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Epothilone A–D and their thiazole-modified analogs as novel anticancer agents*

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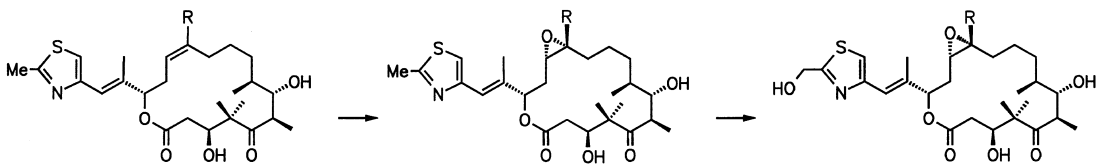
Abstract: Starting from epothilone A–D (**1a–2b**) obtained by large scale fermentation of the myxobacterium *Sorangium cellulosum* the thiazole side-chain was extensively modified by substitution, oxidation and replacement. Metallation afforded the C-19 carbanion **4** which was quenched by various carbon and heteroatom electrophiles to give C-19 substituted epothilones **5**. Thiazole *N*-oxides **9** were obtained by treatment of **2a** and **2b** with *m*-chloroperbenzoic acid and rearranged by acetic anhydride to 21-acetoxy epothilones **10**. Cleavage of epothilones A and B with ozone gave methyl ketones **11** from which carbonyl derivatives **12**, **13**, **14**, and aldol condensation products **16** were prepared. Similarly vinyl boronic acid **17** was obtained and transformed by Suzuki coupling or iodination/Stille coupling to aryl and heteroaryl analogs **15**.

INTRODUCTION

During the past four years epothilones [1] have attracted great interest amongst biologists and synthetic chemists after it was discovered by Bollag *et al*. [2] that their cytotoxic properties are based on the same mode of action as described for paclitaxel (Taxol®) [3], i.e. stabilization of microtubules, arrest of mitosis, and programmed cell death (apoptosis). High activity against multidrug resistant tumor cell lines [2,4] and other favourable properties [1,5] soon promoted epothilones into promising anticancer drug candidates.

In the meantime more than a dozen total syntheses have been published [6,7] and a great variety of structural analogs synthesized *en route* to the natural epothilones A–E (**1a–3a**) to elucidate the structure/activity relationships [6–8].

First results of *in vivo* studies on mouse models were reported by Danishefsky *et al*. In these studies epothilone B (**2b**) demonstrated efficacy which was however accompanied by serious toxic side effects [9]. In contrast, the significantly less active epothilone D (**1b**) showed a promising therapeutic range with good activity against sensitive and multidrug resistant tumors up to the point of complete remission [10].



Scheme 1 Biosynthesis of epothilones A and B (**2a** and **2b**) by epoxidation of epothilones C and D (**1a** and **1b**) and their hydroxylation to epothilones E and F (**3a** and **3b**) by *Sorangium cellulosum*.

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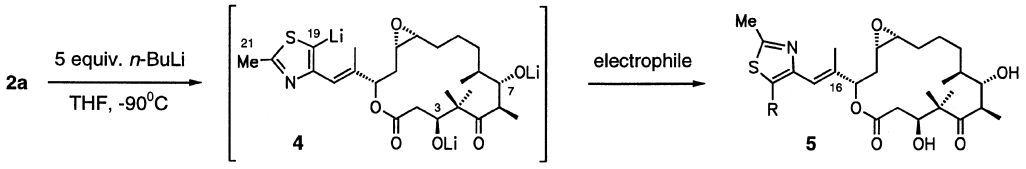
In the search for possible clinically suitable derivatives, we have concentrated our efforts on the isolation of natural structural variants [11] and the chemical modification of the basic epothilones A–D [12] obtained by large scale fermentation of the myxobacterium *Sorangium cellulosum*. During these fermentations the metabolites are released into the culture medium and can be easily extracted and isolated by chromatography [1,11]. The abundant epothilones A (**2a**) and B (**2b**) are obtained in a crystalline state in multigram quantities. Minor constituents are epothilone C and D (**1a**, **1b**), according to feeding experiments the biosynthetic precursors of **2a** and **2b** [13]. In addition small amounts of epothilone E (**3a**) and F (**3b**) are sometimes observed as microbial hydroxylation products of **2a** and **2b** (Scheme 1) [13].

This report describes the chemical modification of the thiazole side-chain of epothilone A–B (**1a–2b**) along three lines: C-19 carbanion formation followed by reaction with electrophiles, *N*-oxidation and rearrangement to C-21 substituted analogs, and cleavage of the C-16/C-17 double bond and introduction of new side-chains.

METALLATION OF C-19 AND REACTION WITH ELECTROPHILES

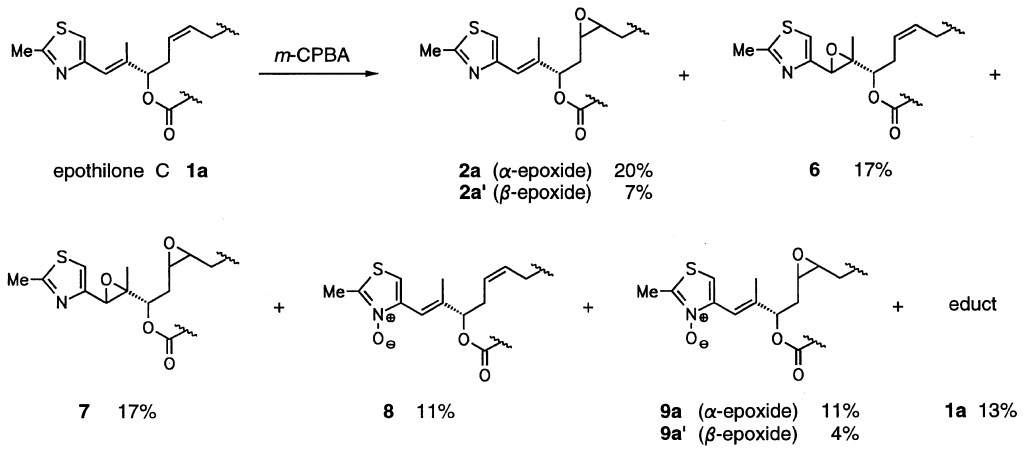
In the course of functional group manipulations we treated epothilone A (**2a**) with strong bases followed by quenching with D_2O. Surprisingly no deuterium was incorporated in α-position of the carbonyl groups nor was significant addition observed of e.g. butyl lithium to the carbonyl or epoxide groups. Instead with 5 equiv. *n*-butyl lithium in THF at −90 °C only the heteroaryl position C-19, and to a small extent the benzylic position C-21, were metallated in addition to the 3-OH and 7-OH groups. After quenching with a variety of electrophiles C-19 substituted epothilones **5** were obtained [12e]. Although only yields of 15–50% of **5** in addition to 20–40% of starting material were achieved after optimization, this one step procedure is extremely valuable in preparing a broad spectrum of analogs for SAR investigations. As all derivatives **5** were essentially inactive in the cytotoxicity cell assay it may be concluded that the conformation of epothilones in the tubulin binding site is restricted to *cis* for 19-H and 16-Me. For steric reasons this conformation cannot be attained in **5** with R bigger than proton. Only some C-21 alkylated analogs were obtained as by-products of the metallation/quenching procedure and found to be biologically active provided the residue introduced was not too big. Therefore a search was started for more general access to C-21 substituted analogs.



N-OXIDATION AND POLONOVSKY-TYPE REARRANGEMENT

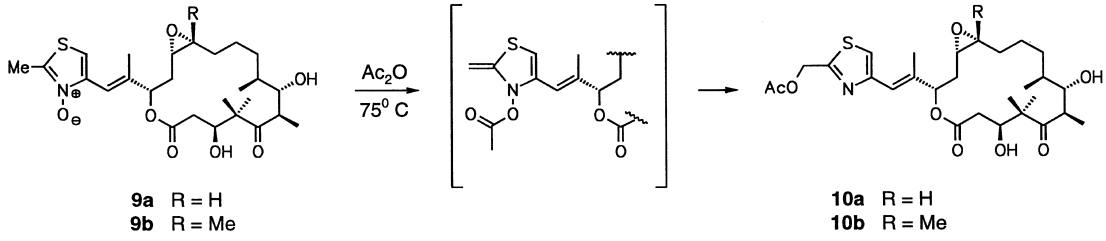
The introduction of heteroatoms at C-21 was achieved by an entirely different route discovered by serendipity. When we investigated the outcome of the epoxidation of epothilone C (**1a**), the last intermediate in some of the ongoing total syntheses of epothilone A (**2a**) at that time [6], we observed complex reaction mixtures by HPLC and ^{1}H-NMR. Particularly with *m*-chloroperbenzoic acid in addition to epothilone A (**2a**), and its β-epoxide isomer **2a′**, we identified two stereoisomers of the 16,17-epoxide **6**, four stereoisomers of the bis-epoxide **7** and three compounds **8**, **9a** and **9a′** with a strong unexpected chromophore ($\lambda_{max} = 236$ nm) [14,15]. The same chromophore developed when we attempted to transform the C-5 ketone of epothilone A or B into a lactone by Bayer-Villiger oxidation [12a].

From analytical data including ^{1}H, ^{15}N-correlated NMR spectra ($\delta_N = 282$ ppm, reference liquid $NH_3 = 0$ p.p.m) the *N*-oxide structure of **9a** was assigned and confirmed by X-ray crystal structure analysis [16]. An advantage is that the *N*-oxides **9a** and **9b** can be prepared in 50% isolated yield directly from epothilone A and B and *m*-chloroperbenzoic acid.



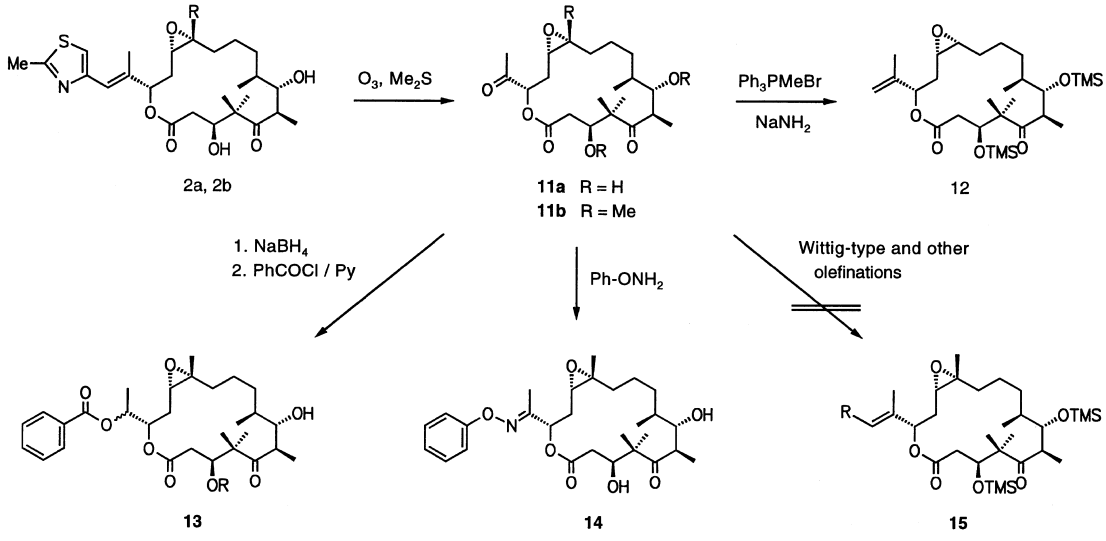
Scheme 2 Reaction products of epothilone C (**1a**) and 1.5 equiv. *m*-chloroperbenzoic acid in CH_2Cl_2 at 50 °C.

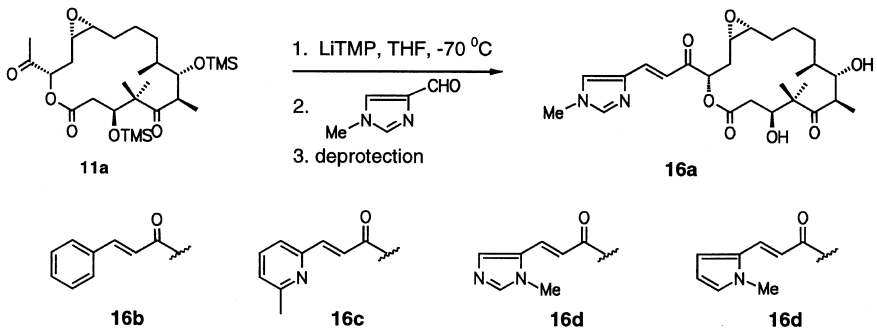
When the *N*-oxides **9a** and **9b** were treated with acetic anhydride at 75 °C a rapid Polonovsky-type rearrangement into the 21-acetoxy epothilones **10a** and **10b** was observed. Cleavage of the acetate esters with dilute ammonia or pig liver esterase gave good yields of epothilone E and F (**3a** and **3b**). Obviously the hydroxymethyl group in **3** can be extensively modified further using standard chemistry.



REPLACEMENT OF THE THIAZONE SIDE-CHAIN

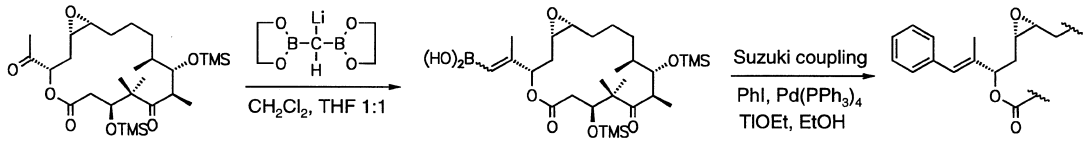
More deep-seated modifications of the side-chain required cleavage of the C-16, C-17 double bond with ozone to give the central intermediate methyl ketone **11a** [12c]. From this common carbonyl derivatives, e.g. *O*-phenyloxime **14**, or, after borohydride reduction, benzoate **13** were obtained. After TMS protection of the hydroxyl groups to **11b** olefination with Schlosser's 'instant ylid' gave exo-methylene derivative



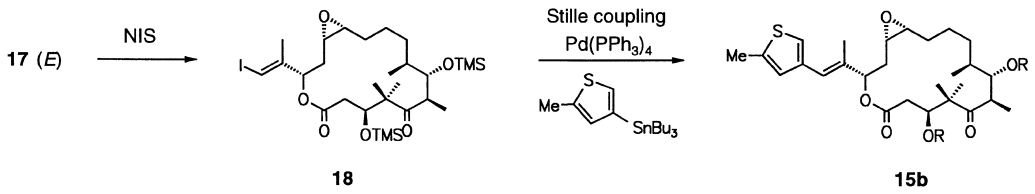


12 in moderate yield. However, in spite of extensive experimentation with various olefination methodologies no higher homologue **15** could be produced.

As this failure was presumably caused by enolisation of the methyl keto group we investigated aldol condensations of **11a**. Using lithium tetramethylpiperidid various aromatic and heterocyclic aldehydes could be condensed to α,β-unsaturated ketones **16** in good to moderate yields. The heterocycles in **16** were hoped to mimic the thiazole side-chain of epothilone, whereas the *N*-methyl urocanyl residue in **16a** might mimic the side-chain of the tubulin inhibitor eleutherobin [17]. However, none of the analogs **16** showed significant activity in the cell toxicity or tubulin assay.



Finally a Wittig-type condensation of **11b** was achieved with a good yield using the less basic bisboryl methyl lithium reagent [18]. The resulting boronic acid **17** (83% of *E*,*Z* mixture 7:3) [19] proved to be a valuable intermediate for chain extension reactions. Thus the *E*-isomer smoothly underwent Suzuki coupling with iodobenzene to phenyl analog **15a** or could be converted with iodosuccinimide into vinyl iodide **18** with retention of configuration [20]. The latter, similarly to published procedures [7b], reacted with tributyltin heterocycles under Stille conditions to e.g. thiophene analog **15b**.



CONCLUSION

In summary, it could be shown that the thiazolyl side-chain of epothilones can be extensively modified in the presence of the various sensitive functional groups of the macrocycle. In some reactions not even the 3,7-hydroxyl groups need protection. Although yields are moderate and sometimes low, a variety of analogs could be prepared from the fermentation products in very few steps.

The structure-activity relationships for this part of the molecule are in line with published data obtained from analogs prepared by total synthesis. Only few modifications are tolerated without significant loss of activity, i.e. replacement of the thiazole by an oxazole ring or introduction of small substituents at C-21. A more detailed evaluation of the biological properties of these derivatives is in progress.

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