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Stereoselective Syntheses and Evaluation of Compounds in the 8-Desmethylepothilone A Series: Some Surprising Observations Regarding Their Chemical and Biological Properties

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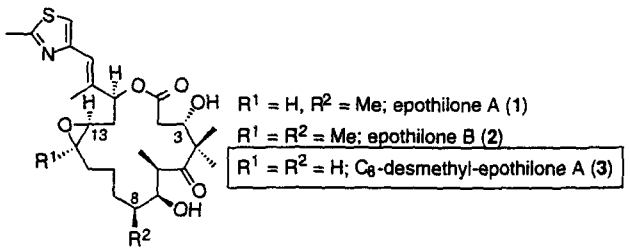
Abstract: The title compounds have been synthesized in a convergent way by recourse to a Weiler type dianion construction. © 1997 Elsevier Science Ltd.

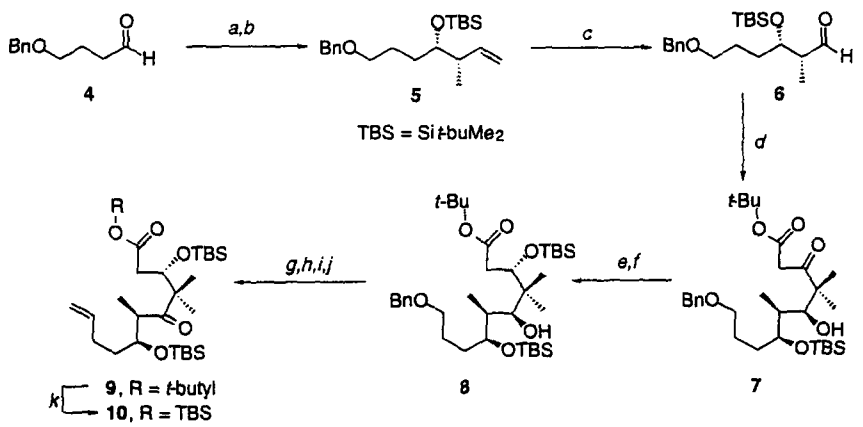
Recently, several groups have described total syntheses of epothilones A (**1**) and B (**2**)[1,2] whose mode of antitumor action closely mimics that of taxol ™.[3] Although taxol™ (Paclitaxel) is a clinically proven drug, its formulation continues to be difficult. In addition, taxol induces the multidrug resistance (MDR) phenotype. Hence, any novel agent that has the same mechanism of action as taxol and has the prospect of having superior therapeutic activity warrants serious study.[4]

A central challenge now is that of generating epothilone analogs that are more effective and more readily synthesized than is the case for **1** and **2**. Though the syntheses of the natural products can provide ample material for preliminary biological evaluation, the prospect of producing adequate amounts of these compounds for full development would be daunting. One particular area where a structural change could bring significant relief from the complexities of the synthesis would be in the deletion of the C_8 methyl group from the polypropionate domain (see goal system **3**). The need to deal with this C_8 chiral center complicates all of the syntheses of epothilone thus far reported. In the context of our own program, deletion of the C_8 methyl group would prompt a major change in synthetic strategy relative to our earlier diene-aldehyde cyclocondensation route.[5]



Asymmetric crotylation[6] (87% ee) of **4** followed by protection led to the TBS ether **5**. The double bond was readily cleaved to give rise to aldehyde **6**. The aldehyde was coupled to the dianion derived from *t*-butyl isobutyrylacetate to provide **7**. The ratio of the C_{5S} (**7** shown here): C_{5R} compound (not shown) is *ca* 10:1. That the Weiler-type β-ketoester dianion chemistry[7] can be conducted in the context of the isobutyryl group prompts several alternate perceptions for still more concise syntheses. Directed reduction of the C_3 ketone of **7** following literature precedents,[8] followed by selective silylation of the C_3 hydroxyl gave a 50% yield of a 10:1 ratio of the required C_{3S} (see compound **8**): to C_{3R} isomer (not shown).[9] The carbinol, produced upon debenzylation, was oxidized to an aldehyde which, following methylenation through a simple Wittig reaction, afforded olefin **9**. Treatment of this compound with TBSOTf provided ester **10** which was used directly in the Suzuki coupling with the vinyl iodide **12** (*vide infra*).

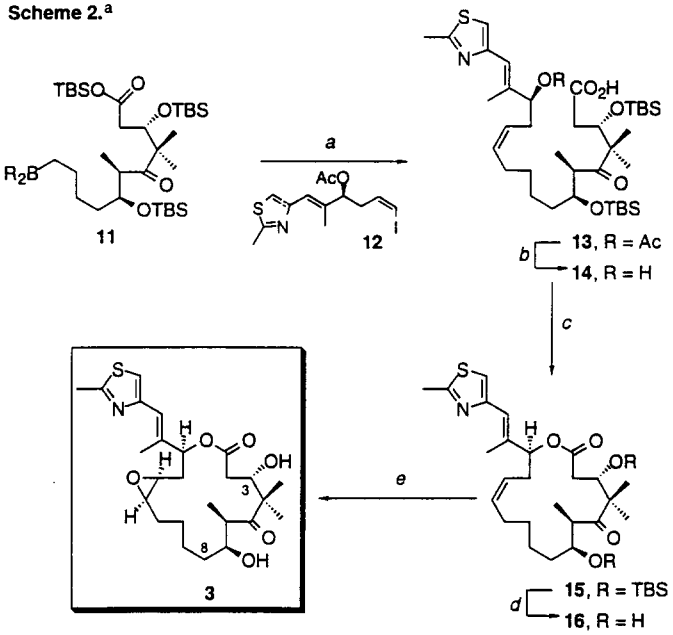
Scheme 1.[a]



[a] a) (*Z*)-Crotyl-B[(-)-Ipc]$_2$, -78°C, Et_2O, *then* 3*N* NaOH, 30% H_2O_2; b) TBSOTf, 2,6-lutidine, CH_2Cl_2 (74% for two steps, 87% ee); c) O_3, CH_2Cl_2/MeOH, -78°C, then DMS, (82%); d) *t*-butyl isobutyrylacetate, NaH, BuLi, 0°C, then **6** (60%, 10:1); e) $Me_4NBH(OAc)_3$, -10°C (50%, 10:1 α/β) or $NaBH_4$, MeOH, THF, 0°C, (88%, 1:1 α/β); f) TBSOTf, 2,6-lutidine, -40°C, (88%), g) Dess-Martin periodinane, (90%); *h*) $Pd(OH)_2$, H_2, EtOH, (96%); *i*) DMSO, *oxalyl chloride*, CH_2Cl_2, -78°C (78%); j) Methyl triphenylphosphonium bromide, NaHMDS, THF, 0°C (85%); k) TBSOTf, 2,6-lutidine, CH_2Cl_2, rt (87%).

The hydroboration of **10** with 9-BBN produced intermediate **11** which, on coupling with the vinyl iodide **12** and *in situ* cleavage of the TBS ester led to **13**. After de-acetylation, the hydroxy acid **14** was in hand. Marcolactonization[10] of this compound produced **15** which, after desilylation, afforded C_8-desmethyldesoxyepothilone (**16**). Finally, epoxidation of this compound with dimethyldioxirane produced the goal structure **3**. The stereoselectivity of epoxidation was surprisingly poor (1.5:1) given that epoxidation of desoxyepothilone A occurred with >20:1 stereoselectivity. *Apparently, the deletion of the C_8 methyl group tilts the conformational distribution of **16** to forms in which the epoxidation by dimethyl dioxirane is less β-selective.*[11]

Scheme 2.[a]



[a] a) $Pd(dppf)_2Cl_2$, Ph_3As, Cs_2CO_3, H_2O, DMF, rt (62%); b) K_2CO_3, MeOH, H_2O (78%); c) DCC, 4-DMAP, 4-DMAP•HCl, $CHCl_3$ (78%); d) HF•pyr, THF, rt (82%), e) 3,3-dimethyl dioxirane, CH_2Cl_2, -35°C (72%, 1.5:1).

Compounds **3** and **16** were tested for cytotoxicity in cell cultures and assembly of tubulin in the absence of GTP. Surprisingly, neither macrolide displayed significant tubulin polymerization.[12] Cytotoxicity studies showed drastically reduced activity in the 8-desmethyl series. Compounds **3** and **16** were approximately 200 times less active than their corresponding epothilone A counterparts (see Table). Recalling earlier SAR findings at both C_3 and C_5, in conjunction with the findings reported here, the polypropionate sector of the epothilones emerges as a particularly sensitive locus of biological function.[2,13] Further studies on the SAR of epothilones congeners, enabled by improved access through synthesis, are ongoing and will be disclosed in due course.

Table 1. *Relative efficacy of epothilone compounds against drug-sensitive and resistant human leukemic CCRF-CEM cell lines.*[a]

Compound	CCRF-CEM IC_{50} (μM)[b]	CCRF-CEM/VBL IC_{50} (μM)[b]	CCRF-CEM/VM_1 IC_{50} (μM)[b]
16	5.00	5.75	6.29
3	0.439	2.47	0.764
epothilone A (1)	0.003	0.020	0.003
desoxyepothilone A	0.022	0.012	0.013
epothilone B (2)	0.0004	0.003	0.002
desoxyepothilone B	0.009	0.017	0.014
taxol®	0.002	3.390	0.002

[a]The cytotoxicities of test compounds were determined by the growth of human lymphoblastic leukemic cells CCRF-CEM, or their sublines resistant to vinblastine and taxol (CCRF-CEM/VBL) or resistant to etoposide (CCRF-CEM/VM-1). XTT-microculture tetrazolium/formazan assays were used.

[b]The IC_{50} values were calculated from 5-6 concentrations based on the median-effect plot using computer software.

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9. Reduction with sodium borohydride afforded a ca. 1:1 mixture of C_3 epimers.
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11. It is interesting to speculate as to whether the effect of the C_8 methyl on the stereoselectivity of epoxidation by dimethydioxirane and the dramatic reduction of biological activity are related. Molecular modeling studies designed to explore this theme are under way.
12. Microtubule protein (MTP) was purified from calf brains by two cycles of temperature dependent assembly and disassembly.[14] In control assembly experiments, MTP (1 mg/mL) was diluted in assembly buffer containing 0.1 M MES (2-(N-morpholino) ethanesulfonic acid), 1 mM EGTA, 0.5 nM $MgCl_2$, 1mM GTP and 3M glycerol, pH 6.6. The concentration of tubulin in MTP was estimated to be about 85%. Assembly was monitored spectrophotometrically at 350 nm, 35°C for 40 min by following changes in turbidity as a measure of polymer mass.[15] Drugs were tested at a concentration of 10 μM, in the absence of GTP. Microtubule formation was verified by electron microscopy. To determine the stability of microtubules assembled in the presence of GTP or drug, turbidity was followed for 40 min after the reaction temperature was shifted to 4°C.
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