ORGANIC LETTERS

2011
Vol. 13, No. 6
1436–1439

Stereoselective Synthesis of 12,13-Cyclopropyl-Epothilone B and Side-Chain-Modified Variants

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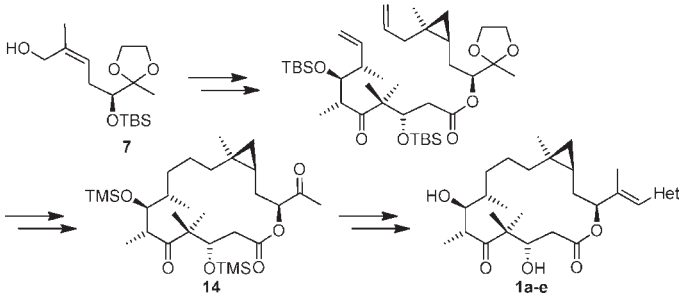
Received January 14, 2011

ABSTRACT



A general strategy has been devised for the stereoselective synthesis of 12,13-cyclopropyl-epothilone B and side-chain-modified variants thereof, which relies on late stage introduction of the heterocycle through Wittig olefination of ketone 14. Formation of the macrocycle was achieved through RCM-based ring closure and introduction of the cyclopropane moiety involved a highly selective Charette cyclopropanation of allylic alcohol 7.

Epothilones are 16-membered bacterial macrolides with potent antitumor activity[1] that have served as lead structures for a series of clinical candidates for cancer treatment (Figure 1).[2] Among these, the lactam analog of epothilone B (Epo B) recently has been approved by the FDA for clinical use in breast cancer patients (ixabepilone, Ixempra).[3]

The chemistry and biology of epothilones have been extensively studied, and numerous analogs or derivatives have been discovered that retain potent *in vitro* activity.[1] Of particular interest is the observation that the 12,13-cyclopropyl (CP) analogs of Epo A and B have been found to be equally potent, or even somewhat more potent than the respective epoxide-based parent compounds.[4] At the same time the replacement of the natural 12,13-epoxide moiety by a cyclopropane ring should lead to enhanced chemical and, in particular, metabolic stability, given the susceptibility of the oxirane ring to undesired chemical transformations[5] and metabolic attack.[6] Based on these considerations, we have become interested in CP-Epo B analogs as active drug moieties for antibody-drug conjugates (ADC)[7] and, thus, have sought efficient synthetic access to such structures. The initial results of this work are described in this communication.[8]

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[‡] University of Bern.

(1) (a) Altmann, K.-H.; Pfeiffer, B.; Arseniyadis, S.; Pratt, B. A.; Nicolaou, K. C. *ChemMedChem* **2007**, *2*, 396. (b) Höfle, G.; Reichenbach, H. Epothilone, a myxobacterial metabolite with promising antitumor activity. In *Anticancer Agents from Natural Products*; Cragg, G. M., Kingston, D. G. I., Newman, D. G., Ed.; Taylor & Francis: Boca Raton, FL, 2005; pp 413.

(2) For a review, see: Coley, H. M. *Cancer Treat. Rev.* **2008**, *34*, 378.

(3) http://www.cancer.gov/cancertopics/druginfo/fda-ixabepilone

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10.1021/ol200114k
Published on Web 02/21/2011

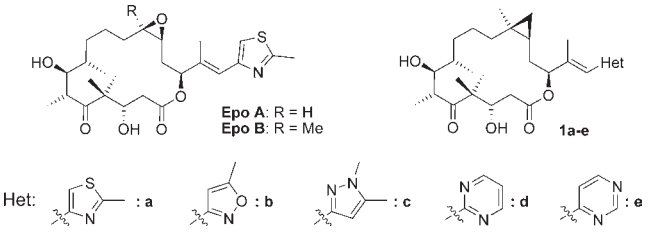
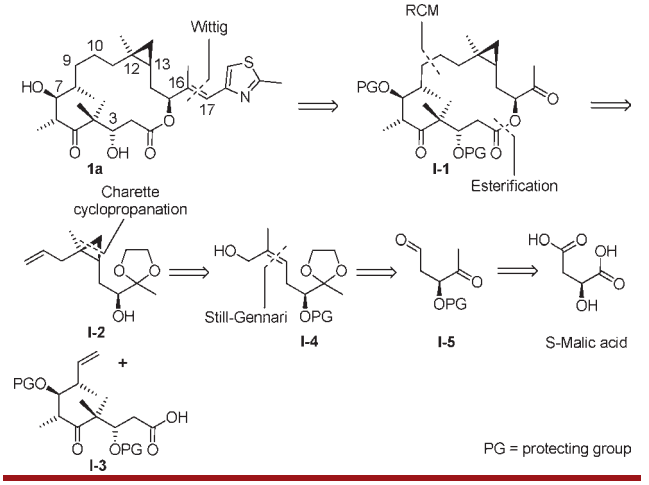


Figure 1. Structures of natural epothilones A and B and of target structures **1a**−**e**.

CP analogs of Epo A and B have been previously prepared by semisynthesis from Epo C and D, respectively, albeit in low yield;[4] CP-Epo A has also been obtained by total chemical synthesis[9] and so have some side-chain-modified variants thereof.[9] In addition, the Nicolaou group has reported a series of side-chain-modified analogs of 12,13-*trans* CP-Epo A[9] and CP-Epo B;[10] in contrast, only two examples of side-chain-modified variants of (*cis*) CP-Epo B are known[11] and no chemical synthesis of CP-Epo B itself has in fact been documented in the literature.[12]

Nicolaou's synthesis of CP-epothilones is based on a convergent strategy that relies on the late stage addition of a side chain vinyl iodide to a C15 aldehyde (epothilone numbering) by means of a Nozaki−Hiyama−Kishi coupling as a key step.[9] This strategy has been instrumental in obtaining analogs for biological testing, but, unfortunately, the Nozaki−Hiyama−Kishi coupling is essentially nonselective, thus leading to a substantial loss of material at a late stage of the synthesis. In an attempt to overcome this limitation we have evaluated an alternative strategy to CP-Epo B analogs, where the heteroaryl-vinyl side chain was to be established in the last step of the synthesis (safe for the final deprotection) through Wittig-type chemistry with a ketone **I-1** (Scheme 1). An analogous approach has been employed by Danishefsky and co-workers in the synthesis of 9,10-dehydro-Epo D[13] and its 26-trifluoromethyl variant (fludelone)[13,14] as well as by Avery and co-workers in their synthesis of Epo A (to generate the epoxidation precursor Epo C);[15] at the same time, Höfle has been unable to re-establish Epo A from the corresponding (epoxide-containing) side chain ketone, in spite of significant optimization attempts.[16,17]

Scheme 1. Retrosynthesis of CP-Epo B **1a**



As illustrated in Scheme 1, ketone **I-1** was to be obtained through ring-closing metathesis (RCM) between C9 and C10 of the desired macrocycle followed by selective double bond reduction. The requisite diene precursor for the macrocyclization reaction would in turn be assembled from alcohol **I-2** and acid **I-3**; the bis-TBS protected version of the latter (Scheme 1, PG = TBS) had been previously synthesized in our laboratory.[18] The stereoselective establishment of the cyclopropane moiety in **I-2** was to be achieved through Charette cyclopropanation of allylic alcohol **I-4**, which would be derived from keto aldehyde **I-5** by means of Still−Gennari olefination and subsequent two-carbon extension. Based on literature precedence it was felt that Still−Gennari olefination of the aldehyde group would be feasible selectively in the presence of the methyl ketone,[19] while the keto group would have to be protected in subsequent steps. Lastly, aldehyde **I-5** was planned to be prepared from *S*-malic acid as a defined source of chirality at C15.

The synthesis of intermediate **11** (i.e., synthon **I-2**) is summarized in Scheme 2. Starting from *S*-malic acid, hydroxy lactone **2** was prepared in a 3-step literature sequence.[20] Treatment of **2** with MeLi gave a mixture of cyclic hemiacetal **3** and the corresponding open chain hydroxy ketone. Synthetically useful conversion of this mixture into aldehyde **4** could only be achieved by

(8) Heterocycles were selected based on previous SAR data on other types of potent side-chain-modified epothilones[1] and the availability of the corresponding epoxides for biological comparison (for **16e** and **d**).

(9) Nicolaou, K. C.; Namoto, K.; Ritzén, A.; Ulven, T.; Shoji, M.; Li, J.; D'Amico, G.; Liotta, D.; French, C. T.; Wartmann, M.; Altmann K.-H.; Giannakakou, P. *J. Am. Chem. Soc.* **2001**, *123*, 9313.

(10) Nicolaou, K. C.; Ritzén, A.; Namoto, K.; Buey, R. M.; Díaz, J. F.; Andreu, J. M.; Wartmann, M.; Altmann, K.-H.; O'Brate, A.; Giannakakou, P. *Tetrahedron* **2002**, *58*, 6413.

(11) Nicolaou, K. C.; Sasmal, P. K.; Rassias, G.; Reddy, M. V.; Altmann, K.-H.; Wartmann, M.; O'Brate, A.; Giannakakou, P. *Angew. Chem., Int. Ed.* **2003**, *42*, 3515.

(12) Based on literature data for two examples 12,13-*trans* configured CP epothilones of type B (methyl group on C12) appear to be significanly less active than the corresponding *cis* derivatives.[10,11]

(13) (a) Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Chou, T.-C.; Dong, H. J.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 10913. (b) Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Chou, T.-C.; Dong, H. J.; Tong, W. P.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 2899.

(14) Chou, T.-C.; Dong, H. J.; Rivkin, A.; Yoshimura, F.; Gabarda, A. E.; Cho, Y. S.; Tong, W. P.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2003**, *42*, 4761.

(15) Hindupur, R. M.; Panicker, B.; Valluri, M.; Avery, M. A. *Tetrahedron Lett.* **2001**, *42*, 7341.

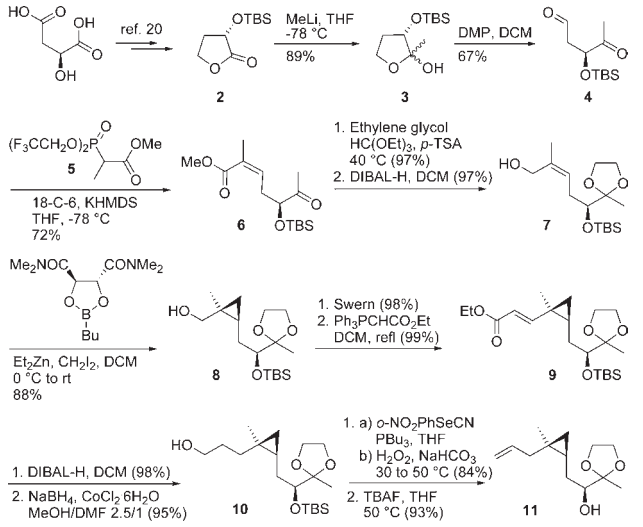
(16) Sefkow, M.; Kiffe, M.; Schummer, D.; Höfle, G. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3025.

(17) The preparation of side-chain-modified analogs of 9,10-dehydro-Epo D from the corresponding ketone has been disclosed in a PCT patent application (WO2004043954 (A2)), without any comments on the stereochemical outcome of the Wittig reactions.

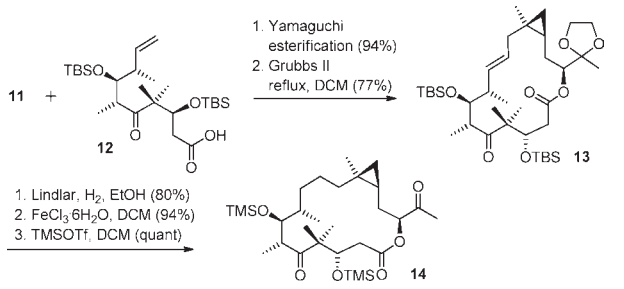
(18) Feyen, F.; Jantsch, A.; Hauenstein, K.; Pfeiffer, B.; Altmann, K.-H. *Tetrahedron* **2008**, *64*, 7920.

(19) Seifert, A.; Vomund, S.; Grohmann, K.; Kriening, S.; Urlacher, V. K.; Laschat, S.; Pleiss, J. *ChemBioChem* **2009**, *10*, 853.

Scheme 2. Synthesis of Building Block **11**



Scheme 3. Assembly of Macrolactone **14**



Dess−Martin oxidation, while all other oxidation methods investigated did not provide any of the aldehyde (PDC, Swern) or gave only low yields (py·SO_3). Subsequent Still−Gennari olefination[21] with phosphonate **5**[22] furnished the desired *Z*-isomer **6** exclusively; the geometry of the double bond was firmly established by means of NOESY experiments. Acetal protection of the ketone functionality in **6** by treatment with ethylene glycol and triethyl orthoformate followed by reduction of the ester moiety with DIBAL-H led to allylic alcohol **7** in excellent overall yield (94% for two steps); the latter underwent highly stereoselective Charette cyclopropanation (dr 18:1) to afford alcohol **8** in high yield (88% for single isomer).[23]

It should be noted that violent explosions have been reported for Charette cyclopropanations carried out on scales of 8 mmol or higher,[23] due to the exothermicity associated with the formation of $Zn(CH_2I)_2$. However, careful temperature control during the addition of CH_2I_2 to the $(Et)_2Zn$ solution allowed the cylopropanation of **7** to be carried out safely also on a larger scale.

With this key reaction successfully implemented, our efforts were then directed toward installing the terminal double bond required for RCM-based macrocyclization. After Swern oxidation of **8**, the resulting aldehyde was subjected to Wittig-olefination with Ph_3PCHCO_2Et to furnish α,β-unsaturated ester **9**. Subsequent reduction of the ester moiety using DIBAL-H followed by reduction of the double bond with $NaBH_4/CoCl_2·6H_2O$[24] provided saturated alcohol **10**. An X-ray crystal structure of the allylic alcohol intermediate obtained from **9** confirmed the predicted stereochemical outcome of the Charette cyclopropanation reaction.[25] Finally, seleniumoxide pyrolysis[26] and TBS deprotection provided alcohol **11** in a total of 12 steps and excellent overall yield (25%) from lactone **2**.

As illustrated in Scheme 3 a high yielding Yamaguchi esterification[27] of alcohol **11** and acid **12** (i.e., **I-3** with PG = TBS, Scheme 1)[18] gave the requisite diene for RCM-based macrocyclization (DCC- or EDCI-based protocols were less effective); this diene underwent smooth RCM in the presence of second generation Grubbs catalyst in refluxing DCM to provide macrolactone **13** as a 12/1 mixture of *E*/*Z* isomers. In contrast, the use of toluene as a solvent only gave traces of product.

Reduction of the C9, C10 double bond in macrolactone **13** proved to be highly challenging, due to simultaneous cyclopropane ring-opening. After extensive optimization, hydrogenation over Lindlar catalyst at a hydrogen pressure of 7.5 bar was found to provide the saturated macrocycle most efficiently (80% yield). While cyclopropane ring-opening could not be avoided completely even under these conditions, it occurred at a much slower rate than double bond reduction.

Unfortunately, we were unable at this stage to accomplish the selective hydrolysis/removal of the cyclic acetal in the presence of the two TBS ethers at C3 and C7, using a variety of different conditions. As a consequence, the reduction product was submitted to global deprotection, which was best achieved with $FeCl_3·6H_2O$.[28]

Not unexpectedly, the resulting (unprotected) ketone was found to be a poor substrate for olefinations with heterocycle-containing phosporanes or phosphonate anions, with target structures **1** being obtained in only low and nonreproducible yields (0−25%). The hydroxyl groups were thus reprotected as TMS ethers to produce the

(20) (a) Denmark, S. E.; Yang, S.-M. *J. Am. Chem. Soc.* **2002**, *124*, 15196. (b) Mulzer, J.; Mantoulidis, A.; Oehler, E. *J. Org. Chem.* **2000**, *65*, 7456. (c) White, J. D.; Hrnciar, P. *J. Org. Chem.* **2000**, *65*, 9129. (d) Green, D. L. C.; Kiddle, J. J.; Thompson, C. M. *Tetrahedron* **1995**, *51*, 2865.

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(22) Phosphonate **5** is accessible from commerically available ethylphosphonic dichloride in a two-step sequence (*cf.* ref 21).

(23) Charette, A. B.; Juteau, H.; Lebel, H.; Molinaro, C. *J. Am. Chem. Soc.* **1998**, *120*, 11943.

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(25) The structure has been deposited in the Cambridge Crystallographic Data Base (deposition number CCDC 808039).

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(29) For experimental details see: Dietrich, S. A.; Lindauer, R.; Stierlin, C.; Gertsch, J.; Matesanz, R.; Notararigo, S.; Díaz, J. F.; Altmann, K.-H. *Chem.—Eur. J.* **2009**, *15*, 10144.

Scheme 4. Synthesis of Target Structures **1a**−**e** (*cf.* Figure 1)

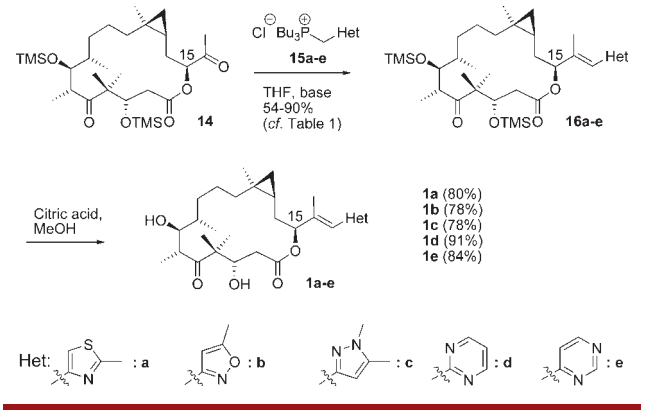

Table 1. Wittig Reactions with Ketone **14**

Wittig salt	base	temperature	product	*E*:*Z*	yield
15a	KHMDS	−78 to −20 °C	**16a**	6:1	67%[a]
15b	KHMDS	−78 to −20 °C	**16b**	17:1	74%[a]
15c	*n*-BuLi	−78 to −20 °C	**16c**	13:1	85%[a]
15d	*n*-BuLi	−78 to 25 °C	**16d**	10:1	90%[b]
15e	*n*-BuLi	−78 to 75 °C	**16e**	7:1	54%[b,c]

[a] Mixture of *E* and *Z* isomers. [b] Mixture of *E* isomer and presumed C15 epimer (see text). [c] 77% based on recovered starting material.

bis-TMS protected macrolactone **14** (i.e., **I-1** with PG = TMS, Scheme 1) in quantitative yield (Scheme 3). As illustrated in Scheme 4 and Table 1, methyl ketone **14** proved to be a highly suitable substrate for heterocycle attachment through Wittig olefination.

In all cases investigated, the reactions proceeded with good selectivity in favor of the desired *E* isomers which could be isolated in acceptable to good yields (Table 1). However, distinct differences were observed between different phosphoranes with regard to selectivity and also reactivity (Table 1).

Thus, while **16a**−**c** were already formed upon warming of the reaction mixture to −20 °C (after deprotonation of the phosphonium salts at −78 °C and addition of **14** at the same temperature), the reaction of **14** with the phosphoranes derived from **15d** and **15e** required temperatures of 25 °C and, quite remarkably, 75 °C, respectively. Attempts to accelerate the reaction of **14** with the phosphorane derived from **15d** by raising the temperature to 75 °C resulted in decomposition.

Noteworthy, after chromatographic separation of **16d** and **16e** from the corresponding *Z* isomers, their ^{1}H and ^{13}C NMR spectra still indicated the presence of an isomeric impurity that could not be separated (*ca.* 12%). We have not established the identity of this impurity, but it is well conceivable that the stereocenter at C15 partially epimerizes under the more forcing conditions of the Wittig reaction required for **16d** and **16e**.

Table 2. Antiproliferative Activity of CP-Epo B Analogs **1a**−**e** (IC_{50} values [nM][a]; after 72 h exposure time)[29]

cell line	**1a**	**1b**	**1c**	**1d**	**1e**
A549	0.70 ± 0.2	0.30 ± 0.07	4.7 ± 0.8	0.90 ± 0.13	1.7 ± 0.15
MCF-7	0.80 ± 0.3	0.40 ± 0.06	8.5 ± 1.5	0.80 ± 0.19	1.9 ± 0.23
HCT116	1.30 ± 0.2	0.30 ± 0.03	3.8 ± 0.7	0.40 ± 0.08	0.90 ± 0.07

[a] IC_{50} values of 0.33, 0.34, and 0.16 nM have been reported for Epo B against the A549, MCF-7, and HCT116 cell lines, respectively.[29]

Deprotection of **16a**−**e** was achieved with citric acid to provide CP-Epo B analogs **1a**−**e** in excellent yields (Scheme 4). For **1d**, the minor isomer formed in the Wittig reaction could be removed by preparative HPLC, whereas **1e** could only be obtained as a 10:1 mixture of the desired stucture and its presumed C15 epimer (and was evaluated as such in cellular experiments).

The antiproliferative activity of CP-Epo B analogs **1a**−**e** was tested against the human cancer cell lines A549 (lung), MCF-7 (breast), and HCT116 (colon) (Table 2).

All compounds are potent inhibitors of cancer cell proliferation, with IC_{50} values in the single digit nM or even sub-nM range. Compared to Epo B, CP-Epo B (**1a**) appears to be 2−8-fold less active; IC_{50} values similar to the corresponding epoxide-based analogs[30] were also observed for **1d**/**1e** (< 3-fold difference in all cases), although **1d** and **1e** showed a tendency for slightly enhanced activity. The most potent compound investigated was isoxazole derivative **1b**, which is in line with previous findings by the Danishefsky group on the activity of isoxazole-containing variants of 9,10-dehydro-12,13-deoxy-epothilones.[31] In light of its sub-nM potency **1b** is an attractive candidate for the construction of ADCs.

In conclusion, we have established an efficient synthesis of CP-based Epo B analogs, which relies on ketone **14** as a highly advanced precursor for the late stage incorporation of the side chain heterocycle; as such this strategy enables convenient access to a wide range of side-chain-modified derivatives. We are currently pursuing the synthesis of further CP-Epo B analogs and of conjugates of such compounds with tumor-targeted antibodies. The results of these studies will be reported in due course.

Acknowledgment. This work was supported by the ETH Research Grant CH1-01 08-3. We are indebted to Dr. Bernhard Pfeiffer (ETHZ) for NMR support, to Kurt Hauenstein (ETHZ) for advice, and to Louis Bertschi from the ETHZ-LOC MS-Service for HRMS spectra.

Supporting Information Available. Synthetic procedures, complete spectroscopic data, ^{1}H and ^{13}C NMR data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(30) These compounds were kindly provided by the Novartis Institute for Biomedical Research in Basel, Switzerland.

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