A Novel Application of a Pd(0)-Catalyzed Nucleophilic Substitution Reaction to the Regio- and Stereoselective Synthesis of Lactam Analogues of the Epothilone Natural Products

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Abstract: Several lactam analogues of the epothilones were prepared using a concise semisynthetic approach starting with the unprotected natural products. Highlighted in this strategy is a novel regio- and stereoselective Pd(0)-catalyzed azidation reaction of a macrocyclic lactone. Subsequent reduction and macrolactamization of the resulting azide acid intermediates provided the desired macrolactams in satisfactory overall yields. The entire three-step sequence was streamlined into a "one-pot" process for the epothilone B-lactam, BMS-247550, which is currently undergoing phase I clinical trials. An initial total synthesis route to prepare the lactam analogue of epothilone C was completed and compared to the more direct semisynthesis approach. All of the lactam analogues were evaluated in vitro and the results are discussed.

Introduction

Epothilones A (**1**, Epo A) and B (**2**, Epo B) are the initial members of a novel family of cytotoxic and antifungal macrolides originally isolated at Gesellschaft für Biotechnologische Forschung (GBF) by Höfle and Reichenbach via fermentation of the myxobacterium *Sorangium cellulosum*.[1] Bollag and co-workers independently isolated these unique natural products and discovered that their cytotoxic effects result from the induction of tubulin polymerization and stabilization of microtubules[2] leading to G_2-M arrest in the cell cycle and ultimately apoptosis.[2a] This mode of action for the epothilones, at least in vitro and presumably in vivo, was shown by the Merck[2a] and GBF[3] groups to be identical to that first described by Horwitz[4] for the clinically successful chemotherapeutic agent Taxol (paclitaxel). More significantly, however, unlike paclitaxel the epothilones are not affected by known mechanisms of resistance such as P-glycoprotein overexpression (multidrug resistance) or mutated tubulin.[2]

Following the initial report by Höfle and co-workers, a number of groups became intrigued by the epothilones' unique structure and impressive biological profile, and thus committed substantial resources toward developing synthetic routes to these natural products[5−10] and related analogues.[11−13] Elegant synthetic strategies incorporating the ring-closing olefin metathesis reaction (RCM), macrolactonization, Suzuki coupling, or ester-

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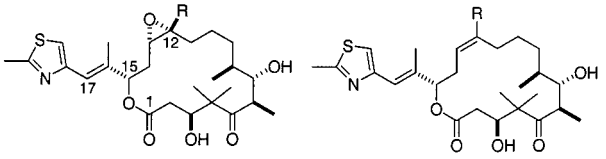
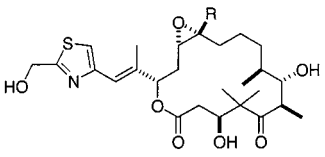
enolate-aldehyde condensation have been developed to complete the total syntheses of these interesting compounds.[5] In addition to these efforts, other members of the epothilone family, namely epothilones C (**3**, Epo C), D (**4**, Epo D), E (**5**, Epo E), and F (**6**, Epo F), were isolated as minor metabolites from the fermentation process[14] or as synthetic intermediates[6−8,10] or via semisynthesis.[13a] Currently, all three of these approaches are being explored by multiple research groups to prepare various epothilone analogues.



1: R = H epothilone A
2: R = Me epothilone B

3: R = H epothilone C
4: R = Me epothilone D



5: R = H epothilone E
6: R = Me epothilone F

Moreover, major pharmaceutical companies have become increasingly interested in the epothilones and/or related analogues as potential chemotherapeutic agents capable of complementing existing anticancer therapies.[15−18] Several factors have made this exciting class of natural products an attractive oncology target, namely: (1) irreversible microtubule stabilization is a clinically validated biological target; (2) epothilones are active in vitro against a number of paclitaxel-resistant tumor cell lines; (3) epothilones exhibit better water solubility than taxanes; (4) epothilones are readily obtained by fermentation; and (5) the epothilone structure is amenable to both total synthesis and semisynthesis. Furthermore, Danishefsky and coworkers demonstrated in vivo that Epo B and Epo D display antitumor effects in both paclitaxel-sensitive and -resistant murine tumor models.[9]

In contrast, our preliminary studies in murine tumor models indicated that at tolerated doses Epo B lacks in vivo activity, resulting from rapid inactivation of the compound in the systemic circulation ($T_{1/2} \approx 40$ min in mouse plasma).[19] It was postulated that this lack of antitumor activity was due to esterase-mediated hydrolysis of the lactone moiety to form an inactive ring-opened species. This hypothesis was supported by the finding that the cell growth inhibitory effects of Epo B could be mitigated by preincubation of the drug substance in murine plasma.

As part of our ongoing research program aimed at preparing metabolically stable epothilone derivatives and establishing a comprehensive structure−activity relationship (SAR) of these analogues,[15,16,19] we have focused on replacing the metabolically labile lactone moiety and altering this portion of the epothilone core. In this article we describe the preparation of several macrocyclic lactam derivatives of the epothilones based on a semisynthetic approach from the corresponding natural macrolides **1** and **2**. In particular, we highlight the concise three-step synthesis of the clinical agent BMS-247550 involving a novel Pd(0)-catalyzed ring-opening reaction of unprotected Epo B as the key step. In addition, we provide a description of our initial attempts to prepare the Epo C-lactam via a total synthesis route. Finally, a discussion of the in vitro biological profiles of these analogues will be provided.

Results and Discussion

Soon after the discovery of the mechanism of action of the epothilones a research collaboration was established between GBF and our laboratories which provided us with access to significant quantities of the epothilone natural products. However, from the outset, we were interested in comparing both total synthesis and semisynthesis approaches, since either strategy would clearly offer distinct advantages. Total synthesis could provide access to a greater number of structurally diverse analogues, and potentially lead to novel, biologically active compounds. Conversely, semisynthesis, while not as flexible, could allow for shorter synthetic routes and provide "unlimited" scale-up potential.

Total Synthesis Approach.[20] We set out to assess the practicality of a total synthesis approach to epothilone analogues from a pharmaceutical development perspective. Our initial strategy to prepare the epothilone lactams was based on a RCM route initially reported by Nicolaou[7] for the syntheses of epothilones A and C.

Thus, thiazole fragment **7**, derived from commercially available L-allylglycine was coupled to the polypropionate acid **8**[7a]

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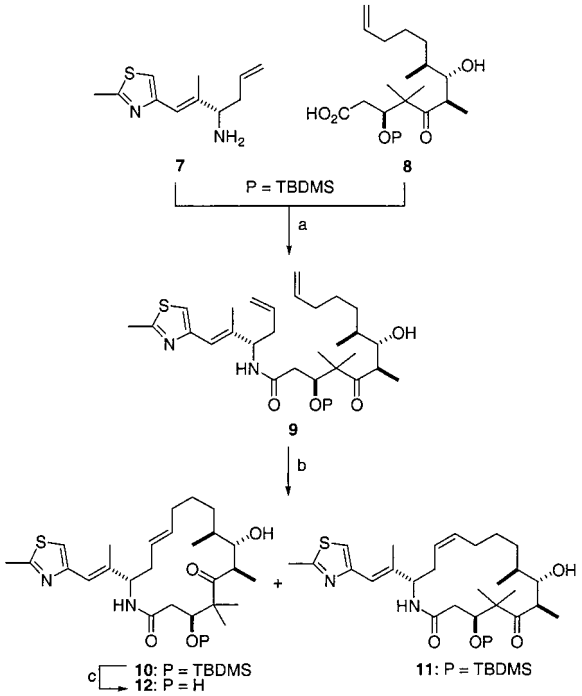
Scheme 1[a]



[a] (a) EDCl, HOBt, DMF, 15 h, 77%; (b) $RuBnCl_2(PCy_3)_2$, C_6H_6 (4 mM), 22 h, 34% *E*-isomer and 7% *Z*-isomer; (c) TFA, CH_2Cl_2, 0 °C, 4 h, sat. aq $NaHCO_3$ solution, 68%.

using standard EDCI−HOBt [EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, HOBt = 1-hydroxybenzotriazole] conditions in DMF at room temperature, to afford olefin metathesis precursor **9** in good yield (Scheme 1). Surprisingly, the RCM reaction[21] of the bis-olefin **9** which was promoted with the Grubbs ruthenium-based catalyst (∼0.7 mol % $RuBnCl_2(PCy_3)_2$) in benzene (0.004 M) at 25 °C (22 h) afforded predominantly the *E*-isomeric macrolactam **10**, along with only a minor amount of the *Z*-olefin **11** in nearly a 5:1 ratio. Subsequent deprotection of the silyl ether of **10** with trifluoroacetic acid in CH_2Cl_2 at 0 °C (4 h) followed by a sodium bicarbonate workup gave the *E*-Epo C-lactam **12**.

While this study demonstrated that the critical RCM reaction was operative, the observed product ratio was unsatisfactory. Based on the poor intrinsic biological activities[19] of the unnatural *E*-like geometric isomers of Epo C and Epo A, and the poor activity observed for *E*-Epo C-lactam **12** (vide infra), it was imperative that we discover a reliable method to access the more desirable *Z*-stereochemistry.

From these preliminary results, it was unlikely that the lengthy RCM approach would provide sufficient quantities of material needed to sustain a significant medicinal chemistry effort, even if conditions could be found that would reverse the stereochemical outcome or allow ring closure of more highly substituted olefins as required for Epo B/D analogues. Although the reaction conditions and alternative catalysts such as the molybdenum-based Schrock catalyst[22] were not examined in detail, it was recognized even in the epothilone literature[6−8a,b,e] that the stereochemical outcome of the RCM process is highly substrate dependent. These discouraging results combined with exciting

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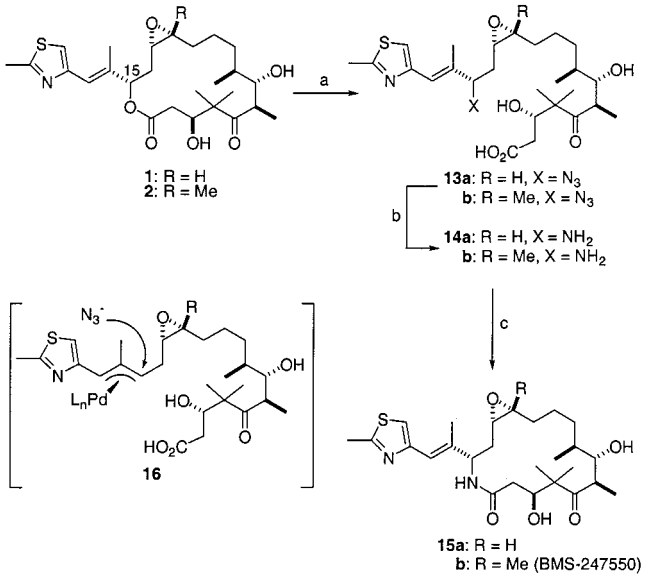
Scheme 2[a]



[a] (a) $Pd(PPh_3)_4$, 10 mol %, NaN_3, degassed THF−H_2O, 45 °C, 1 h, 65−70%; (b) PPh_3, THF, 45 °C for 14 h, then 28% NH_4OH, H_2O, 45 °C for 4 h, or H_2, EtOH, PtO_2, 50 wt %, 10 h, then an additional 25 wt % of PtO_2, 10 h, or PMe_3, THF−H_2O, 25 °C, 2 h, 53−89%; (c) DPPA, $NaHCO_3$, DMF (2.5 mM), 4 °C, 24 h or EDCl, HOBt, MeCN (0.03 M), 25−65%.

developments which had surfaced from our semisynthetic efforts starting with natural product, led us to abandon the total synthesis efforts toward these important lactam derivatives.

Semisynthesis Approach. With access to the natural products, we set out to develop a general strategy to directly convert the lactone to a lactam. Careful analysis of the epothilone structure led to the critical observation that the lactone is actually an allylic ester and may be susceptible to palladium-catalyzed ring-opening to afford an intermediate π-allylpalladium complex (cf. **16**, Scheme 2).[23] This latent species could then be trapped in situ with a "soft" external nucleophile, such as azide to form an allylic azide. If successful, this sequence of events would provide the nitrogen atom necessary at C15 and a readily available coupling partner (carboxylic acid) at C1. Subsequent reduction of the azide followed by macrocyclization would provide the epothilone-lactams. One concern of this strategy, however, was the regio- and stereochemical outcome of the Pd(0)-catalyzed nucleophilic substitution reaction involving a macrolactone substrate, since there was no precedent.

Certainly, the extensively studied palladium-catalyzed substitution of allylic acetates has become an important transformation in organic synthesis since it typically proceeds with a high degree of both regio- and stereochemical control.[23] Depending on the nature of the nucleophile, either overall retention or inversion of configuration is observed for this process. Surprisingly, there have been relatively few examples of palladium-catalyzed substitution reactions of unsaturated lactones in the literature.[24−29] In particular, only two types of systems have

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been explored. In the first case, bridged lactones were shown to undergo ring-opening in the presence of a Pd(0) catalyst to provide intermediate π-allylpalladium complexes which were then rapidly trapped in situ with a variety of nucleophiles.[24−28] Relief of ring strain was clearly the driving force behind these transformations. In the second series, it was shown that unsaturated, fused [3.3.0] bicyclic lactones also undergo Pd(0)-promoted ring-opening to generate disubstituted cyclopentenes.[24b,29] In these examples, the requisite π-allylpalladium intermediates possess an internal nucleophile (carboxylate) which effectively competes with weak, external nucleophiles in an entropy driven process.

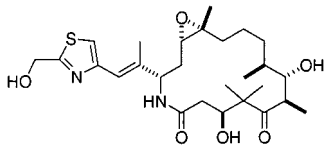
We postulated that a Pd(0)-catalyzed azidation[25a] reaction involving the epothilones should benefit from the entropy associated with a ring-opened macrocycle, forcing the equilibrium toward the formation of an intermediate π-allylpalladium species. We were therefore gratified to discover that when *unprotected* Epo A (**1**) or Epo B (**2**) was treated with a catalytic amount (10 mol %) of tetrakis(triphenylphosphine)palladium-(0) and sodium azide in degassed THF−H_2O at 45 °C (1 h) the azide acids **13a/b** were obtained as single diastereomers in good yield (Scheme 2).

Mechanistically, this reaction can be considered to proceed via *anti*-attack of palladium(0) on the allylic lactone to form an initial π-allylpalladium complex **16**. Subsequent attack by azide at C15, from the opposite face of the palladium/ligands, provides **13** with the desired regio- and stereochemistry (vide infra). In effect, net retention of configuration at C15 was observed. Surprisingly, all of the potentially labile functionality found in the epothilone core, including the epoxide, remained intact. Although not extensively investigated, use of other nucleophiles such as benzylamine resulted in lower yields (∼18%), while methylamine only provided recovered starting material.

Following our success in this critical step, we turned our attention to completing the synthesis of the lactam analogues of the epothilones. The initial reactions were carried out exclusively on the Epo B series. Attempts to chemoselectively reduce the azide acid **13b** to amino acid **14b** via a Staudinger reaction were problematic. Treatment of azide **13b** with triphenylphosphine (2 equiv) in a THF−H_2O (10:1) mixture at 45 °C (14 h) gave the intermediate iminophosphorane as the major product along with a small amount of the desired amino acid **14b**. Addition of ammonium hydroxide to the reaction mixture was required to complete the hydrolysis of this unusually stable intermediate. Alternatively, reduction was accomplished in the absence of base using polymer supported triphenylphosphine or using hydrogenation conditions with stoichiometric amounts of Adams' catalyst (PtO_2). In either case, prolonged reaction times were required. Finally, after further optimization it was found that the highly reactive trimethylphosphine, in the absence of any base, cleanly reduced the azide after a 1−2 h exposure at room temperature.

Several sets of reaction conditions were examined for the final macrolactamization[30] step in the synthesis of Epo B-lactam **15b**. Initially, diphenylphosphoryl azide (DPPA, 4 equiv) and solid sodium bicarbonate (8 equiv) in degassed DMF (2.5 mM) at 0 °C (24 h) were used to promote the macrocyclization of amino acid **14b** to provide the desired lactam **15b**. An X-ray crystal structure of the bis-triethylsilyl ether derivative of lactam **15b** allowed for unambiguous structural assignment, including confirmation of the regio- and C15-stereochemistry which was obtained in the initial Pd(0)-catalyzed reactions (vide supra).[31] In general, the solvents (DMF, CH_2Cl_2, or toluene), reaction time (14−36 h), temperature (−10−25 °C), and concentration (0.05−0.5 mM) had varying effects on the overall yield of the macrocyclization step which never exceeded 40%. At this point we examined alternative coupling reagents which were known to effect macrolactamization reactions,[30,32] such as PyBroP [bromo tripyrrolidinophosphonium hexafluorophosphate], BOP-Cl [bis(2-oxo-3-oxazolidinyl)phosphinic chloride], and HATU [*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate]. In the attempts with PyBrop (PhMe, 25−80 °C), BOP-Cl (CH_2Cl_2, 0−25 °C) or HATU (MeCN−DMF, 25 °C), significant decomposition occurred along with the recovery of minor amounts of starting material or lactam **15b**. However, when amino acid **14b** was treated with EDCI and HOBt or HOAt [1-hydroxy-7-azabenzotriazole] in acetonitrile (0.03 M) at 25 °C (2 h), reproducible yields (∼65%) of the desired macrolactam were obtained.

Epo F-lactam **17** was prepared in the same stepwise fashion as outlined above beginning with readily available epothilone F.[13a] Analogously, *unprotected* Epo F underwent the Pd(0)-catalyzed azidation to afford the intermediate azide acid, albeit in much lower yields than in the cases of epothilones **1** and **2**. Macrolactamization of the subsequent amino acid furnished the desired lactam **17**.



17

We continued to optimize and scale up each of the three steps in the semisynthesis of lactam analogues **15a/b** in order to provide sufficient quantities for biological testing. During this time it became apparent that all of the reagents involved in the sequence were completely compatible with each other. Thus, in an unconventional approach, all three steps were carried out sequentially in a single reaction vessel and without isolation of intermediates (Scheme 3). Remarkably, Epo A and Epo B were converted to the corresponding lactams **15a** and **15b** in acceptable (20−25%) overall yields. In addition, the entire process, including purification, could be accomplished in 1 day.

One obvious issue that needed to be addressed was whether global protection of the molecule would improve the yields in

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(26) Tsuda, T.; Okada, M.; Nishi, S.; Seagusa, T. *J. Org. Chem.* **1986**, *51*, 421.

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(28) (a) Byström, S. E.; Aslanian, R.; Bäckvall, J.-E. *Tetrahedron Lett.* **1985**, *26*, 1749. (b) Genet, J. P.; Balabane, M.; Bäckvall, J.-E.; Byström, S. E. *Tetrahedron Lett.* **1983**, *24*, 2745.

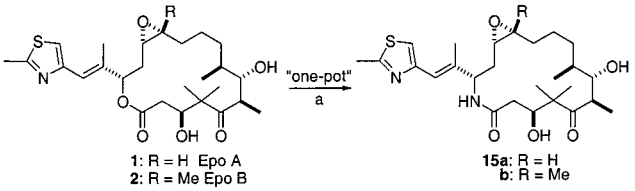
(29) (a) Aggarwal, V. K.; Monteiro, N.; Tarver, G. J.; McCague, R. *J. Org. Chem.* **1997**, *62*, 4665. (b) Aggarwal, V. K.; Monteiro, N. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2531. (c) Aggarwal, V. K.; Monteiro, N.; Tarver, G. J.; Lindell, S. D. *J. Org. Chem.* **1996**, *61*, 1192.

(30) For a comprehensive review on macrolactamization reactions, see: Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243.

(31) Crystallographic data (excluding structure factors) for the compound have been deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication No. CCDC 147158. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: (+44)1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

(32) Trost, B. M.; Ceschi, M. A.; König, B. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1486.

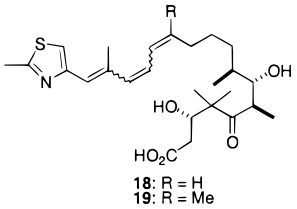
Scheme 3[a]



[a] (a) $Pd(PPh_3)_4$, 10 mol %, NaN_3, degassed THF−H_2O, 45 °C, 20 min, then PMe_3, 1−2 h, then MeCN−DMF (20:1), EDCl−HOBt, 25 °C, 4−12 h, 20−25%.

each reaction. Although this strategy would potentially add two steps to the route, we set out to compare the efficiency of the two sequences. Both hydroxyl groups of Epo B were protected as either the trimethylsilyl or triethylsilyl ethers (10 equiv of trimethylsilyl or triethylsilyl chloride, 15 equiv of *i*-Pr_2EtN, 0.1 equiv of DMAP, CH_2Cl_2, 40 °C, 18 h), and these compounds were subjected to the "one-pot" sequence. In each case only minor improvement in the overall yield (25−35%) was observed, and thus further work using fully protected epothilone derivatives was suspended.

During the attempted semisynthesis of the lactam analogues of Epo C (**3**) and Epo D (**4**), it became clear that there were certain limitations associated with the Pd(0)-catalyzed nucleophilic substitution reaction. Unfortunately, lactones **3** and **4** failed to provide the corresponding azide acids when subjected to our standard reaction conditions. In each case, a complicated mixture of olefin isomers of trienes **18** and **19** was obtained as indicated by ^{1}H NMR.

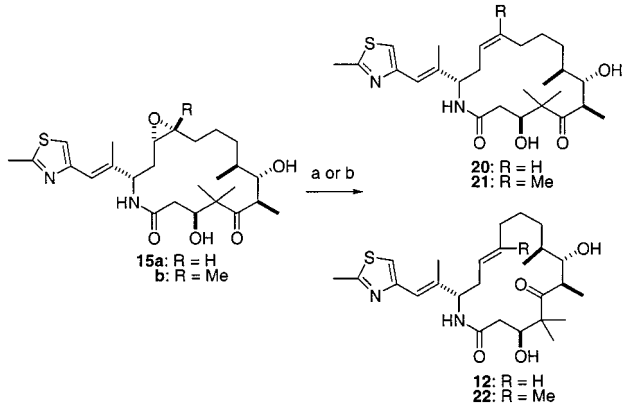


To circumvent this problem, we utilized the stereoselective deoxygenation chemistry recently disclosed by this laboratory to prepare Epo C and Epo D directly from Epo A and B, respectively.[16] Thus, *unprotected* Epo A-lactam **15a** was treated with the titanocene complex[33] derived from $TiCp_2Cl_2$ and magnesium to furnish *Z*-Epo C-lactam **20** along with a small amount of the *E*-isomer **12** (Scheme 4). Interestingly, the identical reaction conditions with unprotected Epo B-lactam **15b** led predominantly to decomposition products. Utilizing conditions reported by Sharpless (WCl_6/*n*-BuLi)[34] for less complex systems, Epo B-lactam **15b** was converted to the lactams *Z*-**21** and *E*-**22** in good yields as a separable 5:1 mixture of geometric isomers. The C12−C13 relative stereochemisty of the major lactam isomers **20** and **21** was determined using 1D and 2D ^{1}H NMR techniques. In the case of lactam **20**, the 2D ^{1}H−^{1}H NOESY NMR spectrum exhibited diagnostic H-12/H-13 and H-11/H-14 NOE cross-peaks, thus indicating that these protons reside on the same side of the olefin (*Z*-relationship). 1D ^{1}H−^{1}H NOE NMR experiments on the Epo D-lactam **21** revealed strong NOE enhancements between H-13 and the C-26 methyl protons (irradiation on either peak provided enhancements). The close proximity of these two groups again establishes the *Z*-configurational assignment.

(33) Schobert, R.; Hoehlein, U. *Synlett* **1990**, *8*, 465.

(34) Sharpless, K. B.; Umbreit, M. A.; Nieh, M. T.; Flood, T. C. *J. Am. Chem. Soc.* **1972**, *94*, 6538.

Scheme 4[a]



[a] (a) R = H: $TiCp_2Cl_2$, Mg(s), THF, 49%, 20:1 ratio of **20** to **12**; (b) R = Me: WCl_6, *n*-BuLi, THF, 56%, 5:1 ratio of **21** to **22**.

Table 1. In Vitro Data for Epothilones A−F and Lactam Analogues

no.	compd	tubulin [a] $EC_{0.01}$ (μM)	HCT-116 [b] IC_{50} (nM)
−	paclitaxel	5.0	2.3
1	Epo A	2.3	3.2
2	Epo B	1.4	0.42
3	*Z*-Epo C	5.0	64
−	*E*-Epo C	21	160
4	*Z*-Epo D	0.80	6.5
−	*E*-Epo D	11	97
5	Epo E	17	6.0
6	Epo F	1.8	0.77
12	*E*-Epo C-lactam	>1100	NT
15a	Epo A-lactam	12	130
15b	Epo B-lactam	3.8	3.6
17	Epo F-lactam	130	120
20	*Z*-Epo C-lactam	110	NT
21	*Z*-Epo D-lactam	5.5	65
22	*E*-Epo D-lactam	>1200	>200

[a] Tubulin polymerization assay performed using the method described in refs 35a and 15b. [b] HCT-116, a human colon carcinoma cell line cytotoxicity assay performed using the method described in ref 35b. NT = not tested.

Biological Testing Results. The in vitro results for the lactam analogues and the natural epothilones[19] are listed in Table 1. In general, the tubulin-polymerizing and cytotoxic potencies of the lactam analogues were clearly inferior to their natural epothilone counterparts. One very important exception to this trend, however, was lactam **15b** (BMS-247550). This semisynthetic analogue of Epo B was in fact comparable to paclitaxel in both in vitro assays. After extensive in vitro testing in a number of different cell lines, BMS-247550 was found to possess a superior biological profile to that of paclitaxel.[19] A second analogue worth highlighting is lactam **21**. This Epo D-lactam performed well in the tubulin polymerization assay, but was 10- and 30-fold less potent than Epo D and paclitaxel, respectively in cell culture.

When evaluated in vivo, BMS-247550 exhibited a broad spectrum of antitumor activity against both paclitaxel-sensitive and paclitaxel-resistant human tumor xenografts grown in athymic mice.[36] In addition, BMS-247550 was found to be superior to the naturally occurring epothilones A−F in preclinical antitumor models. It is postulated that this may be partially attributed to the stabilization of the lactone functionality toward metabolic cleavage. Due to its impressive in vivo profile in several animal models, BMS-247550 was chosen for drug development and clinical investigation.

Summary

After carefully evaluating a total synthesis approach, several metabolically stable lactam analogues of the epothilones were prepared via a semisynthesis route. Highlighted in this novel strategy is a regio- and stereoselective π-allylpalladium nucleophilic substitution reaction which provided a pivotal ring-opened intermediate with a nitrogen substituent at C15 of the epothilone core. Chemoselective reduction of the intermediate azide followed by macrolactamization of the resulting amino acid afforded the desired lactams. In the case of the parent epothilones **1** and **2**, the entire sequence was telescoped into a three step, "one-pot" process providing an efficient and practical approach (20−25% yields) to the corresponding lactams. Further optimization of this sequence is underway and will be reported in due course. To prepare the macrolactams of Epo C and Epo D, it was necessary to employ two highly effective deoxygenation methods to convert the epoxides of **15a/b** to the corresponding olefins. The epothilone-lactams were evaluated in vitro, and one analogue, **15b** (BMS-247550) compared favorably to paclitaxel and Epo B.

Taxanes (e.g., paclitaxel and docetaxel) are among the most effective chemotherapeutic agents used for the treatment of ovarian, breast, and lung cancers. However, many patients develop resistance to taxane therapy, and several additional cancers (e.g., colon and prostate) are refractory to taxanes. BMS-247550 represents a novel "non-taxane" that has the potential to extend the clinical activity of the tubulin-polymerizing class of anticancer agents beyond that demonstrated with taxanes.

Experimental Section

(3*S*,6*R*,7*S*,8*S*)-*N*-(*S*)-[1-(2-Methyl-4-thiazolyl)-2-methyl-1(*E*)-5-hexadien-3-yl]-3-*tert*-butyldimethylsilyloxy-4,4,6,8-tetramethyl-7-hydroxy-5-oxo-12-trideceneamide (9). A solution of amine **7** (88 mg, 0.42 mmol, cf. Supporting Information) in DMF (1.3 mL) at 0 °C was treated sequentially with acid **8**[7a] (0.15 g, 0.35 mmol), 1-hydroxybenzotriazole (49 mg, 0.36 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.10 g, 0.52 mmol). The reaction mixture was gradually warmed to 25 °C, stirred for 15 h, and diluted with H_2O (3 mL). The mixture was extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with aqueous 5% HCl (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and saturated aqueous NaCl (10 mL) and were dried (Na_2SO_4) and concentrated in vacuo. Flash chromatography (SiO_2, 1.5 × 20 cm, 2.5% MeOH−$CHCl_3$) afforded **9** (0.17 g, 77%) as a white foam: $[\alpha]^{22}_D$ −54.1 (*c* 9.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90 (s, 1 H), 6.41 (s, 1 H), 5.83−5.68 (m, 3 H), 5.13−5.07 (m, 2 H), 4.99−4.90 (m, 2 H), 4.52 (dd, 1 H, J = 14.3, 7.1 Hz), 4.41 (dd, 1 H, J = 6.0, 3.8 Hz), 3.39 (s, 1 H), 3.33−3.29 (m, 2 H), 2.69 (s, 3 H), 2.43−2.38 (m, 2 H), 2.33 (dd, 1 H, J = 15.9, 3.3 Hz), 2.12 (dd, 1 H, J = 15.9, 6.0 Hz), 2.06 (s, 3 H), 2.05−1.96 (m, 2 H), 1.76−1.70 (m, 1 H), 1.55−1.42 (m, 2 H), 1.35−1.24 (m, 1 H), 1.21 (s, 3 H), 1.10 (s, 3 H), 1.02 (d, 3 H, J = 6.6 Hz), 0.89 (s, 9 H), 0.83 (d, 3 H, J = 6.6 Hz), 0.11 (s, 3 H), 0.071 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 222.6, 169.8, 164.4, 152.7, 139.1, 138.5, 134.0, 119.4, 118.1, 115.7, 114.2, 74.6, 73.8, 55.4, 54.2, 42.3, 41.5, 37.7, 35.5, 34.2, 32.4, 26.1 (3C), 26.0, 22.6, 19.7, 19.2, 18.1, 16.1, 15.3, 9.6, −4.2, −4.8; HRMS (ESI$^+$) m/z (M^+ + H) calcd for $C_{34}H_{58}N_2O_4SSi$: 619.3965, found: 619.3969.

[4*S*-[4*R,7*S**,8*R**,9*R**,15*R**(*E*)]]-4-*tert*-Butyldimethylsilyloxy-8-hydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)-ethenyl]-1-aza-13(*E*)-cyclohexadecene-2,6-dione (10).** A solution of **9** (17 mg, 27 μmol) in degassed benzene (8.0 mL) was treated with Grubbs' catalyst ([bis(tricyclohexyl-phosphine)benzylidine ruthenium dichloride, Strem Chemicals, 11 mg, 14 μmol) under Ar. The reaction mixture was stirred at 25 °C for 15 h and treated again with an additional portion of catalyst (5.0 mg, 4.5 μmol). After 7 h, the benzene was removed in vacuo, and the black viscous residue was passed through a pad of silica gel (1.0 × 3 cm) eluting with Et_2O (25 mL). The eluent was concentrated in vacuo to afford a separable 5:1 (*E*/*Z*) mixture of geometric isomers. PTLC (SiO_2, 1 mm plate, 2 elutions with a 1:1:1 solution of hexane−toluene−ethyl acetate) afforded the *E*-isomer **10** (5.1 mg, 34%) along with the corresponding *Z*-isomer (1.0 mg, 6.7%). For **10**: LRMS (ESI$^+$) 591.4 (M^+ + H). For the *Z*-isomer **11**: LRMS (ESI$^+$) 591.2 (M^+ + H); (ESI$^-$) 589.3 (M − H)$^-$.

[4*S*-[4*R,7*S**,8*R**,9*R**,15*R**(*E*)]]-4,8-Dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-aza-13(*E*)-cyclohexadecene-2,6-dione (12).** To a 1 dram vial charged with **10** (2.3 mg, 3.9 μmol) in CH_2Cl_2 (0.4 mL) at 0 °C was added trifluoroacetic acid (0.1 mL). The reaction mixture was sealed under a blanket of Ar and stirred at 0 °C. After 4 h, the volatiles were removed under a constant stream of Ar at 0 °C. Saturated aqueous $NaHCO_3$ (1 mL) and EtOAc (1 mL) were added to the residue, and the two layers were separated. The aqueous phase was extracted with EtOAc (4 × 1 mL), and the combined EtOAc layers were dried (Na_2SO_4) and concentrated in vacuo. PTLC (SiO_2, 20 × 10 × 0.025 cm, eluting with 5% MeOH−$CHCl_3$) afforded **12** (1.3 mg, 68%) as a white film: $[\alpha]^{22}_D$ −93.5 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (s, 1 H), 6.44 (s, 1 H), 6.07 (d, 1 H, NH, J = 7.1 Hz), 5.54−5.47 (m, 1 H), 5.38−5.31 (m, 1 H), 4.48−4.43 (m, 1 H), 4.22 (d, 1 H, J = 11.5 Hz), 3.67−3.58 (m, 2 H), 3.29−3.22 (m, 1 H), 3.21 (br d, 1 H, OH, J = 3.8 Hz), 2.71 (s, 3 H), 2.44−2.29 (m, 3 H), 2.28−2.19 (m, 1 H), 2.17 (dd, 1 H, J = 14.5, 1.7 Hz), 2.08 (s, 3 H), 1.95−1.83 (m, 1 H), 1.71−1.62 (m, 2 H), 1.51−1.38 (m, 1 H), 1.32 (s, 3 H), 1.27−1.18 (m, 2 H), 1.17 (d, 3 H, J = 6.8 Hz), 1.06 (s, 3 H), 1.01 (d, 3 H, J = 6.8 Hz); HRMS (ESI$^+$) m/z (M^+ + H) calcd for $C_{26}H_{40}N_2O_4S$: 477.2709, found: 477.2798.

(3*S*,6*R*,7*S*,8*S*,12*R*,13*S*,15*S*)-15-Azido-12,13-epoxy-4,4,6,8,16-pentamethyl-3,7-dihydroxy-17-(2-methyl-4-thiazolyl)-5-oxo-16(*E*)-heptadecenoic acid (13a). A solution of epothilone A (**1**, 2.48 g, 5.02 mmol) and sodium azide (0.489 g, 7.53 mmol) in a degassed THF−H_2O mixture (10:1, 55 mL) was treated with a catalytic amount (0.580 g, 0.502 mmol) of tetrakis(triphenylphosphine) palladium(0) under Ar. The suspension was warmed to 45 °C for 1 h, and the resulting bright yellow, homogeneous solution was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 100 mL). The organic extracts were washed with saturated aqueous NaCl (150 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 4.5 × 30 cm, 95:5 $CHCl_3$−MeOH to 95:5.0:0.5 $CHCl_3$−MeOH−AcOH gradient elution) to afford **13a** (1.84 g, 69%) as a colorless oil: $[\alpha]^{22}_D$ −60.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (s, 1 H), 6.60 (s, 1 H), 4.28−4.24 (m, 1 H), 4.18 (dd, 1 H, J = 8.9, 5.1 Hz), 3.40 (d, 1 H, J = 8.8 Hz), 3.28−3.24 (m, 1 H), 2.88 (dd, 1H, J = 7.8, 3.9 Hz), 2.73 (s, 3 H), 2.48−2.36 (m, 2 H), 2.06 (s, 3 H), 1.92−1.88 (m, 1 H), 1.73−1.61 (m, 1 H), 1.61−1.33 (a series of multiplets, 7 H), 1.31 (s, 3 H), 1.21 (s, 3 H), 1.12 (s, 3 H), 1.06 (d, 3 H, J = 6.8 Hz), 0.86 (d, 3 H, J = 6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 221.9, 175.8, 165.4, 151.2, 136.9, 121.8, 116.5, 74.6, 72.1, 68.3, 57.3, 54.1, 51.9, 40.8, 36.2, 35.2, 32.3, 31.5, 28.1, 23.5, 21.1, 18.6 (2C), 15.3, 14.3, 9.9; HRMS (ESI$^+$) m/z (M^+ + H) calcd for $C_{26}H_{40}N_4O_6S$: 537.2669, found: 537.2745.

(35) (a) Tubulin polymerization assays were performed as described in Swindell, C. S.; Krauss, N. E.; Horwitz, S. B.; Ringel, I. *J. Med. Chem.* **1991**, *34*, 1176. Effective concentration ($EC_{0.01}$) is defined as the interpolated concentration capable of inducing an initial slope of 0.01 OD/min rate and is calculated using the formula: $EC_{0.01}$ = concentration/slope. $EC_{0.01}$ values are expressed as the mean with standard deviation obtained from 3 different concentrations. (b) Cytotoxicity was assessed in HCT-116 human colon carcinoma cells by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium, inner salt) assay as reported in Riss, T. L.; Moravec, R. A. *Mol. Biol. Cell* **1992**, *3* (Suppl.), 184a (Abstract No. 1067).

(36) Complete details of the preclinical efficacy data for BMS-247550 will be published elsewhere. Preliminary preclinical efficacy data was recently presented: Lee, F. Y. F.; Vite, G. D.; Borzilleri, R. M.; Arico, M. A.; Clark, J. L.; Fager, K. L.; Kan, D.; Kennedy, K. A.; Kim, A. S.-H.; Smykla, R. A.; Wen, M.-L.; Kramer, R. A. BMS-247550: An Epothilone Analog Possessing Potent Activity Against Paclitaxel-Sensitive and -Resistant Human Tumors. *Book of Abstracts*, 91st Annual Meeting of the American Association for Cancer Research, San Francisco, CA, April 1−5, 2000; American Association for Cancer Research: Philadephia, PA, 2000; LB-34.

(3*S*,6*R*,7*S*,8*S*,12*R*,13*S*,15*S*)-15-Azido-12,13-epoxy-4,4,6,8,12,16-hexamethyl-3,7-dihydroxy-17-(2-methyl-4-thiazolyl)-5-oxo-16(*E*)-heptadecenoic acid (13b). By using epothilone B(**2**) as the starting material and following the same procedure outlined above for epothilone A, **13b** (1.9 g, 70%) was obtained as a colorless oil: $[\alpha]^{22}_D$ −66.8 (*c* 1.4, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (s, 1 H), 6.61 (s, 1 H), 4.25 (dd, 1 H, J = 7.0, 5.5 Hz), 4.19 (dd, 1 H, J = 9.4, 5.0 Hz), 3.43 (dd, 1 H, J = 8.4, 2.0 Hz), 3.28−3.24 (m, 1 H), 2.86 (dd, 1 H, J = 7.6, 4.4 Hz), 2.73 (s, 3 H), 2.45−2.40 (m, 2 H), 2.06 (s, 3 H), 1.96−1.88 (m, 1 H), 1.70−1.61 (m, 1 H), 1.61−1.33 (a series of multiplets, 7 H), 1.31 (s, 3 H), 1.25 (s, 3 H), 1.13 (s, 3 H), 1.08 (d, 3 H, J = 6.9 Hz), 0.88 (d, 3 H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 222.2, 176.2, 165.5, 151.5, 137.1, 121.9, 116.7, 74.8, 72.3, 68.5, 61.6, 52.1, 41.0, 36.4, 35.5, 33.2, 32.8, 32.4, 22.5, 22.1, 21.2, 18.9, 18.8, 18.7, 15.5, 14.5, 10.2; HRMS (ESI$^+$) *m*/*z* (M^+ + H) calcd for $C_{27}H_{42}N_4O_6S$: 551.2903, found: 551.2916.

(3*S*,6*R*,7*S*,8*S*,12*R*,13*S*,15*S*)-15-Amino-12,13-epoxy-4,4,6,8,16-pentamethyl-3,7-dihydroxy-17-(2-methyl-4-thiazolyl)-5-oxo-16(*E*)-heptadecenoic acid (14a). To a 100 mL round-bottom flask charged with **13a** (0.11 g, 0.20 mmol) and PtO_2 (55 mg, 50 wt %) was added absolute EtOH (30 mL) under Ar. The resulting black mixture was stirred under one atmosphere of H_2 for 10 h, after which time the system was purged with N_2, and an additional portion of PtO_2 (55 mg, 50 wt %) was added. Once again the reaction mixture was stirred under a blanket of H_2 for 10 h. The system was then purged with N_2, and the reaction mixture was filtered through a Celite pad eluting with CH_2Cl_2 (3 × 75 mL). The solvents were removed in vacuo, and the residue was purified by flash chromatography (SiO_2, 1.5 × 10 cm, 95:5.0:0.5 to 90:10:1.0 $CHCl_3$−MeOH−AcOH gradient elution) to afford **14a** (92 mg, 89%) as a foam: $[\alpha]^{22}_D$ −19.0 (*c* 7.7, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.34 (s, 1 H), 6.65 (s, 1 H), 4.17 (dd, 1 H, J = 9.9, 3.0 Hz), 4.02 (dd, 1 H, J = 9.7, 5.1 Hz), 3.58 (dd, 1 H, J = 7.2, 4.0 Hz), 3.33−3.30 (m, 1 H), 2.95−2.89 (m, 2 H), 2.70 (s, 3 H), 2.29 (dd, 1 H, J = 15.2, 3.0 Hz), 2.21−2.10 (m, 2 H), 1.96 (s, 3 H), 1.61−1.05 (a series of multiplets, 8 H), 1.25 (s, 3 H), 1.12 (d, 3 H, J = 6.8 Hz), 1.03 (s, 3 H), 0.95 (d, 3 H, J = 6.8 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 218.8, 175.3, 164.1, 152.0, 137.6, 121.0, 117.5, 75.5, 72.0, 56.8, 55.5, 53.1, 51.7, 43.6, 37.8, 35.1, 30.8, 29.2, 28.0, 23.4, 21.2, 20.9, 18.7, 17.0, 14.6, 13.6; HRMS (ESI$^+$) *m*/*z* (M^+ + H) calcd for $C_{26}H_{42}N_2O_6S$: 511.2842, found: 511.2852.

(3*S*,6*R*,7*S*,8*S*,12*R*,13*S*,15*S*)-15-Amino-12,13-epoxy-4,4,6,8,12,16-hexamethyl-3,7-dihydroxy-17-(2-methyl-4-thiazolyl)-5-oxo-16(*E*)-heptadecenoic acid (14b). A solution of **13b** (0.23 g, 0.42 mmol) in THF (4.0 mL) was treated with H_2O (23 μL, 1.25 mmol) and polymer supported triphenylphosphine (Aldrich, polystyrene cross-linked with 2% DVB, 0.28 g, 0.84 mmol) at 25 °C. The resulting suspension was stirred at 25 °C under Ar (32 h), filtered through a Celite pad, and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2, 1.5 × 10 cm, 95:5.0:0.5 to 90:10:1.0 $CHCl_3$−MeOH−AcOH gradient elution) to afford **14b** (96 mg, 44%) as a colorless oil, which was used directly in the macrolactamization reaction: HRMS (ESI$^+$) *m*/*z* (M^+ + H) calcd for $C_{27}H_{44}N_2O_6S$: 525.2998, found: 525.3000.

Alternatively, to a 100 mL round-bottom flask charged with **13b** (1.9 g, 3.5 mmol) and PtO_2 (0.95 g, 50 wt %) was added absolute EtOH (30 mL) under Ar. The resulting black mixture was stirred under one atmosphere of H_2 for 10 h, after which time the system was purged with N_2, and an additional portion of PtO_2 (0.48 g, 25 wt %) was added. Once again the reaction mixture was stirred under a blanket of H_2 for 10 h. The system was then purged with N_2, and the reaction mixture was filtered through a Celite pad eluting with CH_2Cl_2 (3 × 75 mL). The solvents were removed in vacuo, and the residue was purified as described above to afford **14b** (0.95 g, 53%).

Alternatively, a solution of **13b** (20 mg, 36 μmol) in THF (0.4 mL) was treated with triphenylphosphine (19 mg, 73 μmol) under Ar. The reaction mixture was warmed to 45 °C, stirred for 14 h, and cooled to 25 °C. The resulting iminophosphorane was treated with ammonium hydroxide solution (28%, 0.1 mL), and once again the reaction mixture was warmed to 45 °C. After 4 h, the volatiles were removed in vacuo, and the residue was purified as described above to afford **14b** (13 mg, 70%).

Alternatively, a solution of **13b** (29 mg, 53 μmol) in THF−H_2O (0.5 mL) was treated with trimethylphosphine (1.0 M in toluene, 0.11 mL, 0.11 mmol) under Ar. The reaction mixture was stirred at 25 °C (2 h) and concentrated in vacuo, and the residue was purified as described above to afford **14b** (20 mg, 71%).

[1*S*-[1*R,3*R**(*E*),7*R**,10*S**,11*R**,12*R**,16*S**]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (15b)**. A solution of **14b** (0.95 g, 1.8 mmol) in degassed DMF (700 mL) was treated with solid $NaHCO_3$ (1.2 g, 14 mmol) and diphenylphosphoryl azide (1.6 mL, 7.2 mmol) at 0 °C under Ar. The resulting suspension was stirred at 4 °C for 24 h, diluted with cold phosphate buffer (750 mL, pH = 7) at 0 °C, and extracted with EtOAc (5 × 500 mL). The organic extracts were washed with cold 10% aqueous LiCl (750 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was first purified by flash chromatography (SiO_2, 2.0 × 10 cm, 2−5% MeOH−$CHCl_3$ gradient elution) and then re-purified using a Chromatotron (2 mm SiO_2 GF rotor, 2−5% MeOH−$CHCl_3$ gradient elution) to afford **15b** (0.39 g, 43%) as a colorless oil: $[\alpha]^{22}_D$ −40.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (s, 1 H), 6.71 (d, 1 H, NH, J = 8.1 Hz), 6.56 (s, 1 H), 4.69−4.62 (m, 1 H), 4.28 (d, 1 H, J = 6.6 Hz), 4.06−3.99 (m, 1 H), 3.86−3.81 (m, 1 H), 3.38−3.34 (m, 1 H), 2.82 (dd, 1 H, J = 7.6, 5.7 Hz), 2.71 (s, 3 H), 2.58 (br s, 1 H, OH), 2.43 (dd, 1 H, J = 8.7, 14.5 Hz), 2.34 (dd, 1 H, J = 3.0, 14.5 Hz), 2.14 (s, 3 H), 2.05−1.92 (m, 2 H), 1.82−1.74 (m, 2 H), 1.69−1.40 (m, 5 H), 1.35 (s, 3 H), 1.28 (s, 3 H), 1.18 (d, 3 H, J = 6.8 Hz), 1.14 (s, 3 H), 1.00 (d, 3 H, J = 6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 220.8, 170.9, 165.2, 152.7, 138.1, 119.5, 116.5, 75.4, 73.9, 61.8, 61.4, 54.7, 52.7, 44.2, 40.4, 38.0, 32.1, 31.8, 31.2, 24.0, 23.3, 21.9, 21.5, 19.5, 17.4, 17.3, 15.0; HRMS (FAB) *m*/*z* 507.2892 (M^+ + H) calcd for $C_{27}H_{42}N_2O_5S$, found: 507.2875.

"One-Pot" Procedure: A suspension of epothilone B (**2**, 5.06 g, 9.97 mmol) and sodium azide (0.777 g, 12.0 mmol) in a THF−H_2O mixture (5:1, 96 mL) was degassed for 20 min with nitrogen and treated with a catalytic amount of tetrakis(triphenylphosphine) palladium(0) (Lancaster, 1.2 g, 0.997 mmol) under Ar. The reaction mixture was warmed to 45 °C for 20 min and cooled to 25 °C. The resulting bright yellow, homogeneous solution was directly treated with a 1.0 M solution of trimethylphosphine in THF (24.9 mL, 24.9 mmol) at 25 °C, and the reaction mixture was stirred for 2 h at ambient temperature.[37]

The amino acid mixture was then diluted[38] with MeCN−DMF (20:1, 450 mL), cooled to 0 °C and treated with HOBt (1.35 g, 9.97 mmol) followed by EDCI (4.78 g, 24.9 mmol). The reaction mixture was warmed to 25 °C, stirred for 12 h, and extracted with EtOAc (4 × 200 mL). The organic extracts were washed with H_2O (400 mL), saturated aqueous $NaHCO_3$ (400 mL), and saturated aqueous NaCl (400 mL) and were dried (Na_2SO_4), and concentrated in vacuo. The residue was initially purified by flash chromatography (SiO_2, 5.0 × 25 cm, 2% MeOH−$CHCl_3$) and then further purified by HPLC[39] to afford **15b** (1.2 g, 23% overall yield, >95% purity as judged by HPLC:[40] retention time = 2.01 min) as a white lyopholizate which possessed the same characterization data as listed above.

[1*S*-[1*R,3*R**(*E*),7*R**,10*S**,11*R**,12*R**,16*S**]]-7,11-Dihydroxy-8,8,10,12-tetramethyl-3-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (15a).** By using epothilone A as the starting material and following the "one-pot"

(37) The reaction was monitored by HPLC: YMC S5 ODS (4.6 × 50 mm) column, 0 to 100% **B**, 4 min gradient with 1 min hold time, 4 mL/min flow rate; **A** Solvent: 95% H_2O−5% MeCN−0.1% AcOH, **B** Solvent: 95% MeCN−5% H_2O−0.1% AcOH. The retention times were as follows: For azide acid intermediate **13b**, R_t = 3.35 min; For amino acid intermediate **14b**, R_t = 2.18 min.

(38) Alternatively, at this point the reaction mixture could be concentrated in vacuo and the residual H_2O removed by azeotroping with toluene (the yield remained the same; however, the reaction time was reduced from 12 to 4 h).

(39) HPLC conditions: YMC S-15 ODS (50 × 500 mm) column, 40 to 100% **B** gradient (40 min), 50 mL/min flow rate; **A** Solvent: 95% H_2O−5% MeCN, **B** Solvent: 95% MeCN−5% H_2O. The appropriate fractions were concentrated in vacuo, and the residue was lyophilized from aqueous acetonitrile.

(40) HPLC conditions: YMC S5 ODS (4.6 × 50 mm) column, 20 to 100% **B**, 4 min gradient with 1 min hold time, 4 mL/min flow rate; **A** Solvent: 95% H_2O−5% MeCN, **B** Solvent: 95% MeCN−5% H_2O.

procedure outlined above, **15a** was obtained as a white lyopholizate (18−25% yield): $[\alpha]^{22}_D$ −41.4 (*c* 10, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (d, 1 H, NH, J = 8.1 Hz), 6.88 (s, 1 H), 6.45 (s, 1 H), 4.53 (dd, 1 H, J = 12.5, 5.1 Hz), 4.40 (d, 1 H, J = 6.9 Hz), 3.99−3.94 (m, 1 H), 3.77 (d, 1 H, J = 3.1 Hz), 3.26−3.19 (m, 1 H), 2.98−2.93 (m, 1 H), 2.85−2.71 (m, 2 H), 2.62 (s, 3 H), 2.39 (dd, 1 H, J = 15.2, 9.5 Hz), 2.27 (dd, 1 H, J = 15.2, 2.8 Hz), 2.03 (s, 3 H), 2.02−1.95 (m, 1 H), 1.87−1.78 (m, 1 H), 1.63−1.35 (a series of multiplets, 7 H), 1.27 (s, 3 H), 1.09 (d, 3 H, J = 7.0 Hz), 1.05 (s, 3 H), 0.91 (d, 3 H, J = 7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 221.7, 171.4, 165.6, 153.0, 138.8, 120.1, 116.8, 75.4, 74.8, 58.1, 55.3, 54.2, 52.9, 44.7, 40.7, 37.5, 31.9, 31.4, 27.9, 25.1, 22.6, 22.3, 19.9, 17.9, 17.7, 15.0; HRMS (ESI^+) m/z (M^+ + H) calcd for $C_{26}H_{40}N_2O_5S$: 493.2736, found: 493.2746.

[1*S*-[1*R,3*R**(*E*),7*R**,10*S**,11*R**,12*R**,16*S**]]-7,11-Dihydroxy-8,8,10,12,16-pentamethyl-3-[1-methyl-2-(2-hydroxymethyl-4-thiazolyl)ethenyl]-4-aza-17-oxabicyclo[14.1.0]heptadecane-5,9-dione (17).** To a 10 mL round-bottom flask charged with (3*S*,6*R*,7*S*,8*S*,12*R*,13*S*,-15*S*)-15-azido-12,13-epoxy-4,4,6,8,12,16-hexamethyl-3,7-dihydroxy-17-(2-hydroxymethyl-4-thiazolyl)-5-oxo-16(*E*)-heptadecenoic acid (40 mg, 71 μmol, cf. Supporting Information) and PtO_2 (20 mg, 50 wt %) was added absolute EtOH (3 mL) under Ar. The resulting black mixture was stirred under one atmosphere of H_2 for 10 h. The system was then purged with N_2, and the reaction mixture was filtered through a nylon membrane (washing with 25 mL of MeOH). The solvents were removed in vacuo to afford the intermediate amino acid (29 mg, 76%) as a foam, which was sufficiently pure to use in the next step. LCMS (ESI^+): 541.3 (M^+ + H).

A solution of the amino acid (29 mg, 54 μmol) in degassed DMF (21 mL) was treated with solid $NaHCO_3$ (36 mg, 0.43 mmol) and diphenylphosphoryl azide (46 μL, 0.21 mmol) at 0 °C under Ar. The resulting suspension was stirred at 4 °C for 19 h, cooled to −40 °C, diluted with 25 mL of pH 7 phosphate buffer (carefully adding such that the internal temperature remained below −30 °C), and extracted with EtOAc (4 × 10 mL). The organic extracts were washed with cold 10% aqueous LiCl (25 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified using a Chromatotron (1 mm SiO_2 GF rotor, 2−5% MeOH−$CHCl_3$ gradient elution) to afford **17** (9.1 mg, 34%) as a colorless oil: $[\alpha]^{22}_D$ −23 (*c* 0.06, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1 H), 6.94 (d, 1 H, NH, J = 7.6 Hz), 6.58 (s, 1 H), 4.94 (s, 2 H), 4.69−4.65 (m, 1 H), 4.08 (d, 1 H, J = 7.3 Hz), 3.83 (s, 1 H), 3.39−3.34 (m, 1 H), 2.83 (dd, 1 H, J = 7.1, 6.4 Hz), 2.68 (br s, 1 H), 2.45 (dd, 1 H, J = 14.8, 9.4 Hz), 2.34 (dd, 1 H, J = 14.8, 2.5 Hz), 2.14 (s, 3 H), 2.09−1.96 (m, 2 H), 1.77−1.40 (a series of multiplets, 8 H), 1.37 (s, 3 H), 1.31 (s, 3 H), 1.20 (d, 3 H, J = 6.9 Hz), 1.14 (s, 3 H), 1.02 (d, 3 H, J = 6.9 Hz); ^{13}C NMR (125 MHz, acetone-d_6) δ 217.9, 172.9, 170.8, 154.3, 140.3, 119.3, 117.2, 77.5, 71.7, 63.1, 62.6, 61.5, 54.6, 54.2, 45.8, 40.2, 37.4, 34.3, 34.0, 24.5, 22.9, 22.5, 18.9 (2C), 18.6, 17.1, 15.8; HRMS (ESI^+) m/z (M^+ + H) calcd for $C_{27}H_{42}N_2O_6S$: 523.2842, found: 523.2850.

[4*S*-[4*R,7*S**,8*R**,9*R**,15*R**(*E*)]]-4,8-Dihydroxy-5,5,7,9-tetramethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-aza-13(*Z*)-cyclohexadecene-2,6-dione (20).** To a round-bottom flask was added chopped pieces of magnesium turnings (0.12 g, 5.0 mmol). The reaction vessel was flame-dried under vacuum and cooled under Ar. Bis-(cyclopentadienyl)titanium dichloride (1.25 g, 5.0 mmol) was added followed by anhydrous THF (25 mL). The stirring suspension was evacuated with low vacuum, and the reaction flask was back-filled with Ar. The red suspension became dark and eventually turned a homogeneous deep green after 1.5 h with nearly all of the Mg metal being consumed. An aliquot (6.0 mL, 3.0 equiv) of the 0.2 M titanocene reagent in THF was removed from the solution and cooled to −78 °C under Ar. To this solution was added **15a** (40 mg, 81 μmol). The reaction mixture was stirred at −78 °C for 15 min and diluted with EtOAc (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The mixture was extracted with EtOAc (3 × 10 mL), and the organic layers were washed with brine (25 mL) and were dried (Na_2SO_4) and concentrated in vacuo. The residue was first purified by flash chromatography (SiO_2, 1.5 × 20 cm, 0−5% MeOH−$CHCl_3$ gradient elution) to give a separable 20:1 mixture of *Z*-**20** and *E*-**12**. Further purification of the geometric isomers using a Chromatotron (1 mm SiO_2 GF rotor, 0−5% MeOH−$CHCl_3$ gradient elution) afforded **20** (18.4 mg, 47%) as a colorless oil and **12** (1.0 mg, 2.6%). For **20**: $[\alpha]^{22}_D$ −42.4 (*c* 1.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (s, 1 H), 6.49 (s, 1 H), 6.00 (d, 1 H, NH, J = 7.0 Hz), 5.54−5.51 (m, 1 H), 5.42−5.37 (m, 1 H), 4.42−4.36 (m, 1 H), 4.12−4.06 (m, 1 H), 3.76 (br s, 1 H), 3.59 (d, 1 H, J = 5.5 Hz), 3.14 (q, 1 H, J = 6.9 Hz), 2.92 (d, 1 H, J = 2.6 Hz), 2.70 (s, 3 H), 2.49−2.40 (m, 3 H), 2.29 (dd, 1 H, J = 15.0, 2.7 Hz), 2.05 (s, 3 H), 2.82−2.66 (m, 5 H), 1.31−1.28 (m, 3 H), 1.31 (s, 3 H), 1.19 (d, 3 H, J = 6.9 Hz), 1.11 (s, 3 H), 1.00 (d, 3 H, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 220.7, 170.4, 164.7, 152.5, 139.3, 134.2, 125.2, 118.4, 115.5, 74.4, 73.2, 55.8, 53.0, 42.4, 40.0, 38.5, 33.0, 30.8, 27.6 (2C), 22.3, 20.2, 19.1, 16.9, 15.5, 13.7; HRMS (ESI^+) m/z (M^+ + H) calcd for $C_{26}H_{41}N_2O_4S$: 477.2709, found: 477.2794.

[4*S*-[4*R,7*S**,8*R**,9*R**,15*R**(*E*)]]-4,8-Dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-aza-13(*Z*)-cyclohexadecene-2,6-dione (21).** Tungsten hexachloride (0.76 g, 2.0 mmol) was dissolved in THF (20 mL) and the solution was cooled to −78 °C. *n*-Butyllithium (1.6 M in hexane, 2.5 mL, 4.0 mmol) was added in one portion and the reaction mixture was allowed to warm to 25 °C over 20 min. A 0.1 M solution of the prepared dark green tungsten reagent (13.8 mL, 1.38 mmol) was added to **15b** (0.35 g, 0.69 mmol) in THF (2 mL) at 25 °C. The reaction mixture was stirred at ambient temperature for 30 min, cooled to 0 °C, and then quenched with saturated aqueous $NaHCO_3$ (10 mL). The resulting mixture was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (4 × 30 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo. The inorganics were removed by passing the residue through a silica gel plug (eluting with 19:1 $CHCl_3$-MeOH). The eluent was concentrated in vacuo and the residue was purified by HPLC[39] to afford **21** (0.16 g, 47%) as a white foam along with minor *E*-isomer **22** (0.031 g, 9.1%). For **21**: $[\alpha]^{22}_D$ −44.4 (*c* 5.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (s, 1 H), 6.48 (s, 1 H), 5.99 (d, 1 H, NH, J = 6.4 Hz), 5.13 (dd, 1 H, J = 7.7, 7.5 Hz), 4.36−4.29 (m, 1 H), 4.07−4.01 (m, 1 H), 3.78−3.75 (m, 2 H), 3.14 (q, 1 H, J = 6.9 Hz), 2.98 (d, 1 H, J = 2.5 Hz), 2.70 (s, 3 H), 2.48−2.35 (m, 3 H), 2.23 (dd, 1 H, J = 14.6, 2.6 Hz), 2.07 (s, 3 H), 2.07−2.00 (m, 1 H), 1.82−1.69 (m, 4 H), 1.69 (s, 3 H), 1.32 (s, 3 H), 1.32−1.21 (m, 2 H), 1.19 (d, 3 H, J = 6.9 Hz), 1.12 (s, 3 H), 1.00 (d, 3 H, J = 7.0 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 221.1, 170.5, 164.7, 152.6, 139.6 (2C), 120.6, 118.5, 115.5, 74.2, 73.9, 56.5, 52.9, 42.4, 40.3, 38.3, 32.7, 31.5, 31.3, 25.7, 23.2, 22.8, 20.0, 19.1, 16.6, 15.7, 13.5; HRMS (ESI^+) m/z (M^+ + H) calcd for $C_{27}H_{42}N_2O_4S$: 491.2944, found: 491.2926.

For [4*S*-[4*R,7*S**,8*R**,9*R**,15*R**(*E*)]]-4,8-Dihydroxy-5,5,7,9,13-pentamethyl-16-[1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-1-aza-13-(*E*)-cyclohexadecene-2,6-dione (22)**: white foam, $[\alpha]^{22}_D$ −91.2 (*c* 2.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.94 (s, 1 H), 6.44 (s, 1 H), 6.15 (d, 1 H, NH, J = 8.0 Hz), 5.15−5.06 (m, 1 H), 4.49−4.46 (m, 1 H), 4.24 (dd, 1 H, J = 10.7, 2.0 Hz), 3.80 (d, 1 H, J = 3.5 Hz), 3.58−3.52 (m, 1 H), 3.43−3.39 (m, 1 H), 3.31−3.24 (m, 1 H), 2.70 (s, 3 H), 2.57−2.49 (m, 1 H), 2.39−2.30 (m, 3 H), 2.22−2.17 (m, 1 H), 2.07 (s, 3 H), 1.97−1.88 (m, 1 H), 1.75−1.56 (m, 4 H), 1.63 (s, 3 H), 1.44−1.33 (m, 1 H), 1.33 (s, 3 H), 1.15 (d, 3 H, J = 7.0 Hz), 1.04 (s, 3 H), 0.99 (d, 3 H, J = 6.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 219.6, 171.3, 164.6, 152.5, 139.4, 138.8, 120.3, 117.9, 114.9, 72.0 (2C), 55.1, 53.6, 43.4, 40.2, 39.9, 37.3, 32.8, 30.5, 24.6, 22.3, 19.0, 17.8, 17.2, 16.8, 15.9, 14.0; HRMS (ESI^+) m/z (M^+ + H) calcd for $C_{27}H_{42}N_2O_4S$: 491.2944, found: 491.2943.

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Supporting Information Available: Experimental procedures for the preparation of compounds **7**, **8**, bis-TES **15b**, and the precursor to compound **17**, copies of the ^{1}H and ^{13}C NMR spectra for select compounds, the 2D ^{1}H−^{1}H NOESY NMR spectrum of compound **20**, the 1D ^{1}H−^{1}H NOE NMR spectra of compound **21**, and X-ray crystallographic parameters for the bis-TES ether derivative of compound **15b** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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