we report the total synthesis and biological evaluation of (*S*)-14-methoxyepothilone D **2**, a novel epothilone analogue. The stereochemistry of the C14-substitution was chosen to provide further support for the proposed binding conformation in the C11−C15 region.[7] Moreover, the incorporation of a methoxy group at this position represents a target potentially available through modification to the acyltransferase domain of module 3 in the epothilone PKS gene cluster[8] and the incorporation of an extender unit derived from methoxymalonyl CoA.[9]

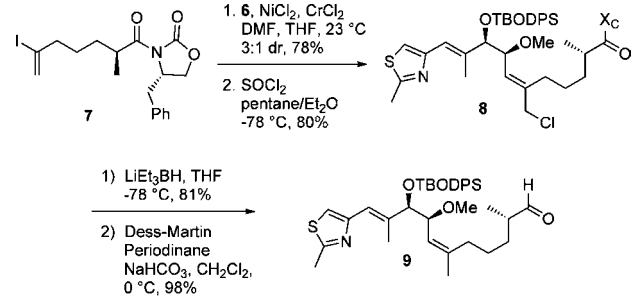
The significant efforts required to develop a fermentation based route could be justified if the biological activity of the target were significant. Thus, a synthetic strategy for the production of (*S*)-14-methoxyepothilone D was developed on the basis of modificatons to our previously reported route to epothilones B and D.[10] This route relied on a sequential Nozaki−Hiyama−Kishi (NHK)[11] coupling−thionyl chloride induced allylic rearrangement[12] to stereoselectively generate the C12−C13 trisubstituted olefin.

Scheme 1. Installation of C14-Methoxy Substituent



The starting thiazole aldehyde fragment **3** was prepared by previously described methods and detailed in Scheme 1.[13] The introduction of the C14 methoxy substituent was accomplished via an asymmetric Brown allylation[14] with lithiated allyl methyl ether in the presence of BF_3•OEt_2 to afford the alcohol **4** as a single diastereomer in >95% ee[15] and 88% yield, Scheme 1. The undersired *syn*-stereochemistry was easily converted to the *anti*-diastereomer via a two-step sequence. First, oxidation of the alcohol was achieved with Dess−Martin periodinane[16] followed by a highly diastereoselective Luche reduction of the α,β-unsaturated ketone.[17] This reduction yielded the optically pure *anti*-diastereomer **5** as a 93:7 mixture of diastereomers in 96% yield. The free hydroxyl group was protected with *tert*-butoxydiphenylsilyl chloride (TBODPSCl), and subsequent oxidative cleavage of the terminal olefin under standard conditions afforded the desired aldehyde fragment **6**.

Scheme 2. Sequential NHK Coupling/Thionyl Chloride Rearrangement for Trisubstituted Olefin Generation



As shown in Scheme 2, fragment coupling of aldehyde **6** with known vinyl iodide fragment **7**, prepared according to a previously reported route,[10] was achieved via a NHK

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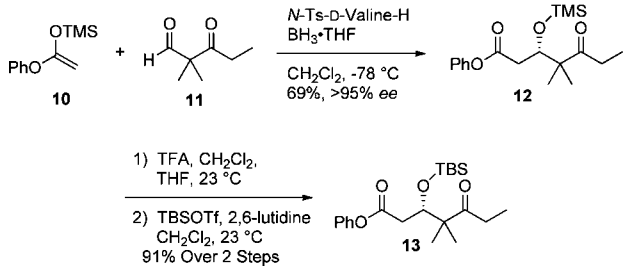
coupling reaction. This key step afforded the desired allylic alcohol as a 3:1 mixture of unassigned and inconsequential diastereomers in 78% yield. Exposure of this mixture to thionyl chloride in ether−pentane provided the desired primary chloride **8** in 80% yield as a single olefin isomer.

Removal of the chiral auxiliary and cleavage of the primary chloride was achieved in a single synthetic operation utilizing $LiEt_3BH$, which was subsequently oxidized with Dess−Martin periodinane to afford the aldehyde **9**. The synthesis of the C1−C6 fragment, Scheme 3, commenced

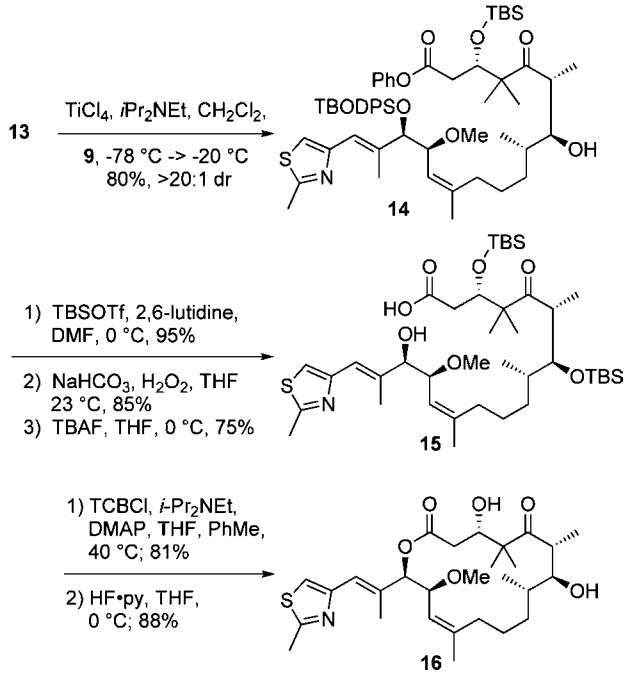
Scheme 3. Synthesis of C1−C6 Fragment



with the readily available silyl ketene acetal **10**[18] and ketoaldehyde **11**.[19] Treatment with Kiyooka's chiral boron reagent[20] (100 mol %) provided the TMS-protected aldol adduct **12** in 69% yield and >95% ee. The TMS group was readily removed with TFA, and the resultant secondary alcohol was protected with TBSOTf in the presence of 2,6-lutidine to afford the C1−C6 fragment **13** in 91% yield.

Aldol coupling between ketone fragment **13** and aldehyde **9** was accomplished by the previously reported, highly

Scheme 4. Selective Aldol and Completion of Synthesis



diastereoselective Ti-mediated coupling conditions.[21] In our system, when **13** was treated with $TiCl_4$ and *i*-Pr_2NEt and then subsequently **9**, the *syn,anti*-aldol product **14** was obtained in 80% yield and as a single diastereomer (Scheme 4). The resultant secondary alcohol was protected as the TBS ether with TBSOTf and 2,6-lutidine in 95% yield. Hydrolysis of the phenyl ester under standard conditions (LiOH in THF/H_2O) provided a small amount of the desired acid, as well as a large amount of C3 elimination product. After screening various conditions, this problem was overcome by the use of milder hydrolysis conditions ($NaHCO_3$ and H_2O_2 in THF), which afforded the desired acid in 85% yield without any formation of a C3-elimination product. Subsequent selective removal of the C15 TBODPS ether was accomplished by careful treatment with TBAF at 0 °C to afford the *seco*-acid **15** in 75% yield. Classic Yamaguchi macrolactonization conditions[22] using trichlorobenzoyl chloride (TCBCl) provided the 16-membered lactone in good yield when heated to 40 °C. Finally, global deprotection was carried out using HF•py to afford *S*-14-methoxyepothilone D **2** in 88% yield.

The biological activity of (*S*)-14-methoxyepothilone D **2** was evaluated against two human tumor cell lines (Table 1)

Table 1. Cytotoxicity of Epothilone Analogues (IC_{50}, nM)

compound	MCF-7	H460
(*S*)-14-methoxyepothilone D **3**	3.7	4.9
epothilone B	0.5	0.3
ixabepilone (Ixempra)	2.1	2.3
paclitaxel (Taxol)	4.7	1.5

in direct comparison to natural epothilone B, ixabepilone, and paclitaxel. The presence of a C12,C13 epoxide as in epothilone B is known to enhance activity by 1 order of magnitude. Ixabepilone is Bristol-Myers Squibb's semisynthetic analogue of epothilone B recently approved by the FDA for advanced breast cancer therapy.[23] (*S*)-14-Methoxyepothilone D retains significant cytotoxic activity on par with both naturally occurring as well as synthetically derived analogues of the epothilones.

(*S*)-14-Methoxyepothilone D represents a conceptually novel example of polyketide analogue design based on an alternative biogenetic pattern of extender units. The significant biological activity observed for this compound provides a foundation to support studies designed to prepare derivatives of this type through fermentation of genetic engineered organisms expressing the modified forms of the epothilone PKS gene cluster and/or precurser-directed biosynthesis. Results along these lines will be reported in due course.

Acknowledgment. Dr. Bandaru Sreenivasulu (Notre Dame) is acknowledged for preliminary studies. Support provided

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

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