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TITLE: Strategy Toward the Total Synthesis of Epothilones A and B

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designated by other documentation.
Having accomplished total syntheses of epothilone A and B, we have been engaged in a new stage of investigation that involves development of efficient strategies for a large scale epothilone preparation and search for more potent analogues. Efficient and processable syntheses of key building blocks of 12,13-desoxyepothilone B (dEpoB) by catalytic asymmetric induction has been achieved. dEpoB is a potent anticancer agent, showing a highly promising therapeutic potential in the currently undergoing phase I study. The syntheses of two epothilone analogues, 15(S)-aza-12,13-desoxyepothilone B and the epimeric 15(R)-aza-12,13-desoxyepothilone B have been accomplished. Tubulin binding and cytotoxicity profiles of these analogues have also been investigated. Another epothilone, 12,13-desoxyepothilone F (dEpoF), was synthesized and evaluated for antitumor potential. The results from an in vitro assay reveal that this new analogue is highly active against various tumor cell lines with a potency comparable to that of dEpoB. In particular, the growth of resistant tumor cells is inhibited by dEpoF at concentrations where paclitaxel (Taxol®) is basically ineffective. A preliminary assessment of its in vivo activity is also promising. The new analogue, containing an additional hydroxyl group at C21, provides advantages over other epothilones in terms of water solubility and can serve as a readily functionalizable handle to produce other useful compounds for pertinent biological studies.
FOREWORD

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Introduction

The epothilones are a family of naturally occurring cytotoxic macrolides that were isolated from the mycobacterium Sorangium cellulosum.\(^1,2\) Despite minimal structural homology with the taxoids, the epothilones manifest biological effects similar to those of paclitaxel (Taxol\(^\text{R}\)) on microtubule and cultured cells.\(^3\) While these two classes of compounds share the same mode of action, the epothilones retain remarkable potency against multidrug resistant tumor cells.\(^4,5\) They may also offer advantages relative to paclitaxel in terms of formulatability. Due to the exciting potential of the epothilones for clinical development, they have attracted considerable attention in cancer research as possible agents for cancer chemotherapy.\(^6\)

![Figure 1. Structure of Epothilones and Desoxyepothilones](image)

1\(a R_1 = H, R_2 = H, \text{Epothilone A}\)
1\(b R_1 = \text{CH}_3, R_2 = H, \text{Epothilone B}\)
1\(c R_1 = H, R_2 = \text{OH}, \text{Epothilone E}\)
1\(d R_1 = \text{CH}_3, R_2 = \text{OH}, \text{Epothilone F}\)
2\(a R_1 = H, R_2 = H, \text{Epothilone C}\)
2\(b R_1 = \text{CH}_3, R_2 = H, \text{Epothilone D}\)
2\(c R_1 = H, R_2 = \text{OH}, \text{Desoxyepothilone E}\)
2\(d R_1 = \text{CH}_3, R_2 = \text{OH}, \text{Desoxyepothilone F}\)
3\(a \alpha-15, \text{epi-Aza-dEpo}\)
3\(b \beta-15, \text{Aza-dEpoB}\)

Given the biological ramifications of the epothilones, they have engendered a great deal of attention from the standpoint of total synthesis. Indeed, several total syntheses of naturally occurring epothilones including the present research in these laboratories have been accomplished.\(^7-21\) Subsequently, syntheses of numerous analogues have served to establish a rather detailed map of the structure-activity relationships (SAR) based on in vitro and in vivo\(^22\) assays.\(^6\) Recently, extensive in vivo experiments demonstrated that the less cytotoxic 12,13-desoxyepothilone B (2b, dEpoB) manifests a more promising therapeutic profile than does epothilone B (1b, EpoB) itself.\(^23,24\) In our studies in mice, the desoxy compound, dEpoB, was well tolerated and is virtually curative against otherwise resistant xenograft
tumors. Although the deoxy derivatives are present as minor components from fermentation, the more biologically significant dEpoB is apparently scarce in that it is a secondary constituent within a family of minor B components from fermentation.

While our previous studies relied entirely on the fully synthetic dEpoB,25,26 our first goal is to develop a practical total synthesis of dEpoB which can furnish material appropriate for a full scale evaluation in humans if findings in higher animals so indicate. Secondly, we hope to continue our search for analogues that may be more potent and readily accessible. In particular, we would like to ascertain the bioprofiles of nitrogen based desoxyepothilone systems (3a and 3b), in macrolactam rather than macrolactone settings. Another interesting candidates are epothilones E and F, each of which possesses a 21-hydroxyl group.14,27,28 In spite of the putative role of the thiazole as a key pharmacophore,29,30 the 21-hydroxyl group does not abrogate activity. Another particularly exciting prospect associated with having a primary hydroxyl group is that of enhanced aqueous solubility, thereby providing major simplifications in issues of formulation. Furthermore, the primary hydroxyl group could be utilized as a staging point for further elaboration. All of these goals require successful syntheses of epothilones and rigorous biological evaluations.
Chemical Syntheses of Epithilone Analogues and Biological Evaluation

1. Practical Synthesis of dEpoB. In our previously described routes to dEpoB,\textsuperscript{25,26} three subunits were built and combined (Figure 2). The route to building block C has already been shown to be amenable to major scale-up. Building block B was prepared by auxiliary-mediated allylation of a suitable propionate derivative. The largest impediment to major scale-up was in the synthesis of the A subunit, which had previously required eight steps, exclusive of generating the required reagents. Several of these maneuvers required rather sophisticated and expensive chemistry, and prospects for serious scale-up of these protocols were daunting. Thus, we engaged in new synthetic developments which would render the A fragment readily available in multigram quantities, utilizing easily processable chemistry. These advances have major favorable consequences for a plant-level synthesis of dEpoB.

![Figure 2. Retrosynthetic Analysis and Epithilone Building Blocks](Image)

The new route for the synthesis of the key vinyl iodide fragment required smooth access to large quantities of phosphine oxide 7. This subunit is easily prepared in two steps on a 100 g scale as shown in Scheme 1. The second subunit required for building the A segment is methyl ketone 8. The condensation between 7 and 8 is conducted in 98% yield on a multigram scale to afford A. However, for this chemistry to be valuable, a straightforward synthesis of 8, amenable to plant-level scale-up, had to be accomplished.
Scheme 1\(^a\)

\[
\begin{align*}
\text{Cl-} & \quad \text{O-Cl} \\
4 \quad + \quad \text{S-NH}_2 & \quad \xrightarrow{a} \quad \text{S-NCl} \quad \xrightarrow{b} \quad \text{S-Ph} \quad \xrightarrow{c} \quad \text{O-OTES} \\
5 & \quad \text{R-OTES} & \quad \text{A}
\end{align*}
\]

\(^a\) (i) acetone, (ii) ZnCl\(_2\), MeOH, reflux, 60%; (b) HOPPh\(_2\), Cs\(_2\)CO\(_3\), Bu\(_3\)Ni, CH\(_2\)Cl\(_2\), 97%; (c) (i) n-BuLi. THF, -78 °C, 30 min, (ii) 8, -78 °C to rt, 98%.

Two such routes have now been developed. In the first approach (Scheme 2) following conversion of 9 to 10, a highly diastereoselective alkylation of lithio 10 with 11 produces 12. Diiodide 11 is available from 2-butynol in two steps, and compound 12 was advanced in three steps to 8. A second route, while somewhat less selective, reaches 8 even more easily, in only four steps via asymmetric dihydroxylation. This synthesis begins with the known reaction of propyne with B-iodo-9-BBN and methyl vinyl ketone to produce 14, which reacts (in multigram scale) with trimethylsilyl iodide to provide an 88:12 (15a : 15b) mixture of silyl enol ether isomers. Asymmetric dihydroxylation of this material, afforded 16 (87% ee, 55% yield in two steps). Finally, silylation of 16 completes the synthesis of ketone 8 and therefore A.

Scheme 2\(^a\)

\[
\begin{align*}
\text{O-POMB} & \quad \xrightarrow{a} \quad \text{O-OTES} \\
9 & \quad \xrightarrow{b} \quad \text{I-OTES} \\
10 & \quad \xrightarrow{c,d} \quad \text{O-OTES} \\
11 & \quad \xrightarrow{e} \quad \text{O-OTES} \\
13 & \quad \xrightarrow{f} \quad \text{15a} & \quad \text{15b} \\
14 & \quad \xrightarrow{g} \quad \text{16}
\end{align*}
\]

\(^a\) (i) TiCl\(_4\), 0 °C, 87%, (ii) TESCl, imidazole, DMF, 84%; (b) LHMDS, 11, -78 °C, 81%; (c) (i) HOAc:THF:H\(_2\)O (3:1:1), (ii) CH\(_3\)ONHCH\(_3\), AlMe\(_3\), (iii) TESCl, DMF, 88% overall; (d) MeMgBr, 0 °C, 93%; (e) 1-9-BBN, hexanes, (ii) methyl vinyl ketone, hexanes, (iii) 3 N NaOH, PhMe, 100 °C, 65%; (f) TMSI, HMDS, CH\(_2\)Cl\(_2\), -20 °C to rt; (g) 1 mol % of OsO\(_4\), AD-mix-, MeSO\(_2\)NH\(_2\), t-BuOH:H\(_2\)O (1:1), 55%-two steps; (h) TESCl, imidazole, DMF, 85%.
With a view to generating fragment B by strictly catalytic asymmetric methods, its synthesis was revisited (Scheme 3). For this purpose, we synthesized subunit 17, which is prepared from isoprene. Asymmetric epoxidation of 17 provides 18, which undergoes reductive cleavage at the more substituted center to furnish diol 19. Following periodate cleavage as shown, building block B is in hand. While this method bypasses recourse to a chiral auxiliary, its ultimate advantage in terms of scale-up to the multigram levels in a plant-type setting awaits demonstration. As our early synthesis, an aldol condensation joins fragments B and C. Subsequently, a palladium-mediated B-alkyl Suzuki merger joins A with B-C. With the carbon skeleton in place, a catalytic Noyori reduction provides the desired stereochemistry at C3 and macrolactonization leads, shortly afterward, to dEpoB.

\textbf{Scheme 3}\textsuperscript{\textordmasculine a}

\begin{align*}
\text{17} & \xrightarrow{a} \text{18} & \text{19} & \xrightarrow{c} \text{B} \\
\end{align*}

\textsuperscript{\textordmasculine a} (a) t-BuOH, Ti(Oi-Pr)\textsubscript{4}, (+)-DET, CH\textsubscript{2}Cl\textsubscript{2}, 98\%, 82\% ee; (b) NaCNBH\textsubscript{3}, BF\textsubscript{2}-OEt\textsubscript{2}, THF, 52\%; (c) NaIO\textsubscript{4}, THF:H\textsubbox{2}O, 81\%.

\section*{2. Synthesis and Evaluation of Aza Analogue of Epothilones}

Next, we examined the feasibility of the new route in the synthesis of the Aza-analogues (3a and 3b). In order to introduce a nitrogen function, we explored the possibility of Mitsunobu substitution at C15 with inversion of stereochemistry. First, asymmetric dihydroxylation of 15 with enantiomeric ligands conveniently afforded both (R)- and (S)-A. Deprotection of A afforded alcohol 20 (Scheme 2). The latter cleanly underwent Mitsunobu inversion to provide azide 21. It was found that a palladium-mediated B-alkyl Suzuki cross-coupling could be conducted with the azide in place. In the context of the C2-C3 enol ether, Staudinger reduction of 23 is possible, leading, to 24 after nitrogen protection with a tBoc group.
With the N-tBoc protective function in place at C15, we could conduct the ruthenium-mediated asymmetric Noyori-type reduction at C3 (Scheme 5). The tBoc and tert-butyl ester groups were cleaved concurrently to afford amino acid 27. A free C3 hydroxyl group also did not interfere with HATU-mediated macro lactamization to afford 28, albeit in moderate yield. Finally, the C7 Troc group was discharged through the action of zinc dust under the influence of sonication, affording the desired 3b. In much the same way, the 15(S) precursor (20) was converted to 15(R)-epi-aza-dEpoB (3a).
The fully synthetic 15-aza-12,13-desoxyepothilone B (3b) and the epimeric analogue (3a) have been tested against a variety of tumor cells in order to evaluate their antitumor activity. Initially, the aza analogues were assayed to determine their relative ability to bind tubulin in comparison to dEpoB. The results indicated that for 3b microtubulin stabilizing activity was maintained, retaining 75% of the activity of dEpoB. However, the epimeric 15-aza-dEpoB analogue (3a) did not appreciably stabilize microtubules within the detection limits of the assay. Hence, a major effect of C15 stereochemistry on the microtubule stabilization properties of the aza agents has been uncovered. Similarly, in cytotoxicity studies, a direct comparison of 15-aza-dEpoB (3b) with dEpoB (2b), using the CCRF-CEM cell line, showed that it was only slightly less potent than dEpoB (4.8 x less active). By contrast, the corresponding 15-epi-aza-dEpoB (3a) analogue displayed severely reduced antitumor activity (21 x less active). Both aza-dEpoB and its corresponding epimer were not active against several benchmark paclitaxel or vinblastine resistant tumor cells (CCRF-CEM/taxol and CCRF-CEM/VBL, respectively) used in earlier studies. By contrast, dEpoB itself is highly active against these resistant lines. In addition, we have successfully epoxidized 3b to produce the corresponding aza-EpoB analogue. This compound has recently been advanced to phase I clinical trials by the Bristol-Myers Squibb company.
3. Synthesis of dEpoF. The preparation of a Left-Wing fragment for the synthesis of dEpoF (2b) has also been explored using the same Wittig type reaction as the key transformation. First, the commercially available ethyl oxamate (29) was condensed with 1,3-dichloroacetone to give 2,4-disubstituted thiazole 30. Subsequent P-alkylation with diphenylphosphine oxide afforded the requisite reagent 31. The olefination reaction of the anion of the phosphine oxide 31 with methyl ketone 8 proceeded smoothly to furnish 32 as a single geometric isomer in good yield. Reduction of the ethyl ester of 6 by Dibal-H followed by protection with TrocCl generated primary alcohol 33 which could then be connected with the Right-Wing fragment BC to give 34 via palladium catalyzed B-alkyl Suzuki coupling reaction. This new route is much shorter and more convergent than the corresponding previous sequence involving 10 linear transformations. In particular, the usage of methyl ketone 8 as a universal hinge conveniently allows for the introduction of diversity to the structure of the thiazole moiety.

![Scheme 6. New Route to the Left-Wing Fragment for the Synthesis of dEpoF](image)

With the successful union of 33 and B-C, stereoselective reduction at C3 was attempted using diketone 34 and C15 free hydroxy derivative of 34 (Scheme 7). Although the Noyori reduction afforded
the desired alcohol 35, C15 methyl ether was produced in an almost equal quantity presumably due to the solvolysis by the solvent methanol. While this very surprising susceptibility to solvolysis of 34 and related congeners at C15 was certainly detrimental to the efficiency of our total synthesis, we moved on to our goal. Having established all of the necessary stereocenters, the tert-butyl ester was unmasked with simultaneous protection of the C3 and C15 alcohols by TESOTf. Selective desilylation of the C15 TES group with methanolic HCl provided 36 setting the stage for cyclization. Maerolactonization of this seco-acid, according to the Yamaguchi protocol,46,47 afforded fully protected lactone 37 in 60–70 % yield. The removal of the two Troc protecting groups was performed through the agency of samarium (II) iodide48 or zinc, both in good yields. Finally, standard fluoride induced removal of the C3 silyl group yielded the desired 12,13-desoxyepothilone F (2d).

Scheme 7a

![Scheme 7](image)

^a a) 5% [Ru(BINAP)Cl2]2•TEA, HCl/MeOH, H3(1200 psi), 78%; b) (i) TESOTf, 2,6-lutidine, CH2Cl2, -78 °C to rt, 8 h, (ii) HCl-CH3OH, 0 °C, 70%; c) 2,4,6-trichlorobenzoyl chloride, (C6H5)3N then 4-DMAP, toluene, slow addition 3 h, 60-70%; d) Zn, AcOH-THF, rt, 1 h, 86% or cat. NiI2/Sml2, THF, -78 to -40 °C, 87%; e) HF•Pyridine, THF 0 °C to rt, 91%.

4. Evaluation of Antitumor Potential of dEpoF. The fully synthetic dEpoF has been evaluated against a variety of cell types in order to evaluate its antitumor potential. As shown in Table 1, dEpoF exhibited high cytotoxicity activity against a range of sensitive and resistant tumor cell lines tested. In particular, high potency and relatively low cross-resistances were observed for dEpoF against sensitive and MDR.
cell lines, respectively. Direct comparison of dEpoF with dEpoB indicates that the new compound possesses a comparable potency. It is noteworthy that dEpoF consistently outperforms other anticancer agents such as taxol, vinblastine, etoposide and actinomycin in inhibiting the growth of MDR tumor cells.

Table 1. Potency of dEpoF, dEpoB and Taxol against various tumor cell growth in vitro

<table>
<thead>
<tr>
<th>Tumor Cell Lines</th>
<th>dEpoF (mM)</th>
<th>dEpoB (mM)</th>
<th>Taxol (mM)</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human T-cell AL Leukemia</td>
<td>0.0027</td>
<td>0.0095</td>
<td>0.0021</td>
<td>0.00063b, 0.290c</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>0.047 (17.4 x)</td>
<td>0.017 (1.8 x)</td>
<td>4.140 (1971 x)</td>
<td>0.3324 (527 x)</td>
</tr>
<tr>
<td>CCRF-CEM/VBL100</td>
<td>0.0049 (1.8 x)</td>
<td>0.014 (1.5 x)</td>
<td>0.0066 (3.18 x)</td>
<td>3.444 (117 x)</td>
</tr>
<tr>
<td>CCRF-CEM/VM1</td>
<td>0.0053 (2.0 x)</td>
<td>0.0162 (1.7 x)</td>
<td>0.120 (57 x)</td>
<td></td>
</tr>
<tr>
<td>CCRF-CEM/Taxol</td>
<td>0.0017</td>
<td>0.0019</td>
<td>0.0135</td>
<td>0.00025d</td>
</tr>
<tr>
<td>Hamster Lung Fibroblasts</td>
<td>0.0136 (8.0 x)</td>
<td>0.0073 (3.8 x)</td>
<td>0.583 (43.2 x)</td>
<td>0.001534 (61.2 x)</td>
</tr>
<tr>
<td>DC-3F</td>
<td>0.0223 (13.1 x)</td>
<td>0.0288 (15.2 x)</td>
<td>20.19 (1496 x)</td>
<td>0.4092 (1637 x)</td>
</tr>
<tr>
<td>DC-3F/ADII</td>
<td>0.0021</td>
<td>0.0036</td>
<td>0.0029</td>
<td></td>
</tr>
<tr>
<td>Human CM Leukemia</td>
<td>0.0042</td>
<td>0.0221</td>
<td>0.0394</td>
<td>0.00184e</td>
</tr>
<tr>
<td>K562</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human Mammary Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MX-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a) Cell growth inhibition was measured by XTT tetrazonium assay after 72 h incubation for cell growth as described previously in ref. 23. The values were determined with six to seven concentrations of each drug using a computer program. The cross-resistance are shown in parentheses. b) Vinblastin (VBL). c) Etoposide (VP-16). d) Actinomycin D (AD). e) Epothilone B (EpoB).

We then turned our attention to the in vivo efficacy of dEpoF. Thus, the therapeutic effect of dEpoF was evaluated in athymic mice bearing a human leukemia K562 xenograft. The animal experiments were performed according to the slow IV infusion protocol developed in our previous studies.23,24 As depicted in Figure 2, treatment of the mice with dEpoF (30 mg/kg) readily induced reduction in the size of tumor to the point of remission. While the preliminary in vivo results with this sensitive tumor clearly look promising, more revealing experiments are necessary to assess its full promise.

With the encouraging biological results, we next examined the aqueous solubility of dEpoF using an HPLC-based method.49 Indeed, dEpoF was found to be 2.5 times more water soluble than dEpoB. Although literature estimates of paclitaxel aqueous solubility vary considerably,50 it has been noted that epothilones are approximately 30 times more water soluble than paclitaxel.1 Considering these observations and the problems associated paclitaxel administration, the present analogue appears to be a promising candidate that may bring significant improvement in the formulation of the active drug.
Figure 3. Therapeutic effect of dEpoF in nude mice bearing human leukemia K562 xenograft

Tumor size in nude mice bearing human leukemia K562 tumor

Control (n=3)
dEpoF 30 mg/kg (n=3)

Day after tumor implantation
Key Research Accomplishments

- Development of a practical route for the plant scale preparation of desoxyepothilone B (dEpoB).
- Synthesis of dEpoB to provide sufficient amounts for higher animal studies.
- Successful total syntheses of new 21-hydroxy, 15-aza- and epi-aza desoxy epothilone analogues.
- In vitro antitumor evaluation of the new analogues.
- In vivo evaluation of dEpoF.
- Discovery of desoxyepothilone F (dEpoF) as a potent anticancer agent.
Reportable Outcomes


Conclusions

In summary, a highly efficient and practical route for dEpoB systems has been developed. Using this synthetic route, various epothilone analogues have been produced through total syntheses to provide sufficient amounts for biological studies that will support a full and searching evaluation of dEpoB and other promising epothilones at the clinical level. The feasibility of converting an oxygen at C15 to a nitrogen by Mitsunobu inversion reaction in seco dEpoB systems gives access to various novel structural variants of the system. Aza systems, however, do not appear to be as clinically promising as dEpoB in the preliminary screening. Another epothilone analogue that possesses an additional hydroxy group at C21 has also been synthesized. The strategy based on convergent merger of the two key fragments in the current synthesis of dEpoF, compared to the dEpoB synthesis, proved less efficient due to the unexpected acid induced susceptibility. Nevertheless, the in vitro and in vivo tumor growth inhibition experiments demonstrated the hydroxy analogue possesses high antitumor activity. Given the promising in vivo profile of the closely related dEpoB, the need for further investigation with dEpoF is readily apparent. Overall, our successful chemical synthesis of these epothilone derivatives has made it possible to study all too important clinical aspects of epothilones, which otherwise would have been difficult.
References


Appendices

- Reprints of published papers.
- Curriculum vitae
En Route to a Plant Scale Synthesis of the Promising Antitumor Agent 12,13-Desoxyepothilone B

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Abstract

Efficient and processable syntheses of key building blocks of the antitumor agent 12,13-desoxyepothilone B (dEpoB) by catalytic asymmetric induction are herein described.

Interest in the epothilone family of natural products on the part organic chemists was incited, no doubt, by the promising biological profiles of the A and B isomers, particularly the latter.1,2 These compounds seem to share a common tubulin-centered mechanistic framework with paclitaxel, yet offer potential advantages in terms of formu-

As early as 1997, using our then intricate (though stereospecific) academic type synthesis, enough epothilone B was garnered to show for the first time that the initial Merck reports3 on the favorable in vitro biological profiles of epothilones were extendable to human tumors transplanted in murine hosts in a xenograft setting.4 However, during these


investigations, some potentially serious toxicity problems were uncovered using agent 2 (Figure 1). These findings cast

![Chemical structures](image)

Figure 1. Epothilones.

some doubt as to whether exploitable therapeutic indices could be found with epothilone B (EpoB). During this era, we demonstrated that much of the toxicity of EpoB could be abrogated through the use of 12,13-desoxyepothilone B (dEpoB, 3).\(^6,10\) Vast superiority of dEpoB relative to EpoB and to paclitaxel has been demonstrated in a variety of competitive in vivo settings, and the results have been published elsewhere.\(^2,11\) Presently, dEpoB has advanced to toxicity and efficacy studies in dogs, en route to a full-scale clinical evaluation.\(^15\)

Since our laboratory lacks access to fermentation-derived epothilones, total synthesis constituted our only recourse to produce material for biological investigations. Indeed, all of the in vivo evaluations have been conducted on fully synthetic dEpoB. The present goal is to develop a practical total synthesis of dEpoB which can furnish material appropriate for a full-scale evaluation in humans if, as expected, findings in higher and larger animals so indicate.

In our previously described improved routes to dEpoB,\(^13,14\) three subunits were built and combined. The route to building block C (see Figure 2) has already been shown to be

![Chemical structures](image)

Figure 2. Epothilone building blocks.

Amenable to major scale-up. As previously described by Overman,\(^15\) building block B was prepared by auxiliary-mediated alkylation of a suitable propionate derivative, using methodology first promulgated by Evans and associates.\(^16\) The largest impediment to major scale-up was in the synthesis of the A subunit, which had previously required eight steps, exclusive of generating the required reagents. Several of these maneuvers required rather sophisticated and expensive chemistry, and prospects for serious scale-up of these protocols were daunting. The disclosure herein now describes new synthetic developments which render the A fragment readily available in multigram quantities, utilizing easily processable chemistry. These advances have major favorable consequences for a plant-level synthesis of dEpoB.

The new route for the synthesis of the key vinyl iodide fragment required smooth access to large quantities of phosphine oxide 7. This subunit is in fact easily prepared in two steps on a 100 g scale as shown in Scheme 1.\(^17\) The second subunit required for building the A segment is methyl ketone 8 (vide infra). The Horner-like condensation\(^18\) between 7 and 8 is conducted in 98% yield on a multigram scale to afford A. However, for this chemistry to be valuable, a straightforward synthesis of 8, amenable to plant-level scale-up, had to be accomplished.

Fortunately, two such routes have now been developed. In the first approach (Scheme 2) following conversion of 9

![Chemical structures](image)

Scheme 2

a) (i) acetone, (ii) ZnCl₂, MeOH, reflux, 60%; (b) HOPPh₃, Cs₂CO₃, Bu₅NL, CH₂Cl₂, 97%; (c) 7, n-BuLi, THF, −78 °C, 30 min, (ii) 8, −78 °C to rt, 98%.


(12) This work was done in collaboration with Dr. T.-C. Chou, Dr. W. Tong, Dr. O. O’Connor, and Dr. J. Bertinato at the Skag-Retinger Institute for Cancer Research.


to 10 (84%),\textsuperscript{19,20} a highly diastereoselective alkylation of lithium 11 with 10 produces 12 (>25:1 de) in 81% yield.\textsuperscript{19,20} As was previously reported,\textsuperscript{2,3} diiodide 11 is available from 2-butynol in two steps as shown. Finally, compound 12 was advanced in three steps to B by recourse to the Weinreb amide 13.\textsuperscript{2,3}

A second route, while somewhat less selective, reaches 8 even more easily, in only four steps via asymmetric dihydroxylation\textsuperscript{23} (Scheme 3). This synthesis begins with the known reaction of propyne with B-iodo-9-BBN and methyl vinyl ketone to produce 17,\textsuperscript{24} which reacts (in multimgram scale) with trimethylsilyl iodide to provide an 88:12 (18a:18b) mixture of silyl enol ether isomers 18.\textsuperscript{25} Asymmetric dihydroxylation\textsuperscript{23} of this material, under the conditions shown, afforded 19 (87% ee, 55% yield—two steps). Finally, triethylsililation of 19 completes the synthesis of keto 8 and therefore A.

With a view to generating fragment B by strictly catalytic asymmetric methods, its synthesis was revisited (Scheme 4).

For this purpose, we synthesized subunit 20, which is prepared from isoprene by known chemistry.\textsuperscript{26} Asymmetric epoxidation\textsuperscript{27} of 20 provides 21, which undergoes reductive cleavage\textsuperscript{28} at the more substituted center to furnish diol 22. Following periodate cleavage as shown, building block B is in hand. While this method bypasses recourse to a chiral auxiliary, its ultimate advantage in terms of scale-up to the multigram levels in a plant-type setting awaits demonstration.

As previously described,\textsuperscript{13,14} a novel aldol condensation joins fragments B and C. Subsequently, a palladium-mediated B-alkyl Suzuki\textsuperscript{29} merger joins A with B–C. With the carbon skeleton in place, a catalytic Noyori reduction\textsuperscript{30} provides the desired stereochemistry at C3 and macrocyclization leads, shortly afterward, to dEpoB.\textsuperscript{31} While we always remain open to possibilities for still greater practicality, we are now already confident that compound availability through total synthesis will support a full and searching evaluation of dEpoB and other promising epothilones at the clinical level.\textsuperscript{31}


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On the Total Synthesis and Preliminary Biological Evaluations of 15(R) and 15(S) Aza-dEpoB: A Mitsunobu Inversion at C15 in Pre-Epitholine Fragments

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ABSTRACT

The syntheses of two epitholine analogues, 15(S)-aza-12,13-desoxyporpholine B and the epimeric 15(R)-aza-12,13-desoxyporpholine B, are described. A Mitsunobu inversion was utilized for elaboration of pre-epitholine fragments to the corresponding macrolactam. Tubulin binding and cytotoxicity profiles of these analogues are presented.

The previous Letter set forth the background of the epitholine project from our perspective. In particular, it described a key asymmetric dihydroxylation reaction. We took recourse to AD mix-α (see 3 → 4) to reach the 15(S) enantiomer (5a), and shortly thereafter dEpoB (1). Not surprisingly, recourse to AD mix-β leads to the antipodal 15(R) stereoseries (see compound 6) en route to 7 and 7a (Scheme 1). With compounds 5a and 7a in hand, we wondered about the possibility of displacement of the C-15 hydroxyl centers, thereby enabling the introduction of nitrogen-based nucleophiles. Assuming strict inversion of configuration could be realized, it was hoped that the 15(R) alcohol (7a) would lead eventually to a 15(S)aza analogue of dEpoB (2) while the 15(S) alcohol (5a) would pave the way for reaching the epimeric 15(R)aza series (vide infra 17). In this way, we could ascertain the biosynthetic pathways for such new dEpoB systems, in macrolactam rather than macrolactone settings. We further hoped to evaluate the consequences of permuting the C-15 stereochemistry on the tubulin binding and cytotoxicity profiles. Finally, we hoped to evaluate the capacity of these


aza compounds to function in the context of otherwise resistant tumors. An account of the chemical synthesis of our targets and their preliminary evaluation is provided herein.

Deprotection of 7 as shown afforded alcohol 7a (Scheme 2). The latter underwent Mitsunobu inversion, as indicated.

Eventually, a passage was navigated. It was found that a palladium-mediated B-alkyl Suzuki cross-coupling could be conducted with the azide in place (see 8 → 9). In the context of the C2–C3 enol ether, Staudinger reduction of 9 is possible, leading, after nitrogen protection, to 10.

By contrast, in a related azido substrate 11 containing the C3 ketone, Staudinger reduction en route to 12 could be conducted in our hands in only 18% yield (Scheme 3).

Reduction of the azide to the amine is apparently complicated by Schiff base formation (inter- or intramolecular) between the in situ generated 15-amine functionality and the 3-keto group.

With the N-Boc protective function in place at C15, we could conduct the ruthenium-mediated asymmetric Noyori-type reduction at C3 (see transformation 10 → 14, Scheme 4). By contrast, the corresponding reduction at the stage of 11 was not successful. Apparently the azide function is not compatible with the strongly acidic Noyori reduction protocol, though it does not seem to be converted to the amino group under these conditions.

With 14 in hand, the Boc and tert-butyl ester groups were cleaved concurrently to afford amino acid 15. This reaction could be conducted in the presence of a free C3 hydroxyl group. This group also did not interfere with HATU-mediated macro lactamization to afford 16, albeit in only 50% yield. Finally, the C7 Troc group was discharged through the action of zinc dust under the influence of sonication, affording the desired 2. In much the same way, the 15(S) precursor (5) was converted to 15(R)-epi-aza-dEPOB (17).

The fully synthetic 15-aza-12,13-desoxyepothilone B (2) and the epimeric 15-aza-12,13-desoxyepothilone B (17) analogues have been tested against a variety of tumor cells in order to evaluate their antitumor activity. Initially, the aza

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(5) Mitsunobu attempts on late-stage epothilone constructs were low yielding.


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Scheme 4. Synthesis of Aza-dEpoB (2)

![Chemical Structures]

Conditions: a) p-TsOH, acetone, 82%; b) [Ru(BINAP)Cl]2, Et3N, 0.12M HCl/MeOH, H2, 1200 psi, 78%; c) TFA, CH2Cl2; d) HATU, H2O, DIPEA, CH2Cl2, 50%; e) zinc dust, HOAc, sonication.

Similarly

3 AD mix-α

4

analogue (2 and 17) were assayed to determine their relative ability to bind tubulin in comparison to dEpoB. The results indicated that for 15-aza-dEpoB (2) microtubulin stabilizing activity was maintained, retaining 75% of the activity of dEpoB. However, the epimeric 15-aza-dEpoB analogue (17) did not appreciably stabilize microtubules within the detection limits of the assay. Hence, a major effect of C15 stereochemistry on the microtubule stabilization properties of the aza agents has been uncovered. Similarly, in cytotoxicity studies, a direct comparison of 15-aza-dEpoB (2) with dEpoB (1), using the CCRF-CEM cell line, showed that it was only slightly less potent than dEpoB (4.8× less active). By contrast, the corresponding 15-epi-aza-dEpoB (17) analogue displayed severely reduced antitumor activity (21× less active). We also noted that, both aza-dEpoB (2) and its corresponding epimer (17) were not active against several benchmark paclitaxel or vinblastine resistant tumor cells (CCRF-CEM/taxol and CCRF-CEM/VBL, respectively) used in earlier studies. By contrast, dEpoB itself is highly active against these resistant lines. In addition, we have successfully epoxidized 2 to produce the corresponding aza-EpoB analogue. This compound has recently been advanced to phase I clinical trials by the Bristol-Myers Squibb company.

In summary, the feasibility of carrying out an oxygen to nitrogen Mitsunobu inversion in seco dEpoB systems portends access to various novel structural variants of the system. For the moment, however, aza systems 2 and 17 do not appear to be as clinically promising as dEpoB.

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