Insights into Long-Range Structural Effects on the Stereochemistry of Aldol Condensations: A Practical Total Synthesis of Desoxyepothilone F

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Abstract: A processable total synthesis of a potent antitumor agent, desoxyepothilone F (dEpoF, 21-hydroxy-12,13-desoxyepothilone B, 21-hydroxyepothilone D), has been accomplished. The route is highly convergent. The new technology has also been applied to a total synthesis of 12,13-desoxyepothilone (dEpoB). The crucial point of departure from previous syntheses of dEpoB and dEpoF involves presentation of the C1−C11 sector for Suzuki coupling with C3 in reduced form. Hitherto, the required *S* stereochemistry at C3 had been implemented via reduction of a keto function after Suzuki coupling. Whereas that chemistry worked quite well in a synthesis of dEpoB, it was not transferable to a high-yielding synthesis of dEpoF. The reduction of the keto group at C3 via a Noyori protocol after Suzuki coupling had proved to be very difficult. In our current approach, two consecutive aldol reactions are used to fashion the acyl sector. In the first aldol condensation, C6 becomes attached to C7. Following protection at C7, a two-carbon acetate equivalent is used to join C2 and C3 with very high asymmetric induction at C3. Only after this center has been implemented is the Suzuki reaction conducted. This major advance allowed us to synthesize dEpoF in a straightforward fashion. These findings found ready application in the total synthesis of dEpoB. Another part of the study involved analysis of the factors associated with aldol condensations joining C6 to C7. In the work described herein, the consequences of the status of C3 in promoting the C6−C7 aldol coupling are probed in detail. Dramatic stereochemical long-range effects uncovered during the study are described, and a working model to explain these effects has emerged.

Introduction

The clinical successes of paclitaxel (Taxol) and docetaxel (Taxotere) in the treatment of cancer has spurred searches for other agents that inhibit the growth of tumors through similar mechanisms of action.[1] The cytotoxicity of Taxol (and possibly its mode of action at the level of clinical application) arises from its ability to induce apoptosis via tubulin polymerization and stabilization of microtubule assemblies.[2] The tubulin-based mechanism of action, originally discovered by Horwitz,[3] has been recognized to be operative in several naturally occurring nontaxoids such as discodermolide,[4] eleuthesides,[5] laulimalides,[6] and epothilones.[7]

While of unquestioned clinical value, Taxol is far from being an ideal drug. Its marginal aqueous solubility necessitates recourse to formulation vehicles such as cremophores that pose their own risks and management issues.[8] Moreover, Taxol is vulnerable to deactivation through multiple drug resistance (MDR). Resistance apparently arises through overexpression of P-glycoprotein (P-gp) and via tubulin mutation at the binding site.[9]

Among naturally occurring microtubule modulators, the recently discovered epothilones have evoked much excitement among scientists in various disciplines. These cytotoxic macrolides originated from a cellulose-degrading mycobacteria of the genus *Sorangium* and were found to possess remarkable antitumor activities.[10] In particular, epothilone B (**1b**, EpoB)

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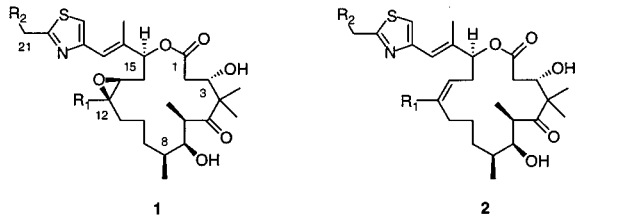

1

1a R_1 = H, R_2 = H, Epothilone A
1b R_1 = CH_3, R_2 = H, Epothilone B
1c R_1 = H, R_2 = OH, Epothilone E
1d R_1 = CH_3, R_2 = OH, Epothilone F

2

2a R_1 = H, R_2 = H, Epothilone C
2b R_1 = CH_3, R_2 = H, Epothilone D
2c R_1 = H, R_2 = OH, Desoxyepothilone E
2d R_1 = CH_3, R_2 = OH, Desoxyepothilone F

Figure 1. Structure of epothilones.

exhibits significantly higher cytotoxicity than does Taxol against various cancer cell lines. Furthermore, epothilones are apparently not susceptible to deactivation through MDR and maintain activity against resistant tumor cell lines.

We note that epothilones have also recently been constructed by heterologous expression of the appropriate biosynthesis genes in productive microorganisms.[11] The problem of epothilone supply may well be resolved by conventional fermentation augmented by emerging biotechnology. It is also possible that eventually, other useful analogues could be produced by this biotransformation protocol (Figure 1).

Given the promising in vitro data associated with epothilones, given several challenging features in their molecular frameworks vis-à-vis chemical synthesis, and given the uncertainties as to the availability of homogeneous epothilones in bulk via fermentation, these drugs have attracted significant attention from the community of synthetic chemists. Indeed, a variety of total syntheses have been accomplished.[12] Our exercises, initially launched from the perspective of total synthesis,[13] enabled a rather detailed SAR mapping of the drugs and allowed for dissection of epothilone bioactivity patterns into discrete zones of varying tolerances to structural modification.[14]

Using totally synthetic derived drug, our laboratory was the first to conduct in vivo evaluations of epothilone B.[15] When it was found that EpoB itself exhibited a worrisome toxicity profile (which to our estimation could result in an unacceptably narrow therapeutic index), we turned to the 12,13-desoxy compound in the hope of providing greater margins of potential opportunity. From combined total synthesis and SAR research, it had been shown that dEpoB is significantly less potent in vitro than EpoB. It is nonetheless, highly cytotoxic and robust with respect to MDR deactivation.Our original rationale for pursuing the 12,-13-deoxy congeners at the time arose from a conjecture that the enormous cytotoxicity of EpoB itself may be a consequence of two components. With the epoxide intact, there could well be a general cytotoxicity component (perhaps arising from the capacity of the system to function as a DNA alkylating agent). In addition we theorized that there might be a more specific mode of action (for instance, tubulin binding) which could be of potential therapeutic value against transformed cells and perhaps tumor masses. Our hope was that the nontumor-specific cytotoxicity, vested in the epoxide linkage of EpoB, would be substantially abrogated in dEpoB which would nonetheless retain the mechanistically specific tumor-directed cytotoxicity.

Indeed, in vivo experiments based on various mouse models have consistently demonstrated that dEpoB possesses remarkable therapeutic potential and is essentially curative against various sensitive and resistant tumors in xenografts. Due to its impressive in vivo profile, dEpoB has been advanced through toxicology evaluations in dogs, in expectation of human trials anticipating its deployment as an anticancer drug.[16] The excellent preclinical in vivo successes realized with dEpoB do not necessarily prove the validity of our dissection of its cytotoxicity into a tumor selective component as well as nonspecific toxicity arising from the epoxide linkage. At the very minimum, this hypothesis served as a valuable working model in prompting our interest in the 12,13-desoxy series. The full accounting of the superior performance of dEpoB remains an intriguing subject, and clearly merits further investigation.

In building upon the "12,13-desoxy concept" it was appropriate to evaluate the in vivo efficacy of other such agents. In this spirit we pursued 21-hydroxylated versions of 12,13-desoxy-epothilones such as dEpoE (**1c**) and dEpoF (**1d**).[17] As indicated by in vitro assays, 21-hydroxylated epothilones such as EpoE and EpoF do not forfeit the activities of the A and B systems.[18] Since EpoB is significantly more potent than EpoA, we naturally placed dEpoF at a higher priority than dEpoE. Furthermore, we anticipated that the 21-hydroxyl group of dEpoF might provide advantageous properties relative to the 21-methyl group of dEpoB in terms of aqueous solubility and might serve as a handle for further chemical elaborations. While a semi- and total

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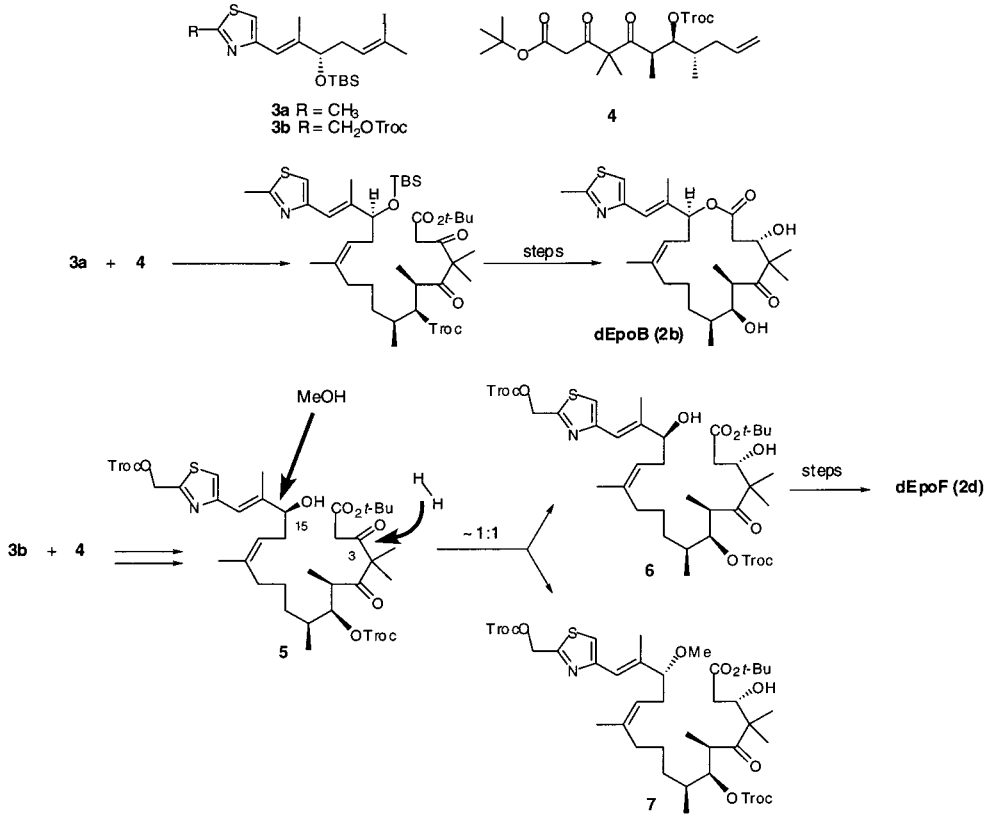
Scheme 1. Previous Total Synthesis of dEpoB and dEpoF



synthesis of the epothilones bearing a 21-hydroxy group has recently been disclosed,[18,19] our interests are focused rather specifically on homogeneous end products with *Z*-double bonds in the B (12-methyl) series. Apparently no such product had been previously synthesized and evaluated as to usefulness in chemotherapy. Having no access to such compounds through fermentation, we were obliged to accomplish the preparation of dEpoF by total synthesis[20] if our proposed studies were to go forward.

In approaching this goal, the starting point of our thinking was our semi-practical synthesis of dEpoB,[13e] which has produced multigrams of the drug, enabling extensive preclinical studies.[15] Naively, we thought that extension of the dEpoB strategy could readily deliver the desired 21-hydroxy analogue. However, as will be discussed later, our initial synthesis suffered a serious setback due to the unexpected behavior of the structurally altered thiazole in dEpoF. To understand the nature of the dEpoF problem as it developed, we first focus on the key *B*-alkyl Suzuki reaction by which the C11−C12 bond of dEpoB had been established.

The key coupling step conducted between **3** and **4**, maintained the *Z*-geometry of the C12−C13 linkage. After the required Suzuki coupling, the required *S*-steroechemistry at C3 was implemented by asymmetric reduction of the less hindered C3 ketone using protocols developed by Noyori.[21] To achieve the reduction of the C3 ketone with hydrogen in the dEpoB synthesis, we required recourse to 5% $[Et_2NH_2][\{(R\text{-BINAP})\text{-RuCl}\}_2Cl_3]$ as the catalyst in methanol under strongly acidic conditions (methanolic HCl). Fortunately, the precursor to dEpoB proved to be stable to these harsh conditions.

Turning to dEpoF, it did indeed prove possible to synthesize the required **5**. However, following deprotection of the TBS ether, attempted reduction of the C3 ketone under the conditions described above, led to extensive competitive solvolysis at C15 with formation of the methyl ether **7**. The ratio of **6**:**7** was not reproducible, and yields of the desired **6** oscillated in the 25−50% percent range (Scheme 1).

The precise reasons as to why substitution at C21, by an electron-withdrawing oxygen-based group, exerted such a profound effect in favoring methanolysis at C15 are still not clear. Nonetheless, the concurrent solvolysis reaction constituted a crippling blow to our plans to produce substantial amounts of dEpoF by total synthesis. This difficulty became a particularly pressing matter when the dEpoF, which we did produce, turned out to be a highly promising anticancer agent on the basis of in vitro assays as well as in the context of in vivo settings in xenografts.[20]

Accordingly, it emerged that to reach dEpoF by total synthesis, it would be necessary to present the acyl sector for Suzuki coupling *with C3 already reduced*. Needless to say, attempts to conduct the Noyori reduction at the stage of **4** were not possible due to reduction of the double bond. This raised the significant question as to how we could concisely generate a structure of the type **9** for Suzuki coupling. Potential solutions to this problem by recourse to substances of appropriate configuration arising from the "chiral pool" were entertained.

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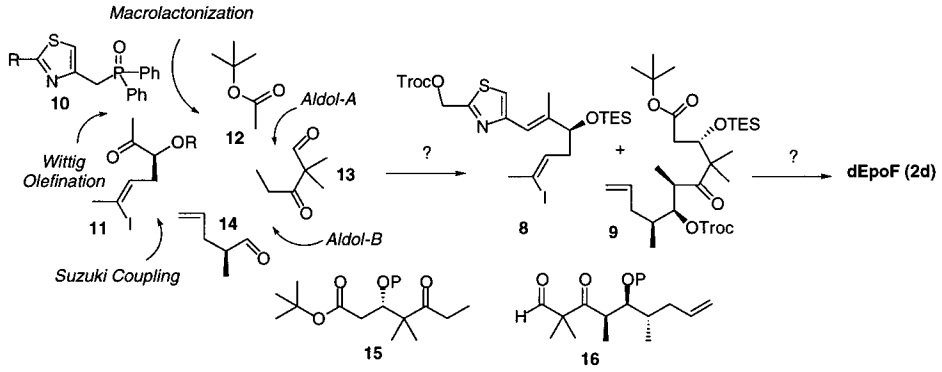
Scheme 2. New Synthetic Plan for 12,13-Desoxyepothilone F



However, such forays held out, to us, the prospect of being quite lengthy. In addition to solving the C3 problem, we also hoped to deal with several other awkward elements arising in our previous syntheses of dEpoF and dEpoB. An account of our findings in accomplishing effective total syntheses of both agents and our newly gained insights in the fashioning of the C2−C3 and C6−C7 bonds in the acyl sector are provided below.

Synthetic Plan. In planning a strategy to circumvent the serious problems encountered in our earlier synthesis of dEpoF, primary focus was placed on the development of a sequence in which carbon 3 of the coupling component, containing the terminal vinyl group (and subsequently the boryl function) would be presented for Suzuki coupling with C3 already in reduced form and of appropriate (*S*) configuration. In this way, the problematic Noyori reduction, which results in concurrent solvolysis at C15, would be avoided. In the context of such an effort, we also hoped to develop a more convergent and modular route to both the acyl and alkyl sectors. These two key fragments would be merged by a *B*-alkyl Suzuki coupling reaction and would be advanced to a macrolactone by a Yamaguchi macrocyclization (in a fashion similar to the previous syntheses of dEpoB[13e] and dEpoF[20]). It was first necessary to gain comfortable access to the *O*-alkyl sector **8**. In our earlier efforts, the route to this compound was lengthy and processable only with considerable difficulty. As an alternative, we envisioned reaching **8** via condensation of a Horner-like reagent (cf. **10**) and ketone **11**. While a phosphine oxide of general type **10** (R = CH_3) had been successfully employed for our syntheses of EpoA and EpoB,[22] de novo synthesis of a C21 functionalized version would now be required to reach dEpoF. Our approach to ketone **10** stemmed from the recognition that the critical iodoalkene functionality could be accessed by the stereoselective iodination of a proper alkyne precursor (vide infra) (Scheme 2). In this way, the serious problems associated with the complicated and low-yielding (albeit *Z*-selective) iodoethylenation practiced earlier would be resolved. Clearly, we would also require a maximally concise route to ketone **11**. It would be particularly critical to integrate efficient access to both the C12−C13 iodoalkene and the C15 (protected alcohol) functionalities in to a program which also deals effectively with the substituted thiazole issue.[23]

For the synthesis of the *O*-acyl wing **9**, the largest issue was that of gaining access to the required (*S*)-configured C3, without benefit of a C3 ketone reduction (via a Noyori protocol) and without a lengthy excursion which would be required if we were to exploit the chiral pool of starting materials. Instead, we anticipated recourse to two aldol condensations eventually joining keto aldehyde **13**, acetate **12** and aldehyde **14**. As we contemplated these two aldol condensations, two obvious sequencing variations presented themselves. In one case, we would first couple **13** to **14**. The only inducing element to control the C6−C7 stereochemistry would be the chirality of aldehyde **14**. In our recent synthesis of dEpoB,[13b] it had been shown that this α-stereogenic center provides remarkable facial stereochemical guidance in aldol condensation with a related achiral enolate. Following suitable protection at C7 and deprotection at C3, **9** would be in hand. Needless to say, a device would be necessary to ensure highly stereoselective formation of the (*S*)-configured C3, in the course of the second aldol condensation.

An alternative possibility contemplated coupling of **12** + **13**, first giving rise to **15**. Again, this bond-forming sequence would require a control element to ensure formation of the required C3(*S*) configuration in **15**. The second stage, aldol condensation, would be conducted between **15** and aldehyde **14**. In considering this second aldol condensation we leave unspecified whether C3 would be protected or unprotected. One influencing element would be the α-methyl stereogenic center at C6 in **14** in conjunction with its C10−C11 double bond to control the sense of the C6−C7 bond formation. We also anticipated that the configuration at C3 could have consequences for the second aldol condensation. This type of possibility had intervened in the Schinzer synthesis of epothilones, although only in a specific case where C1 was also present in fully reduced form.[24] The findings stemming from our total synthesis plans and a detailed investigation of the consequences of a prebuilt C3 stereogenic center on the stereochemical sense of C6−C7 bond formation will also be related.

A New Synthetic Route to the *O*-Alkyl Fragment. Our new synthesis commenced with the preparation of methyl ketone **11** by two independent sequences. The first approach employed an asymmetric catalytic oxygenation method to install the C15 center and iodoboration of an alkyne to implement the (*Z*)-alkene geometry (Scheme 3). In practice, propyne (**17**) reacted with *B*-iodo-9-BBN, and the resultant vinyl borane was added to methyl vinyl ketone to furnish ketone **18**.[25] Subsequent treatment of this compound with TMSI/HMDS afforded an 88:12 mixture

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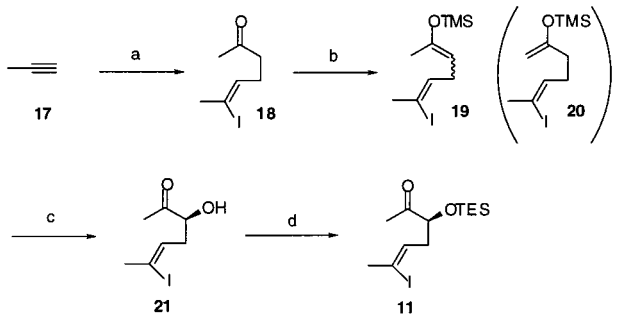
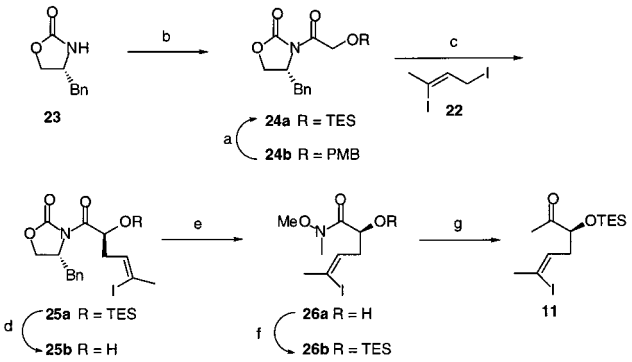
Scheme 3. Catalytic Asymmetric Route to Ketone **11**[a]



[a] Reagents and conditions: (a) i) 9-BBN-I, hexanes, ii) methyl vinyl ketone, iii) 3 N NaOH, toluene, 100 °C, 65%; (b) TMSI-HMDS, CH_2Cl_2, −20 °C to rt; (c) 1 mol % OsO_4, AD-mix-α, $MeSO_2NH_2$, *t*-BuOH−H_2O (1:1), 55% for two steps; (d) TESCl, imidazole, DMF, 85%.

Scheme 4. Stereoselective Alkylation Route to Ketone **11**[a]

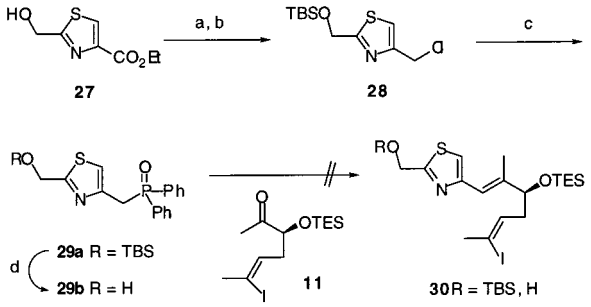


[a] Reagents and conditions: (a) i) $TiCl_4$, CH_2Cl_2, DIPEA, 87%, ii) TESCl, imidazole, DMF, 84%; (b) i) glycolic acid, TESCl, NaH−TEA, ether, 0 °C, then *t*-BuCOCl, −78 °C, ii) *n*-BuLi, −78 °C to rt, 38∼41%; (c) LHMDS, −78 °C, THF, 81%; (d) AcOH:H_2O:THF (3:1:1), 86%; (e) i) CH_3ONHCH_3, $AlMe_3$, CH_2Cl_2 ii) TESCl, imidazole, DMF, 88%; (g) MeMgBr, 0 °C, 93%.

of two silyl ether regioisomers **19** and **20**. Asymmetric dihydroxylation of the mixture, using AD-mix-α, generated hydroxyketone **21** in 55% yield and in 87% enantiomeric excess.[26] It is noteworthy that it was possible to sustain the potentially vulnerable iodoalkene functionality during the osmium-mediated dihydroxylation.[27] Finally, triethylsilylation of **25** produced **11**, completing the sequence in only four steps.

To develop a more enantioselective route, we explored the possibility of an asymmetric alkylation reaction to establish the C15 configuration (Scheme 4). Carbon−carbon bond formation, using a chiral glycolate enolate as a nucleophile, had appeal since the required (*Z*)-2,4-diiodo-2-butene (**22**) could be readily prepared from 2-butyn-4-ol.[28] Previously, asymmetric synthesis of glycolates had been accomplished by oxygenation of the "chiral enolate" (i.e., one armed with an auxiliary acyl group) rather than by alkylation.[29] Examples involving the alkylation of a glycolate of the general type **24** tended to require a robust

Scheme 5. Attempted Preparation of the *O*-Alkyl Wing[a]



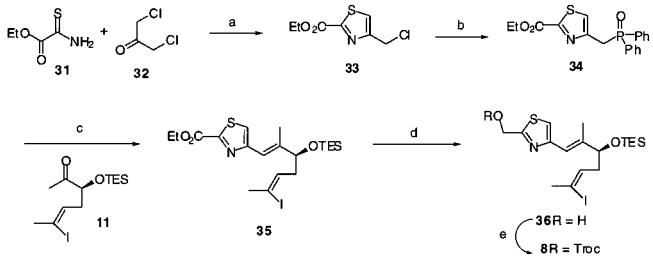
[a] Reagents and conditions: (a) TBSCl, imidazole, DMF, 99%; (b) i) Dibal-H, CH_2Cl_2, 65%, ii) CCl_4, PPh_3 84%; (c) i) $HPPh_2$, *n*-BuLi, THF, ii) H_2O_2, 33%; (d) TBAF, THF, rt, 60%.

O-protecting group.[30] Applied to our case, deprotection of a stable protected alcohol, given the sensitive iodoalkene moiety, seemed to be questionable. Hence, we examined the feasibility of using silyl-protected **24** in these alkylations.[31] Synthesis of the silylated glycolate began with the known PMB derivative **24b** which was obtained from **23** in three steps.[32] It was found later that **23** could be converted to **24a** in multigram scales, albeit in moderate yields, by a one-flask procedure involving in situ formation of a mixed anhydride.[33] Remarkably, treatment of lithio **24a** with diiodide **22** at −78 °C afforded the desired **25a** as a single isomer (>98% de) in good yield. Interestingly, strong dependence of the yield on the nature of the silyl-protecting group was observed. For example, only trace amounts, at best, of the alkylation product could be obtained using other silyl-protecting groups (e.g., R = TMS, TBS, or TBDPS in **24**). In these cases, the chiral auxiliary **23** was cleaved, and a complicated mixture of intractable products was formed. Thus, the TES function was preferentially used as the protecting device, and the asymmetric alkylation of **24a** could be routinely performed in multigram scales to give enantiopure **25a**. After removal of the TES group, the formation of Weinreb amide **26a**[34] was effected by simultaneous detachment of the chiral auxiliary. Subsequent protection and Grignard addition then afforded **11**.

With the requisite ketone **11** in hand, we turned our attention to the preparation of Horner-like compound **10**. Using the known 2-substituted thiazole derivative **27**,[35] phosphine oxide **29a** was uneventfully advanced through the intermediacy of chloride **28** by standard transformations (Scheme 5). Surprisingly, the condensation between lithio **29a** and **11**, however, led to the recovery of both reactants rather than to the formation of the desired **30**. Under a variety of different conditions, including the employment of dilithio **29b** as the nucleophile, the formation of **30** could not be realized. These results stand in sharp contrast to those encountered in our synthesis of the corresponding

(25) Satoh, Y.; Serizawa, H.; Hara, S.; Suzuki, A. *J. Am. Chem. Soc.* **1985**, *107*, 5225.
(26) (a) Sharpless, K. B.; Amberg, W.; Beller, M.; Chen, H.; Hartung, J.; Kawanami, Y.; Lubben, D.; Manoury, E.; Ogino, Y.; Shibata, T.; Ukita, T. *J. Org. Chem.* **1991**, *56*, 4585. (b) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768. (c) Hashiyama, T.; Morikawa, K.; Sharpless, K. B. *J. Org. Chem.* **1992**, *57*, 5067.
(27) Fattori, D.; de Guchteneere, E.; Vogel, P. *Tetrahedron Lett.* **1989**, *52*, 7415.
(28) Gras, J.-L.; Kong Win Chang, Y.-Y.; Bertrand, M. *Tetrahedron Lett.* **1982**, *23*, 3571.
(29) For example, see: Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346.
(30) For examples, see: (a) Jung, J. E.; Ho, H.; Kim, H.-D. *Tetrahedron Lett.* **2000**, *41*, 1793. (b) Paterson, I.; Bower, S.; McLeod, M. D. *Tetrahedron Lett.* **1995**, *36*, 175. (c) Cardillo, G.; Orena, M.; Romero, M.; Sandr, S. *Tetrahedron* **1989**, *45*, 1501. (d) Kelly, T. R.; Arvanitis, A. *Tetrahedron Lett.* **1984**, *25*, 39.
(31) Reference 23. During our study, an independent investigation on this subject appeared. Crimmins, M. T.; Emmmitte, K. A.; Katz, J. D. *Org. Lett.* **2000**, *2*, 2165.
(32) Evans, D. A.; Kaldor, S. W.; Jones, T. K.; Clardy, J.; Stout, T. J. *J. Am. Chem. Soc.* **1990**, *112*, 7001.
(33) Cossío, F. P.; Palomo, C. *Tetrahedron Lett.* **1985**, *26*, 4239.
(34) (a) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815. (b) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989.
(35) Ciufolini, M. A.; Shen, Y. C. *J. Org. Chem.* **1997**, *62*, 3804.

Scheme 6. Synthesis of the *O*-Alkyl Wing via Horner Condensation[a]

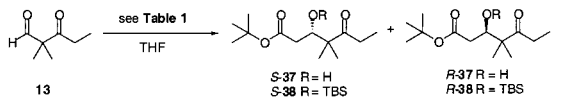


[a] Reagents and conditions: (a) toluene, 110 °C, 2 h, 96%; (b) $HOPPh_2$, Cs_2CO_3, cat. TBAI, CH_2Cl_2, rt, 48 h, 82%; (c) LHMDS, THF, −78 °C, 52%; (d) 2.5 eq Dibal-H, CH_2Cl_2, 0 °C, 98%; (e) Cl_3CCH_2OCOCl, Pyridine, CH_2Cl_2, 86%.

segment required for EpoB. In that case, excellent yields were achieved using a 2-methylthiazole derivative (R = CH_3 in **10**).[22,23]

The failure of the needed Horner reaction led us to seek an alternative approach in which the C21 hydroxy function could emerge after the olefination reaction (Scheme 6). Accordingly, ethyl thiooxamate (**31**) was condensed with 1,3-dichloroacetone (**32**) to provide 2,4-disubstituted thiazole **33** in excellent yield. Subsequent Arbuzov reaction using Ph_2POEt gave **34** in moderate yield. Fortunately, direct *P*-alkylation with $HOPPh_3$ generated **34** in good yield. Upon treatment with LHMDS, thiazole **34** and ketone **11** condensed smoothly to furnish **35** as a single geometric isomer. Finally, reduction of ester **35** with Dibal-H generated alcohol **36** which was protected with a Troc group to yield the desired *O*-alkyl moiety. The new route is significantly shorter and more convergent than the previous sequence[20] which involved 10 linear transformations.

New Synthetic Route to the *O*-Acyl Fragment. Having successfully prepared the *O*-alkyl building block **8** in quantity, our attention was turned to the development of a route to the *O*-acyl fragment **9**. As described earlier, use of the segment used in our previous synthesis (cf. **4**), led to a surprising solvolysis problem at C15 during the Noyori reduction. *Hence, the major point of improvement was focused on the establishment of the C3 carbinol center prior to the Suzuki merger*. Our investigation commenced with attempts to realize an asymmetric Reformatsky reaction (eq 1, Table 1).

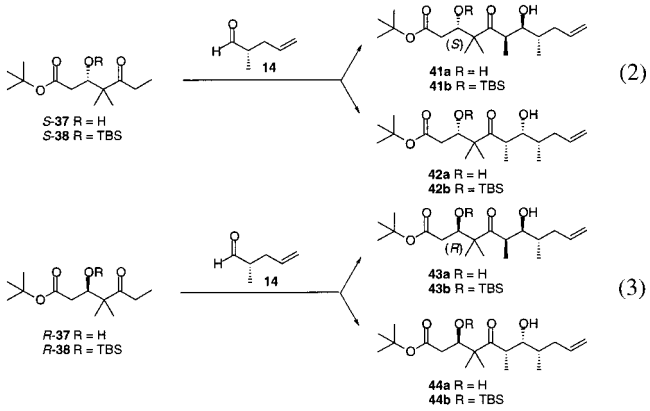


(1)

Addition reactions of "zincated" *tert*-butyl bromoacetate to keto aldehyde **13** were performed in the presence of chiral amino alcohol **39**.[36] The reactions gave rise to the expected aldol adduct in excellent yields. Unfortunately, the enantioselectivities did not rise to synthetically useful levels (entries 1 and 2). While decreasing the reaction temperature increased the enantioselectivities significantly, reactions conducted this way suffered from poor conversions (entry 3).

Accordingly, the classical Reformatsky inspired reactions were set aside. Fortunately, a high degree of stereocontrol was achieved by the addition reaction of a chiral titanium enolate derived from *tert*-butyl acetate to the aldehyde. Following literature protocol,[37] both enantiomers of β-hydroxy *tert*-butyl ester **37** were prepared in good yields with excellent enantioselectivities (entries 4 and 5). The ee and sense of the absolute stereoselectivity was determined by derivatization of **37** to the corresponding Mosher's ester, and corroborated by subsequent events in our program.[38]

Having found a method to generate **37** in high enantiomeric excess, we set out to investigate the double stereo-differentiation aldol reaction.[39] To use protected **37** as the nucleophile, both enantiomeric aldol adducts were converted to corresponding TBS ether antipodes **38**. Unfortunately, the attempted reaction between lithio **38** with **14**, however, could not be effected in meaningful yield due to the sensitivity of the β-silyloxy system to elimination. Thus, we took recourse to a less basic, titanium enolate of **38** ($TiCl_4$, *i*-Pr_2NEt, CH_2Cl_2, −78 °C) for the same aldol reaction (eqs 2 and 3).[40] Indeed, both (*S*)-**38** and (*R*)-**38** underwent the aldol condensation, giving mixtures of diastereomers in moderate yields (**41b**:**42b** = 3.5:1, 52% and **43b**:**44b** = 1:3.0, 40%). The stereochemical outcome of the major diastereomers, **41b** and **44b**, from each reaction indicated that the configuration of C3 rather than C8 had a larger effect on the sense of the newly formed C6 and C7 centers. However, the degree of the selectivity (*dr* = 3∼3.5:1) in these reactions was lower than that of our previous reaction (*dr* = 6.5:1), suggesting that neither enantiomer of **38** benefited from matching chirality in **14**. While the explanation for the puzzling lack of "matching" in these alkylation reactions will require more revealing experiments, the results must, to some extent, reflect differences arising from altering the cation (titanium rather than lithium). The special "γ,δ-unsaturated aldehyde effect" was most pronounced with lithium as the counterion.[41]



The difficulty of conducting the aldol reaction with a lithium enolate of **38** led us to investigate the feasibility of condensation using dilithio **37** as a nucleophile. Indeed, the reaction of the dianion of **37** with aldehyde **14** did not induce β-elimination, and the condensation proceeded smoothly.[42] When dilithio (*S*)-**37** was treated with homochiral **14**, there was obtained a 2:3 mixture of diastereomers **41a** and **42a** in 53% yield. Extensive

(36) (a) Soai, K.; Takahashi, K. *J. Chem. Soc., Perkin Trans 1* **1994**, 1257. (b) Soai, K.; Niwa, S. *Chem. Rev.* **1992**, *92*, 833.

(37) Duthaler, R. O.; Herold, P.; Lottenbach, W.; Oretle, K.; Reidiker, M. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 495.

(38) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.

(39) For examples of aldol reactions of β-hydroxy ketones, see: (a) Luke, G. P.; Morris, J. *J. Org. Chem.* **1995**, *60*, 3013. (b) Evans, D. A.; Calter, M. A. *Tetrahedron Lett.* **1993**, *34*, 6871. (c) McCarthy, P. A.; Kageyama, M. *J. Org. Chem.* **1987**, *52*, 4681.

(40) Evans, D. A.; Urpí, F.; Somers, T. C.; Clark, J. S.; Bilodeau, M. T. *J. Am. Chem. Soc.* **1990**, *112*, 8215.

(41) (a) Harris, C. R.; Kuduk, S. D.; Balog, A.; Savin, K.; Danishefsky, S. J. *Tetrahedron Lett.* **1999**, *40*, 2267. (b) The use of other enlolates (K, Na, Mg, Al, and B) instead of Li for the aldol reaction gave inferior results (dr = 2∼4:1). Harris, C. R.; Kuduk, S. D.; Lee, C. B. Unpublished results in these laboratories.

(42) Martin, V. A.; Murray, D. H.; Pratt, N. E.; Zhao, Y.-b.; Albizati, K. F. *J. Am. Chem. Soc.* **1990**, *112*, 6965.

Table 1. Asymmetric Synthesis of C3 Stereogenic Center

Entry	Nucleophile	Additive	Temp (°C)	%Yield[a]	er[b] (*S*-**37**:*R*-**37**)
1	*t*-BuOCCH$_2$Br, Zn, TMSCl	**39**	0	91	3 : 1
2	*t*-BuOCCH$_2$Br, Zn, TMSCl	**39**	-40	93	5 : 1
3	*t*-BuOCCH$_2$Br, Zn, TMSCl	**39**	-78	22	11 : 1
4	*t*-BuOCCH$_3$, LDA	L-**40**, R = L-DIPGF[c]	-78	80	> 20 : 1
5	*t*-BuOCCH$_3$, LDA	D-**40**, R = D-DIPGF[d]	-78	85	> 1 : 20

[a] Isolated yields. [b] er = enantiomeric ratio determined by ^{1}H NMR integration of appropriate signals of the corresponding Mosher's ester. [c] L-DIPGF = 1,2:5,6-di-*O*-isopropylidene-α-L-glucofuranos-3-*O*-yl. [d] D-DIPGF = 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranos-3-*O*-yl.

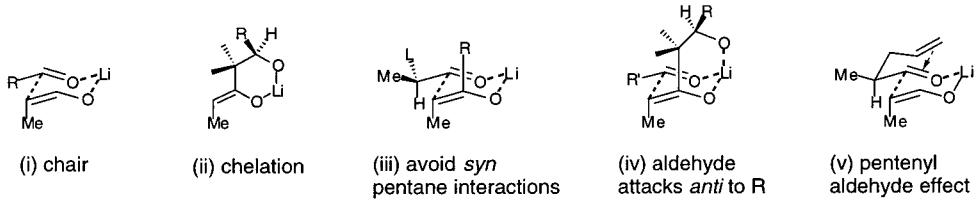


Figure 2. Factors governing the aldol reaction.

chemical and spectroscopic correlation studies revealed that the major diastereomer **42a** corresponded to a C6−C7 bis-*epi* version of **9** (vide infra). Thus, the desired isomer **41a** whose configurations were those required to reach "natural" epothilones constituted the minor product. We then performed the corresponding reaction using the enantiomeric ketone, (*R*)-**37**. Remarkably, this reaction gave rise to a single diastereomer in 88% yield. Subsequent correlation studies verified that the stereochemistry of the C6 and C7 centers corresponded to those of "natural" epothilones. The C3 center was, of course, configured as *R* rather than *S*. Therefore, the use of **43a** as a precursor of the *O*-acyl wing **9** would necessitate a subsequent inversion at C3. Attempts to conduct Mitsonobu type inversions were not rewarding.

Despite the undesired outcome of this attempt, the results from the dianion aldol reaction provided potentially useful information for understanding the principles underlying double stereo-differentiation aldol reactions, and the matter was pursued in some detail. The C3 protected series, such as in Schinzer's case,[24] required the (*S*) configuration at C3 to deliver C6(*R*),-C7(*S*) configurations in a related aldol reaction (employing a C8(*S*)-aldehyde structurally very similar to **14**). In contrast, the combination of (*R*) chirality at C3 and (*S*) chirality at C8 led to the required C6(*R*),C7(*S*) configurations in our dianion aldol reactions. The disparity between the two systems in terms of a "matching" and "mismatching" pair, strongly suggested that the transition state of the aldol reaction of the C3 unprotected (alkoxide) system is very different from that of the C3 protected system.

To explain the drastic influence of the C3 protection status on the stereochemical outcome and the remarkable selectivity in the aldol reaction between (*R*)-**37** and aldehyde **14**, various considerations pertinent to the transition state (Figure 2) must be taken into account. We propose that the factors governing such aldol condensation include; (i) the chairlike transition state which leads to a *syn*-aldol product,[43] (ii) chelation of the lithium counterion by the β-alkoxido oxygen, (iii) a *syn* relationship between the α-proton of the aldehyde and the optimal presentation of the C5 methyl group of the enolate to minimize a developing *syn* pentane interaction,[44] (iv) a preference for the aldehyde to attack the enolate *anti* to the large resident R group, and (v) the special γ,δ-unsaturated aldehyde effect[41] which tends to orient the pendent allyl group for a favorable interaction with the carbonyl group of the aldehyde, thus guiding the enolate attack *anti* to such organized framework of the aldehyde. Note that factor (v) reinforces the facial selectivity of the aldehyde influenced by factor (iii).

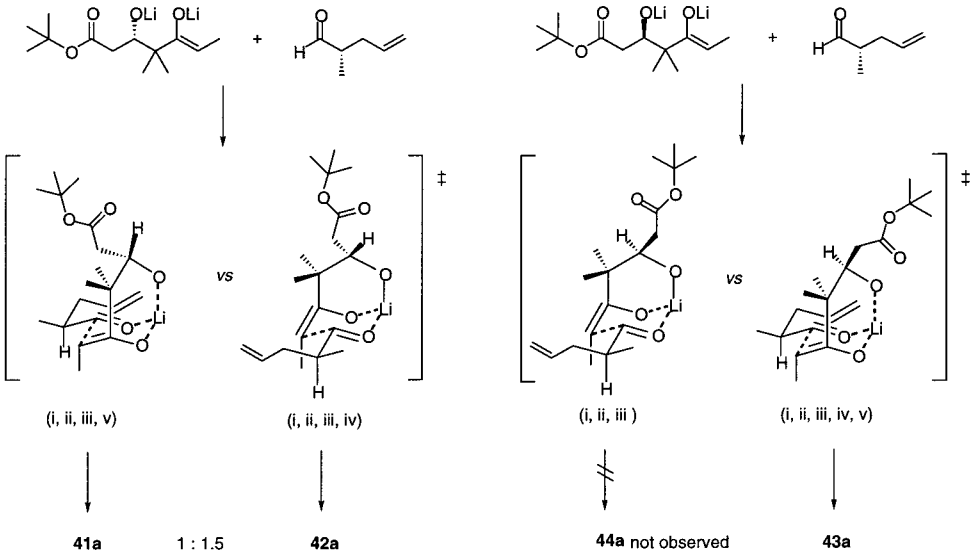
Taking all ofthese preferences into consideration, we analyzed the structure of transition states leading to the formation of **41a**−**44a** (Scheme 7).[45] While the relative contribution of each factor to transition state stabilization cannot be weighted, we note the reaction of (*R*)-**37** with **14** incorporates each of the energy-lowering elements we propose in the transition state. Thus, the reaction between these two compounds constitutes a "matched" event, giving rise to **43a** with complete diastereoselectivity. On the other hand, the transition state for the formation of the alternative (nonobserved) diastereomer **44a**, would lack both favorable elements (iv) and (v). In a similar manner, the combination

(43) (a) Heathcock, C. H.; Pirrung, M. C.; Lampe, J.; Buse, C. T.; Young, S. D. *J. Org. Chem.* **1981**, *46*, 2290. (b) Frater, G. *Tetrahedron Lett.* **1981**, *22*, 425. (c) Seebach, D.; Wasmuth, D. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 971. (d) Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737. (e) Williard, P.; Salvino, J. M. *Tetrahedron Lett.* **1985**, *26*, 3931.

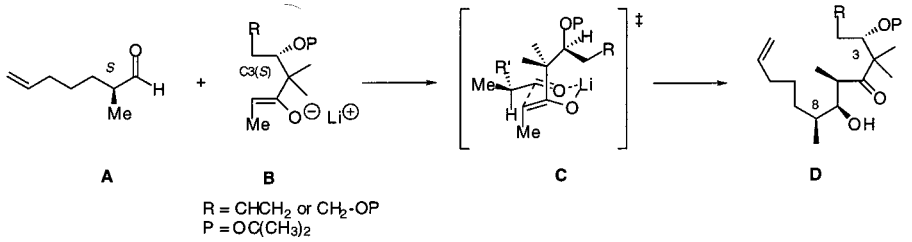
(44) Roush, W. R. *J. Org. Chem.* **1991**, *56*, 4151.

(45) For the preliminary communication, see: Wu, Z.; Zhang, F.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2000**, *39*, 4505.

Scheme 7. Proposed Transition States for Dianion Aldol Reactions

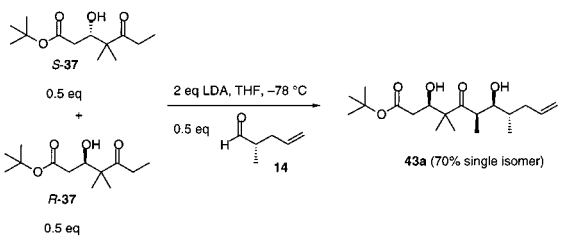


Scheme 8. Schinzer-Type Aldol Reaction



of dilithio (*S*)-**37** with **14** to produce **41a** or **42a**, although incorporating the features (i−iii), leads to suboptimal transition states, lacking either (iv) or (v). To reach **41a**, the aldehyde must attack the enolate *syn* to the large group, contrary to the preferred arrangement (factor iv). The alternative facial mode which leads to **42a** would require a "trade off" between features (iii) and (v), since the transition state either forfeits the favorable overlap of the terminal alkene with the aldehyde carbonyl or accepts the unfavorable *syn* pentane interaction.

To explore the concepts implied in Figure 2 and in Scheme 7, a critical experiment was conducted. Since our interpretation of the outcome of the results described above clearly indicates the presence of matched and mismatched events, it seemed reasonable that there could be measurable kinetic implications. *Accordingly, we performed an aldol reaction in a setting where the homochiral aldehyde **14** could face internal competition between the two enantiomeric enolates.* When the racemic enolate corresponding to both antipodes of **37** was treated with a limiting concentration (0.5 equiv) of homochiral aldehyde **14** at −78 °C, the reaction generated only **43a** as the product, virtually free of other diastereomers (**41a**, **42a**, and **44a**) in 70% yield (eq 4). Therefore, our hypothesis as to the matching−mismatching issues in the fascinating aldol reaction has been supported at the kinetic level.



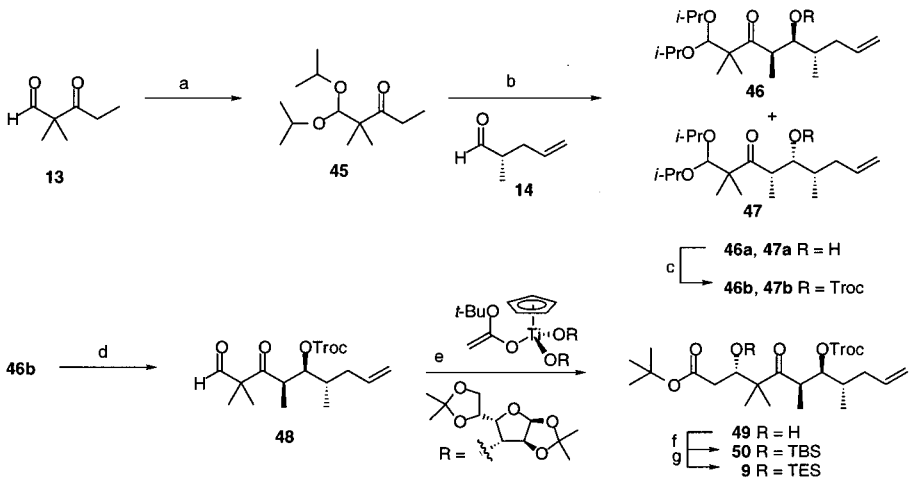
(4)

We have not conducted our own experiments with Schinzer-type aldol condensations where the oxygens at C3 and C1 are co-protected as a cyclic acetonide (Scheme 8). However, we note that in the Schinzer C3 protected series,[24] it is the C3(*S*) configuration in **B** which is required to induce the C6(*R*),C7(*S*) configurations. This result is in contrast to our C3 unprotected case, reported above, wherein the C3(*R*) antipode is the one which induces the C6(*R*),C7(*S*) configurations. We propose that the C3−OR bond is orientated differently in the two enolates and that this key divergence accounts for the opposite senses of long-range asymmetric induction of the protected (cf. **B**) versus unprotected enolates (see dilithio *S*-**37**). We propose that in **B** (as opposed to **37**), the C3−OR group is positioned *anti*-periplanar to the C4−C5 bond in the transition state **C**. This orientation in the C3(*S*) enolate would favor the attack of the C8(*S*) aldehyde **A** *syn* to the C3 hydrogen rather than *syn* to the large C1−C2 moiety. This bias would give rise to the C6-(*R*),C7(*S*) configuration in the product **D**. While the orientation of the C3 alkoxy substituent in the transition state is not established with certainty, it is well to note that the aldol reaction of chiral β-alkoxy enolates have been shown to exhibit strong 1,5-asymmetric induction.[46]

Although the double stereo-differentiating aldol process proved highly informative, the use of **43a** in a total synthesis would, as noted above, require a nonstraightforward inversion at the C3 center of the product. Thus, we sought an alternative sequence to **9** wherein sequential "substrate-directed" and "reagent-controlled" aldol reactions would establish the requisite configurations at C6, C7, and C3 (Scheme 9).

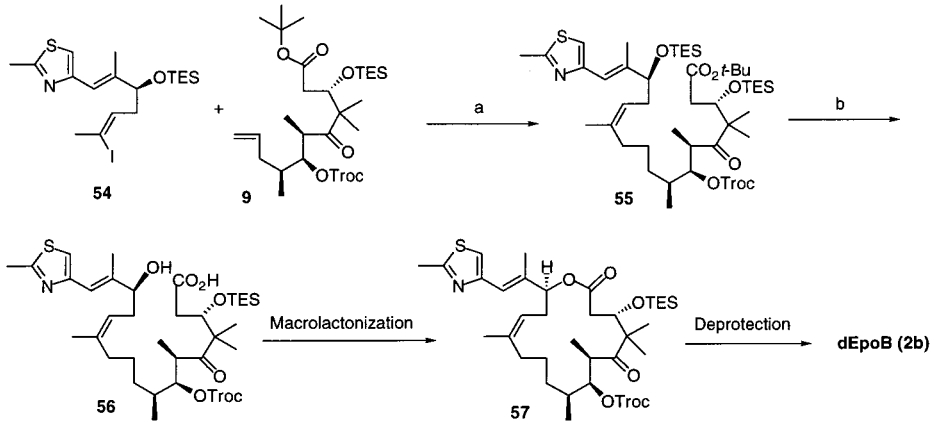
(46) For a related issue in the aldol reaction of ketones, see: (a) Paterson, I.; Gibson, K. R.; Oballa, R. M. *Tetrahedron Lett.* **1996**, *37*, 8585. (b) Evans, D. A.; Coleman, P. J.; Côté, B. *J. Org. Chem.* **1997**, *62*, 788. (c) Tanimoto, N.; Gerritz, S. W.; Sawabe, A.; Takeshi, N.; Filla, S. A.; Masamune, S. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 673.

Scheme 9. New Synthetic Route to the *O*-Acyl Wing[a]



[a] Reagents and conditions: (a) $CH(OiPr)_3$, *i*PrOH, cat. TsOH, 88%; (b) LDA, −78 °C, 85%, **46**:**47** = 4:1; (c) TrocCl, Pyridine, CH_2Cl_2, 0 °C, 99%; (d) H_2O/THF, cat. TsOH, 88%; (e) THF, 89%, dr > 20:1; (f) TBSOTf, 2,6-lutidine, CH_2Cl_2, −78 °C, 81%; (g) TESCl, imidazole, DMF, 96%.

Scheme 10. Application to the Total Synthesis of dEpoB[a]

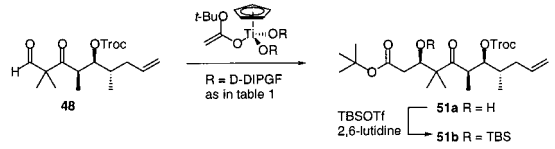


[a] Reagents and conditions: (a) i) 9-BBN-H, THF (ii) $PdCl_2$(dppf), $AsPh_3$, DMF−THF−H_2O, rt, 2 h, 72%; (b) TESOTf, 2,6-lutidine, CH_2Cl_2, −78 °C to rt, 8 h, (ii) HCl−CH_3OH, THF, 0 °C, 69%.

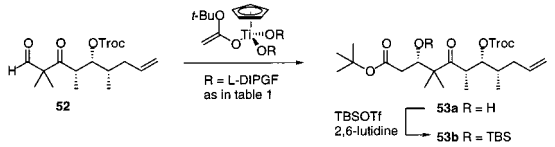
Toward this end, keto aldehyde **13** was protected as a diisopropyl acetal, and the resultant ketone **45** was subjected to an aldol reaction with **14**. Upon deprotonation and reaction of **45** with **14**, smooth condensation gave rise to a 4:1 mixture of aldol adducts **46a** and **47a**. While this ratio was somewhat lower than that of our previous route (6.5∼5.5:1),[13e,16,45] the major diastereomer **46a** was very easily separated by flash chromatography and protected as a Troc group. Indeed, there was a significant advantage in this route in that the aldol condensation, unlike our earlier cases where C3 corresponded to an enol ether,[13e] went to completion. Furthermore, the reaction conditions are much less demanding technologically, since now the coupling is conducted at −78 °C rather than at −120 °C in the previous synthesis.

Hydrolysis of the diisopropyl acetal group of **46b** under acid catalysis gave keto aldehyde **48**, setting the stage for the second aldol reaction. Following the same "titano" *tert*-butyl ester method using a glucose derived auxiliary as in eq 1, the desired C3(*S*)-**49** was obtained in high diastereoselectivity (dr > 20:1). Protection of the C3 alcohol with a silyl group finally afforded the *O*-acyl wing **9** and the TBS derivative **50** whose spectral and chromatographic properties were identical to previously obtained material from other programs in these laboratories.[13e]

For the purpose of correlation studies, keto aldehyde **48** was treated with an enantiomeric *tert*-butyl titanoacetate to afford **51a**, whose TBS ether **51b** correlated with the aldol product **43** from the earlier studies (eqs 5 and 6). Similarly, the minor isomer **47** was correlated. Compound **53**, arising from **52**, corresponded to the previously encountered **42**.



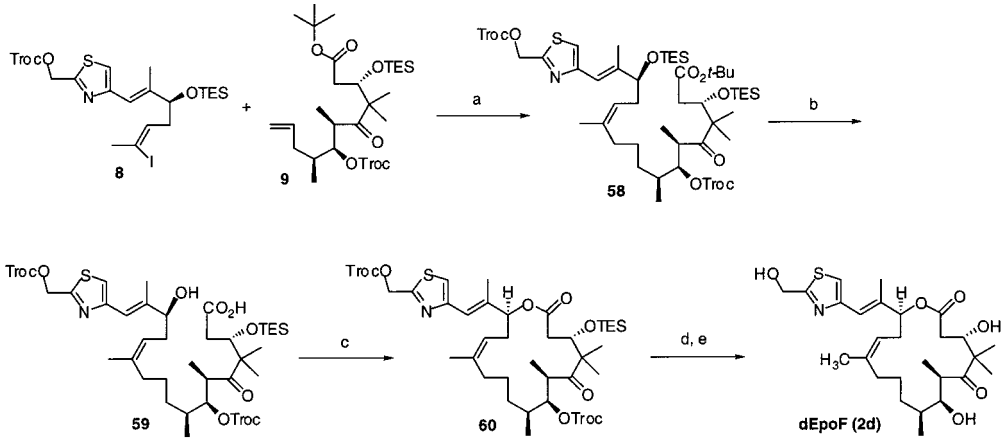
(5)



(6)

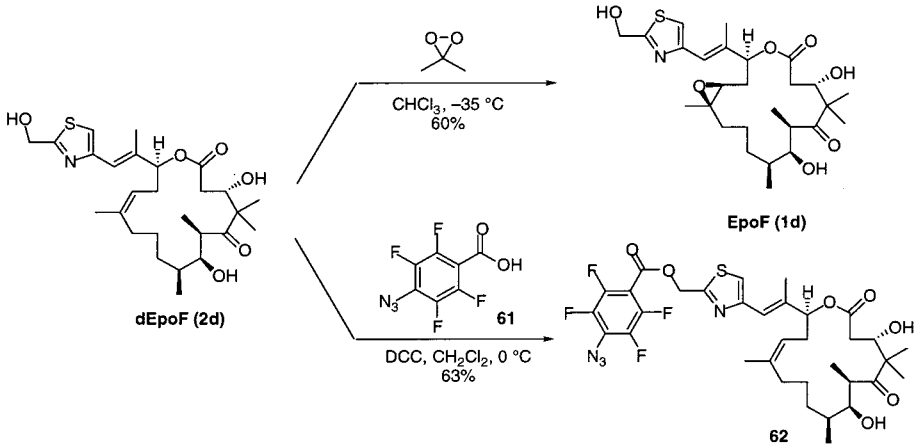
In addition to these correlation studies, the newly prepared **9** was utilized in the formal total synthesis of dEpoB as illustrated in Scheme 10. The *B*-alkyl Suzuki coupling with the *O*-alkyl segment for EpoB series **54** proceeded to yield **55**. Subsequently, treatment of **55** with TESOTf followed by selective desilylation afforded hydoxyacid **56** which had been advanced to dEpoB (**2b**) and EpoB (**1b**) in our previous syntheses. Accordingly, these studies unambiguously established the stereochemical

Scheme 11. Completion of the Total Synthesis of dEpoF[a]



[a] Reagents and conditions: (a) i) 9-BBN-H, THF (ii) $PdCl_2$(dppf), $AsPh_3$, DMF−THF−H_2O, rt, 8 h, 89%; (b) TESOTf, 2,6-lutidine, CH_2Cl_2, −78 °C to rt, 8 h, (ii) HCl−CH_3OH, 0 °C, 78%; (c) 2,4,6-trichlorobenzoyl chloride, $(C_2H_5)_3N$ then 4-DMAP, toluene, slow addition 3 h, 60−70%; (d) Zn, AcOH−THF, rt, 1 h, 90% (e) HF·pyridine, THF 0 °C to rt, 91%.

Scheme 12. Synthesis of EpoF and Photoffinity Labeled dEpoF



outcomes of the various aldol reactions and constitute an alternate total synthesis of dEpoB. The ultimate choice between the "titanoacetate"-based route to the C3(*S*) series in the acyl sector described above relative to the post-Suzuki (C3 keto → C3(*S*) hydroxy) route practiced previously will depend on issues of costing and on susceptibility to scale-up.

Finally, and most importantly, we applied these findings to the total synthesis of dEpoF (Scheme 11). As explained above, a much improved total synthesis of this compound was critical if it were to advance to clinical evaluation. At this stage it seemed that the key synthetic issues had indeed been favorably resolved. The proposed *O*-alkyl and acyl building blocks were now available in multigram quantities with a clear closing strategy in place.

Fragments **8** and **9** were conjoined via the *B*-alkyl Suzuki protocol to provide the *seco* ester **58**. Conversion of the *tert*-butyl ester **58** to the TES ester followed by acid-catalyzed selective desilylation provided **59**, which awaited macrolactonization. At this point, detailed spectral correlations of **59** confirmed its identity with the previously synthesized intermediate. Following the same sequence as was used in our earlier synthesis,[20] hydroxyacid **59** was cyclized to the fully protected macrolactone **60**. Finally, sequential removal of the Troc and TES groups afforded 12,13-desoxyepothilone (**2d**, dEpoF) which again proved identical in all respects with the previously synthesized dEpoF.

Following completion of the synthesis of dEpoF, we also accomplished the synthesis of epothilone F (**1d**, EpoF) itself by epoxidation at the 12,13-alkene (Scheme 12). Treatment of the synthetic dEpoF with 2,2-dimethyldioxirane (DMDO) induced the formation of the 12,13-epoxide with natural stereochemistry (dr > 15:1) to afford EpoF. Spectroscopic data and the observed $[\alpha]_D$ −26.5 (*c* 0.35, MeOH) corresponded well to the naturally occurring EpoF, lit.,[18] $[\alpha]_D$ −27.4 (*c* 0.5, MeOH). As we had hoped at the outset, the 21-hydroxyl group did increase the aqueous solubility of dEpoB by a factor of 2.5 as measured by a HPLC method.[47] In addition, its utility as a staging point for further functionalization was demonstrated. The condensation of unprotected dEpoF with azidoacid **61** was performed under the agency of DCC to afford the photoaffinity labeled dEpoF **62**. While the tubulin-binding assay had shown that dEpoF retains 90% activity of dEpoB, the aroylated derivative **62** did not induce tubulin polymerization,[48] thus underscoring the subtle nature of the thiazole region in effecting tubulin binding.

The fully synthetic dEpoF was first tested against various cell types to evaluate its antitumor potential. As shown in Table 2, dEpoF showed high cytotoxic activities against a broad range of sensitive and resistant tumor cell lines. In particular, dEpoF retained high potency and low cross-resistances against MDR cell lines and consistently outperformed other non-epothilone anticancer agents such as paclitaxel, vinblastine, etoposide, actinomycin, and adriamycin. These properties of dEpoF are

(47) Swindell, C. S.; Krauss, N. E.; Horwitz, S. B.; Ringel, I. *J. Med. Chem.* **1992**, *34*, 1176. Also see ref 20.

(48) Horwitz, S. B. Personal communication.

Table 2. Potency of dEpoF, dEpoB, and Taxol against Various Tumor Cell Growth in Vitro

	IC_{50} (μM)[a]			
tumor cell Lines	dEpoF	dEpoB	Taxol	Others
		Human T-cell AL Leukemia		
CCRF-CEM	0.0027	0.0095	0.0021	0.00063[b], 0.0290c
CCRF-CEM/VBL_{100}	0.047 (17.4 x)	0.017 (1.8 x)	4.140 (1971 x)	0.332[b] (527 x)
CCRF-CEM/VM_1	0.0049 (1.8 x)	0.014 (1.5 x)	0.0066 (3.18 x)	3.44[c](117 x)
CCRF-CEM/Taxol	0.0053 (2.0 x)	0.0162 (1.7 x)	0.120 (57 x)	
		Hamster Lung Fibroblasts		
DC-3F	0.0017	0.0019	0.0135	0.00025[d]
DC-3F/ADX	0.0136 (8.0 x)	0.0073 (3.8 x)	0.583 (43.2 x)	0.00153[d] (61.2 x)
DC-3F/ADII	0.0223 (13.1 x)	0.0288 (15.2 x)	20.19 (1496 x)	0.4092[d] (1637 x)
		Human Promyelocytic Leukemia		
HL-60	0.0007	0.0031	0.0011	
		Human CM Leukemia		
K562	0.0021	0.0036	0.0029	
		Human Prostate Adenocarcinoma		
PC-3	0.0119	0.0209	0.0280	
		Human Colon Adenocarcinoma		
HT-29	0.0014	0.0048	0.0016	
		Human Mammary Adenocarcinoma		
MCF-7	0.0069	0.0040	0.0024	0.081[e], 0.0094[b]
MCF-7/Adr	0.0097 (1.4 x)	0.00715 (1.4 x)	0.0135 (5.6 x)	0.280 (3.5 x), 0.025 (26.6 x)
		Human Mammary Carcinoma		
MX-1	0.0042	0.0221	0.0394	0.00184[f]
		Human Ovary Carcinoma		
SK-OV-3	0.0051	0.0035	0.0038	0.0016[f]
UL-3-C	0.0048	0.0021	0.0016	0.00037[b], 0.00058f
UL-3-B/Taxol	0.0067 (1.4 x)	0.0070 (3.3 x)	0.0107 (6.7 x)	0.00310 (8.4 x)[b], 0.00124 (2.1 x)[f]

[a] Cell growth inhibition was measured by XTT tetrazonium assay after 72 h incubation for cell growth as described previously in ref 15. The values were determined with six to seven concentrations of each drug using a computer program. The cross-resistances are shown in parentheses. [b] Vinblastin (VBL). [c] Etoposide (VM_1, VP-16). [d] Actinomycin D (AD). [e] Adriamycin (Adr). [f] Epothilone B (EpoB).

closely comparable to those of the highly promising antitumor agent, dEpoB. Striking in vivo comparisons of dEpoF with a current clinical candidate already in phase I trials will be presented elsewhere.[49]

Summary

A markedly improved total synthesis of the promising antitumor agent, dEpoF has been accomplished. The new synthesis features a convergent and modular nature strategy with strong stereoselectivities at each step. The conciseness of the syntheses of the key intermediates readily allows for large-scale preparation and easy structural variation in each synthetic segment.

For the synthesis of the *O*-alkyl wing of dEpoF, the Horner-like condensation conveniently conjoined the thiazole moiety and the segment possessing the critical C15 stereochemistry and (*Z*)-12,13-alkene function. The stereoselective aldol reactions en route to the *O*-acyl wing were investigated in some detail. It was demonstrated that a subtle variation in factors affecting the transition state has a remarkable influence on the long-range transmission of stereochemical information. In these studies, the implications of stereochemical matching of components in an aldol condensation have been probed at the kinetic level. A remarkable instance of the consequences of molecular recognition in covalent bond formation has been demonstrated via kinetic resolution.

Given the high degree of intolerance found in the SAR map of the polypropionate region,[13b] the new *O*-acyl wing fragment may serve as a widely applicable intermediate for accessing various analogues. The in vitro and in vivo evaluations of dEpoF[49] attest to the impressive potential of 12,13-desoxyepothilones in the B series.

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Supporting Information Available: Procedures for the synthesis and characterization data of all new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA010039J

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