Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 3025–3030

BIOORGANIC &
MEDICINAL CHEMISTRY
LETTERS

Oxidative and reductive transformations of epothilone A

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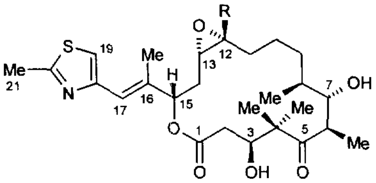
Received 27 May 1998; accepted 2 October 1998

Abstract: The C7 hydroxy group of cytotoxic epothilone A was selectively oxidized using PDC. A selective oxidation of the C3 hydroxy group was accomplished with $Me_2S/(PhCO_2)_2$ after *in situ* protection of C7–OH. Reduction of epothilone A or of a C5, C7 dioxo derivative with $NaBH_4$ proceeded at the C5 carbonyl group. Oxidation and hydrogenation of the C16–C17 double bond proved to be difficult but it was easily cleaved with ozone and the resulting keto derivative was transformed to epothilone analogs with different side chains. © 1998 Elsevier Science Ltd. All rights reserved.

Since our initial reports,[1] the highly cytotoxic epothilones A (**1a**) and B (**1b**) (Scheme 1) have gained much attention by either biologists and medicinal[2] and synthetic organic chemists. The race for the first total synthesis immediately started and in less than six month after appearence of our articles the first synthesis of epothilone A was published by Danishefsky *et al.*[3a] To date three groups have successfully completed their syntheses of epothilone A and/or B.[3-5] Recently Danishefsky *et al.*[6] and Nicolaou *et al.*[7] reported that they obtained and identified a variety of synthetic derivatives of epothilones during their syntheses of the natural products. Furthermore, a route to a synthetic epothilone library was presented in the latter paper.[7a] This prompted us to communicate our efforts on modification of epothilones A and B obtained by fermentation.

Scheme 1 Epothilone A (**1a**) and B (**1b**).



1a, R = H, Epothilone A
1b, R = Me, Epothilone B

In this and the accompanying letter we wish to present some of our results on derivatization of all functional groups of epothilones (**1**), except the lactone and the thiazole moiety. For clarity, we devided the molecule in two regions of action: 1) oxidative and reductive modifications of the „southern hemisphere“ including the exocyclic double bond and 2) transformations of the C12–C13 epoxide function. In this paper we describe our studies on regioselective variations of the oxidation state at C3–C7 and C16–C17 of epothilone A (**1a**).

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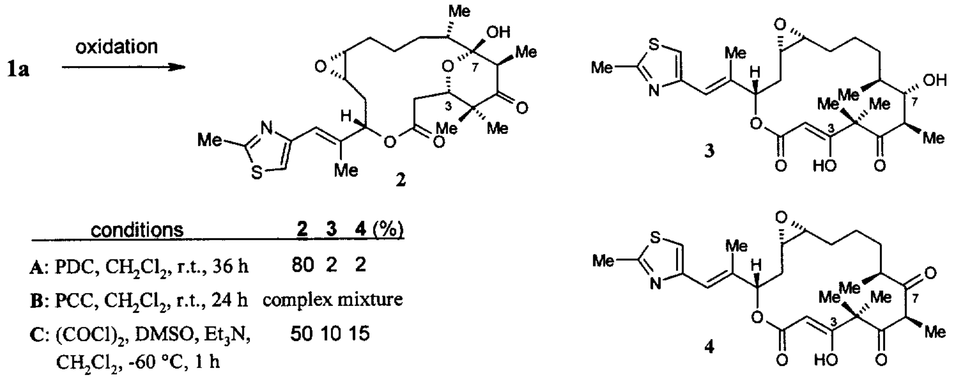
PII: S0960-894X(98)00545-9

The epothilones contain two secondary hydroxy groups with similar stereochemical environment. Both occupy a pseudoaxial position in the macrocycle and form no hydrogen bond to carbonyl groups.[1a] Therefore, we did not expect a strong preference for one of the two hydroxy groups under any alcohol oxidizing conditions. Indeed, treatment of epothilone A (**1a**) with the acidic pyridinium chlorochromate (PCC)[8] afforded a complex, unseparable product mixture. The neutral pyridinium dichromate (PDC),[9] instead, oxidized regioselectively the hydroxy group at C7 either in dichloromethane or DMF.[10] Surprisingly, the product isolated in over 80% yield was not the expected bis-oxo derivative but its hemiacetal **2** (δ_{C7} = 104.1 ppm in $CDCl_3$). The immediate formation of an acetal apparently protected the C3 hydroxy group from further oxidation.[11] Only traces (less than 2%) of the C3 mono oxidized derivative **3** or the double oxidized compound **4** were detected by HPLC-MS of the crude reaction mixture (Scheme 2).[12]

Oxidation of **1a** using the Swern protocol [$(COCl)_2$, DMSO, Et_3N][13] was much less selective, producing **2**, **3** and **4** in 50, 10 and 15% yield along with some other, not identified compounds. Interestingly, all derivatives having a C3 oxo group exhibit keto-enol tautomerism with a predominant enol form (^{1}H NMR; $CDCl_3$:enol: $\delta_{2\text{-}H}$ = 5.41 ppm; δ_{OH} = 12.10 ppm; keton: *AB* $\delta_{2\text{-}H}$ 3.41 and 3.70 ppm, J_{AB} = 16.2 Hz).

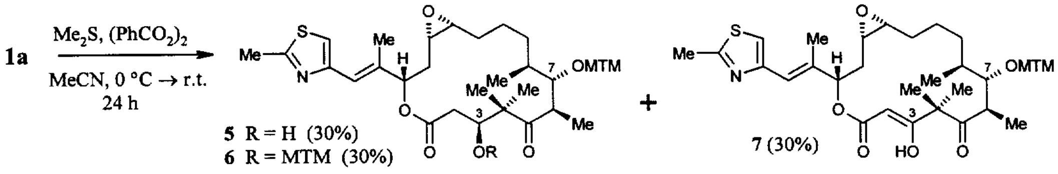
Scheme 2 Oxidation of the hydroxy groups at C3 and C7.



conditions	2 3 4 (%)
A: PDC, CH_2Cl_2, r.t., 36 h	80 2 2
B: PCC, CH_2Cl_2, r.t., 24 h	complex mixture
C: $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -60 °C, 1 h	50 10 15

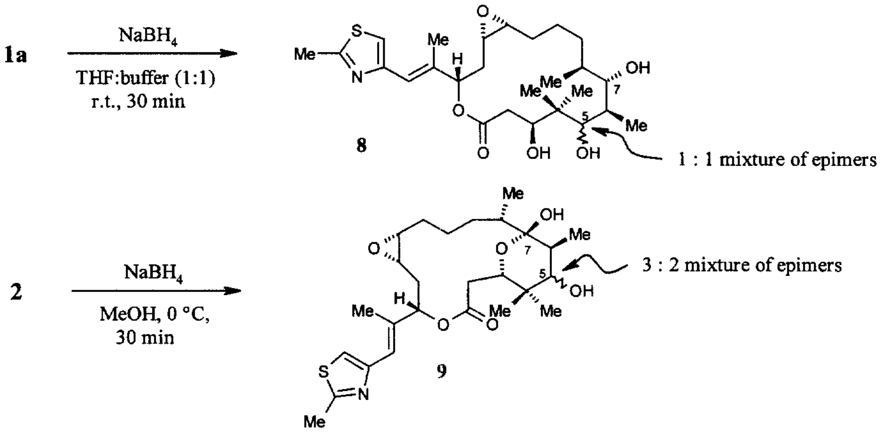
A selective C3 oxidation of epothilone A was accomplished using a different approach. With the intention to protect both hydroxy groups as their methylthiomethyl (MTM) ether, epothilone A (**1a**) was treated with a mixture of dimethylsulfide and dry dibenzoyl peroxide in acetonitrile.[14] However, a very slow etherification rate of the C3 hydroxy group made it amenable for oxidation, a known side reaction of this method for MTM ether formation, while the C7 position was already completely blocked as the MTM ether (Scheme 3). Thus, we obtained three major compounds, mono and bis MTM ethers **5** and **6** and *via in situ* protection of C7-OH the C3-oxo derivative **7** (2:3 mixture of keto and enol form), each in ca. 30% isolated yield.

Scheme 3 Selective protection of the C7 hydroxy group.



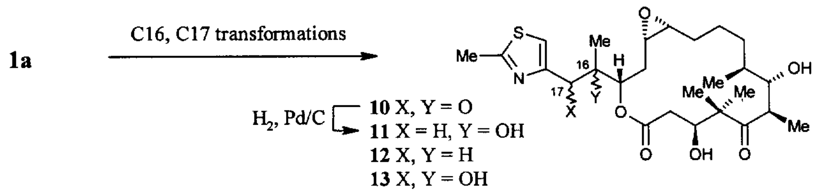
Reduction of the C5 oxo group of epothilone **1a** proceeded smoothly with sodium borohydride in a THF/pH 7 buffer solvent system providing alcohol **8** as an approximately 1:1 mixture of epimers in 70% yield (Scheme 4). Without pH control the reaction mixture soon turned basic and rapid saponification of the lacton moiety occured (halflife at pH 13 is less than 10 min). Exposure of hemiacetal **2** to similar conditions ($NaBH_4$, MeOH) reduced regioselectively the C5 oxo group affording alcohol **9** (85%) as an easily seperable 3:2 mixture of epimers. No products containing a reduced C7 moiety were found although, according to the ^{1}H NMR spectrum of **2**, about three percent of the 7-keto form are present under these reaction conditions.

Scheme 4 Reduction of the C5 carbonyl group of epothilone **1a** and dioxo derivative **2**.



The exocyclic trisubstituted double bond of the epothilones connects the aromatic thiazole group with the macrocycle. The resulting stereoelectronic effects significantly decrease the reactivity of this double bond. For example, bromine in CCl_4 did not attack the double bond, although one equivalent of the halogen was consumed.[15] Nevertheless, epoxidation of the double bond was achieved either with *m*-chloroperbenzoic acid (*m*-CPBA)[16] (CH_2Cl_2, r.t., 1 h) or with dimethyldioxirane (DMDO)[17] (acetone, 0 °C → r.t., 5 h affording bis-epoxide **10** as a 3:2 mixture of the epimers, albeit in low yields (10% for *m*-CPBA, 25% for DMDO).[18,19] Hydrogenolysis of the benzyl-like C17–O bond of the C16–C17 epoxide **10** with 10% palladium on charcoal (EtOH, r.t., 90 h) gave quantitatively the alcohols **11** (Scheme 5) but hydrogenation of the C16–C17 double bond of epothilone A (**1a**) was more difficult. Under several conditions (H_2 and **a**: Pt/C, AcOH; **b**: PtO_2, MeOH; **c**: Pd/C, MeOH; **d**: Pd/C, EtOH or N_2H_4, H_2O_2[20] or Bu_3SnH, $ZnCl_2$, $Pd(PPh_3)_4$[21] or Bu_3SnH, AIBN[22]) either no reaction occured or complex reaction mixtures were formed. Only with 10% Pd/C (EtOH, r.t., 36 h) the saturated epothilone analogs **12** could be isolated in 8% yield as a 2:1 mixture of epimers.[23]

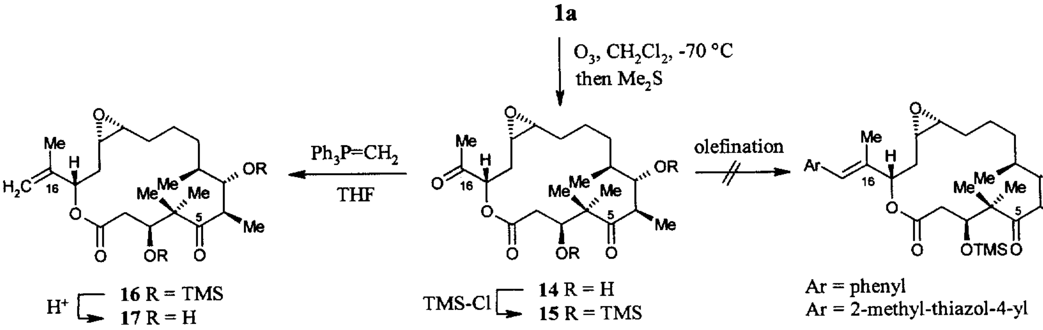
Scheme 5 Oxidative and reductive transformations of the C16–C17 double bond of epothilone **1a**.



Dihydroxylation of epothilone A (**1a**) under standard conditions (cat. OsO_4, N-methylmorpholine-N-oxid, *t*-BuOH/THF/water 8:3:1, r. t.)[7b,24] required 20 h to go to completion affording diol **13** as a 5:3 mixture of epimers in 80% yield. To our surprise, the minor epimer could not be purified on reversed phase HPLC because it undergoes rapid transesterfication to a C1–C16 or a C1–C17 macrolacton in protic solvent mixtures.

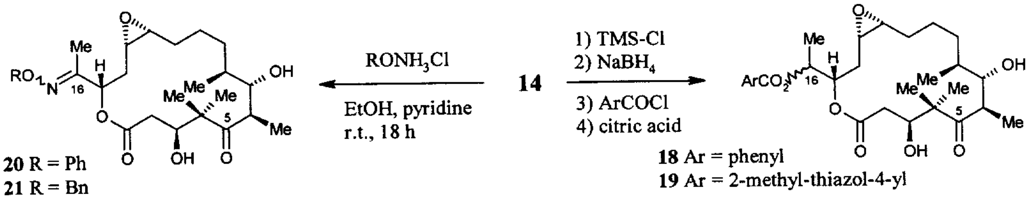
Ozonolysis (O_3, CH_2Cl_2, –70 °C, 10 min, then Me_2S) of epothilone A (**1a**)[25] proceeded smoothly affording ketone **14**. The hydroxy groups of **14** were immediately protected as trimethylsilyl ether (TMS-Cl, Et_3N, DMAP, CH_2Cl_2, r.t., 18 h) providing bis-TMS ether **15** in 70% overall yield (Scheme 6). Several attempts to reconstruct the double bond from the C16 keto group by olefination reactions were undertaken. Unfortunately, the only successful reaction was the methylenation of ketone **15** (→ **16**) using the commercially available „instant ylide".[26] Subsequent cleavage of the TMS ether (citric acid, MeOH, 65 °C, 1 h) produced derivative **17** in 15% overall yield. All other experiments either to rebuild epothilone A using a recently published procedure[27] (2-methyl-thiazol-4-yl-diphenylthioacetal, $Ti(Cp)_2Cl_2$, Mg, $P(OEt)_3$, THF, r. t., 30 min) or to construct a phenyl analog of epothilone under crown-ether catalysis[28] ($PhCH_2P(O)(OEt)_2$, NaH, 15-crown-5, THF, 0 °C → r. t., 20 h) failed to give the desired products.

Scheme 6 Side chain modification of **1a** *via* olefination reaction.



However, with ketone **14** some other transformations at the C16 carbonyl group were carried out. Regioselective reduction of the C16 keto group of TMS-protected ketone proceeded with $NaBH_4$ in MeOH at room temperature affording a 1:1 mixture of alcohol epimers (90%), which were (poorly) acylated with benzoylchloride or 2-methyl-thiazole-4-carbonylchloride and pyridine/dimethylaminopyridine (DMAP) in CH_2Cl_2. Cleavage of the TMS ethers (citric acid, MeOH, 80 °C) provided the esters **18** and **19** in 10 and 20 % overall yield (Scheme 7). On the other hand, treatment of the ketone with phenyloxy- or benzyloxyammoniumchloride and pyridine in EtOH at room temperature gave the corresponding oximes **20** and **21** in 50–60% yield as an approximately 2.5:1 mixtures of *E* and *Z* isomers (Scheme 7).

Scheme 7 Oxime and ester derivatives from C5, C16-diketone **14**.



All derivatives were tested against the L 929 mouse fibroblast cell line.[1b] Modifications of the C16–C17 double bond resulted in a 50–200 times loss (IC_{50} between 100 and 500 ng/ml) in activity, whereas changes at the macrocycle were more dramatic (IC_{50} between 1000 and 4000 ng/ml, exception acetal **3** (200 ng/ml)).

In conclusion, we have demonstrated, that regioselective oxidations at either C3 or C7 of epothilone A can be achieved with simple reagents. Reduction of epothilone or its C7 oxo product **2** with sodium borohydride always occurs preferentially at the C5 carbonyl but with poor stereoselection (d.r.: 50:50 – 60:40). The C16–C17 double bond of **1a** can be easily oxidized, but hydrogenation was difficult and succeeded only as a side reaction with H_2 and 10% Pd/C in EtOH. However, ozonolysis of the double bond of epothilone A proceeded smoothly and the resulting keto group was transformed to epothilone analogs with different side chains (oximes, esters).

ACKNOWLEDGEMENT

This paper is dedicated to Professor Dieter Seebach (Eidgenössische Technische Hochschule Zürich) on the occasion of his 60th birthday.

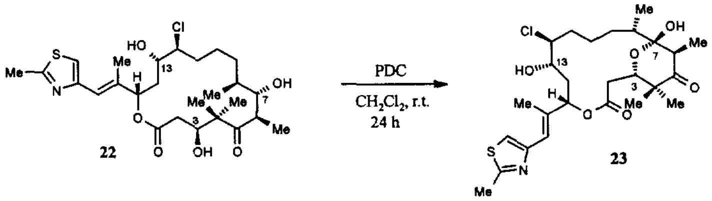
We are grateful to Dr. K. Gerth for fermentation and H. Steinmetz for isolation of epothilones and Dr. F. Sasse for biological tests. We thank Dr. V. Wray, B. Jaschok-Kentner and C. Kakoschke for recording NMR spectra, and A. Meier, S. Pohlan and A. Ritter for HPLC/MS analyses.

References and Notes

1. a) Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H.; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567–1569; b) Gerth, K.; Bedorf, N.; Höfle, G.; Irschik, H.; Reichenbach, H.; *J. Antibiot.* **1996**, *49*, 560–563.
2. For some recent articles about the biological activity of epothilones, see: a) Kowalski, R. J.; Giannakakou, P.; Hamel, E.; *J. Biol. Chem.* **1997**, *272*, 2534–2541; b) Wolff, A.; Technau, A.; Brandner, G.; *Int. J. Oncol.* **1997**, *11*, 123–126; c) Mühlradt, P. F.; Sasse, F.; *Cancer Res.* **1997**, *57*, 3344–3346.
3. a) Balog, A.; Meng, D.; Kamenecka, T.; Bertinato, P.; Su, D.-S.; Sorensen, E. J.; Danishefsky, S. J.; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801–2803; b) Su, D.-S.; Meng, D.; Bertinato, P.; Balog, A.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B.; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 757–759; c) Meng, D.; Bertinato, P.; Balog, A.; Su, D.-S.; Kamenecka, T.; Sorensen, E. J.; Danishefsky, S. J.; *J. Am. Chem. Soc.* **1997**, *119*, 10073–10092.
4. a) Yang, Z.; He, Y.; Vourloumis, D.; Vallberg, H.; Nicolaou, K. C.; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 166–168; b) Nicolaou, K. C.; Sarabia, F.; Ninkovic, S.; Yang, Z.; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 525–527; c) Nicolaou, K. C.; He, Y.; Vourloumis, D.; Vallberg, H.; Roschangar, F.; Sarabia, F.; Ninkovic, S.; Yang, Z.; Trujillo, J. I.; *J. Am. Chem. Soc.* **1997**, *119*, 7960–7973; d) Nicolaou, K. C.; Ninkovic, S.; Sarabia, F.; Vourloumis, D.; He, Y.; Vallberg, H.; Finlay, M. R. V.; Yang, Z.; *J. Am. Chem. Soc.* **1997**, *119*, 7974–7991.
5. Schinzer, D.; Limberg, A.; Bauer, A.; Böhm, O. M.; Cordes, M.; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 523–524.
6. a) Meng, D.; Su, D.-S.; Balog, A.; Bertinato, P.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B.; *J. Am. Chem. Soc.* **1997**, *119*, 2733–2734; b) Balog, A.; Bertinato, P.; Su, D.-S.; Meng, D.; Sorensen, E. J.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B.; *Tetrahedron Lett.* **1997**, *38*, 4529–4532; c) Su, D.-S.; Balog, A.; Meng, D.; Bertinato, P.; Danishefsky, S. J.; Zheng, Y.-H.; Chou, T.-C.; He, L.; Horwitz, S. B.; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2093–2096.
7. a) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Ninkovic, S.; Sarabia, F.; He, Y.; Vourloumis, D.; Zhang, Z.; Li, T.; Giannakakou, P.; Hamel, E.; *Nature* **1997**, *387*, 268–272; b) Nicolaou, K. C.; Vourloumis, D.; Li, T.; Pastor, J.; Winssinger, N.; He, Y.; Ninkovic, S.; Sarabia, F.; Vallberg, H.; Roschangar, F.; King, N. P.; Finlay, M. R. V.; Giannakakou, P.; Verdier-Pinard, P.; Hamel, E.; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2097–2103; c) Nicolaou, K. C.; Ninkovic, S.; Finlay, M. R. V.; Sarabia, F.; Li, T.; *Chem. Commun.*

1997, 2343–2344; d) Nicolaou, K. C.; Sarabia, F.; Ninkovic, S.; Finlay, M. R. V.; Boddy, C. N. C.; *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 81–84; e) Nicolaou, K. C.; He, Y.; Roschangar, F.; King, N. P.; Vourloumis, D.; Li, T.; *ibid.* **1998**, *37*, 84–87; f) Nicolaou, K. C.; Vallberg, H.; King, N. P.; Roschangar, F.; He, Y.; Vourloumis, D.; Nicolaou; C. G.; *Chem. Eur. J.* **1997**, *3*, 1957–1970; g) Nicolaou, K. C.; Sarabia, F.; Finlay, M. R. V.; Ninkovic, S.; King, N. P.; Vourloumis, D.; He, Y.; *ibid.* **1997**, *3*, 1971–1986.

8. Corey, E. J.; Suggs, J. W.; *Tetrahedron Lett.* **1975**, 2647–2650.
9. Corey, E. J.; Schmidt, G.; *Tetrahedron Lett.* **1979**, 399–402.
10. The preference for the C7 hydroxy group was also observed with chlorohydrin **22**. Despite an additional hydroxy group at C13, only C7 reacted with PDC to give hemiacetal **23** as the sole product (60%).



11. Careful inspection of the ^1H NMR spectrum of hemiacetal **2** revealed, that it containes approximately 5% of the bis-keto form.
12. The tri-oxo derivative **4** might be a result of initial C3-oxidation to compound **3**, which cannot close to an acetal and, thus, allowing oxidation of the remaining hydroxy group.
13. Mancuso, A. J.; Swern, D.; *Synthesis* **1981**, 165–185.
14. Medina, J. C.; Salomon, M.; Kyler, K. S.; *Tetrahedron Lett.* **1988**, *29*, 3773–3776. Exclusion of moisture was very important for short reaction times. Epothilone containing traces of water, needed 24 h at room temperature to go to completion, whereas dried material reacted within two hours.
15. With bromine in $CCl_4/CDCl_3$ the C12–C13 bromohydrin (3:1 mixture of regioisomers) was isolated in 45% yield caused by the formation of HBr during the reaction although *no* electrophilic substitution products could be detected. Using powdered sodium bicarbonate as buffer the C19-bromo analog of **1a** was obtained in 40% yield through an electrophilic bromination of thiazole moiety.
16. a) *Encyclopedia of Reagents for Organic Synthesis*, Paquette, L. A.(Ed.); John Wiley & Sons, Chichester, **1995**, p. 1192–1198 and references cited therein. See also refs 4–7 for its application on epothilones.
17. *ibid.*, p. 2061–2064, for preparation of DMDO, see: Adam, W.; Bialas, J.; Hadjiarapoglou, L.; *Chem. Ber.* **1991**, *124*, 2377–2379, for the reaction of epothilone C see also references 4c and 7b.
18. The major product of these reactions, which was isolated in up to 50% yield is an epothilone analog bearing an oxygen at the thiazole nitrogen, see, for similar examples, reference 4b.
19. Epothilone B (**1b**) gave upon epoxidation with *m*-CPBA up to 10% of the bis-epoxide and about 50% of a compound related to the side product obtained with epothilone A (**1a**).
20. For a recent review, see: Pasto, D. J.; Taylor, R. T.; *Organic Reactions* **1991**, *40*, 91–155.
21. a) Four, P.; Guibé, F.; *Tetrahedron Lett.* **1982**, *23*, 1825–1828. b) Cortese, N. A.; Heck, R. F.; *J. Org. Chem.* **1978**, *43*, 3985–3987.
22. Hutchinson, J.; Pattenden, G.; Meyers, P. L.; Searle, G. D.; *Tetrahedron Lett.* **1987**, *28*, 1313–1316.
23. The dominant (expected) reaction was the reductive cleavage of the allylic lactone at C15 followed by hydrogenation of the C16–C17 double bond.
24. VanRheenen, V.; Kelly, R. C.; Cha, D. Y.; *Tetrahedron Lett.* **1976**, 1973–1976.
25. Höfle, G.; Kiffe, M.; (GBF), Offenleg. DE 195 42 986 A1, **1997** [Chem. Abstr. **1998**, *127*, 50474]. Ozonolysis of epothilone B (**1b**) gave the corresponding keton in 50% yield.
26. Schlosser, M.; Schaub, B.; *Chimia* **1982**, *36*, 396–397.
27. The advantage of this procedure are the non-basic conditions in an attempt to avoid possible basic decomposition of the substrate. For a discussion, see: Horikawa, Y.; Watanabe, M.; Fujiwara, T.; Takeda, T.; *J. Am. Chem. Soc.* **1997**, *119*, 1127–1128.
28. Baker, R.; Sims, R. J.; *Synthesis* **1981**, 117.