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Total Syntheses of [17]- and [18]Dehydrodesoxyepothilones B via a Concise Ring-Closing Metathesis-Based Strategy: Correlation of Ring Size with Biological Activity in the Epothilone Series

Alexey Rivkin,[†] Jon T. Njardarson,[†] Kaustav Biswas,[†] Ting-Chao Chou,[‡] and Samuel J. Danishefsky*[,†,||]

Laboratory for Bioorganic Chemistry, Preclinical Pharmacology Core Facility and Analytical Pharmacology Core Facility, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021, and Department of Chemistry, Columbia University, Havemeyer Hall, New York, New York 10027

s-danishefsky@ski.mskcc.org

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A convergent ring-closing metathesis strategy has been employed for the highly concise syntheses of 10,11-dehydro-13,14-[17]desoxyepothilone B ([17]ddEpoB) and 10,11-dehydro-14,15-[18]desoxyepothilone B ([18]ddEpoB), which are 17- and 18-membered ring homologues of 10,11-dehydro-12,13-desoxyepothilone B ([16]ddEpoB or epothilone 490). We have demonstrated that the ring-closing metathesis (RCM) provides [17]ddEpoB or [18]ddEpoB with a high level of stereocontrol in the generation of the desired olefin in the products. These analogues were evaluated for antitumor activity. The results from the in vitro assays revealed that the [17]ddEpoB analogue is highly active against various tumor cell lines with a potency comparable to that of [16]ddEpoB. This is the first example of a 17-membered ring macrolactone epothilone that has retained its antitumor activity. In contrast, the biological data revealed that [18]ddEpoB is significantly less active than either [17]ddEpoB or the parent [16]ddEpoB.

Introduction

The promotion of mitotic arrest by microtubulin stabilization has been at the forefront of the search for new leads in cancer chemotherapy for the past decade.[1] The most well known microtublin stabilization agent is paclitaxel (Taxol), which is currently one of the front-line drugs used for the treatment of cancer.[2] Despite this success, Taxol is not necessarily the ultimate drug, due to its susceptibility to multidrug resistance and the formulation difficulties arising from its lack of solubility in aqueous media.[3] These limitations drive the search for novel microtubulin stabilization agents that possess the potent activity of taxol but have a better therapeutic profile as well as increased water solubility. In the past decade, several microtubulin-stabilization agents have been discovered and studied with respect to overcoming the inherent limitations of paclitaxel.[1] Of these, the epothilones have been recognized as primary alternatives to paclitaxel in the light of their greater water solubility and their potent activity against multi-drug-resistant cancer lines (Figure 1).[4] To date, several total syntheses of the naturally occurring epothilones have been accomplished.[5] Subsequently, the preparation of hundreds of analogues allowed for the establishment of a detailed map of the structure−activity relationships based on in vitro and in vivo assays.[6]

While chemical modifications have been reported for many positions on the epothilone macrolide framework, the effects of ring size with respect to cytotoxic activity have been only briefly studied. The syntheses of 14-, 15-, 17-, and 18-membered ring analogues of epothilone A have been reported by Nicolaou and co-workers.[7] These analogues, with the exception of one, had relatively weak tubulin binding activity in comparison to the [16]-epothilone A (**1a**). The only exception was the [18]-desoxyepothilone A, which revealed slightly lower tubulin polymerization activity in comparison to [16]epothilone A (**1a**). To provide further insight into the correlation between ring size of the epothilones and their corresponding biological activity, we elected to synthesize the

* To whom correspondence should be addressed.
[†] Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute.
[‡] Preclinical Pharmacology Core Facility, Sloan-Kettering Institute.
[||] Department of Chemistry, Columbia University.

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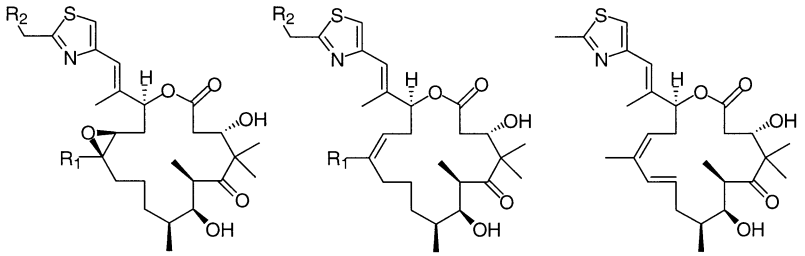


1

1a R_1 = H, R_2 = H, Epothilone A
1b R_1 = CH_3, R_2 = H, Epothilone B
1c R_1 = H, R_2 = OH, Epothilone E
1d R_1 = CH_3, R_2 = OH, Epothilone F

2

2a R_1 = H, R_2 = H, Epothilone C
2b R_1 = CH_3, R_2 = H, Epothilone D ([16]dEpoB)
2c R_1 = H, R_2 = OH, Desoxyepothilone E
2d R_1 = CH_3, R_2 = OH, Desoxyepothilone F

2e

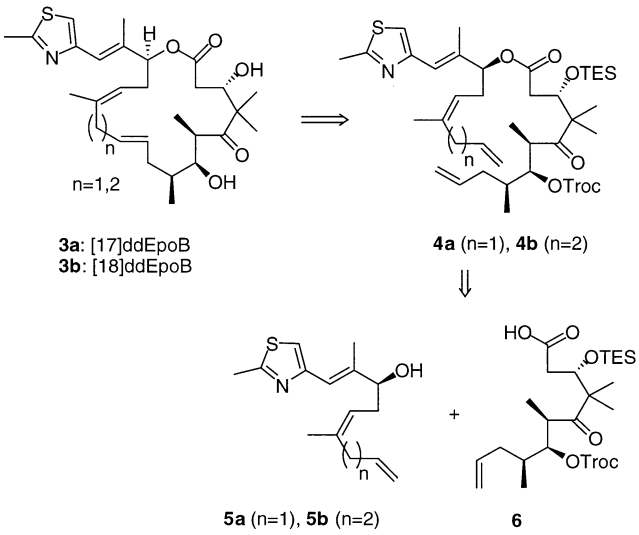
2e 10,11-Dehydroepothilone D ([16]ddEpoB)

FIGURE 1. Structure of [16]epothilones and [16]desoxyepothilones.

corresponding 17- and 18-membered ring homologues of [16]ddEpoB (**2e**) and evaluate their antitumor activity.

We have been actively involved in total syntheses and structure −activity relationship studies of the epothilones for some years.[8] Our epothilone program has led to the development of [16]dEpoB (**2b**) as a drug candidate, currently in human trials. The high demand for substantial quantities of [16]dEpoB (**2b**) for earlier preclinical studies and the lack of in-house access to fermentation-derived material in our research setting prompted our efforts to access the epothilones by total synthesis. We have previously shown how we addressed and solved the challenges associated with devising a concise, modular, and efficient route to [16]ddEpoB (**2e**), a synthetic precursor of [16]dEpoB (**2b**), based on a ring-closing metathesis-based strategy.[9] As previously reported, **2e** is highly active against various tumor cell lines with a potency comparable to that of [16]dEpoB (**2b**). More importantly, the availability of [16]ddEpoB (**2e**) provided an opportunity for the synthesis of many new analogues of dEpoB **2b** that could not have been prepared using earlier synthetic routes. The disclosure herein describes the total synthesis 17- and 18-membered ring homologues of **2e**. While these syntheses draw from the previously described approaches, a significant modification was required if we were to attain the flexibility to control the ring size of the macrolactone via late-stage variations. Preliminary biological evaluations of the novel compositions synthesized are provided below.

SCHEME 1. Application of RCM Strategy toward the Synthesis [17]- and [18]ddEpoB



3a: [17]ddEpoB
3b: [18]ddEpoB

4a (n=1), **4b** (n=2)

5a (n=1), **5b** (n=2)

6

A highly convergent strategy, related to that employed in the synthesis of [16]ddEpoB (**2e**),[9] was used. Accordingly, fragments of similar complexity served as key building blocks (Scheme 1). We envisioned that the acyl sector **6**[9] could serve as the polypropionate domain and the alkyl sector **5a** or **5b** would be prepared in a few steps from a known intermediate.[9] The union of the two fragments **5a** (**5b**) and **6** would be initiated through an esterificiation and consummated via a subsequent ring-closing metathesis. Finally, cleavage of the protecting

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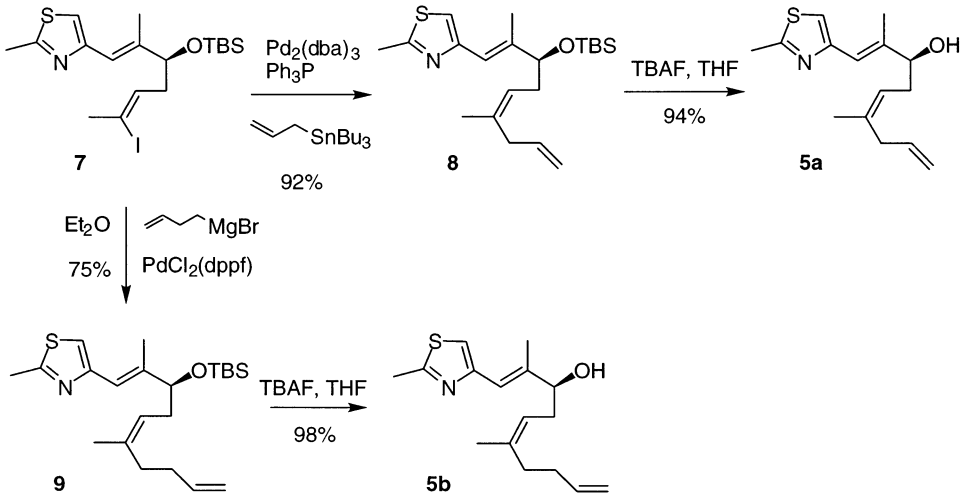
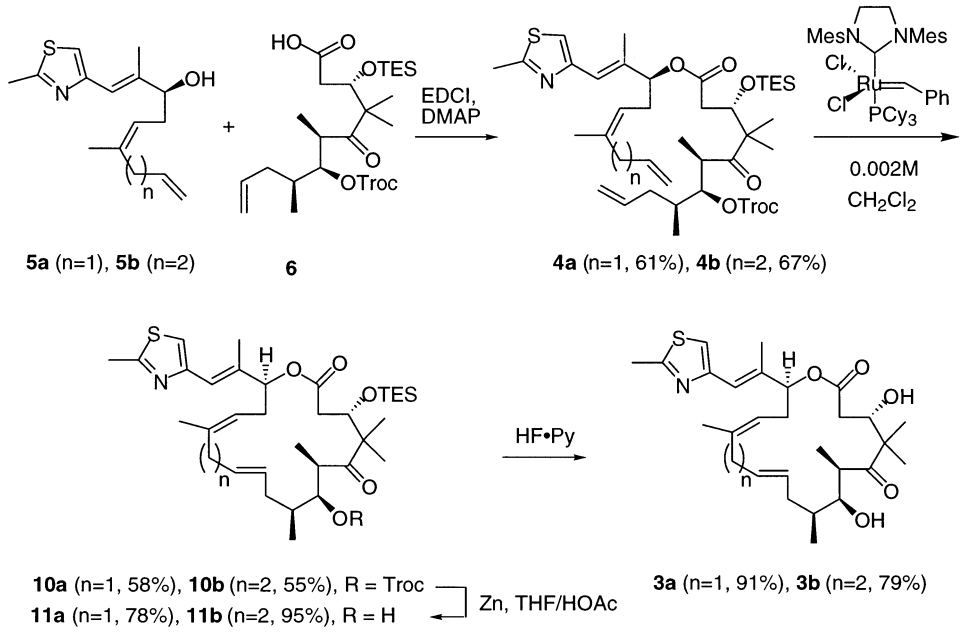
SCHEME 2. Preparation of Left Fragments 5a and 5b



SCHEME 3. Synthesis of [17]ddEpoB and [18]ddEpoB



groups would provide the desired 17- and 18-membered ring homologues (**3a** and **3b**) of **2e**.

Results and Discussion

The synthesis of the 17- and 18-membered ring homologues commenced with the conversion of the previously reported vinyl iodide **7**[10] to the corresponding 1,4-diene **5a** and 1,5-diene **5b** (Scheme 2). Reaction of vinyl iodide **7**, with allyltributyltin under Stille conditions, afforded the desired 1,4-diene **8** in 92% yield. Correspondingly, reaction of vinyl iodide **7** with butenylmagnesium bromide under the Tamao−Kumada−Corriu palladium(0)-mediated coupling conditions[11] provided the desired 1,5-diene **9** in 75%. It seems likely that this reaction could be extended toward the synthesis of alternative unconjugated dienes, which could allow for the synthesis of even larger ring analogues. Finally, treatment of 1,4-diene **8** and 1,5-diene **9** with tetra-*n*-butylammonium fluoride accomplished deprotection of the secondary alcohol in high yield.

Esterification of the resultant allylic alcohols **5a** and **5b** with C_1–C_{10} acid fragment **6** provided the corresponding RCM cyclization precursors in 61% (**4a**) and 67% (**4b**) yields, respectively (Scheme 3). The ring-closing metathesis reaction of 1,4-diene **4a** was then carried out using the second-generation Grubbs catalyst[12] in methylene chloride, which provided, as in our earlier study,[9] exclusively the trans isomer **10a** in a yield of 58%. Using the same RCM reaction conditions with the 1,5-diene **4b** provided exclusively the trans isomer **10b** in 55% yield,

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TABLE 1. In Vitro Cytotoxicities (IC_{50}) with Tumor Cell Lines[a]

tumor cell lines[b]	IC_{50} (μM)[a] [17]ddEpoB (**3a**)	[18]ddEpoB (**3b**)	[16]ddEpoB (**2e**)	dEpoB (**2b**)
CCRF−CEM	0.040	0.322	0.025	0.011
CCRF−CEM/VBL_{100}	0.126	0.870	0.091	0.015
CCRF−CEM/VM_1	0.055	ND[c]	0.035	0.016
CCRF−CEM/Taxol	0.053	0.508	0.032	0.007

[a] XTT assay following 72 h inhibition. [b] CCRF−CEM is a human T-cell acute lymphoblastic leukemia cell line. The CCRF−CEM/VBL_{100}, CCRF−CEM/VM_1, and CCRF−CEM/Taxol cell lines all overexpress P-glycoprotein and display a multidrug resistance phenotype to MDR-associated oncolytics.[8] [c] None detected.

along with recovered starting material. Finally, reductive cleavage of the 2,2,2-trichloroethoxycarbonyl protecting group with zinc and acetic acid followed by deprotection of triethylsilyl ether with HF−pyridine led to the [17]- and [18]ddEpoB (**3a** and **3b**).

The fully synthetic [17]- and [18]ddEpoB have been evaluated against a variety of cell types to determine their antitumor potential. As shown in Table 1, [17]-ddEpoB (**3a**) exhibited high cytotoxic activity against a variety of sensitive and resistant tumor cell lines. Direct comparison of [17]ddEpoB (**3a**) with the previously reported [16]ddEpoB (**2e**) indicates that the new compound possess comparable potency. In contrast, the data reveal that [18]ddEpoB (**3b**) is significantly less active than either [17]ddEpoB (**3a**) or [16]ddEpoB (**2e**).[7] Preliminary model studies have indicated that there is only a small difference between the overall configuration of [17]ddEpoB (**3a**) and [16]ddEpoB (**2e**), whereas there is a large difference between the overall configuration of [18]ddEpoB (**3b**) and [16]ddEpoB (**2e**). Thus, we suggest that the alterations in overall configuration in the case of [18]ddEpoB (**3b**) may have led to the distortion of the essential pharmacore and the reduction of antitumor activity.

In summary, the key step that controls the eventual ring size of the ultimate epothilone is a cross coupling of vinyl iodide under mediation by Pd(0). The total syntheses of [17]ddEpoB (**3a**) and [18]ddEpoB (**3b**) have been achieved using a strategy based on a convergent merger of two major fragments by an esterification and subsequent ring-closing metathesis.[13] Application of the second-generation Grubbs catalyst in the ring-closing metathesis in the synthesis of [17]- and [18]ddEpoB (**3a** and **3b**) gave exclusively the trans olefinic isomer. It seems likely that the ring-closing metathesis strategy described herein can be extended toward the synthesis of higher ring homologues of the epothilones.

The in vitro tumor growth inhibition experiments demonstrated that the new [17]ddEpoB (**3a**) analogue possesses high in vitro antitumor activity, which is comparable to that of [16]ddEpoB (**2e**). This represents the first example of a 17-membered ring epothilone macrolide that has antitumor activity similar to the 16-membered natural products. The dramatic diminution in activity of [18]ddEpoB (**3b**) gives further support toward the limited tolerance of the pharmacophore to distortion. Further investigations with [17]ddEpoB (**3a**) are currently underway.

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Supporting Information Available: Experimental details and ^1H and ^{13}C NMR spectra for all characterized compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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