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Derivatization of the C12–C13 functional groups of epothilones A, B and C

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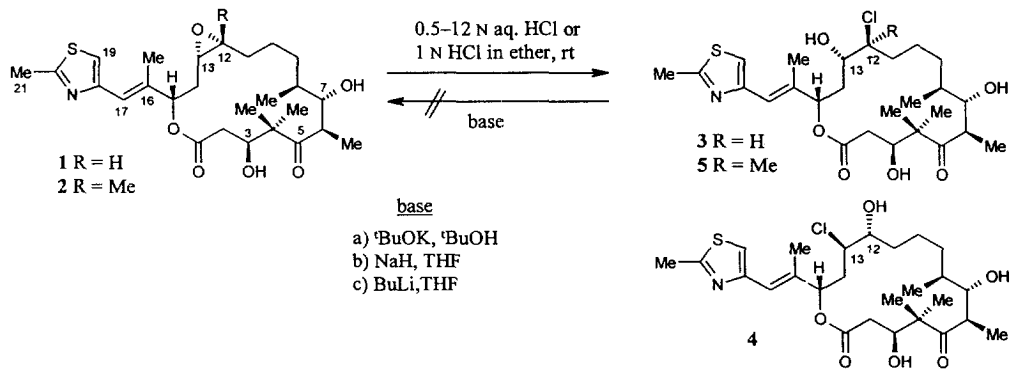
Abstract: Epothilone A reacted with hydrohalic acids to C12–C13 halohydrin regioisomers (ratios: 2:1 – 4:1), whereas epothilone B gave under the same conditions the isomerically pure C12 halo C13 hydroxy derivative. With non-nucleophilic Brønstedt acids and with Lewis acids a highly solvent dependent product distribution and some unexpected rearrangement products were observed. Epothilone C bearing a double bond between C12 and C13 was regioselectively dihydroxylated or hydrogenated at that position.

Epothilones[1] are currently of great interest because of their potential as anticancer agents. Thus, we have initiated a program for derivatization of epothilones to study its reactivity and structure activity relationship. In the preceeding letter, we described regioselective transformations of the oxidation state of C3, C5, C7 and of the exocyclic double bond. This paper deals with ring-opening reactions of the C12–C13 epoxide moiety of epothilone A (**1**) and B (**2**) induced by Brønstedt or Lewis acids and modifications of the C12-C13 double bond of epothilone C (**17**).

Dissolving epothilone A (**1**) in THF/aqueous HCl or in 1.0 M HCl in ether afforded in less than 20 minutes the chlorohydrin[2] regioisomers **3** and **4**[3] (ratios: 2:1 – 4:1, 60–80%) along with up to 20% of a mixture of C12–C13 diol diastereoisomers[4] (**9**, **10**, shown in Scheme 2). The ratio of isomers depends on the concentration of the acid. Higher concentrations of HCl usually gave lower selectivity. On the other hand, epothilone B (**2**) produced under the samer conditions the isomerically pure 12-chloro-13-hydroxy analog **5** in over 80% isolated yield.[5]

Scheme 1 Epoxide opening of epothilone A (**1**) and B (**2**) with hydrochloric acid.



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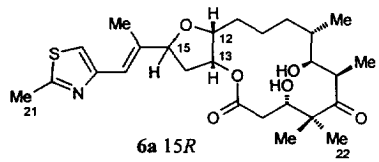
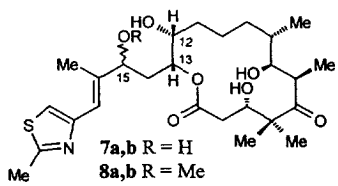
Unexpectedly, all attempts to reconvert the chlorohydrins **3–5** to epoxides **1** and **2** under basic conditions (*t*-BuOK, *t*-BuOH, 50 °C; NaH, THF, r. t.; *n*-BuLi, THF, –70 °C) failed. The macrocycle in the chlorohydrins presumably relaxed by rotation around the C12–C13 bond positioning OH and Cl in a *cisoid* orientation from which elimination of HCl cannot occur.

Treatment of epothilone A and B with non-nucleophilic Brønstedt acids [e. g. H_2SO_4, trifluoroacetic acid (TFA)] or with Lewis acids like $BF_3{\cdot}Et_2O$ resulted in much more complex product mixtures. Schemes 2 and 3 show all isolated and identified derivatives, obtained from the reaction of **1** and **2** with acids under conditions listed in Tables 1 and 2.

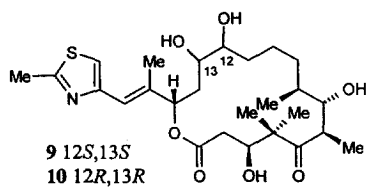
Scheme 2 Products obtained by the Brønstedt and Lewis acid catalyzed epoxide opening of epothilone A (**1**).



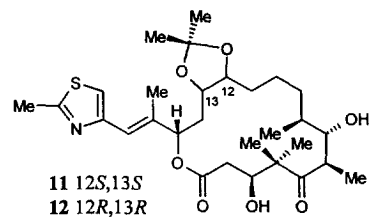
6a 15*R*
6b 15*S*



7a,b R = H
8a,b R = Me



9 12*S*,13*S*
10 12*R*,13*R*



11 12*S*,13*S*
12 12*R*,13*R*

Table 1. Conditions and product distribution of the reactions of **1** with Brønstedt and Lewis acids.

entry	conditions	yield (%)[a]								
		6a	**6b**	**7a**	**7b**	**8a**	**8b**	**9**	**10**	and
1	0.65 M TFA, acetone, 50 °C, 10 h	50	35	-	-	-	-	-	-	
2	0.65 M TFA, water, 23 °C, 48 h	-	-	12	6	-	-	55	15	
3	0.40 M TFA, MeOH, 23 °C, 68 h	12	18	4	4	15	10	-	-	
4	1.0 M H_2SO_4, water, 23 °C, 30 min[b]	-	-	24	6	-	-	48	12	
5	0.04 M $BF_3{\cdot}Et_2O$, Et_2O/THF, 23 °C, 8 h	30	20	23	15	-	-	-	-	
6	0.04 M $BF_3{\cdot}Et_2O$, acetone, 23 °C, 24 h	43	27	4	4	-	-	4	5	**11** (5)
7	0.16 M $BF_3{\cdot}Et_2O$, MeOH, 23 °C, 72 h	19	20	-	-	10	6	-	-	
8	0.04 M $BF_3{\cdot}Et_2O$, $CH_2(OMe)_2$, 23 °C, 72 h	10	-	-	-	-	-	-	-	MOM ether (70)[c,d]
9	0.1 M $LiClO_4$, benzene, 55 °C, 20 h	no reaction								recovered **1**

[a] Yields of isolated product. [b] Addition of THF or MeOH produced more of the undesired diols **8a,b**. [c] Several mono, di and tri MOM ether were detected by HPLC-MS. [d] Combined yield, based on integration of the corresponding HPLC peaks.

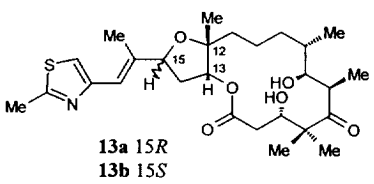
Epothilone A (**1**) on treatment with TFA in acetone (entry 1) gave after 20 h at 50 °C two products with different polarities than the one of the starting material but the same molecular mass (493) according to HPLC-MS. To establish the structure of these new compounds 1D and 2D NMR data have been evaluated. They revealed – to our surprise – that both products are epimers of a macrobicycle (**6a**, **6b**) in which the lacton unit has migrated to C13.[6] The unusual difference in polarity of the two epimers is caused by an intramolecular hydrogen bond between C3-OH and the thiazole nitrogen of the less polar epimer **6a**.[7]

In 5% aqueous TFA solution **1** dissolved within a few minutes at room temperature (entry 2). TLC of the colourless solution showed one sharp spot which corresponded with the most polar TLC-spot obtained in the reactions of epothilone with aqueous HCl solutions. The ^{1}H NMR spectrum of purified material (reversed phase HPLC, MeOH:H_2O 6:4, one peak!) revealed the presence of four similar compounds in a ratio of 4.0 : 1.0 : 1.0 : 0.5 with identical molecular masses (511 = epothilone + water). Separation of this mixture was possible by reversed phase HPLC in THF:H_2O 1:3 affording the expected diols **9** and **10** and the rearranged diols **7a**, **7b**,. The same compounds were obtained with H_2SO_4 in water (entry 4), although formation of the rearranged products was favoured.[8]

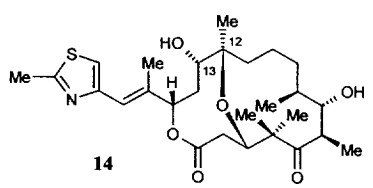
In methanol epothilone A (**1**) was transformed with either TFA or $BF_3{\cdot}Et_2O$ exclusively to the rearranged analogs **6**, **7** and **8** (entry 3 and 7). $BF_3{\cdot}Et_2O$ in Et_2O/THF or acetone (entry 5 and 6) afforded predominantly **6** along with diols **7**. With acetone as solvent, diols **9** and **10** and acetonide **11**[9] were obtained as minor components. The only detectable compound of known structure in the reaction of **1** with $BF_3{\cdot}Et_2O$ in $CH_2(OMe)_2$ was derivative **6a** beside several mono, di and tri MOM ether of the starting material or of diol intermediates (entry 8). No reaction was observed with lithium perchlorate as Lewis acid (entry 9).[10]

With epothilone B (**2**), aqueous solutions of TFA or H_2SO_4 provided the diastereomerically pure diol **16** in 75 and 45% yield, respectively. Only traces of C13 rearranged derivatives **15** or other isomers were detected by HPLC-MS (entry 2 and 3). This result is similar to that obtained with aqueous HCl solutions and displays the different reactivities of a second *vs.* tertiary carbon in **2**.[5] In TFA,acetone or $BF_3{\cdot}Et_2O$,THF/Et_2O two other compounds beside the rearranged derivatives **13a** and **13b** were isolated having the same molecular masses as the starting material. One of them was identified as the tricyclus **14** with an internal ether group between C3 and C12. The structure of the other compound remained uncertain (entry 1 and 4).

Scheme 3 Products obtained by the Brønstedt and Lewis acid catalyzed epoxide opening of epothilone B (**2**).



13a 15*R*
13b 15*S*



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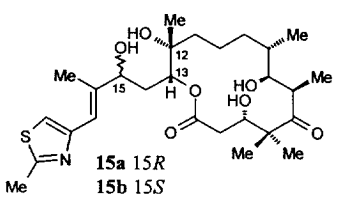

15a 15*R*
15b 15*S*

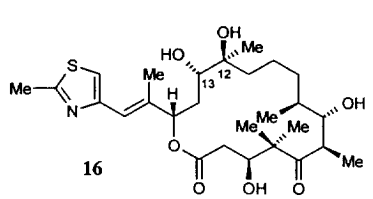

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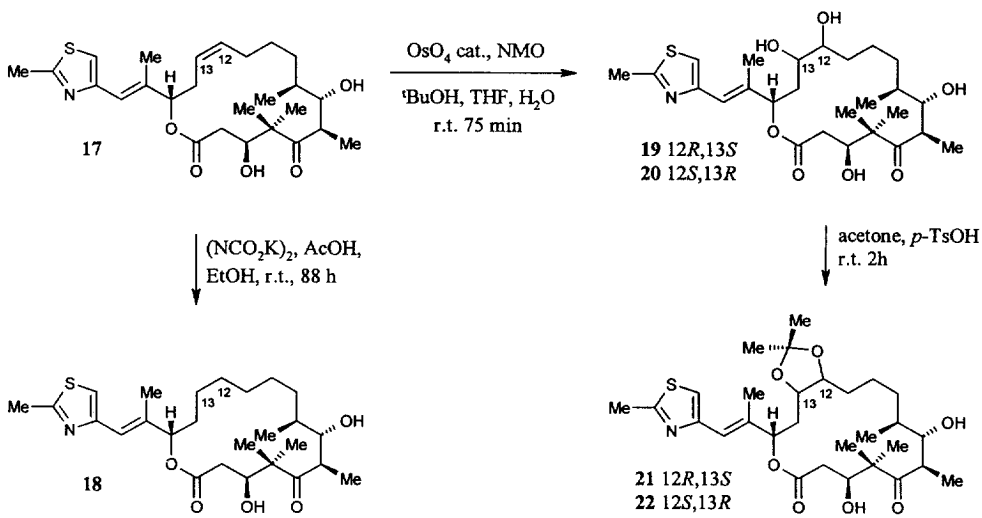
Table 2. Conditions and product distribution of the reactions of **2** with Brønstedt and Lewis acids.

entry	conditions	yield (%)[a]						
		13a	**13b**	**14**	**15a**	**15b**	**16**	and
1	0.65 M TFA, acetone, 50 °C, 2 h	30	15	25	1	1		C7-C12 ether?[b]
2	0.65 M TFA, water, 23 °C, 3.5 h						75	
3	1.0 M H_2SO_4, water/THF, 60 °C, 2 h						45	
4	0.04 M $BF_3{\cdot}Et_2O$, Et_2O/THF, 23 °C, 8 h	18	12	8	13	2		C7-C12 ether?[b]

[a] Yields of isolated products. [b] Not enough material was available for a complete struture analysis.

In epothilone C (**17**) two double bonds (C12–C13 and C16–C17) are present. The reduced reactivity of the C16–C17 double bond towards oxidation or reduction (see the preceeding letter) makes a regioselective functionalization of the C12–C13 double bond in this epothilone possible.[2,11] Thus, treatment of **17** with diimine,[12] generated by decomposition of the potassium salt of diazocarboxylic acid with AcOH at room temperature, produced regioselectively the C12–C13 saturated derivative **18** in 60% yield (98% based on recovered starting material). Dihydroxylation of **17** (cat. OsO_4, NMO, ^tBuOH, THF, H_2O, r. t., 75 min)[13] also occured with high preference for the electron rich C12–C13 double bond, although progress of the reaction must be monitored steadily to avoid overoxidation. The isomeric *cis*-diols **19** and **20** were obtained as a 2:1 mixture in 62% combined yield.[14] Suqsequent acetalization (acetone, cat. *p*-TsOH, r. t., 2 h, 90%) of the epimeric mixture gave the acetonides **21** and **22** which were easily separated by reversed phase HPLC.

Scheme 4 Regioselective dihydroxylation and reduction of the C12–C13 double bond in epothilone C (**17**).



All derivatives described in this paper show only weak cytotoxic activity against the L 929 mouse fibroblast cell line[1b] (70 – 4000 times less than epothilone A). Particularly low activities were observed with all rearranged

products (IC_{50} between 2000 and 4000 ng/ml) and with compounds having a flexible C12–C13 bond. For example, diol **9** has an IC_{50} value of >4000 ng/ml, the corresponding acetonide **11** an IC_{50} value of 150 ng/ml and epothilone C (**17**) an IC_{50} value of 100 ng/ml.

In summary, we have presented acid catalyzed ring opening reactions of the epoxide moiety of epothilone A and B. The product distribution was highly dependend on the nature of the acid and the solvent. An unexpected lactone rearrangement occured during some of those reactions. Epothilone C was dihydroxylated with OsO_4 and saturated with diimine at the C12–C13 double bond regioselectively.

ACKNOWLEDGEMENT

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

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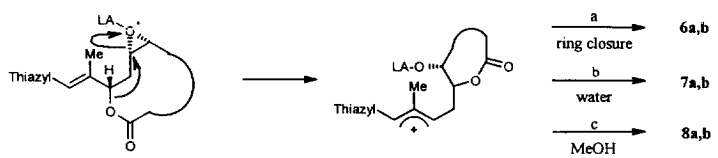
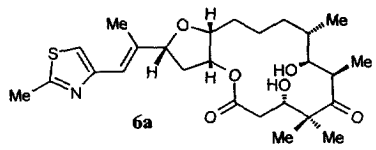
References and Notes

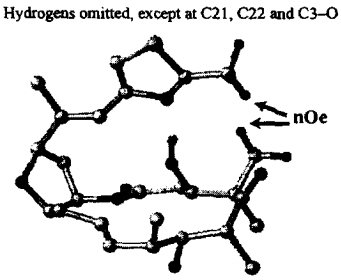
1. a) Höfle, G.; Bedorf, N.; Steinmetz, H.; Schomburg, D.; Gerth, K.; Reichenbach, H.; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567–1569; b) Gerth, K.; Bedorf, N.; Höfle, G.; Irschik, H.; Reichenbach, H.; *J. Antibiot.* **1996**, *49*, 560–563.
2. Höfle, G.; Kiffe, M.; (GBF), Offenleg. DE 195 42 986 A1, **1997** [Chem. Abstr. **1998**, *127*, 50474]; Höfle, G.; Kiffe, M.; (GBF), Offenleg. WO 97 19086, **1998** [Chem. Abstr. **1998**, *127*, 81289]. The bromohydrin and iodohydrin congeners were obtained „accidently" by treatment of **1** with bromine or iodine in $CCl_4/CDCl_3$ in 45 and 25% yield, respectively, and with similar regioselectivity (ratio: ~75:25 in favour of C12–Br(I) and C13–OH).
3. The regioisomers were separated on reversed phase HPLC (C_{18}, THF:H_2O 3:7) and identified through the COSY spectra of their triformyl esters which allowed to distinguish between the C12 and C13 formiate.
4. Concentrated aqueous HCl in THF allmost and 1.0 M HCl in Et_2O completely excluded the formation of diol congeners.
5. The tertiary center at C12 of epothilone B clearly favours heterolytic ring opening of the epoxide at this position under acidic conditions, following by an attack of the nucleophile on the opposite side of the hydroxy group. In epothilone A only the steric and electronic differences between C12 and C13 are weaker.
6. This rearrangement can be easily explained as shown in the graphic below: Lewis acid activation of the epoxide weakens the bonds between the oxygen and C12 and C13, respectively. Assisted by the carboxyl group, which is in an good steric position to migrate, the C13–O bond breaks and a C13 lacton is formed under inversion of the stereocenter. This leaves a cation stabilized through the double bond and the thiazole moiety. Intramolecular ring closure in aprotic solvents or trapping of the cation with protic solvents then lead to the products **6**, **7**, and **8** as mixtures of epimers at C15.



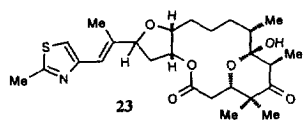
7. Evidence for the hydrogen bond are: 1) the ^{1}H NMR shift of the C3-OH (5.9 ppm in **6a** and 3.0 ppm in **6b**), 2) the nOe between C21–H (methyl group at the thiazole moiety) and C22–H (methyl group at C5), and 3) a decreased polarity (less than that of epothilone A (**1**) and **6b**). This hydrogen bond gives the molecule a scorpion-like shape (shown below) and allows the specification of the unkown asymmetric center at C15 (*R* for **6a** and *S* for **6b**).



6a



8. The ratio of diols **9**/**10** *vs.* rearranged diols **7a**/**7b** is also dependent of organic co-solvents and of the temperature. Higher concentrations of organic solvent and higher temperature lead to more rearranged products **7a**,**b**.
9. Acetonides **11** and **12** were more conveniently prepared in a two step sequence: 1) hydrolysis of **1** in aqueous sulfuric acid to the diols **9** and **10** as described above, and 2) acetalization (acetone, cat. *p*-TsOH, r. t., 90 min) in good overall yield (>80%).
10. We found that Dess-Martin oxidation of **1** (periodinane, CH_2Cl_2, r. t., 3 weeks) produced the rearranged and C7 oxidized analog **23** in 75% yield, see D. B. Dess, J. C. Martin, *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287.



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11. For example, bromine in presence of powdered sodium bicarbonate as buffer produced the C19-bromo analog of epothilone **1** in 40% yield.
12. For a recent review, see: Pasto, D. J.; Taylor, R. T.; *Organic Reactions* **1991**, *40*, 91–155.
13. VanRheenen, V.; Kelly, R. C.; Cha, D. Y.; *Tetrahedron Lett.* **1976**, 1973–1976.
14. For a discussion about the stereoselectivity on epoxidation of epothilone C, see: 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) Yang, Z.; He, Y.; Vourloumis, D.; Vallberg, H.; Nicolaou, K. C.; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 166–168; c) Höfle, G.; *et al.*, *Angew. Chem. Int. Ed. Engl.*, in preparation.