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Synthesis of Cryptothilone 1, the First Cryptophycin–Epothilone Hybrid

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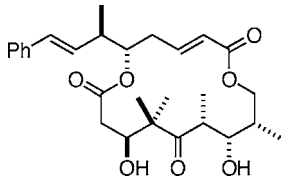
ABSTRACT



A hybrid structure was synthesized in which one portion is characteristic of a cryptophycin and a second sector bears the signature of an epothilone. The hybrid, in contrast to parent cryptophycin and epothilone systems, showed no effect on the tubulin assembly reaction.

Cryptophycins and epothilones are cytotoxic natural products of widely different origin, the one emanating from a blue−green alga[1] and the other from a soil bacterium.[2] Interestingly, they both possess tubulin binding properties that inhibit cell proliferation at mitosis.[3,4] Cryptophycins are believed to bind to the ends of microtubules and, like vinblastine and certain other antimitotic agents, they disrupt the polymerization process by which α,β-tubulin heterodimers condense into aggregates.[5]

Epothilones, on the other hand, are known to bind to an interior region of the microtubule at a site close to that which complexes taxol.[6] This site is believed to be located on the β-tubulin subunit in a location adjacent to the neighboring protofilament. The epothilones and taxol coordinate to microtubules in a manner that reduces the rate of α/β-tubulin dissociation by serving as a bracketing device. This arrangement stabilizes and augments the proportion of tubulin polymer and prevents nuclear division, leading in turn to apoptosis ("programmed cell death"). Thus, although both cryptophycins and epothilones are antimitotic agents which block cell proliferation by interfering with mitotic spindle division between the metaphase and anaphase, they operate at different sites on tubulin.

A comparison of the structures of cryptophycins and epothilones reveals intriguing similarities as well as one significant difference. Figure 1 shows the structure of natural cryptophycin 4 and *trans*-epothilone C, a synthetic analogue known to have tubulin polymerization properties similar to

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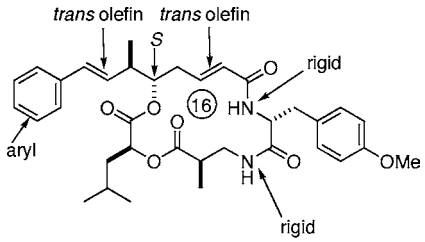
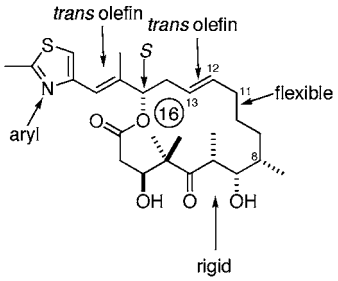


1, Cryptophycin 4



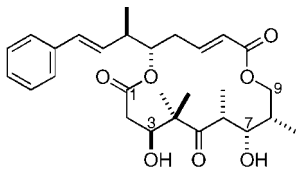
2, *trans*-Epothilone C

Figure 1. Structural homology of a cryptophycin and an epothilone.

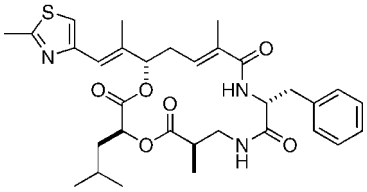
the natural 12,13-cis isomer. Common to both structures are (i) a 16-membered ring, (ii) an aryl substituent attached to a conjugated double bond (which is epoxidized in some cryptophycins), (iii) a methyl substituent at or in close proximity to the conjugated double bond, (iv) (*S*) configuration at the oxygen substituent to which the lactone carbonyl is attached, and (v) an alkene (which is epoxidized in epothilones A and B) separated from the acyloxy carbon by one methylene unit. On the other hand, there is one region of the macrocycle perimeter that is different in these two structures. The C8−C11 segment of epothilones is relatively flexible, whereas the cryptophycin sector that would superimpose on this set of four atoms is quite rigid due to the two amide linkages. Hydrogen bonding in this peptidic section of the cryptophycin perimeter imposes a conformation that is not matched in epothilones.

A hybrid structure that incorporates selected features of **1** and **2** and which also complies with the homologies (i−v) noted above appeared to be a possible probe for investigating the different tubulin binding modes characteristic of each of the parent structures. Issues that could be addressed by this approach include a better definition of the particular structural motifs responsible for binding of cryptophycins and epothilones to different regions of tubulin and whether the distinction is due to recognition by the tubulin receptor of a peptidic domain in one case and a lipophilic sector in the other. Other questions such as whether cryptophycins and epothilones can be made to exchange binding sites on tubulin by structural modification or whether structures can be generated which endow a hybrid molecule with cytotoxic properties found in both parents (or perhaps in neither ancestor) can also be addressed, in principle, by this strategy.

In a first attempt to answer these questions, two "cryptothilone" hybrids were conceived (Figure 2). Cryptothilone



3, Cryptothilone 1



4, Cryptothilone 2

Figure 2. Hybrid structures of a cryptophycin and an epothilone.

1 (**3**) contains the upper half of cryptophycin 4 and a lower half common to most epothilones. Cryptothilone 2 (**4**) would reverse this architecture by connecting the lower half of cryptophycin 4 (**1**) with an upper sector found in natural epothilones. Herein, we report the synthesis of **3** and the results of initial experiments designed to test its effect on tubulin assembly.

Our previous studies on the synthesis of cryptophycins[7,8] and epothilones[9−12] laid the groundwork for our approach to the hybrid system **3**. Alcohol **5** was prepared in five steps from the known aldehyde **6**[13] and was oxidized to aldehyde **7** with Dess−Martin periodinane (Scheme 1). A Wittig reaction of **7** with phosphorane **8** afforded (*E*)-α,*β*-unsaturated ester **9** in near quantitative yield, but attempts to remove the *tert*-butyl ester from **9** with trifluoroacetic acid produced an intractable mixture. The use of trimethylsilyl triflate for this ester cleavage was more successful and gave carboxylic acid **10** in excellent yield. However, this left us with the problem of cleaving the C5 secondary TBS ether selectively in the presence of two additional secondary TBS ethers located in the epothilone sector after the coupling to produce our cryptophilone precursor (vida infra). Although this had been accomplished in high yield with TBAF in our route to epothilone D,[11] the strategy had failed completely in our approach to certain epothilone analogues.[12]

Therefore, an alternative route from **9** was pursued in which the silyl ether was first cleaved with TBAF to give hydroxy ester **11** (Scheme 2). Subsequent treatment with ice-cold trifluoroacetic acid then gave hydroxy acid **12**. This

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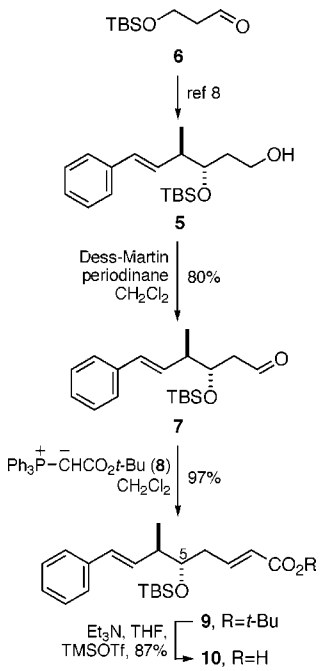
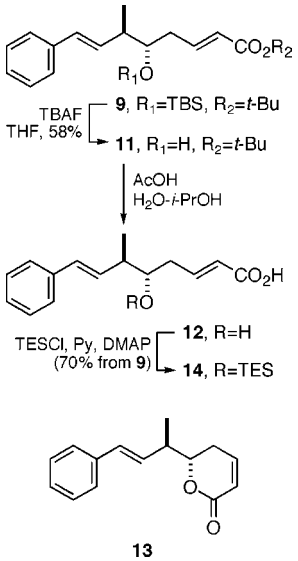
Scheme 1. Synthesis of a Cryptophycin Sector of Cryptothilone 1



highly polar substance was unstable under a variety of conditions, including those contemplated for coupling of **12** with a segment corresponding to C1−C9 of the epothilone nucleus. One of the substances formed from **12** was δ-lactone **13** resulting from (*E*)-to-(*Z*) isomerization of the conjugated olefin. In light of this problem, a milder route to hydroxy acid **12** was sought, and it was found that exposure of **9** to acetic acid in aqueous 2-propanol resulted in cleavage of both the silyl ether and the *tert*-butyl ester to give **12**. To carry this carboxylic acid forward for coupling with an

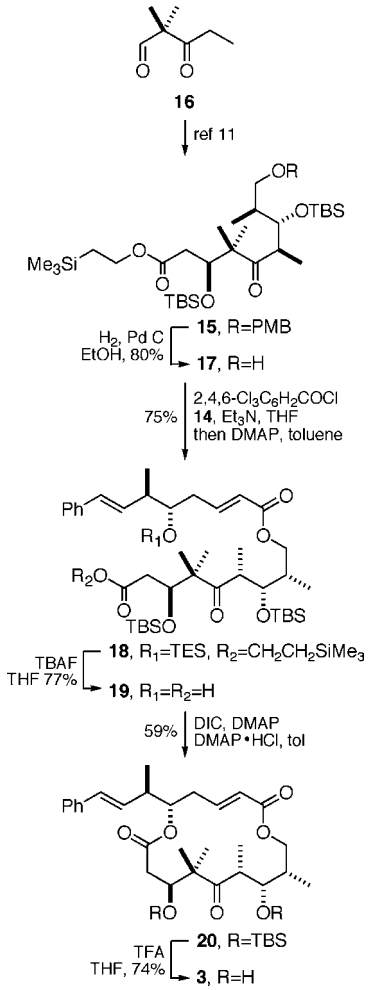
Scheme 2. Coupling Precursor for a Cryptophycin Sector of Cryptothilone 1



epothilone segment, the hydroxyl group of **12** was masked as its triethylsilyl ether **14**.

The epothilone sector required for assembling **3** was derived from **15**, prepared from keto aldehyde **16** in six steps as described previously.[11] Hydrogenolysis of the *p*-methoxybenzyl ether of **15** yielded alcohol **17** (Scheme 3), but

Scheme 3. Coupling of Cryptophycin and Epothilone Sectors of Cryptothilone 1



attempts to esterify this alcohol with carboxylic acid **14** employing carbodiimide activation gave a low yield of the desired product. Finally, the Yamaguchi protocol,[14] in which **14** was activated with 2,4,6-trichlorobenzoyl chloride and the resultant anhydride treated with **17** in the presence of DMAP and hot toluene, afforded ester **18** in good yield. Previous experience gleaned from our studies on epothilones had demonstrated that *tert*-butyldimethylsilyl ethers at C3 and C7 are not cleaved with TBAF, and it was therefore possible to remove both the TES ether and the trimethylsilylethyl ester from **18** with this reagent.

It was assumed that hydroxy acid **19** would lactonize to **20** under the same conditions we had used to close the 16-

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membered lactone of epothilone D and various analogues, but this proved to be a false hope. Exposure of **19** to the Yamaguchi protocol[14] led to an intractable mixture from which no lactone could be isolated. Fortunately, Keck−Steglich macrolactonization[15] of **19** was successful and delivered **20** in acceptable yield. Final cleavage of the pair of TBS ethers from **20** with trifluoroacetic acid gave cryptothilone 1 (**3**).

Preliminary experiments with cryptothilone 1 (**3**) in which crytophycin 1 and taxol (substituted for epothilone A) were used as controls showed that hybrid molecule **3** had no effect on the rate of tubulin assembly into microtubules. Both cryptophycin 1 and taxol were active under the experimental conditions used. The fact that **3** showed no acceleration of tubulin polymerization or depolymerization at concentrations up to 40 μM implies that this substance has no affinity for either the cryptophycin or the taxol/epothilone binding site on tubulin. However, further experiments including cell line assays are necessary before the concept of a structural motif based around these hybrid molecules as cytotoxic agents can be dismissed. One of those experiments will entail the synthesis of a second cryptothilone **4**.

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Supporting Information Available: Experimental procedures, characterization data, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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