Cover Picture

Teresa Carlomagno,* Marcel J. J. Blommers,* Jens Meiler, Wolfgang Jahnke, Thomas Schupp, Frank Petersen, Dieter Schinzer, Karl-Heinz Altmann, and Christian Griesinger*

The powerful antitumor agents, the epothilones, bind to tubulin and interfere with microtubule dynamics. Until now, no high-resolution structure for the tubulin–epothilone complex was available. NMR spectroscopy studies provide a high-resolution structure of epothilone bound to tubulin and show two major conformational changes of epothilone relative to the free conformation. These results correlate well with chemical-modification data. These findings and NMR techniques are discussed in the Communications by Carlomagno and co-workers on pp. 2511 – 2517.

Structure of Bound Epothilone

The High-Resolution Solution Structure of Epothilone A Bound to Tubulin: An Understanding of the Structure–Activity Relationships for a Powerful Class of Antitumor Agents**

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Most of the drugs available today for the treatment of cancer are based on the inhibition of cell proliferation and induction of cell death by apoptosis. At the molecular level, the majority of cytotoxic agents interfere with DNA function. However, a prominent subclass of compounds, including paclitaxel (taxol), exert their cytotoxic activity by perturbing microtubule dynamics. In 1993, Höfle, Reichenbach, and coworkers reported the isolation and characterization of a novel class of cytotoxic polyketide macrolides from the myxobacterium *Sorangium cellulosum*, which were named epothilones (**1**).[1,2] The interest in these natural products immediately soared when it was found that epothilones are microtubule stabilizers and that they inhibit cell proliferation through a mechanism of action analogous to that of the renowned clinical anticancer drug taxol.[3] Epothilones exhibit extraordinary antiproliferative activity in vitro and they efficiently induce cell death in paclitaxel-resistant tumor cell lines at up to 5000-fold

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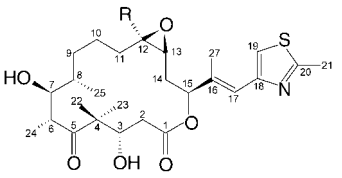

1a: R = H (epothilone A)
1b: R = CH_3 (epothilone B)

lower concentrations than taxol.[3–5] In addition, they are more soluble than taxol in water,[2,4] which allows delivery in vivo with non-chremaphor-containing formulation vehicles, thus eliminating formulation-based side effects (major hypersensitivity reactions (HSR)).[6] The potential clinical utility of epothilones is supported by in vivo experiments with epothilone B in a variety of nude mouse human-tumor models.[4,7]

Shortly after the elucidation of the absolute stereochemistry of epothilone B by a combination of X-ray crystallography and chemical degradation studies,[2] routes for the total synthesis of the epothilones were devised.[8–10] To date, more than 20 total syntheses of epothilones A and B have been published.[8–14] At the same time several hundred analogues have been prepared and their biological activity investigated, which has led to a remarkably comprehensive understanding of the structure–activity relationships (SAR) for epothilone-derived structures.[11,12,15,16] Recently, the structure of a 2D tubulin polymer sheet in complex with docetaxel (taxotere) was solved at 3.7-Å resolution by electron crystallography.[17] However, in the absence of information on the bioactive conformation of epothilones any 3D model of the tubulin–epothilone complex remains uncertain.[18–23]

Herein we describe the conformation of epothilone A bound to tubulin, as determined by NMR spectroscopy in aqueous solution. This structure provides the first piece of experimental information on the active conformation of this class of microtubule stabilizers. The conformational differences observed between the X-ray crystal structure and the tubulin-bound conformation of epothilone A allow the rationalization of the biological data available on the tubulin-polymerization activity of several epothilone derivatives.

Both epothilones A and B, the two major naturally occurring epothilone variants, displace ^{3}H-taxol from tubulin ($K_i = 1.4$ and 0.71 μM for epothilone A and epothilone B, respectively),[5] indicating that the tubulin-binding sites of the epothilones and taxol overlap.

The high molecular weight of the tubulin–epothilone complex (~100 kDa) renders a tight complex a difficult target for NMR-based structural studies. However, if the binding of the ligand to the receptor is weak ($K_d > 10$ μM, $K_{off} > 100$ Hz), transferred nuclear Overhauser enhancement (trNOE)[24] and transferred cross-correlated relaxation (trCCR)[25,26] methods can be employed to unravel the conformation of epothilones in complex with tubulin. When a ligand is in fast exchange with the receptor, its spectroscopic properties result from the weighted average of those of the free and the bound conformation. NOE interactions and CCR rates are directly proportional to the correlation time of the molecule, and their average is governed by the contribution of the bound conformation, as a result of the considerably larger correlation time of the complex relative to the free ligand. Fortunately, the kinetic and thermodynamic properties of the complex between epothilone A and tubulin are in the desirable range for trNOE and trCCR experiments, as indicated by the negative sign of the NOE cross-peaks in a 100:1 mixture of epothilone A with tubulin. The trNOE and trCCR data are in agreement with a K_d in the range of 10–100 μM. Evidence for *specific* binding of epothilone A to tubulin is provided by the restoration of the NOE spectrum of free epothilone A upon addition of epothilone B to the mixture, which proves the quantitative displacement of epothilone A from tubulin by the tighter binder epothilone B. The existence of specific and transient binding of epothilone A to tubulin enables the structural analysis of the active conformation of the epothilones by NMR.

A crucial provision for the structural investigation of the epothilone–tubulin complex in solution is the prevention of tubulin polymerization. Electron microscopy and gel electrophoresis show that substitution of Mg^{2+} with Ca^{2+}[27] and D_2O as solvent[28] are sufficient to prevent tubulin polymerization. Samples were stable for approximately one week.

The tubulin-bound conformation of epothilone A was calculated from 46 interproton-distance restraints and seven torsion-angle restraints measured for a 0.5 mM solution of epothilone A in water in the presence of 5 μM tubulin. The distance restraints were derived from transferred NOE experiments. To filter out spin-diffusion-mediated peaks, only those signals with opposite sign to the diagonal peaks in a transferred ROESY experiment were taken into account. The dihedral-angle restraints were obtained by measuring CH–CH dipolar–dipolar and CH–CO dipolar–CSA transferred CCR rates for 60–70% ^{13}C-labeled epothilone A. The transferred cross-correlated relaxation (trCCR) experiments[25,26] were indispensable to obtain a unique description of the bound conformation, as more than one structure of the macrolide ring is compatible with the same H–H distance set (NOE intensities). The seven trCCR rates defined the torsion angles O1-C1-C2-C3, C1-C2-C3-C4, C2-C3-C4-C5, C5-C6-C7-C8, C12-C13-C14-C15, C13-C14-C15-O1, and C14-C15-C16-C17. One particular problem in the determination of the bound conformation of epothilone was the stereospecific assignment of the two methyl groups at C4, which turned out to be opposite to the published assignment (see Experimental Section for details).[2]

The tubulin-bound conformation of epothilone A is shown in green in Figure 1 and is compared with the free (unbound) conformation of epothilone A determined by X-ray crystallography,[29] which is shown in gray. We chose to compare the tubulin-bound conformation of epothilone with the X-ray structure and not with the solution structure available in CD_2Cl_2, because of the extensive flexibility of epothilone in solution in the absence of tubulin.[30] However, the most populated conformer in solution is indeed very similar to the X-ray conformer. The position of the thiazole nitrogen atom (blue in Figure 1) and of the 3-OH group (red), which are important for the delineation of a pharmocophore model, change significantly upon binding.

A comparison of the torsion angles of epothilone A in the tubulin-bound (green) and in the free (gray) conformation

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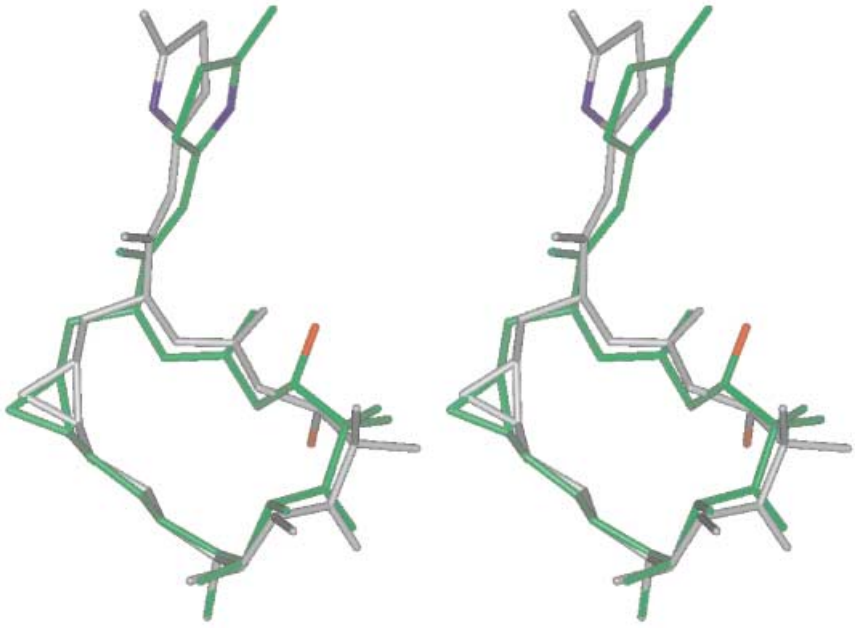

Figure 1. Stereoview of a comparison of the tubulin-bound conformation of epothilone A in aqueous solution (green) with the free conformation determined by X-ray crystallography[29] (gray). Hydrogen atoms have been removed for clarity. The conformational change that occurs in the O1–C6 region is clearly visible and is associated with a significant shift in the position of the 3-OH group (in red). A second key feature of the bound structure is the 180° change in the orientation of the side chain, which liberates the nitrogen atom (in blue) of the thiazole ring from the bulky Me27 group and makes it accessible to hydrogen-bonding donors.

(Table 1) reveals two major changes: the first occurs in the O1–C6 region and the second affects the orientation of the thiazole ring with respect to the C16–C17 double bond. In the lower region of the macrocycle, both cross-correlated relaxation rates (see preceding paper) and NOE data define the conformational change of the dihedral angle C2-C3-C4-C5, which switches from a *gauche*+ to a *gauche*− conformation, while the variations of the dihedral angles O1-C1-C2-C3 (+*anti*-periplanar to −*anti*-clinal) and C3-C4-C5-C6 result from the adjustment of the macrocycle to the modified torsion around the C3–C4 bond. The observed conformational change in the O1–C6 region of the ring primarily affects the position of the protons at C2, and of the 3-OH group. The two protons at C2 move towards the inner part of the ring. At the same time the oxygen atom attached to C3, which points to the inside of the macrocycle in the free conformation, moves towards the exterior by 3.8 Å (i.e. it now points away from the macrocycle). Neither the C5-C6-C7-C8 dihedral angle, determined by trCCR, nor the C10–C15 region exhibit any significant conformational change upon binding to tubulin. The latter finding corroborates a recent study in which the conformation of the macrocycle in the epoxide region is investigated with analogues of epothilone B and D.[31]

The second significant difference between the free and tubulin-bound conformations of epothilone occurs in the side chain that bears the thiazole ring. The C16-C17-C18-C19 dihedral angle changes from an *anti*-periplanar to a *syn*-periplanar conformation. This finding is substantiated by a strong NOE interaction between the protons of Me27 and H19 together with a significant weakening of the NOE interaction between H17 and H19 compared with that observed for epothilone A in aqueous solution and in the absence of tubulin. The eclipsed rotamer is energetically disfavored as a result of the steric hindrance between Me27 and H19. However, in this conformation, the nitrogen atom of the thiazole ring becomes more accessible for potential hydrogen-bonding formation with functional groups of proteins, which may more than offset an intrinsically disfavored epothilone side-chain conformation.

The tubulin-bound conformation of epothilone described herein is consistent with the wealth of chemical modification data available for this class of compounds. Epothilones are quite sensitive to chemical modifications in the C4–C8 region. The inversion of the stereochemistry at C3 also results in a considerable loss of potency in the induction of tubulin polymerization.[32] On the other hand, α,β-unsaturated (*trans* C2–C3 double bond) analogues of epothilones A and B are almost as active as the natural products. A very recent report by Vite and co-workers also showed that the replacement of the hydroxy group at C3

Table 1: Comparison of the dihedral angles of the tubulin-bound and the free conformations of epothilone A.

Dihedral angle	Tubulin-bound conformation [°]	Free conformation [°]
C1-C2-C3-C4	−152.5 ± 0.2	165.4
C2-C3-C4-C5	−51.7 ± 0.1	73.0
C3-C4-C5-C6	−43.0 ± 1.8	−75.9
C4-C5-C6-C7	156.4 ± 0.5	145.6
C5-C6-C7-C8	−70.0 ± 0.8	−64.0
C6-C7-C8-C9	−74.8 ± 0.3	−79.2
C7-C8-C9-C10	164.1 ± 0.8	159.0
C8-C9-C10-C11	−171.9 ± 0.4	176.8
C9-C10-C11-C12	−178.0 ± 0.4	174.8
C10-C11-C12-C13	−129.2 ± 0.5	−112.8
C11-C12-C13-C14	4.1 ± 0.1	−1.9
C12-C13-C14-C15	76.3 ± 0.1	93.9
C13-C14-C15-O1	−62.6 ± 1.0	−82.6
C14-C15-O1-C1	179.5 ± 0.5	159.6
C15-O1-C1-C2	176.3 ± 1.3	174.2
O1-C1-C2-C3	−124.3 ± 1.2	156.7
C14-C15-C16-C17	−129.7 ± 1.3	−118.5
C15-C16-C17-C18	180.0 ± 0.2	176.0
C16-C17-C18-N	180.0 ± 0.3	−7.6

The values given for the tubulin-bound conformation result from averaging over the ten lowest-energy conformations. The values given for the free conformation result from crystal structure analysis of epothilone A in methyl *tert*-butyl ether ($CH_3OC(CH_3)_3$) solvate.[29] This conformation, as well as crystal packing, is nearly identical to that of epothilone B,[2] although the two compounds crystallize from different solvents.

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with a cyano moiety leads to a compound that retains high biological activity.[33]

The hydroxy group attached to C3 is one of the biologically most relevant sites. In the free conformation, this OH group points towards the inner part of the ring and its oxygen atom is not easily accessible for the formation of external hydrogen bonds; conversely, in the tubulin-bound conformation, the 3-OH group points outwards, which makes the oxygen atom a potentially better acceptor of hydrogen bonding from tubulin side chains. This observation is consistent with chemical modification data, which underline the importance of the stereochemistry at C3 and therefore of the position of the 3-OH group. On the other hand, the fact that α,β-unsaturated epothilone analogues are almost as active as the natural compound indicates that the OH group is not crucial for tight binding to tubulin. This finding does not necessarily contradict the hypothesis of the existence of hydrogen bonding between the 3-OH of epothilone and tubulin side chains, as a double bond can also function as an electron donor.

The second profound conformational change observed upon binding of epothilone to tubulin concerns the orientation of the thiazole side chain, which undergoes a 180° rotation around the C17–C18 bond to liberate the nitrogen atom of the thiazole ring from the hindrance of the methyl group C27. It might be speculated that the nitrogen atom is now better available for hydrogen-bonding formation with a tubulin side chain. The potential role of the nitrogen atom as an hydrogen-bonding acceptor is corroborated by chemical modification data: the tubulin polymerization activity is retained upon substitution of the thiazole ring with a 2-pyridyl moiety, whereas the incorporation of a 3-pyridyl residue causes a notable decrease in the biological activity.[21] The recognition that the nitrogen atom of the thiazole ring may be involved in a hydrogen bond is highly relevant, as it might change the way of projecting new pharmacophore models for the orientation of epothilone in the tubulin-binding pocket. To date, all pharmacophore models are based on the attempted overlapping of the thiazole ring of epothilone with either the 2-OCOPh or the C3′-Ph of taxol. Nevertheless, the indubitable relevance of the nitrogen atom of the thiazole ring as an hydrogen-bonding acceptor opens the way to new pharmacophore models in which the nitrogen atom overlaps with a hydrogen-bonding acceptor in taxol. This new working hypothesis is currently under investigation in our laboratory.

So far, the design and synthesis of new potent analogues of epothilones was based on the available crystal structure of free epothilone. This has led to the discovery of candidates with promising properties, which, however, are still closely related to the parent compound. Potent analogues with structures deviating more strongly from the natural products might have a higher potential for alterations in the overall pharmacological profile. In addition, such compounds might be synthetically more readily accessible than closely related analogues of the natural products. The design of such analogues will be greatly aided by the knowledge described herein about the conformation of epothilone in its tubulin-bound state.

Experimental Section

All NMR experiments were performed on Bruker DRX 600 MHz or Avance 700 MHz spectrometers. The NOE constraints used to calculate the structure of the tubulin-bound conformation of epothilone were obtained from "transferred-NOE" experiments for a solution of epothilone A (0.5 mM) and tubulin (5 μM) in D_2O at mixing times of 100, 200, and 300 ms. The tr-ROESY experiment had a mixing time of 60 ms with CW irradiation at a field strength of 3500 Hz. CH–CH cross-correlated relaxation rates (trCCR-rates) for the determination of the C1-C2-C3-C4, C5-C6-C7-C8, C12-C13-C14-C15, and C13-C14-C15-C16 torsion angles were measured in HCCH correlation experiments, which were adapted from the original pulse program[34] to achieve the desired selectivity on the various epothilone moieties. The O-C1-C2-C3 torsion angle was measured in a dipole-CSA (CSA = chemical-shift anisotropy) trCCR experiment that was adapted from that used to measure CCR rates in the protein backbone.[35] The C2-C3-C4-C5 and C14-C15-C16-C17 torsion angles were measured with the help of a newly developed trCCR experiment.[36]

Tubulin was obtained from cytoskeleton (bovine brain, T238). The protein was dialyzed against calcium phosphate (3 mM), pH 7.0, lyophilized, and dissolved in D_2O prior to each measurement. All the trCCR experiments were acquired for a solution of ^{13}C-labeled epothilone A (0.5 mM) and tubulin (5 μM) in D_2O. ^{13}C-labeled epothilone was obtained by shake flask fermentation with the myxobacterium *Sorangium cellulosum* So ce90 by using a high-epothilone-producing mutant, BCE99/41 (Novartis strain collection). The fermentation was performed by growing the strain in medium (1000 mL) containing ^{13}C-labeled starch ($^{13}C_6$, 98%; 20 g) as carbohydrate source. Yields: epothilone A (20 mg) and epothilone B (15 mg), incorporation rate: 60–70% ^{13}C.

The assignment of the two diastereotopic methyl groups had to be established as it was essential to determine the dihedral angle around the C3–C4 bond by cross-correlated relaxation and consequently detect the large conformational change that takes place in this region upon binding to tubulin. Owing to the paucity of protons around the C3–C4 bond and to the conformational averaging present in this region for epothilone in solution in the absence of tubulin, the stereospecific assignment of the methyl groups could not be carried out by a combination of NOE interactions and $^3J_{CH}$ coupling constants. Therefore, we relied on the combination of NOE experiments, $^3J_{C,H}$ and $^3J_{C,C}$ couplings constants ($^3J_{C2,C5}$, $^3J_{C2,C22}$, and $^3J_{C2,C23}$) measured for epothilone B in the absence of tubulin at −20 °C in dimethylformamide. Under these conditions, the macrocycle can assume two conformations around the C3–C4 bond. Two pictures are compatible with the measured set of $^3J_{C,H}$ ($^3J_{C22,H3} \approx {}^3J_{C5,H3} > {}^3J_{C23,H3}$): 1) C22 = pro-*R*, C23 = pro-*S*, and a mixture of *gauche*+ and *trans* conformations around the C3–C4 bond; 2) pro*S*-C22, pro*R*-C23, and a mixture of *gauche*+ and *gauche* − conformations around the C3–C4 bond. The measured set of $^3J_{C,C}$ couplings ($^3J_{C2,C5} < {}^3J_{C2,C23} < {}^3J_{C2,C22}$) unequivocally confirms alternative 2. The resulting stereospecific assignment is opposite to that previously published.[2]

The structure of the tubulin-bound conformation of epothilone was determined by using the simulated annealing protocol, including NMR restraints, of the X-PLOR program.[37] The force field was adapted to include atom types occurring in epothilone A. Bond lengths and angles were taken from the X-ray structure of the free conformation. In addition, the force field included 46 NOE restraints and seven dihedral angles derived from trCCR rates. From a random starting point, an initial energy minimization of 50 steps, a high-temperature phase (32.5 ps, 6500 steps, 2000 K) and two cooling phases (25.0 ps, 5000 steps, 2000 K → 1000 K/10.0 ps, 2000 steps, 1000 K → 100 K) were performed. The closest local minimum was reached by applying a final energy minimization of 200 steps. This protocol was repeated 100 times. Within the ten lowest energy structures, all dihedral angles have a standard deviation of less than 2° and the RMSD of the positions of the heavy atoms is 0.044 Å.

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[1] G. Höfle, K. Bedorf, K. Gerth, *Chem. Abstr.* **1993**, *120*, 52841.
[2] G. Höfle, N. Bedorf, H. Steinmetz, D. Schomburg, K. Gerth, H. Reichenbach, *Angew. Chem.* **1996**, *108,* 1671 – 1672; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1567 – 1569.
[3] 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 – 2333.
[4] K.-H. Altmann, M. Wartmann, T. O'Reilly, *Biochim. Biophys. Acta* **2000**, *1470*, M79-M91.
[5] J. Kowalski, P. Giannakakou, E. Hamel, *J. Biol. Chem.* **1997**, *272*, 2534 – 2541.
[6] E. K. Rowinsky, *Annu. Rev. Med.* **1997**, *48*, 353 – 374.
[7] K.-H. Altmann, G. Bold, G. Caravatti, N. End, A. Florsheimer, V. Guagnano, T. O'Reilly, M. Wartmann, *Chimia* **2000**, *54*, 612 – 621.
[8] A. Balog, D. Meng, T. Kamenecka, P. Bertinato, D.-S. Su, E. J. Sorensen, S. J. Danishefsky, *Angew. Chem.* **1996**, *108*, 2976 – 2978; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2801 – 2803.
[9] Z. Yang, Y. He, D. Vourloumis, H. Vallberg, K. C. Nicolaou, *Angew. Chem.* **1997**, *109*, 170 – 173; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 166 – 168.
[10] D. Schinzer, A. Limberg, A. Bauer, O. M. Böhm, M. Cordes, *Angew. Chem.* **1997**, *109*, 543 – 544; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 523 – 524.
[11] K. C. Nicolaou, F. Roschangar, D. Vourloumis, *Angew. Chem.* **1998**, *110*, 2120-2153; *Angew. Chem. Int. Ed.* **1998**, *37*, 2014 – 2045.
[12] C. R. Harris, S. J Danishefsky, *J. Org. Chem.* **1999**, *64*, 8434 – 8456.
[13] J. Mulzer, H. J. Martin, M. Berger, *J. Heterocycl. Chem.* **1999**, *36*, 1421 – 1436.
[14] K. C. Nicolaou, A. Ritzen, K. Namoto, *J. Chem. Soc. Chem. Commun.* **2001**, 1523 – 1535.
[15] K.-H. Altmann, M. Wartmann, T. O'Reilly, *Biochim. Biophys. Acta* **2000**, *1470*, M79-M91.
[16] M. Wartmann, K.-H. Altmann, *Curr. Med. Chem. - Anti-cancer Agents* **2002**, *2*, 123 – 148.
[17] E. Nogales, S. G. Wolf, K. H. Downing, *Nature* **1998**, *391*, 199 – 203.
[18] J. D. Winkler, P. H. Axelsen, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2963 – 2966.
[19] M. Wang, X. Xia, Y. Kim, D. Hwang, J. M. Jansen, M. Botta, D. C. Liotta, J. P. Snyder, *Org. Lett.* **1999**, *1*, 43 – 46.
[20] I. Ojima, S. Chakravarty, T. Inoue, S. Lin, L. He, S. B. Horwitz, S. D. Kuduk, S. J. Danishefsky, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 4256 – 4261.
[21] K. C. Nicolaou, R. Scarpelli, B. Bollbuck, B. Werschkun, M. M. A. Pereira, M. Wartmann, K. H. Altmann, D. Zaharevitz, R. Gussio, P. Giannakakou, *Chem. Biol.* **2000**, *7*, 593 – 599.
[22] P. Giannakakou, R. Gussio, E. Nogales, K. H. Downing, D. Zaharevitz, B. Bollbuck, G. Poy, D. Sackett, K. C. Nicolaou, T. Fojo, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 2904 – 2909.
[23] L. He, P. G. Jagtap, D. G. I. Kingston, H.-J. Shen, G. A. Orr, S. B. Horwitz, *Biochemistry* **2000**, *39*, 3972 – 3978.
[24] F. Ni, *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, *26*, 517 – 606.
[25] T. Carlomagno, I. C. Felli, M. Czech, R. Fischer, M. Sprinzl, C. Griesinger, *J. Am. Chem. Soc.* **1999**, *121*, 1945 – 1948.
[26] M. J. J. Blommers, W. Stark, C. E. Jones, D. Head, C. E. Owen, W. Jahnke, *J. Am. Chem. Soc.* **1999**, *121*, 1949 – 1953.
[27] F. Solomon, *Biochemistry* **1977**, *16*, 358 – 363.
[28] G. Chakrabarti, S. Kim, M. L. Gupta, Jr., J. S. Barton, R. H. Himes, *Biochemistry* **1999**, *38*, 3067 – 3072.
[29] G. Rihs, H. R. Walter, unpublished results.
[30] R. E. Taylor, J. Zajicek, *J. Org. Chem.* **1999**, *64*, 7224 – 7228.
[31] R. E. Taylor, Y. Chen, A. Beatty, D. C. Myles, Y. Zhou, *J. Am. Chem. Soc.* **2003**, *125*, 26 – 27.
[32] K. C. Nicolaou, D. Vourloumis, T. Li, J. Pastor, N. Winssinger, Y. He, S. Ninkovic, F. Sarabia, H. Vallberg, F. Roschangar, N. P. King, M. Ray, V. Finlay, P. Giannakakou, P. Verdier-Pinard, E. Hamel, *Angew. Chem.* **1997**, *109*, 2181 – 2187; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2097 – 2103.
[33] A. Regueiro-Ren, K. Leavitt, S.-H. Kim, G. Höfle, M. Kiffe, J. Z. Gougoutas, J. D. DiMarco, F. Y. F. Lee, C. R. Fairchild, B. H. Long, G. D. Vite, *Org. Lett.* **2002**, *4*, 3815 – 3818.
[34] I. C. Felli, C. Richter, C. Griesinger, H. Schwalbe, *J. Am. Chem. Soc.* **1999**, *121*, 1956.
[35] D. W. Yang, R. Konrat, L. E. Kay, *J. Am. Chem. Soc.* **1999**, *121*, 11938 – 11940.
[36] See following manuscript in this issue: T. Carlomagno, V. M. Sanchez, M. J. J. Blommers, C. Griesinger, *Angew. Chem.* **2003**, *115*, 2619 – 2621; *Angew. Chem. Int. Ed.* **2003**, *42*, 2515 – 2517.
[37] A. T. Bruenger, *X-PLOR A System for X-ray Crystallography and NMR* (Yale University Press, New Haven), **1992**.

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