Fourth International Electronic Conference on Synthetic Organic Chemistry (ECSOC-4), www.mdpi.org/ecsoc-4.htm, September 1-30, 2000

[C0024]

Synthetic Epothilone Analogs with Modifications in the Northern Hemisphere and the Heterocyclic Side-Chain - Synthesis and Biological Evaluation

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Received: 7 August 2000 / Uploaded: 10 August

Introduction

Epothilones A and B **I** (*Fig. 1*) are naturally occurring 16-membered macrolides, which are produced by the myxobacterium *Sorangium cellulosum*. [1] Although these compounds do not share any obvious structural similarities with the prominent anticancer drug paclitaxel (Taxol®), they exhibit a similar biological profile *in vitro*, including the ability to inhibit microtubule depolymerization and to induce apoptosis in human cancer cell lines with sub-nM IC50's. [2] [3] Epothilone B is a 3-20-fold more potent inhibitor of human cancer cell growth than paclitaxel and unlike paclitaxel is also effective against multidrug-resistant cell lines. Epothilone B has demonstrated potent *in vivo* antitumor activity [3] in and is currently undergoing Phase **I** clinical trials by Novartis.

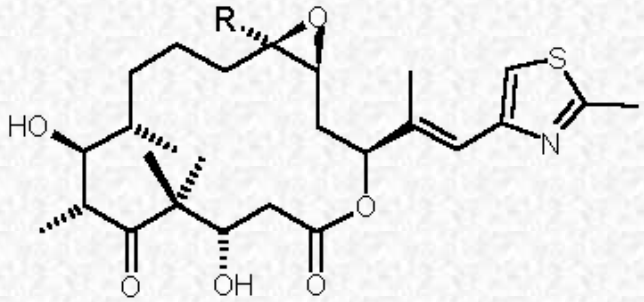
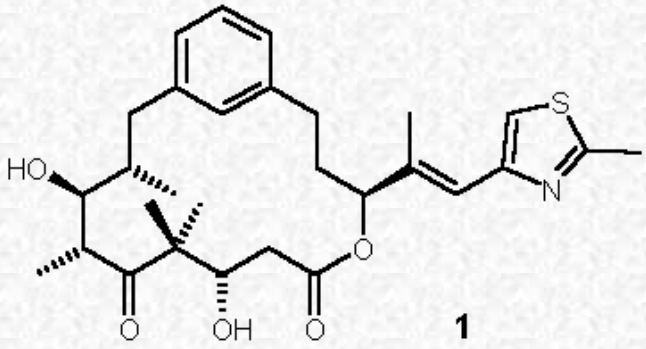


Figure 1: Structures of Epothilones A (R = H) and B (R = CH_3)

The biological profile of natural epothilones has triggered substantial synthetic efforts directed at the exploration of the SAR of this class of natural products and the discovery of analogs with an improved pharmacological profile. [4] This has led to a rather comprehensive understanding of the SAR of epothilones within an unusually short (given the complexity of these molecules) time period after the disclosure of their absolute stereochemistry in mid-1996. [5] Among others, three of the most relevant SAR features include the facts that (i) the presence of a C12-C13 epoxide moiety is not absolutely required for potent microtubule stabilization and profound antiproliferative activity (the epoxide may be replaced by a simple C12-C13 double bond), (ii) analogs incorporating a *trans* epoxide or *trans* olefin structure at C12/C13 appear to be almost equipotent with the corresponding *cis* isomers, and (iii) the replacement of the thiazole ring either by an oxazole or various pyridine [6] moieties is well tolerated. Within the context of these general aspects of the epothilone SAR this paper addresses three specific questions each of which is related to one of the above types of modifications:

- The potent biological activity of deoxyepothilones (incorporating a C12-C13 *cis* double bond in place of the epoxide moiety) [4] raises the possibility that the

introduction of conformational constraints in other parts of the macrocyclic framework could lead to potent analogs without a C12-C13 epoxide or even a C12-C13 olefinic double bond. This question was addressed in target structure **1**, which incorporates a *meta*-substituted phenylene moiety between C-9 and C-13, a region of potentially high conformational flexibility in the natural products. Based on an epothilone pharmacophore model developed in our laboratories, [7] the presence of this phenylene moiety was expected to stabilize the bioactive conformation of epothilones. This is illustrated in *Fig. 2*, which shows an overlay between a low energy conformation of **1** and the purported bioactive conformation of epothilone B.



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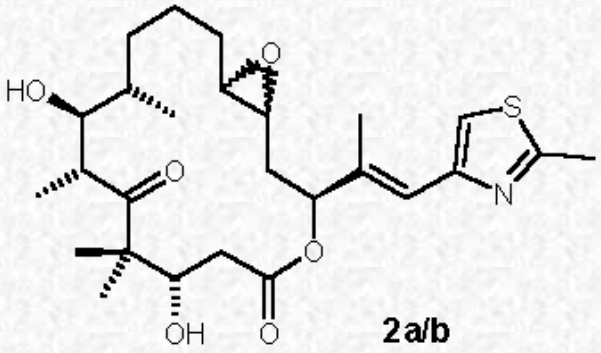
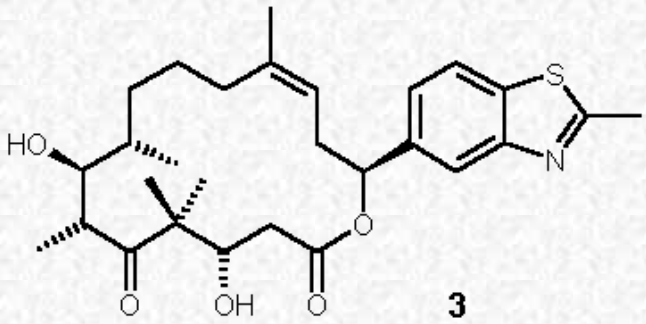
- The synthesis of *trans*-epothilones A **2** has previously been described in the literature by Nicolaou *et al*. [8] and the activity of "*trans*-epothilone A" against human ovarian and breast cancer cell lines was reported to be only marginally lower than that of epothilone A itself. [9] However, this active compound was obtained as the result of a non-selective epoxidation reaction with the corresponding *trans*-olefin precursor and the stereochemistry of the epoxide moiety was not established. In order to clarify the relationship between epoxide stereochemistry and biological activity in *trans*-epothilones A we have developed a stereoselective route to both *trans*-epothilone A isomers and we have determined their biological activity.



2a/b

- Based on the observation that the conformation of the epothilone side-chain both in the X-ray crystal structures of epothilones A/B [5][10] as well as in our pharmacophore model is characterized by a co-planar arrangement of the thiazole ring with the olefinic double bond between C16 and C18, we have designed a new type of side-chain modified epothilone analog which is exemplified by target structure **3**. In this benzothiazole-based analog the C16-C18 olefinic double bond is made part of a six-membered aromatic ring which also incorporates C17, C19 and C22 of the original epothilone structure, thus leading to a perfect co-planar arrangement of the C16-C18 "double bond" and the thiazole ring and reducing the inherent conformational entropy of the heterocyclic side-chain moiety.



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Results and Discussion

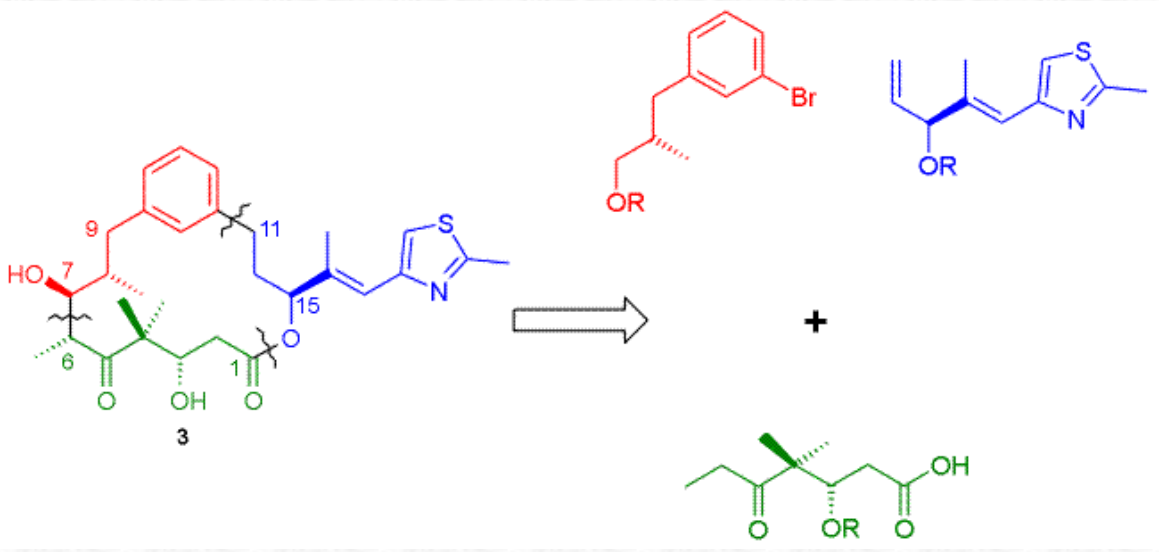
1. Retrosynthesis

The retrosynthetic analysis for target structures **1** - **3** is summarized in *Schemes 1A* (**1**) and *1B* (**2**, **3**). In all three cases the key steps for construction of the macrocyclic skeleton involve *Yamaguchi* macrolactonization, the build-up of the requisite

seco-acid through aldol reaction between the C7-C15 aldehyde and the dianion of the O-protected C1-C6 b-hyroxy acid fragment, and the assembly of the C7-C15 aldehyde through the appropriate type of Pd(0)-catalyzed coupling reaction. In the case of **2a**/**b** macrolactonization and deprotection were to be followed by stereoselective epoxidation of the *trans* C12-C13 double bond. This approach in essence represents a combination of the strategies developed by Schinzer *et al*. [11] and Nicolaou *et al*. [4a] for the synthesis of the natural products.

Scheme 1

A



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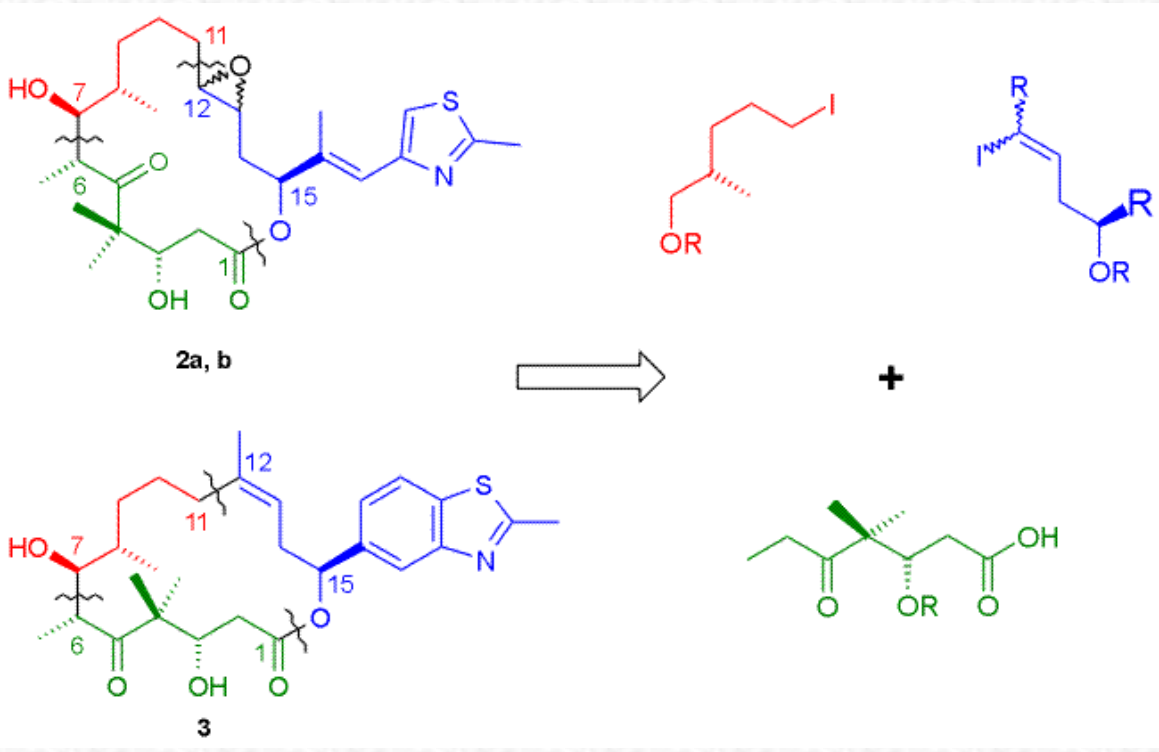
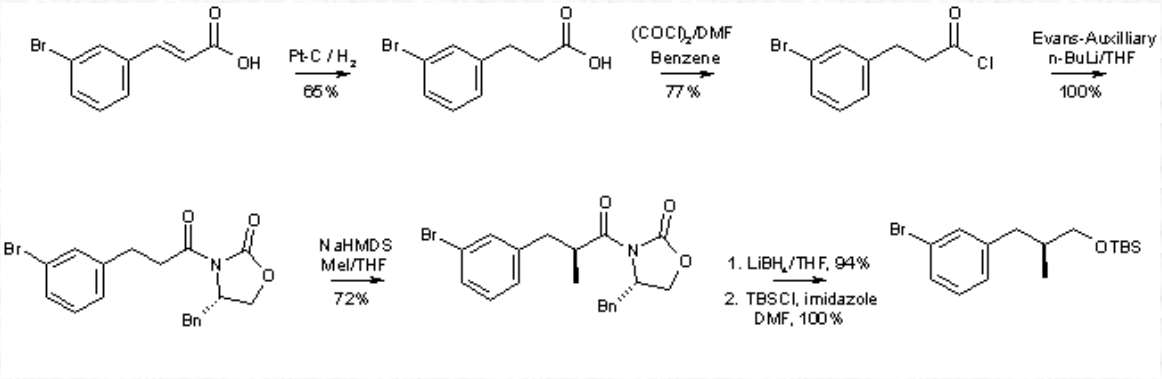


2. Synthesis of Conformationally Constrained Epothilone Analog 1

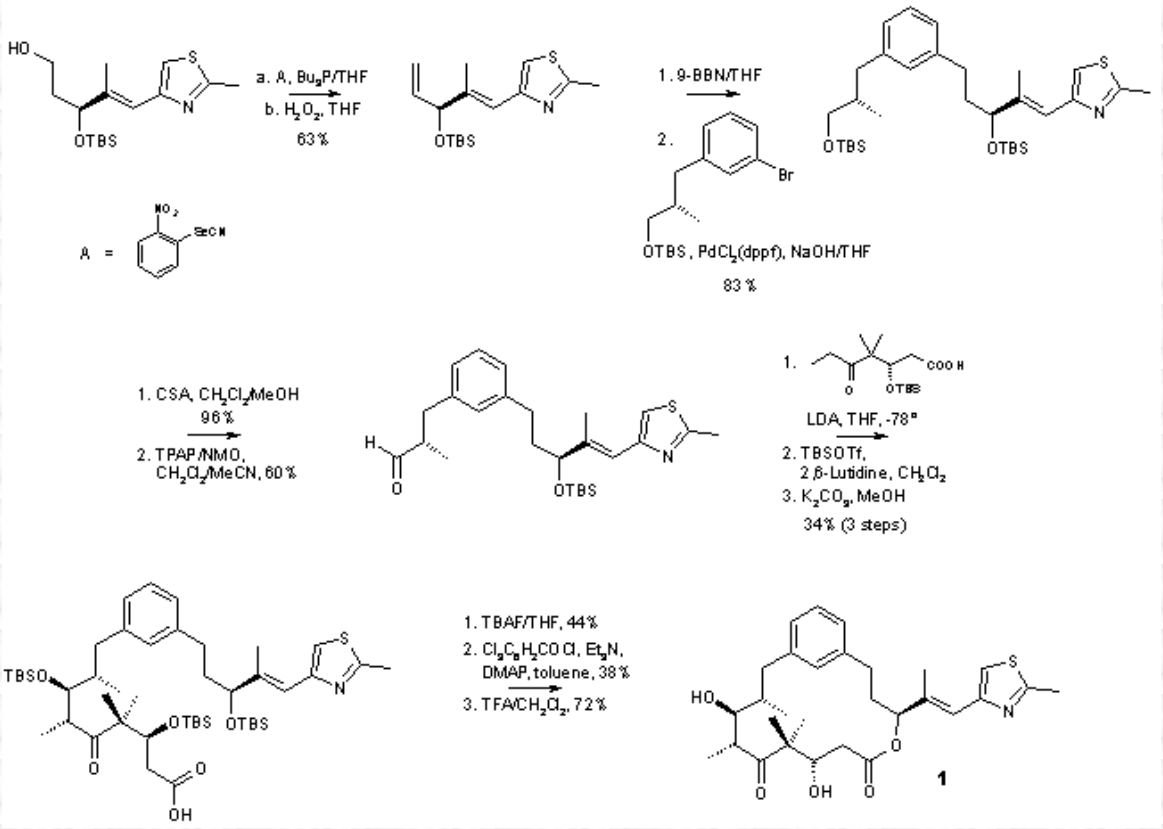
The synthesis of epothilone analog **1** is summarized in *Schemes 2* and *3*. The protected C7-C12 fragment (red structure in *Scheme 1*, R = TBS) was obtained from commercially available 3-bromocinnamic acid as the starting material, using *Evans* chemistry to establish the chiral center at C8 (epothilone numbering, *Scheme 2*)) . The critical junction between fragments C7-C12 and C13-C15 was achieved through alkyl *Suzuki* coupling between the C7-13 aryl bromide and a C13-

C15 terminal olefin in excellent 83% yield. Transformation of this coupling product to the aldehyde and subsequent aldol reaction with the dianion of the O-protected b-hydroxy acid comprising the C1-C6 fragment of epothilones yielded the desired *anti*-Felkin *syn* aldol product with 5:1 selectivity. The b-hydroxy acid (green structure in *Scheme 1*, R = TBS) was prepared as suggested by de Brabander *et al*. [12] However, it should be noted that contrary to what is reported in ref. [12], the preparation of the desired (*3S*)-enantiomer requires the use of the (***2R***)-bornane-10,2-sultam as chiral auxiliary. The synthesis was completed by *Yamaguchi* macrolactonization, which proceeded in moderate yield, followed by deprotection with TFA/CH_2Cl_2.

Scheme 2: Epothilone Analog* 1 *- Synthesis of the C7-C12 Fragment



Scheme 3: Epothilone Analog* 1 *- Synthesis of the C13-C15 Fragment and Construction of the Macrocycle

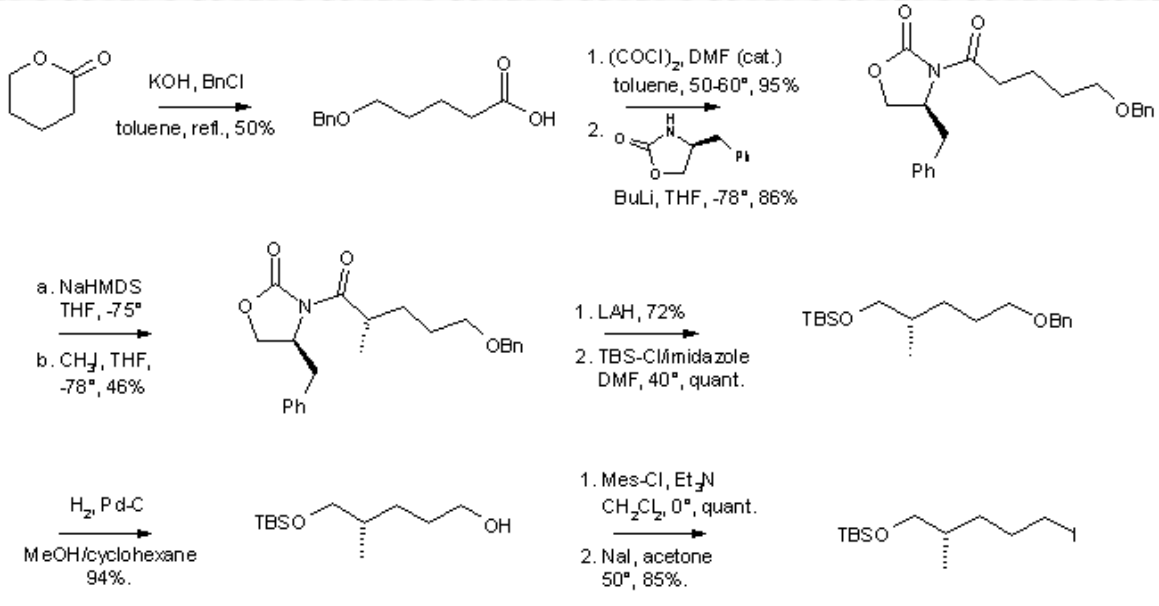


3. Synthesis of *Trans*-Epothilones A 2a/b

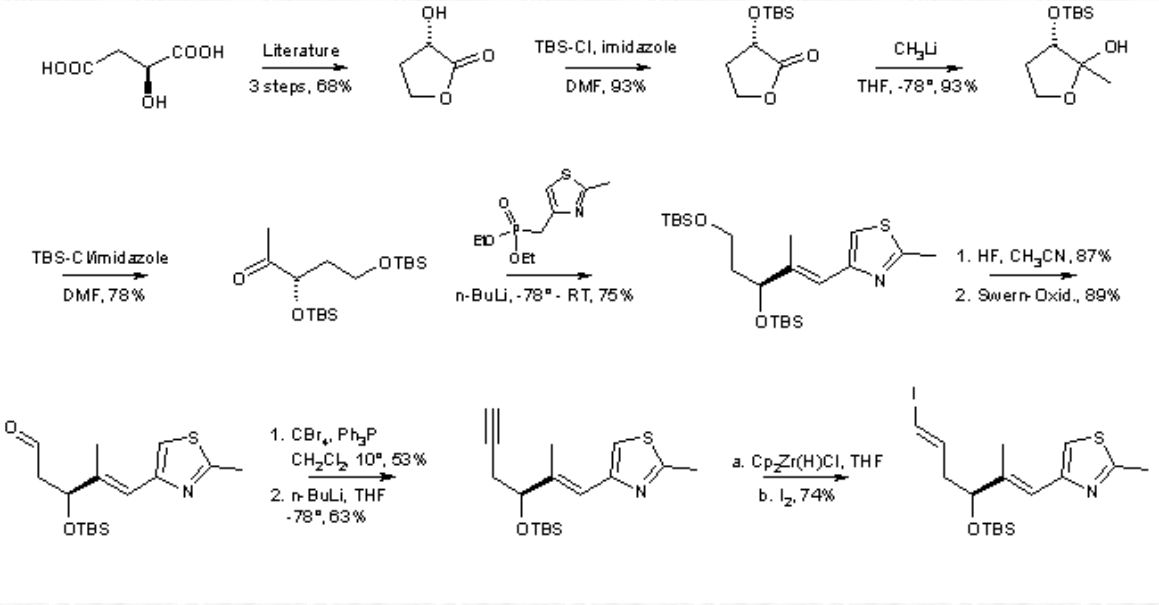
The stereoselective synthesis of *trans*-epothilones A is summarized in *Schemes 4 – 7.* The strategies for the preparation of the C7-C11 (*Scheme 4*) and C12-C15 (*Scheme 5*) fragments are closely related to those devised by Schinzer *et al*. [9] and either involve *Evans* chemistry or a chiral pool starting material ((*S*)-malic acid; fragment C12-C15, *Scheme 5*) to establish the required chiral centers. The coupling of fragments C7-C11 and C12-C15 in this case was achieved through Pd(0)-catalyzed reaction between the C12-C15 *trans* vinyl iodide and the zincate derived from the C7-C11 alkyl iodide in 64% yield (*Scheme 6*). The most critical step in the preparation of *trans*-epothilones A proved to be the chemo- and

stereoselective epoxidation of the C12-C13 *trans* double bond, which was finally accomplished using fructose derived epoxidation catalysts developed by *Shi* [13] (*Scheme 7*). The stereochemistry of the epoxidation products was unambiguously established by X-ray crystallographic analysis of compound **2a**.

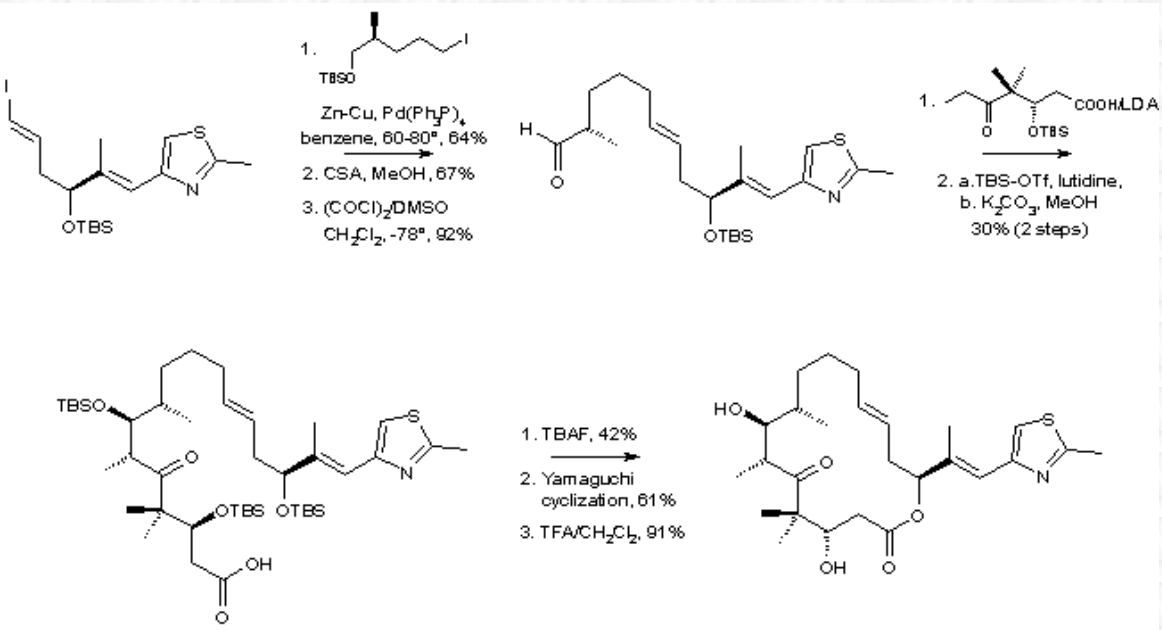
Scheme 4: Trans-Epothilones A - Synthesis of the C7-C11 Fragment



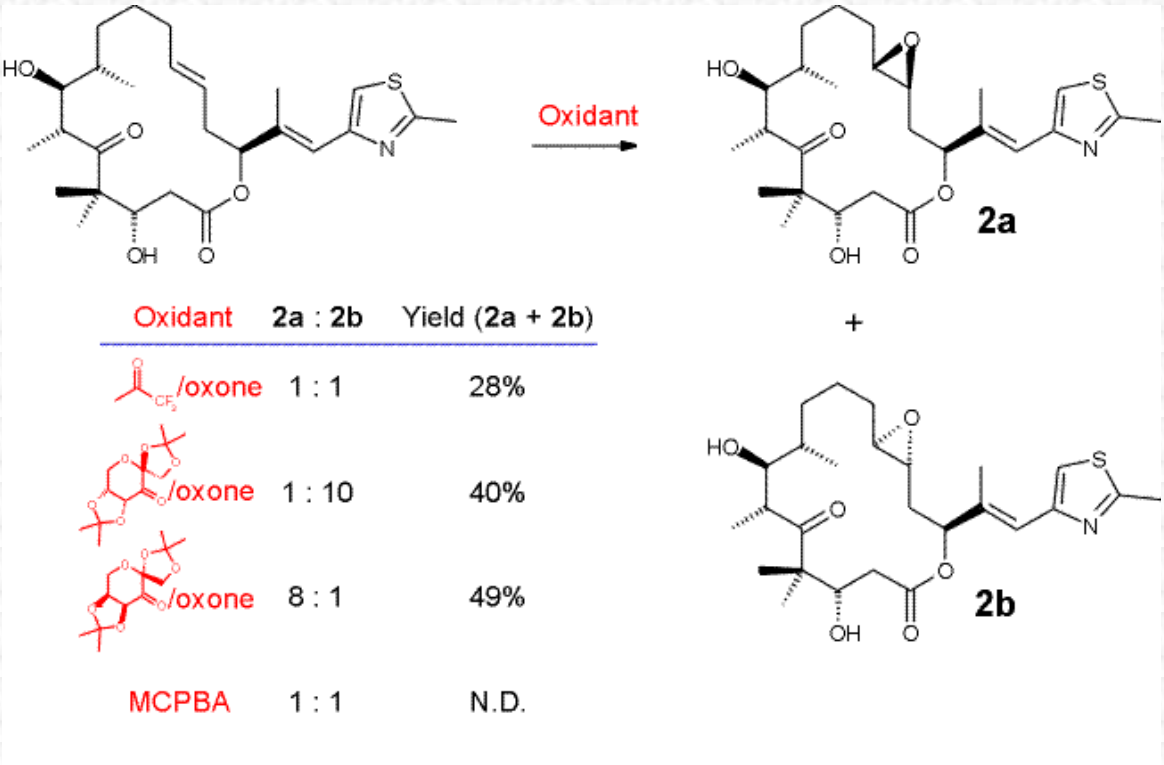
Scheme 5: Trans Epothilones A - Synthesis of the C12-C15 Fragment



Scheme 6: Trans-Epothilones A - Synthesis of the Olefin Precursor



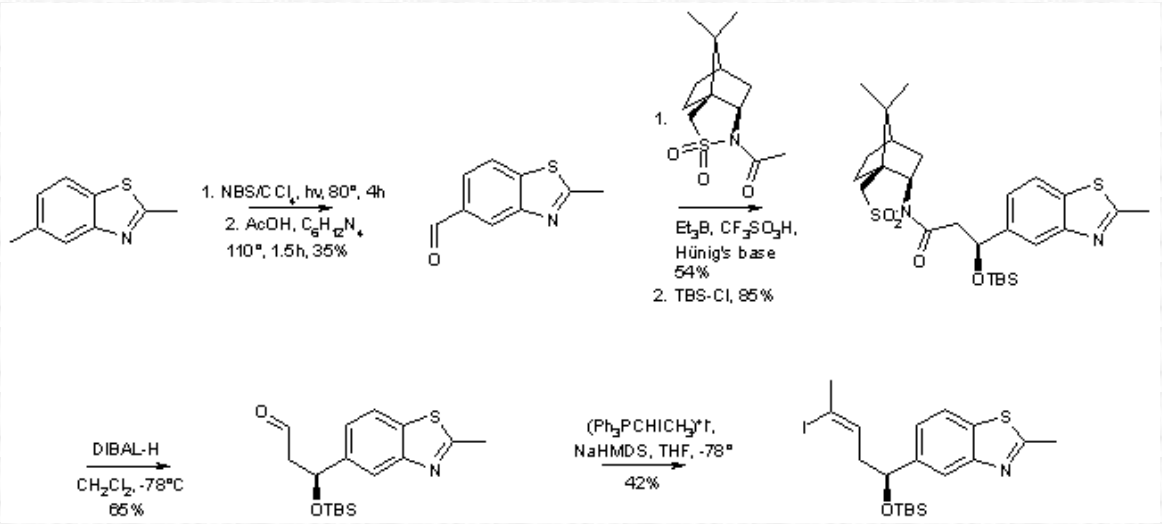
Scheme 7: Trans-Epothilones A – Epoxidation Selectivity



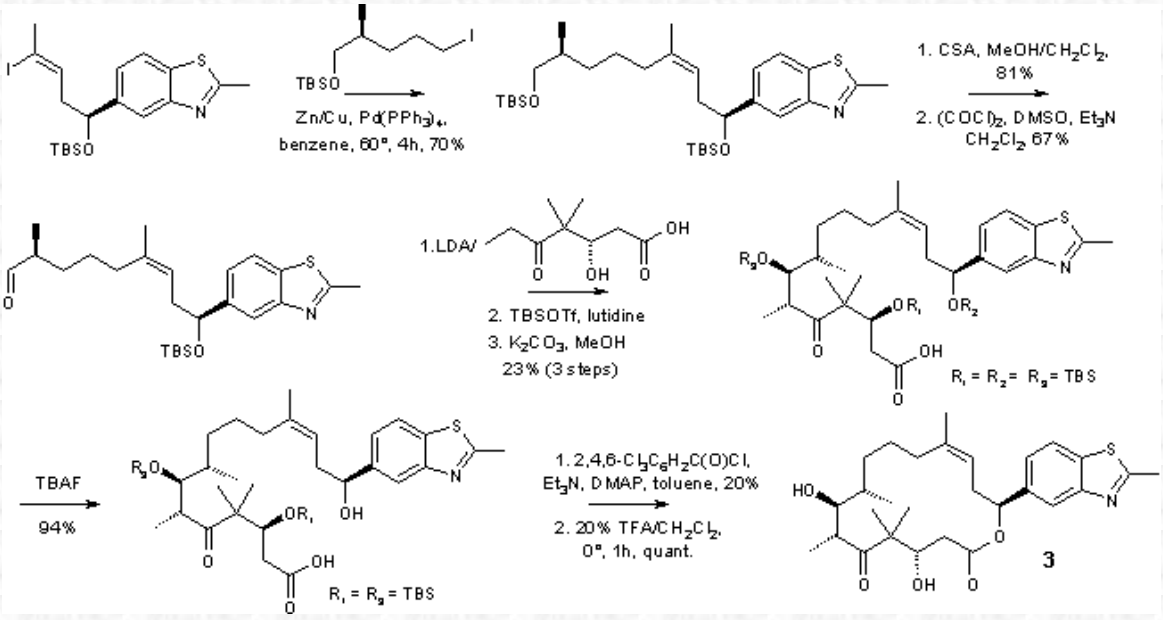
4. Synthesis of Side-chain modified Deoxyepothilone B Analog 3

The synthesis of side-chain modified deoxyepothilone B analog **3** is summarized in *Schemes 8 and 9*. The retrosynthetic analysis outlined in *Scheme 1* called for a C12-C15 *cis*-vinyl iodide as one of the coupling components for the construction of the macrocycle. This compound was obtained through aldol reaction between 2-methylbenzothiazole-5-carbaldehyde and (2R)-N-acetylbornane-10,2-sultam (*ca*. 4/1 selectivity), followed by O-protection, reduction to the aldehyde and Wittig-olefination [14] (*Scheme 8*). The following construction of the macrocycle mirrors the chemistry employed for the synthesis of **2a/b**, except that a significantly lower yield was obtained in the macrolactonization step, which was accompanied by the formation of significant amounts of the cyclic dimer (*Scheme 9*).

Scheme 8: Deoxyepothilone B analog* 3 *– Synthesis of the C12-C15 Vinyl Iodide



Scheme 9: Deoxyepothilone B analog* 3 *– Construction of the macrocycle



5. Biological Activity

Epothilone analogs **1**, **2a/b**, and **3** were assessed for their ability to induce tubulin polymerization *in vitro* (as a measure for their microtubule stabilization potential) and to inhibit the growth of human cancer cells, either in a drug-sensitive or a drug-resistant background. The results of these studies are summarized in *Table 1*.

Table 1: Biological Activity of Epothilone Analogs.

		Growth Inhibition (IC50 [nM])[b]	
Epothilone analog	Tubulin Polymerization (%)[a]	KB-31[c]	KB-8511[d]
1	< 10	> 1000	> 1000
2a	85	1.00	0.87

2b	< 10	523	305
3	79	0.45	0.23
Epothilone A	65	2.15	1.91
Epothilone B	85	0.20	0.20
Deoxyepothilone B	93	2.70	1.44
Paclitaxel (Taxol®)	44	2.84	615

[a]Induction of tubulin polymerization at 2 mM compound concentration relative to the effect of 25 mM Epothilone B, which is defined as the 100% level. [b]Cancer Cell growth inhibition was assessed after a 72h exposure period. [c]Human epidermoid cancer cell line, which is sensitive to growth inhibition by most common anticancer agents. [d]P-glycoprotein (P-gp)-overexpressing subline of the KB-31 parental line, which is resistant to treatment with paclitaxel.

Epothilone analog **1** proved to be devoid of any appreciable biological activity. This may either indicate that our pharmacophore model does not appropriately reflect the bioactive conformation of epothilones; alternatively, the additional steric bulk associated with the phenylene moiety could conceivably more than outweigh any possible activity gain arising even from proper conformational stabilization. With regard to *trans*-epothilones A the data in *Table 1* clearly demonstrate that **2a** is at least equipotent with, if not more potent than epothilone A itself, while its diastereoisomer **2b** is 500-fold less potent. Likewise, **3** exhibits significantly higher biological activity than its parent compound deoxyepothilone B. Based on these *in vitro* data **2a** as well **3** represent promising candidates for further profiling *in vivo*.

6. Acknowledgement

The skilled technical assistance by K. Hauenstein, W. Vetterli, J. Koppler, and M. Lartigot is gratefully acknowledged. N. van Campenhout and P. Furet are thanked for the development of the epothilone pharmacophore model.

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