SUBSTITUTIONS AT THE THIAZOLE MOIETY OF EPOTHILONE

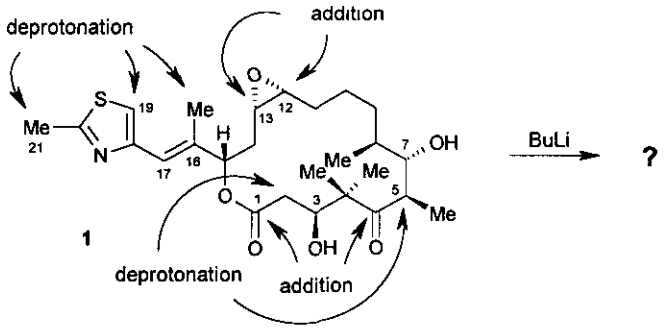
Michael Sefkow* and Gerhard Höfle

Gesellschaft für Biotechnologische Forschung mbH, Abt. Naturstoffchemie, Mascheroder Weg 1, D-38124 Braunschweig, Germany

Abstract – Epothilone A is metalated at low temperature with an excess of butyllithium preferentially at C19 of the thiazole moiety. After addition of various carbon and heteroatom electrophiles the corresponding substitution products were obtained. Some of them have similar cytotoxic activity than the starting material.

Epothilones are currently of great interest due to their potential as anticancer agents.[1,2] In previous papers we reported various derivatizations of the macrocycle and of the adjacent double bond of epothilones.[3] Modification of the thiazole moiety of epothilone A (**1**) *via* a selective deprotonation-electrophile addition sequence should easily access a variety of analogs to study structure activity relationships, although it was difficult to predict whether strong nucleophilic bases like BuLi deprotonate the thiazole group regioselectively (Scheme 1).

Scheme 1 *Possible reaction sites of epothilone A (1) with nucleophilic bases.*



Thus in a first experiment, epothilone A (**1**) was treated with 5 equivalents of BuLi in THF at –90 °C. The solution soon turned orange and after 15 minutes acetic acid-d_4 was added. TLC of the pale yellow reaction mixture showed just starting material and two minor spots. After usual workup and chromatographic separation three fractions were obtained in 70, 10 and 5% yields.

* New address: Universität Potsdam, Institut für Organische Chemie, Am Neuen Palais 10, D-14469 Potsdam, Germany, e-mail: sefkow@rz.uni-potsdam.de, FAX: +49 331 977 1399.

Comparison of the ^{1}H NMR spectra of **1** and the isolated epothilone revealed an incorporation of only one deuterium (at C19) in about 30% (confirmed by HPLC-MS). No deuterium was detected at C21, indicating that deprotonation occurred under kinetic control at –90 °C, because metalation of C21 should be favoured under thermodynamic conditions.[4] Only one addition product of BuLi to **1** could be identified. This compound was an open chain butyl ketone (5%) resulting from an attack of BuLi to the lactone unit.[5,6]

Epothilone A (**1**) was then lithiated at –90 °C for 10–15 min and reacted with a variety of electrophiles. Interestingly, carbon electrophiles gave not only C19 modified derivatives (**2.x**) but also C21 alkylation products (**3.x**) as minor components. On the other hand, very hard electrophiles gave only the C7 esters (**4.x**) (Scheme 2 and Table 1).

Scheme 2 *Epothilone derivatives obtained by electrophile addition to metalated 1.*

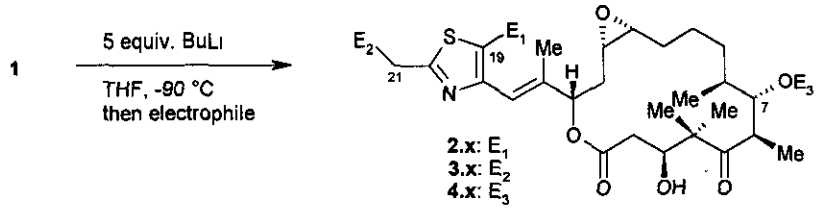


Table 1 *Product distribution of the reaction of lithiated 1 with various electrophiles.*

entry (x)	electrophile	product	yield (%)[a] 2.x	3.x	4.x
1	CCl_4	–Cl	50 (76)	–	–
2	NBS	–Br	15 (50)	–	–
3	I_2	–I	25 (50)	–	–
4	$EtO_2CN{=}NCO_2Et$	$-N(CO_2Et)NHCO_2Et$	50 (83)	–	–
5	Me_2S_2	–SMe	25 (63)	–	–
6	O_2	–OOH	traces[c]		–
7	$PhSO_2N(O)CHPh$	–OH	traces[c]		–
8	$(PhCO_2)_2$	–COPh	–	–	11 (73)
9	$NO^+BF_4^-$	–NO	–	–	8 (75)
10	$PhSO_2N{=}CHPh$	$-CH(Ph)NHSO_2Ph$[b]	40 (66)	~7 (12)[c]	–
11	PhCHO	$-CH(OH)Ph$[b]	40 (66)	8 (13)	–
12	EtI[d]	–Et	–	8 (40)	–
13	MeI[d]	–Me	30 (60)	6 (12)	–
14	$HCONMe_2$	–CHO	30 (63)[e]	–	–
15	Me_3SiCl	$-SiMe_3$	35 (65)	–	–

[a]Yields of isolated products (in parentheses based on recovered starting material). [b]1:1 mixture of diastereoisomers at C1'. [c]Based on HPLC–MS. [d]Warmed to –70 °C prior to addition of electrophile and to –30 °C prior to quenching. [e]Contaminated with a compound of unknown structure (neither **3.13** nor **4.13**).

Halogenation of epothilone A (**1**) exclusively occurs at C19 if the lithiated species is quenched with either elemental iodine (→ **2.3**) or *N*-bromosuccinimide (→ **2.2**)[7] or tetrachloromethane (→ **2.1**)[8] in up to 50%

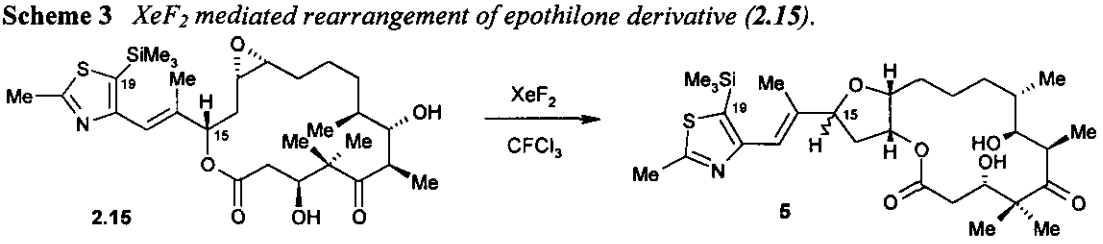
yield (entries 1–3). Surprisingly, no electrophilic fluorination reagent was found to successfully introduce a fluorine at C19, although several of the new reagents developed in the last years were tested.[9] A C19 nitrogen and a sulfur analog of epothilone was obtained using neat diethyl azodicarboxylate (→ **2.4**) or dimethyl disulfide (→ **2.5**) as electrophile in 50 and 25% yield (entries 4 and 5).

On the other hand, oxygenation of the lithiated thiazole group was found to be as difficult as fluorination. With molecular oxygen or Davis' oxaziridine[10] only traces of compounds having the expected molecular masses of 525 and 509 were detected by HPLC-MS, and dried dibenzoylperoxide as electrophile afforded only the C7 benzoylated compound (**4.8**) albeit in low yield (entries 6–8). Esterfication at C7 was also observed when lithiated **1** was quenched with nitroso tetrafluoroborate (→ **4.9**, 8%)[12] (entry 9).

A different result was obtained when carbon electrophiles were employed (entries 10–13). In most cases not only the C19 (**2.x**) but also the C21 (**3.x**) alkylated derivatives were isolated in ratios of 4–5 : 1 (**2.x** : **3.x** based on ^{1}H NMR analysis of the crude products).[13] Benzylidenetoluenesulfonimine and benzaldehyde produced the C19 derivatives (**2.10**) and (**2.11**) in 40% yield as a 1:1 mixture of epimers at C1'. Only the benzaldehyde adducts (**2.11**) and (**3.11**) were separated, affording pure alcohol (**3.11**) in 8% yield.[14] Alkylations of C19 and C21 were performed with EtI and MeI in 8 (**3.12**), 30 (**2.13**) and 6% (**3.13**) yields. Interestingly, with EtI the C21 alkylation product (**3.12**) was formed exclusively. Carbonylation of C19 was achieved with DMF providing aldehyde (**2.14**) in about 30% yield but this material was contaminated with an unkown compound (4%) which is not the C21 aldehyde. In order to increase the yield of the C21 alkylation products we intended to block C19 reversibly with a suitable electrophile in a first metalation/addition sequence. After a second deprotonation/addition procedure alkylation should occur exclusively at C21.[15] Thus, C19 was protected with a trimethylsilyl group as described and, subsequently, another five equivalents of BuLi were added. The reaction mixture was then quenched with MeI but only the C19 TMS derivative (**2.15**) was isolated in 35% yield (entry 13).

With the TMS compound (**2.15**) an alternative route to a C19 fluorinated epothilone derivative seemed to be possible: According to a recently published paper, aryltrimethylsilanes are cleaved to the corresponding aryl fluorides with XeF_2 in fluorinated solvents like $CFCl_3$.[16] Unfortunately, XeF_2 gave with compound (**2.15**) in $CFCl_3$ not the desired fluoro derivative but acted as *Lewis* acid and epimers of rearrangment product (**5**) similar to those described previously by us[3b] were isolated in 50% yield (Scheme 3).

Scheme 3 *XeF_2 mediated rearrangement of epothilone derivative (2.15).*



All compounds of the **2.x** series with modification at C19 exhibit only weak cytotoxic activity (IC_{50} 1000 – 2000 ng/mL) against the L 929 mouse fibroblast cell line.[1b] On the other hand, functionalization at C21 is much less critical. Thus, **3.11**, **3.12** and **3.13** gave IC_{50} values of 10, 50 and 10 ng/mL, respectively, compared with 4 ng/mL for epothilone A (**1**). C7-Nitrite (**4.8**) was also very active (IC_{50} 15 ng/mL).

ACKNOWLEDGEMENT

This paper is dedicated to Prof. 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.

FOOTNOTES AND REFERENCES

1. (a) G. Höfle, N. Bedorf, H. Steinmetz, D. Schomburg, K. Gerth, and H. Reichenbach, *Angew. Chem., Int. Ed. Engl.*, **1996**, *35*, 1567; (b) K. Gerth, N. Bedorf, G. Höfle, H. Irschik, and H. Reichenbach, *J Antibiot*, **1996**, *49*, 560.
2. For some recent reports on that subject, see: (a) D. Meng, P. Bertinato, A. Balog, D.-S. Su, T. Kamenecka, E. J. Sorensen, and S. J. Danishefsky, *J. Am. Chem. Soc.*, **1997**, *119*, 10073; (b) K. C. Nicolaou, F. Sarabia, S. Ninkovic, M. R. V. Finlay, and C. N. C. Boddy, *Angew. Chem., Int. Ed. Engl.*, **1998**, *37*, 81; c) K. C. Nicolaou, Y. He, F. Roschangar, N. P. King, D. Vourloumis, and T. Li, *ibid.*, **1998**, *37*, 84; for a review, see: K. C. Nicolaou, F. Roschangar, and D. Vourloumis, *Angew. Chem., Int. Ed. Engl.*, **1998**, 37, 2014.
3. (a) M. Sefkow, M. Kiffe, D. Schummer, and G. Höfle, *Bioorg. Med. Chem. Lett.*, **1998**, *8*, in press; (b) M. Sefkow, M. Kiffe, and G. Höfle, *ibid.*, **1998**, in press.
4. For a discussion of thermodynamic vs. kinetic control of metalation of 2-methylthiazoles, see: J. Liebscher in Houben-Weyl, *Methoden der Organischen Chemie*, Bd. E8b, Thieme Stuttgart, **1993**, p. 309. For procedures of a selective metalation of substituted thiazoles, see: G. Knaus and A. I. Meyers, *J. Org. Chem.*, **1974**, *39*, 1189, 1192.
5. The second fraction was still a mixture of at least two compounds whose structures remain unknown.
6. Epothilone (**1**) did not reacted with five equivalents of MeMgBr in Et_2O/THF between –80 and 0 °C. Warming to room temperature resulted in a complex product mixture. HPLC-MS analysis of the crude material revealed several peaks having a molecular mass of 509 (epothilone A + CH_4) as a result of addition reactions to several functional groups.
7. The same compound was obtained in about 30% yield, if epothilone was treated with elemental bromine in presence of $NaHCO_3$ ($CDCl_3$, 0 °C).
8. In one experiment, **1** was metalated and accidently quenched with a 0.1 M solution of iodine in CCl_4 producing the C19 chloro and C19 iodo analogs in a ratio of 3.2:1 and in over 70% combined yield. For a general use of CCl_4 for halogenations of carbanions, see: R. T. Arnold and S. T. Kulenovic, *J. Org. Chem.*, **1978**, *43*, 3687.
9. For recent overviews of electrophilic fluorine reagents see: (a) L. S. German and S. Zemskov, *New Fluorinating Agents in Organic Synthesis*, Springer, Berlin, **1989**; (b) R. Bohlmann in *Organic Synthesis Highlights II*, H. Waldmann *Ed.*, VCH-Verlag, Weinheim, **1995**.
10. F. A. Davis, J. Lamendola, Jr., U. Nadir, E. W. Kluger, T. C. Sedergran, T. W. Panunto, R. Billmers, R. Jenkins, Jr., I. J. Turchi, W. H. Watson, J. S. Chen, and M. Kimura, *J. Am. Chem. Soc.*, **1980**, *102*, 2000.
11. The structures of the compounds with the masses 525 and 509 were not identified. Our assumption is based on the deprotonation behaviour of epothilone, the mass peak and the UV spectra of the products.
12. The preference for C7 oxyanion over C19 carbanion is probably an effect of the hardness of electrophiles (R. G. Pearson, *J. Chem. Educ.*, **1987**, *64*, 561).
13. The regioisomers (**2.x**) and (**3.x**) were only separable by reversed phase HPLC in THF/H_2O solvent systems. Other solvent mixtures or chromatographic procedures failed to separate the isomers.
14. According to the ^1H NMR spectrum the product isolated is diastereomerically pure. Either the second epimer was separated during workup or no splitting of signals can be observed in this case.
15. For a similar example, see ref. 4, p. 268.
16. A. P. Lothian and C. A. Ramsden, *Synlett*, **1993**, 753.

Received, 31st August, 1998