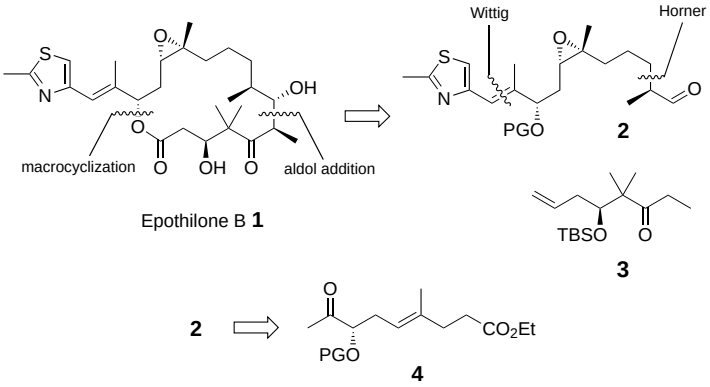
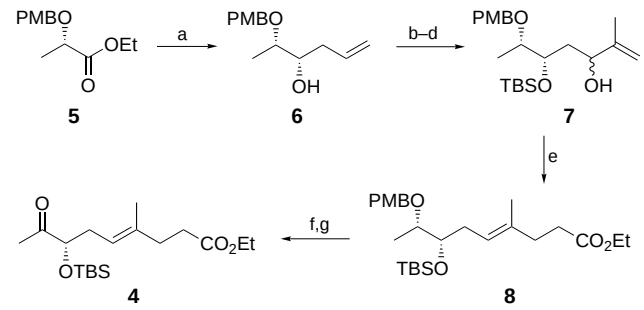
How Stable Are Epoxides? A Novel Synthesis of Epothilone B**

Harry J. Martin, Martina Drescher, and Johann Mulzer*

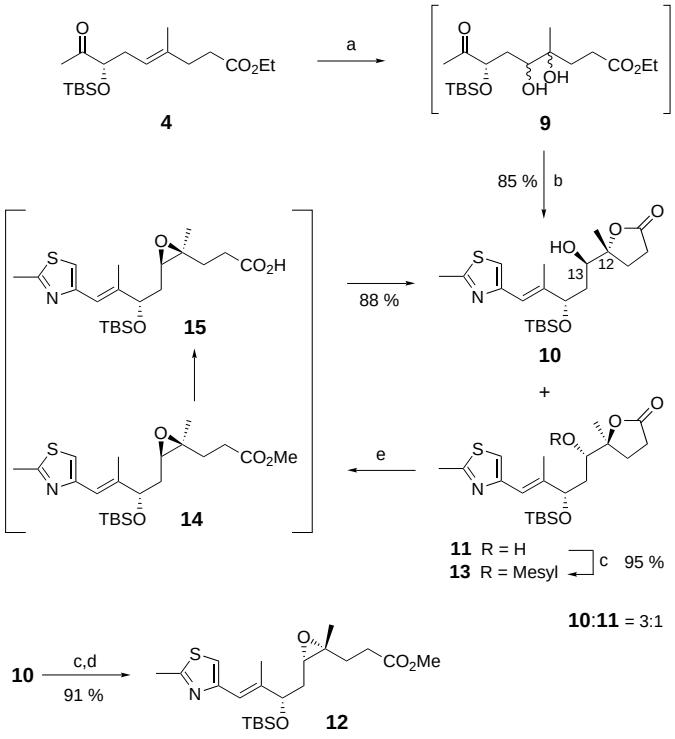
Epothilone B (**1**), a novel antitumor agent,[1] features a trisubstituted epoxide as a central structural element, whose precise contribution to the biological activity is not yet clear.[2] In all syntheses of **1** so far,[3] the corresponding (*Z*)-olefin (epothilone D) was epoxidized in the last step with diastereoselectivies between 4:1 and 20:1 in favor of the desired β isomer. This strategy has been chosen obviously to avoid undesired additions to a presumably labile epoxide. We wanted to test the alleged lability of the epoxide by a new synthesis in which the epoxide is introduced at a very early stage and then deliberately carried through the hardships of a multistep synthesis. The key step of our synthesis featured an aldol addition of epoxyaldehyde **2** to the known ketone **3**[3b] (Scheme 1). Fragment **2** was to be obtained from the (*E*)-enoate **4** readily available from (*S*)-ethyllactate **5** (Scheme 2) which was reduced to the aldehyde and submitted to a chelate-controlled allyl addition to give **6**. Chain elongation produced allylic alcohol **7** which was used in a Johnson Claisen rearrangement to furnish (*E*)-olefin **8** as a single stereoisomer. In the conversion of the ester **4** into the key building block **2** it is particularly important to avoid the introduction of O^- and OH functions in γ-position to one of the epoxide carbon atoms, since, as previous studies have shown, otherwise opening of the epoxide is inevitable. Removal of the *p*-methoxybenzyl (PMB) group and Swern oxidation delivered ketone **4**, which on asymmetric dihydroxylation[4] furnished diol **9** as an inseparable mixture of isomers. Without purification, this mixture was converted into olefins **10** and **11** by a Wittig reaction. After chromatographic separation, **10** was mesylated and treated with potassium carbonate in methanol to give the desired epoxide **12** (Scheme 3). In contrast to the highly (*E*)-selective (>98:2)



Scheme 1. Retrosynthetic analysis. PG = protective group, TBS = *tert*-butyldimethylsilyl.



Scheme 2. Synthesis of (*E*)-olefin **4**: a) 1 equiv DIBAL, CH_2Cl_2, $-78\,^\circ C$, 2 h, 1 equiv $MgBr_2 \cdot Et_2O$, then 2 equiv Allyl–MgBr, -78 to $20\,^\circ C$, 12 h, 92%, diastereoselectivity 9:1; b) 1.4 equiv TBSCl, 4 equiv imidazole, DMF, $22\,^\circ C$, 24 h, 98%; c) O_3, CH_2Cl_2/MeOH (15:1) $-78\,^\circ C$, then PPh_3, 96%; d) isopropenyl–MgBr, THF, $-10\,^\circ C$, 45 min, 89% as a 1:1 mixture of diastereomers; e) 8 equiv triethylorthoacetate, $C_2H_5CO_2H$ (catalytic amount), xylene, $140\,^\circ C$, 12 h, 95%, only *E* isomer; f) 1.1 equiv DDQ, CH_2Cl_2/H_2O (19:1), 0.5 h, 94%; g) 2.5 equiv $(COCl)_2$, 4 equiv DMSO, 6 equiv NEt_3, $-78\,^\circ C$, 97%. DIBAL = diisobutylaluminum hydride; DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone; DMSO = dimethyl sulfoxide; PMB = *p*-methoxybenzyl.



Scheme 3. Synthesis of the *cis*-epoxide **12**: a) AD-mix-β, methanesulfonamide, *t*BuOH/H_2O (1:1), room temperature, 20 h; b) 2.2 equiv (2-methylthiazol-4-ylmethyl)-tributylphosphonium chloride, 2.2 equiv KHMDS, THF, $-78\,^\circ C$, 0.5 h, then **9**, -78 to $35\,^\circ C$, 5 min, 78–85% over two steps, **10**:**11** ca. 3:1; c) 2 equiv MsCl, 2.5 equiv NEt_3, CH_2Cl_2, $-15\,^\circ C$, 3 h; d) 2 equiv K_2CO_3, MeOH, 0.75 h, 91% over two steps; e) 2 equiv K_2CO_3, MeOH, room temperature, 1 h; 2 equiv K_2CO_3 MeOH/H_2O, 36 h; extraction with aqueous HCl (1N)/CH_2Cl_2, then silica gel, CH_2Cl_2, 3 d, 88%. KHMDS = potassium bis(trimethylsilyl)amide; Ms = mesyl = methanesulfonyl.

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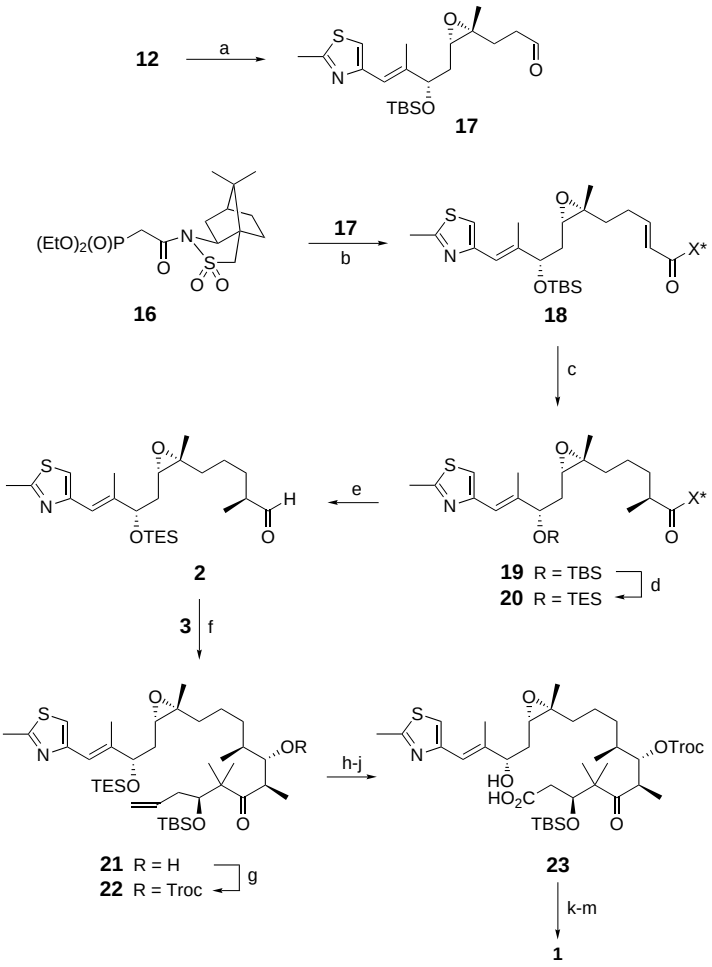
Wittig reaction, the dihydroxylation was much less diastereoselective (3:1). To recover stereochemically misdirected material, **11** was converted into **10** by a double inversion at C12 and C13. Hence, **11** was first converted into the mesylate **13**, which was successively treated with potassium carbonate, water, and 1N HCl (Scheme 3) to give stereoisomerically pure **10** via the nonisolated intermediates **14** and **15**. By this procedure, olefin **4** was converted into the desired (12*R*,13*S*)-epoxide **12** without stereochemical loss. To complete the synthesis of fragment **2**, we used a Horner olefination followed by auxiliary-controlled introduction of the methyl group at C8 (Scheme 4). Thus epoxide **12** was reduced to aldehyde **17**, which was treated with chiral phosphonate **16**[5] in the presence of lithium hydroxide (prepared in situ from BuLi/THF/H_2O) to give the enoylsultam **18** (X* = sulfane residue) in good yield. 1,4-Hydride addition with L-selectride furnished the enolate which was trapped with methyl iodide to give **19** with excellent diastereoselectivity (≥99:1, HPLC analysis).[6] The proper choice of the protective group (PG) for the 15-OH function was particularly important as this PG had to be stable through all transformations up to the macrolactonization. On the other hand, the PG should be removable in the presence of the epoxide at any stage of the sequence. This requirement was extremely hard to fulfil in view of the imminent formation of a 15,12-oxolane ring. A TBS protective group proved insufficient in this respect as its removal proceeded only with 40% yield. However, after conversion of the 15-OTBS derivative **19** into the 15-OTES analogue **20**, all remaining synthetic steps could be carried out in high yield. Specifically, the reductive removal of the auxiliary with DIBAL furnished aldehyde **2** which was treated with the lithium enolate of ketone **3** to give the aldol adduct **21** with a diastereoselectivity of >95:5 (NMR and HPLC analysis). Troc-protection of the 7-OH function gave **22**, which was converted into seco acid **23** by oxidation of the terminal olefin to the aldehyde, followed by 15-O-desilylation and Pinnick oxidation of the aldehyde. Yamaguchi macrolactonization of **23** and removal of the Troc protective group and the 3-OTBS protective group furnished diastereomerically pure **1**.

The epoxide, introduced early into the molecule, has proven its stability under the following conditions: 1) reductive (neutral (DIBAL), ionic (selectride), and metallic (Zn)); 2) oxidative (osmium tetroxide/sodium periodate, sodium chlorite); 3) basic (fluoride in aprotic solvents, DMAP, LDA, enolates). In this respect, it is remarkable that carbon and oxygen anions do not open the epoxide, even when they are generated in a 1,5-relationship to the epoxide; 4) electrophilic (acylation with an acyl chloride in the Yamaguchi reaction). Of all reagents applied, only dilute acid has led to (in this case desired) epoxide opening (**15** → **10**). Apart from providing us with the valuable information that epoxides are by no means such highly reactive intermediates as postulated,[7] the early introduction of the epoxide has been of advantage in the overall synthesis of **1**. For instance, the formation of thiazole-N-oxides previously observed in the *m*-chloroperoxybenzoic acid epoxidation of the C12–C13 double bond[8] has been avoided as well as the formation of the (12*S*,13*R*)-epoxide which is hard to separate from the correct



Scheme 4. Synthesis of epothilone B (**1**) by aldol addition and macrolactonization: a) 1.1 equiv DIBAL, CH_2Cl_2, −95 to −80 °C, 1 h, 93%; b) 1.1 equiv BuLi, Et_2O, 0 °C, 1.1 equiv H_2O (in THF), then **16**, 5 min, room temperature, then **17**, 30 min, room temperature, 92%; c) 1.4 equiv L-selectride, THF, −78 to −60 °C, 0.5 h, 8 equiv HMPA, 12 equiv MeI, −78 to 20 °C, 16 h, 78%; d) 1.) 4 equiv TBAF, THF, room temperature, 3 h; 2.) TESCl, NEt_3, cat. DMAP, room temperature, 1 h, 85% over 2 steps; e) 2 equiv DIBAL, CH_2Cl_2, −95 to −80 °C, 1 h, 93%; f) 1.5 equiv LDA, 1.5 equiv **3**, −78 to −40 °C, −78 °C then **2**, 15 min, 92%, diastereoselectivity 95:5; g) 6 equiv 2,2,2-trichloroethoxy chloroformiate (TrocCl), 18 equiv pyridine, CH_2Cl_2, 20 °C, 0.5 h, 94%; h) 1.) 0.05 equiv OsO_4, 1 equiv NMO, THF/*t*BuOH/H_2O (5:5:1), room temperature, 16 h; 2.) 3 equiv $NaIO_4$, EtOH/H_2O (4:1), 22 °C, 1 h; i) HF·pyridine, pyridine, THF, room temperature, 0.5 h; j) $NaClO_2$, NaH_2PO_4, *t*BuOH/2,2-dimethyl-2-butene (2:1), room temperature, 1 h, 63% for four steps; k) 2.0 equiv 2,4,6-trichlorobenzoyl chloride, 2.5 equiv NEt_3, room temperature, 1 h, then added slowly (1 h) to a solution of 8 equiv DMAP in toluene (0.002 M in seco acid), 0.5 h room temperature, 65%; l) 80 equiv Zn, 100 equiv NH_4Cl, 80 °C, 20 min; m) HF·pyridine, pyridine, 30 °C, 7 d, 62% for 2 steps. L-Selectride = lithium-tri-*sec*-butylboranate; HMPA = hexamethyl phosphoric acid triamide; TBAF = tetrabutylammonium fluoride; TES = triethylsilyl; DMAP = *N*,*N*-dimethyl-4-aminopyridine; LDA = lithium diisopropylamide; NMO = *N*-methylmorpholine-*N*-oxide.

stereoisomer. Additionally, the diastereocontrol of the aldol addition with the epoxyaldehyde is significantly better than it is with the olefinic aldehyde.[3b,f,g] The use of ester **4** as an intermediate allows the application of the Claisen rearrangement as an efficient chain-elongation procedure. Also, it is possible to introduce additional double bonds after the epoxide has been generated which may be useful for the

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preparation of novel epothilone derivatives. On the other hand, the necessary exchange of the 15-O-silyl protective groups is a clear disadvantage. As to the biological role of the epoxide in epothilone B (**1**), it appears doubtful that it is opened under physiological conditions because of the high stability of the oxirane. Rather, the epoxide may interact with the receptor unchanged or may be used in an intramolecular hydrogen bridge with the 3-OH function to generate a favorable conformation.[9]

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[1] a) Isolation of the epothilones: a) G. Höfle, N. Bedorf, K. Gerth, H. Reichenbach (GBF), DE-B 4138042, **1993** [*Chem. Abstr.* **1993**, *120*, 52841]; b) antitumor activity: D. M. Bollag, P. A. McQueney, J. Zhu, O.Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C. M. Woods, *Cancer Res.* **1995**, *55*, 2325; c) review of the chemistry and biology of the epothilones: K. C. Nicolaou, F. Roschangar, D. Vourloumis, *Angew. Chem.* **1998**, *110*, 2120; *Angew. Chem. Int. Ed.* **1998**, *37*, 2014.

[2] a) D.-S. Su, A. Balog, D. Meng, P. Bertinato, S. J. Danishefsky, Y.-H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 2178; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2093; b) K. C. Nicolaou, N. Wissinger, J. Pastor, S. Ninkowic, F. Sarabia, Y. He, D. Vourloumis, Z. Yang, T. Li, P. Giannakakou, E. Hamel, *Nature* **1997**, *387*, 268.

[3] a) D.-S. Su, D. Meng, P. Bertinato, A. Balog, E. J. Sorensen, S. J. Danishefsky, Y. H. Zheng, T.-C. Chou, L. He, S. B. Horwitz, *Angew. Chem.* **1997**, *109*, 775; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 757; b) K. C. Nicolaou, S. Ninkovic, F. Sarabia, D. Vourloumis, Y. He, H. Vallberg, M. R. V. Finlay, Z. Yang, *J. Am. Chem. Soc.* **1997**, *119*, 7974; c) D. Meng, P. Bertinato, A. Balog, D.-S. Su, T. Kamenecka, E. J. Sorensen, S. J. Danishefsky, *J. Am. Chem. Soc.* **1997**, *119*, 10073; d) S. A. May, P. A. Grieco, *Chem. Commun.* **1998**, 1597; e) D. Schinzer, A. Bauer, J. Schieber, *Synlett* **1998**, 861; f) A. Ballog, C. Harris, K. Savin, X.-G. Zhang, T.-C. Chou, S. J. Danishefsky, *Angew. Chem.* **1998**, *110*, 2821; *Angew. Chem. Int. Ed.* **1998**, *37*, 2675; g) J. Mulzer, A. Mantoulidis, E. Öhler, *Tetrahedron Lett.* **1998**, *39*, 8633; h) J. D. White, R. G. Carter, K. F. Sundermann, *J. Org. Chem.* **1999**, *64*, 684.

[4] review: H. C. Kolb, M. S. VanNieuwenzhe, K. B. Sharpless, *Chem. Rev.* **1994**, *94*, 2483.

[5] *ent*-**15**: W. Oppolzer, D. Dupuis, G. Poli, T. M. Raynham, G. Bernardinelli, *Tetrahedron Lett.* **1988**, *29*, 5885.

[6] W. Oppolzer, G. Poli, *Tetrahedron. Lett.* **1986**, *27*, 4717.

[7] a) Review: J. Gorzynski Smith, *Synthesis* **1983**, 629; epoxides as intermediates in natural product syntheses: b) Monensin: G. Schmid, T. Tukuyama, K. Akasaka, Y. Kishi, *J. Am. Chem. Soc.* **1979**, *101*, 259; c) lonomycin A: D. A. Evans, A. M. Ratz, B. E. Huff, G. S. Sheppard, *J. Am. Chem. Soc.* **1995**, *117*, 3448; d) brevetoxin: K. C. Nicolaou, *Angew. Chem.* **1996**, *108*, 644; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 589. Regarding the stability of "early" epoxides in total synthesis see: e) E. J. Corey, B. B. Snider, *J. Am. Chem. Soc.* **1972**, *94*, 2549; f) E. J. Corey, J. P. Dittami, *J. Am. Chem. Soc.* **1985**, *107*, 256; g) D. A. Evans, A. S. Kim, R. Metternich, V. J. Novack, *J. Am. Chem. Soc.* **1998**, *120*, 5921.

[8] G. Höfle, N. Glaser, M. Kiffe, H. J. Hecht, F. Sasse, H. Reichenbach, *Angew. Chem.* **1999**, *111*, 2090; *Angew. Chem. Int. Ed.* **1999**, *38*, 1971.

[9] M. Wang, X. Xia, Y. Kim, D. Hwan, J. M. Jansen, M. Botta, D. C. Liotta, J. P. Snyder, *Org. Lett.* **1999**, *1*, 43.

Identification of Toxic 2,4-Decadienal in Oxidized, Low-Density Lipoprotein by Solid-Phase Microextraction

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9-Hydroxy-10,12-octadecadienoic acid (9-HODE) induces the liberation of interleukin-1β (IL-1β) together with α,β-unsaturated aldehydes, especially 2,4-decadienal, from macrophages.[1] IL-1β in turn stimulates the proliferation of smooth muscle cells.[2, 3] This process is regarded as being connected to atherogenesis,[1] since particularily high levels of IL-1β were detected in atherosclerotic plaques.[4] 2,4-Decadienal was detectable only in trace quantities after copper(II) ion induced air oxidation of low-density lipoprotein (LDL).[1] In addition, this detection required long separation procedures and preparation of the 2,4-dinitrophenylhydrazone derivative.[1, 5]

A detection method which needs neither sample procession nor derivatization is solid-phase microextraction (SPME).[6, 7] It also avoids the formation of artifacts by handling. Electron impact mass spectrometry (EI-MS) is excellent for the characterization of α,β-unsaturated aldehydes. We used the combination of SPME/EI-MS to obtain insight into the events proceeding in the artificial oxidation of LDL: Blood samples were withdrawn from volunteers and LDL was isolated immediately.[9] The LDL thus obtained was oxidized by air after addition of catalytic amounts of $CuSO_4$. Samples were collected in time intervals and analyzed by GC/MS. The measurement of the compounds present is achieved by determining the total ion current. Such a chromatogram is reproduced in Figure 1.

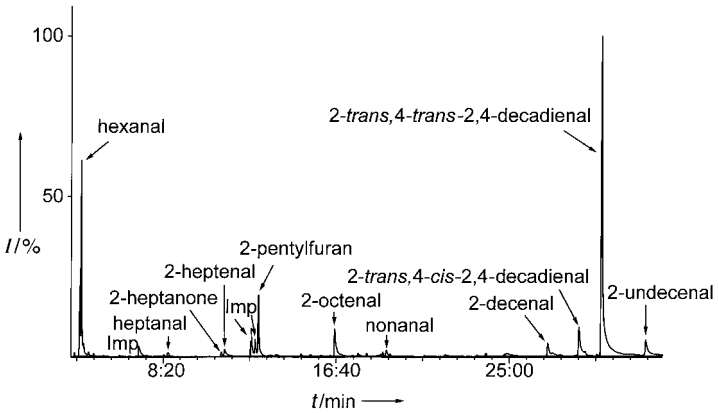

Figure 1. Reconstituted ion current chromatogram (RIC) of a LDL sample after 24 h oxidation with 50 μM $CuSO_4$ solution at 37 °C.

LDL contains different amounts of individual antioxidants which are consumed first. Therefore, some time (lag-time) is required before lipid peroxidation starts.[10] This lag-time also

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