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Synthesis and Evaluation of a Linkable Functional Group-Equipped Analogue of the Epothilones

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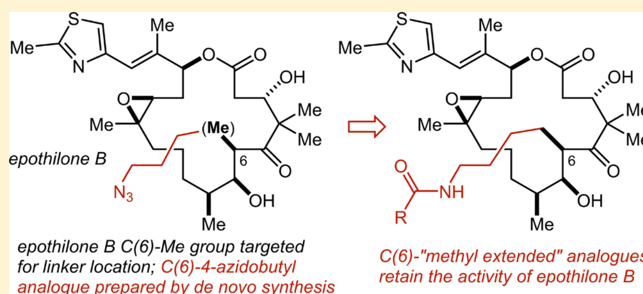
Supporting Information

ABSTRACT: An approach to the validation of a linker strategy for the epothilone family of microtubule-stabilizing agents is reported. An analogue of epothilone B in which the C(6) methyl group has been replaced with a 4-azidobutyl group has been prepared by total chemical synthesis, and amides derived from the azido group have been shown to retain the activity of the parent compound. These results set the stage for an evaluation of the potential of the epothilones to serve as the drug component of antibody–drug conjugates and other selective tumor cell-targeting conjugates.



KEYWORDS: *Epothilones, microtubules, antibody–drug conjugates, linker, methyl extension*

Among the large group of microtubule-stabilizing agents (MSAs) known to exert their antimitotic activity by binding to the luminal taxane binding site on the β-tubulin subunits of microtubules, the epothilone family of natural products[1−3] constitute perhaps the most promising (Figure 1).

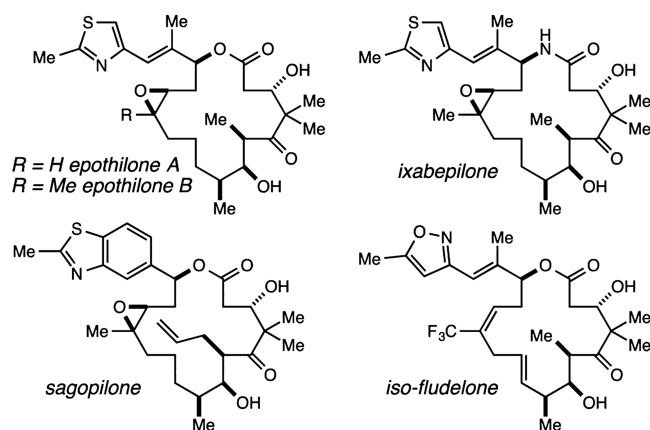


Figure 1. Potent MSAs epothilone A and B, ixabepilone, sagopilone, and iso-fludelone.

Ixabepilone,[4,5] the lactam analogue of epothilone B, was approved by the FDA in 2007 for use in the treatment of particularly aggressive and otherwise unresponsive forms of metastatic breast cancer, while several other fully synthetic analogues (e.g., sagopilone,[6−8] iso-fludelone[9]) have recently undergone or are currently undergoing clinical evaluation. Because epothilone B, sagopilone, and iso-fludelone, among others, are possessed of subnanomolar potencies against a variety of cell lines including some taxol-resistant cell lines, we have become interested in establishing a validated linker strategy for this family of anticancer agents. Such a linker strategy would allow investigation of the potential of these compounds to serve as the drug component of antibody–drug conjugates (ADCs)[10−12] and other selective tumor cell targeting conjugates.

In order to prepare conjugates, the most basic requirement for the drug is that it must have a functional group (most straightforwardly an unhindered alcohol, a thiol, or an amine) that may be used selectively in synthetically mild and efficient conjugation reactions. With a traceless or self-immolative linker strategy this may be the only requirement, and two potent semisynthetic epothilone analogues equipped with a suitably reactive alcohol or amine (e.g., **1**[13] and **2**,[14] Figure 2a) have been reported and usefully employed in simple conjugation (acylation) reactions by the Bristol-Myers Squibb group in the course of their efforts to develop an epothilone–folic acid conjugate.[14,15] An important additional design consideration for us, however, was to leave open the possibility of exploring conjugates wherein some portion of the linker may be retained (for example, to allow for the incorporation of additional functionality into the drug) in the active drug entity that is released from the conjugate following lysozomal degradation of the antibody. This adds the rather more stringent requirement that the conjugatable functionality must be located in a region of the drug that is solvent-exposed when the drug is bound in the receptor such that relatively large groups may be

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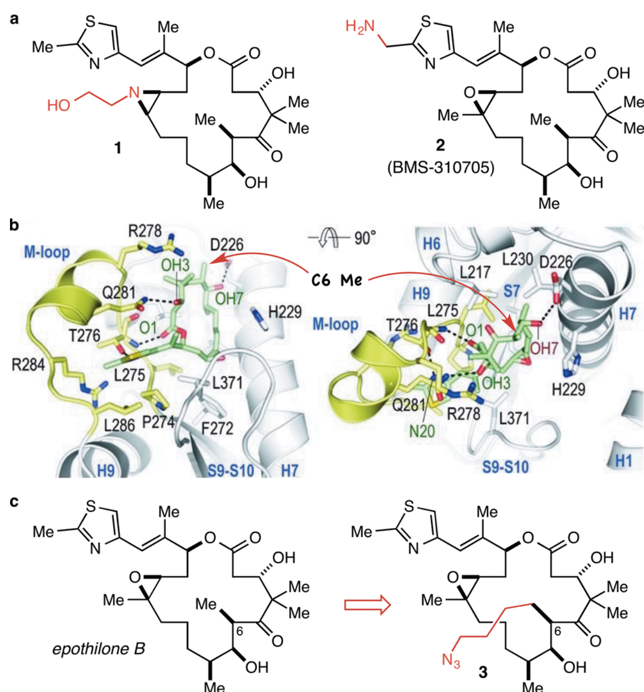

Figure 2. (a) Two previously described analogues of the epothilones that may be used in conjugation reactions with a traceless or self-immolative linker strategy. (b) The crystal structure of epothilone A bound in the taxane binding site reveals that the C(6) methyl group is solvent exposed, consistent with available SAR data. Reprinted with permission from ref 17. Copyright 2013 AAAS. (c) C(6)-methyl extended analogue **3** is predicted to be approximately equipotent with the parent natural product.

incorporated in the drug structure without any deleterious impact on its potency. So as to accomplish this while also perturbing the steric and electronic structure of the drug as little as possible, we decided to use the "methyl extension" strategy that we reported recently,[16] which entails the identification of a solvent-exposed methyl group and the *de novo* synthesis of the analogue wherein the methyl group is replaced with a functional group-equipped linear alkyl group. To identify a suitable methyl group, we examined the recently reported high-resolution structure of epothilone A bound in the taxane binding site of an ($\alpha\beta$-tubulin)$_2$-RB3-tubulin tyrosine ligase complex (Figure 2b).[17] This structure reveals that the C(6) methyl group is solvent exposed and uninvolved in any interactions with the receptor, consistent with the observation that sagopilone, with an allyl group at C(6), is approximately equipotent with epothilone B.[6] In this way, we decided to synthesize the C(6)-4-azidobutyl epothilone B analogue **3** (Figure 2c) and evaluate it and amides derived therefrom for potency.

We have developed two conceptually related syntheses of the C(1)−C(9) fragment of the epothilone family of natural products[18,19] based on our silylformylation/crotylsilylation/Tamao oxidation methodology[20] we developed for the step-economical and efficient syntheses of complex polyketide fragments. The requisite starting material for the synthesis of the C(6)-4-azidobutyl analogue, homopropargyl alcohol **4**, was prepared using our recently reported synthesis[19] (see the Supporting Information file for details), and we began by examining our second-generation approach in which the three parts of the sequence were carried out in a stepwise fashion (Figure 3a).[19] As shown, this sequence was successful in delivering the desired C(1)−C(9) fragment **10** with a 4-chlorobutyl group installed at C(6). Unfortunately, however, the aldehyde crotylation step (**7** + **8** → **9**),[21] which had worked well in the C(6)-methyl series, proceeded with low (3.5:1) diastereoselectivity and was inefficient, presumably due to the extreme steric hindrance of aldehyde **7**. As a result, we were able to isolate the desired product (**9**) in only 37% yield. Given that one of the primary intended benefits of the second-generation stepwise sequence was to allow for high levels of diastereoselectivity in the crotylation event, we decided to evaluate the first-generation tandem and one-pot version of the sequence (Figure 3b). This was carried out according to our previous report[18] and produced **10** as the major product of a 7:1.3:1 mixture of diastereomers in 44% combined yield from **4**. Though due to the added steric hindrance of the C(6)-4-chlorobutyl group this sequence, too, proceeded with reduced efficiency relative to the version with the C(6)-methyl substrate, it also proceeds in just two pots, and because of this extraordinary step-economy, we were able to rapidly produce multigram quantities of **10** using this route.

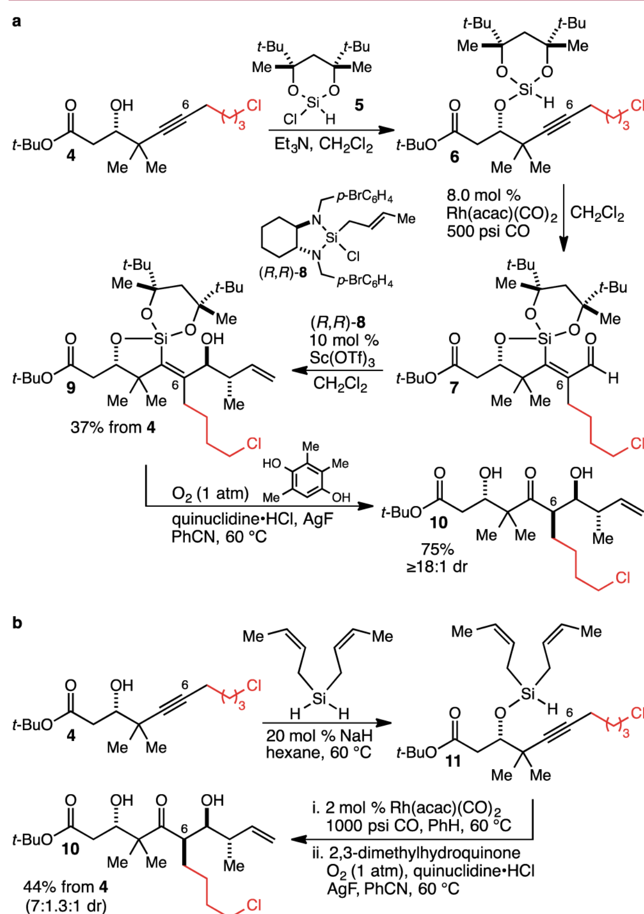

Figure 3. (a) Synthesis of the C(1)−C(9) fragment of the epothilones with a 4-chlorobutyl group installed at C(6) using our second generation approach (ref 19). (b) Synthesis of the C(1)−C(9) fragment of the epothilones with a 4-chlorobutyl group installed at C(6) using our first generation approach (ref 18).

To complete the synthesis of the target compound **3**, we adapted the chemistry[22] pioneered by the Danishefsky team in the course of their development of the fludelone family of epothilone analogues.[23] As shown in Figure 4, coupling of acid

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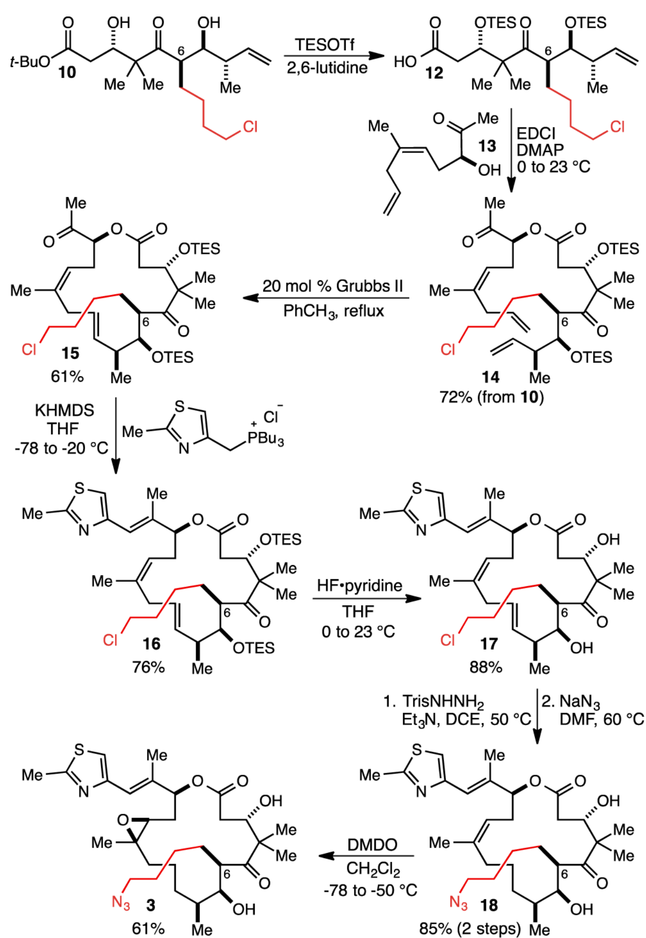
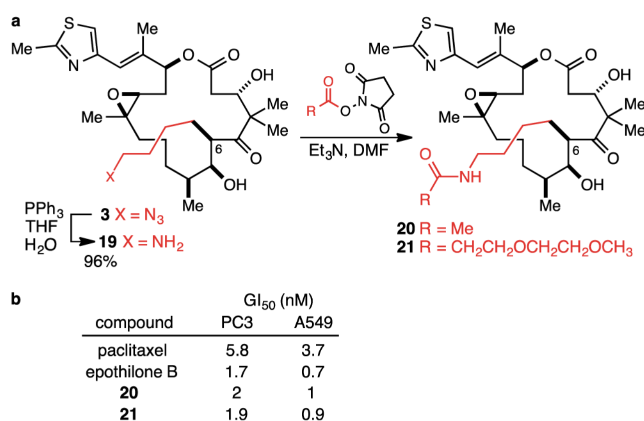


Figure 4. Completion of the synthesis of the C(6)-4-azidobutyl epothilone B analogue **3** according to the Danishefsky strategy.

12 and alcohol **13** proceeded smoothly and was followed by ring-closing metathesis with the second generation Grubbs catalyst[24] and Wittig reaction to install the thiazole side chain. Removal of the triethylsilyl (TES) protecting groups was followed by selective reduction of the C(9)−C(10) alkene and displacement of the chloride with azide. Finally, epoxidation of the macrocyclic alkene with dimethyldioxirane according to Danishefsky's procedure[25] gave the C(6)-4-azidobutyl epothilone B analogue **3**.

With access to **3** secured, we explored various linking/conjugation strategies. While strain-accelerated azide−alkyne cycloaddition reactions with cyclooctynes[26,27] proved straightforward and efficient, we also found that the azide could be reduced using the classical Staudinger reaction,[28] and the derived amine (**19**) smoothly acylated with *N*-hydroxysuccinate esters (Figure 5). This was judged to be the more convenient and straightforward approach, and in short order, amides **20** and **21** were produced as models for linker equipped analogues of epothilone B. Amides **20** and **21** were assayed for cell growth inhibition against the PC3 (prostate) and A549 (lung) cell lines along with paclitaxel and epothilone B as positive controls, and gratifyingly, they were found to be approximately equipotent with epothilone B (Figure 5b).

Guided by the high-resolution structure of epothilone A bound in the taxane binding site (Figure 2b), we applied the methyl extension approach[16] to design the C(6)-4-azidobutyl epothilone B analogue **3** and synthesized it using our previously developed routes to the C(1)−C(9) fragment of the



b

compound	GI_{50} (nM) PC3	A549
paclitaxel	5.8	3.7
epothilone B	1.7	0.7
20	2	1
21	1.9	0.9

Figure 5. (a) Synthesis of C(6)-4-aminobutyl epothilone B (**19**) and conjugation reactions to produce model linker-epothilone B constructs **20** and **21**. (b) Cell growth inhibition GI_{50} values for **20** and **21** (values are the average over two−four experiments; see the Supporting Information for details).

epothilones[18,19] and Danishefsky's fragment coupling and end-game strategies.[22,23,25] Amides **20** and **21** were then produced and evaluated in an *in vitro* cell growth inhibition assay and found to be approximately equipotent with epothilone B. These results thus constitute a proof-of-concept result toward a validated linker strategy for the epothilone family of MSAs, and set the stage for a full exploration of linker structure−activity relationships and ultimately an evaluation of the potential of these clinically validated compounds to serve as the drug component of ADCs and other selective tumor cell-targeting conjugates. Current efforts are focused on those objectives, as well as on a streamlined and more efficient and selective method for the synthesis of the C(1)−C(9) fragment of the epothilones with a 4-chlorobutyl group installed at C(6).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00131.

Experimental procedures, characterization data, and NMR spectra (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

Biographies

James Leighton received his Ph.D. from Harvard University in 1994 working under the direction of Professor David Evans. After a National Science Foundation Postdoctoral Fellowship with Professor Eric Jacobsen at Harvard University, he joined the faculty at Columbia University as an Assistant Professor in 1996. He was promoted to the rank of Associate Professor in 1999 and to the rank of Professor in 2004. His research interests are in organic chemistry, with a particular

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current focus on the synthesis and development of bioactive polyketide natural products.

Dan Sackett received his Ph.D. in Molecular and Cellular Biology from Brown University. His work since has continued his focus on cell division and microtubule biochemistry and biophysics, beginning with Post Doctoral research at the NIADDKD, NIH. His work continued in the National Cancer Institute until, in 1999, he joined the Program in Physical Biology at the NICHD. He is currently Senior Researcher in the Division of Basic and Translational Biophysics at the NICHD, NIH, where his research focuses on biochemistry and pharmacology of tubulin and biophysics of microtubules and microtubule arrays.

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