Antibiotic Resistance

DOI: 10.1002/ange.200604232

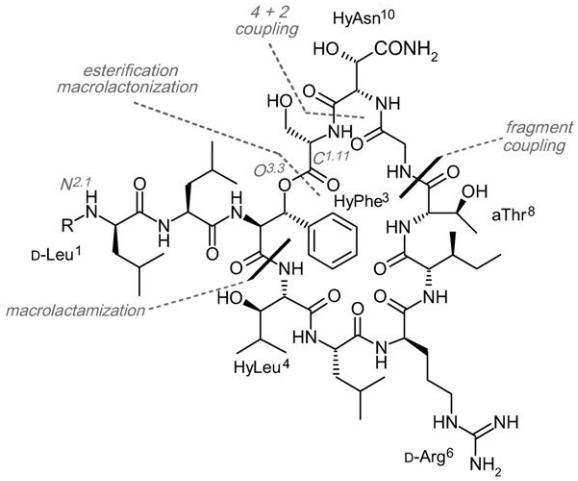
Structure and Total Synthesis of Lysobactin (Katanosin B)**

Franz von Nussbaum, Sonja Anlauf, Jordi Benet-Buchholz, Dieter Häbich, Johannes Köbberling, László Musza, Joachim Telser, Helga Rübsamen-Waigmann, and Nina A. Brunner*

Bacterial infections increasingly evade standard treatments as resistance to multiple antibiotics is growing. Multiresistant pathogens are no longer a problem restricted to hospitals.[1] New antibacterial lead structures[2] are urgently needed to guarantee future therapeutic efficacy. With advanced technologies in screening, chemistry, and genomics (the genomics revolution),[3] we possess new tools to transform natural products into valuable antibacterial drugs (chemical postevolution).[4] A flexible and efficient total synthesis is among the most powerful means to foster this process.

The cyclodepsipeptide[5] lysobactin (**1**, also known as katanosin B) was isolated independently from bacteria (*Lysobacter* sp.)[6] and from a strain related to the genus *Cytophaga* (Scheme 1).[7] The natural depsipeptides pose a significantly greater challenge for synthesis than regular peptides because of their nonribosomal motifs, such as hydroxylated or D-configured amino acids.[8] In particular, macrocyclic depsipeptides are demanding and often sensitive synthetic targets.[9] For many years, various research groups have tried to gain synthetic access to the natural lariat structure **1**,[10,11] but, to the best of our knowledge, a complete total synthesis has not been published so far.

Lysobactin has excellent in vitro activity against a wide range of Gram-positive bacteria which are increasingly associated with life-threatening infections, including multiresistant variants such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).[14] Substantial in vivo efficacy was shown in a mouse sepsis model.[7] The bacterial cell wall precursor lipid II has been assumed to be the target of **1**. Inhibition of the bacterial transglycosylation process would be based on molecular recognition of **1** and lipid II, which are binding partners of comparable molecular weights.[14]



Scheme 1. Lariat structure of lysobactin (**1**), consisting of a dipeptidic linear segment (D-Leu[1]–Leu[2]) and a nonapeptidic cyclic segment (HyPhe[3]–Ser[11]). The depsipeptide **1** contains various nonribosomal amino acids. Atom-description indices based on amino acid residue and then atom number. HyLeu = *threo*-hydroxyleucine;[12] aThr = allothreonine; HyAsn = *threo*-β-hydroxyasparagine;[13] HyPhe = *threo*-β-hydroxyphenylalanine, Boc = *tert*-butoxycarbonyl, Cbz = benzyloxycarbonyl.

We envisioned lysobactin to be a biologically promising but synthetically demanding lead structure. Although gram amounts of **1** were accessible through fermentation,[15] for the purpose of comprehensive chemical postevolution,[4] full synthetic control of the whole molecule well beyond semisynthesis was our goal. The feasibility of a synthetic de novo approach in this unexplored class should be proven with a flexible and efficient total synthesis.

Until now, as is the case for the majority of sensitive macrocyclic depsipeptides, no crystal structure has been reported for **1**, and two reported structures of **1** have been a matter of debate.[7,16] Although total synthesis is a classical way to confirm the structure of a natural product,[17] the 15 stereogenic centers left too many unaccountable options. Thus, unambiguous clarity of the structure of **1** was necessary prior to any attempt at synthesis.

[*] Dr. F. von Nussbaum, S. Anlauf, Dr. J. Benet-Buchholz,[+] Dr. D. Häbich, Dr. J. Köbberling, Dr. L. Musza,[+] Dr. J. Telser
Bayer HealthCare AG
Medicinal Chemistry Europe
42096 Wuppertal (Germany)
Fax: (+49) 202-36-9694797
E-mail: franz.nussbaum@bayerhealthcare.com
Prof. Dr. H. Rübsamen-Waigmann, Dr. N. A. Brunner
AiCuris GmbH & Co. KG
Bayer Pharma- und Chemiepark
Friedrich-Ebert Strasse 475
42117 Wuppertal (Germany)

[+] J.B.-B.: X-ray crystallography. L.M.: NMR solution structure.

[**] We are grateful to our colleagues C. Fehér, S. Heald, P. Schmitt, and S. Seip for NMR analyses. G. Schiffer determined the minimal inhibitory concentrations. A. Mayer-Bartschmidt and M.-A. Brüning carried out the fermentations. W. Schröder helped us with small-scale peptide analyses. J. Ragot resynthesized compound **2**. We thank A. Göller, J. Schamberger, and M. Lobell for fruitful discussions about the 3D structure. We are indebted to H. Wild and H. Haning for their continuous support of this project and to H. Schirok and N. Griebenow for a critical revision of the manuscript.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

Parallel crystallization studies were carried out with natural **1** in aqueous solvent systems. Even at this stage, opening of the macrolactone ring turned out to be the omnipresent threat in lysobactin chemistry. Under basic or neutral conditions, cleavage of the ester group readily occurred (**1**→**4**, pH 7, $t_{1/2} \approx 10$ h). The inherent need to use acidic conditions restricted the choice of applicable chemical manipulations and purification protocols throughout the entire project. However, a solution of **1** in acidic methanol (pH ≈ 3) proved to be stable over a prolonged period of time. Fortunately, a crystal structure[18] was obtained that unquestionably verified the sequence originally published by Tymiak et al.[16] For the first time, the instructive 3D architecture of **1** was at our disposal (Figure 1) and could serve as a basis for prospective chemical operations. Held in place by a rigid cyclic peptide backbone that is stabilized by multiple hydrogen bonds, the side chains give **1** a highly organized, globular appearance. Hydrophobic and hydrophilic domains can clearly be distinguished. A transannular charge–π interaction clamps the aromatic moiety of HyPhe[3] and the D-Arg[6] guanidinium group. While the lariat-type 2D structure of **1** suggests a flexible linear segment that points into the solvent, in fact, the two *N*-terminal leucin moieties are in tight contact with the rigid peptidic backbone fixed by four hydrogen bonds and several van der Waals interactions.

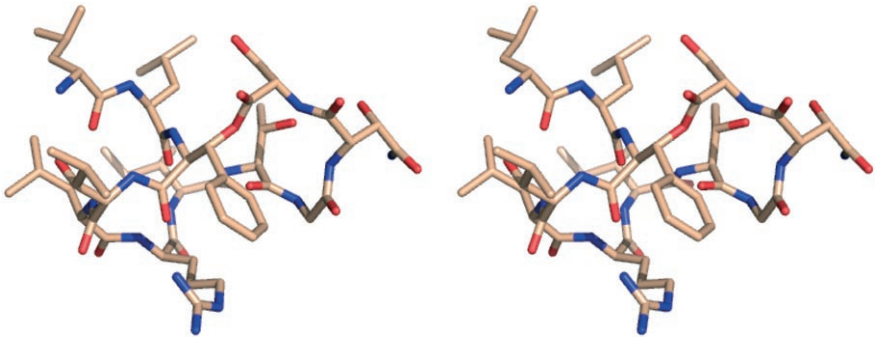


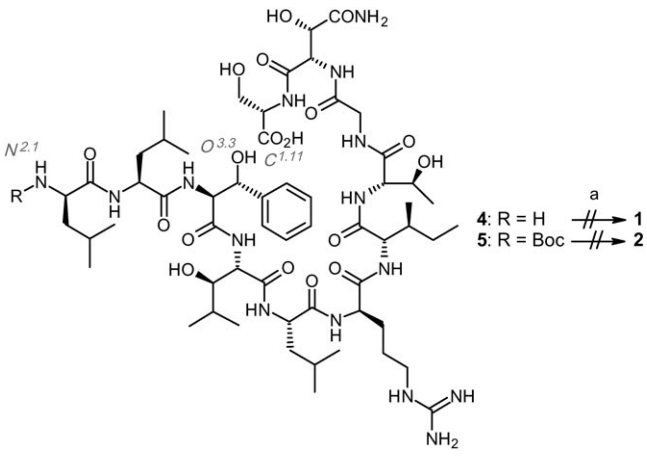
Figure 1. Crystal structure of **1**, stereoview. Hydrogen atoms have been removed for clarity; O red, N blue.

To complement the biological and the chemical relevance of the solid-state structure, an NMR solution structure was determined. Conformational flexibility was only observed near the *N*-terminal D-Leu[1] residue. ROESY and NOESY experiments gave the through-space ^{1}H–^{1}H distances, which, together with torsion angles from coupling constants, demonstrated that the solution structure of **1**, particularly for the large cyclic segment, was basically the same as in the solid state.

At that point our target structure was defined. Yet, several hurdles remained to be overcome en route to **1**, which included: 1) mode and site of cyclization, 2) ester formation, and 3) ester preservation in the presence of a plethora of nucleophilic functional groups. Cyclization of the 28-membered ring was expected to be the most delicate task. Fortunately, **1** is a rigid molecule with little conformational flexibility in solution and in the solid state (Figure 1). Preorganization was expected to result in favorable cyclization kinetics, provided that most groups that would affect the conformational constraint were in place.[19] Therefore, unprotected side chains were expected to exert a beneficial influence. Avoiding protection coincided well with our aim to design a short and economic synthesis.

The biosynthesis of several cyclic depsipeptides proceeds through a macrolactonization step.[20] With natural material **1** at hand, it was straightforward to evaluate this cyclization method in a semisynthetic way (Scheme 2). We tried to recyclize the ring-opened lysobactin **4** as well as the corresponding $N^{2.1}$-Boc derivative **5** under a number of esterification conditions (**4**→**1**, **5**→**2**). Although anhydro species were observed, HPLC analysis of our reaction mixtures by co-injection with **1** and semisynthetic **2**[21] showed that condensation had not occurred between the $C^{1.11}$ and $O^{3.3}$ sites. Too many hydroxy groups had competed with the rather unreactive secondary alcohol at HyPhe[3]. Accordingly, macrolactamization rather than macrolactonization appeared to be the method of choice: the superior reactivity of an amine should outrival competing nucleophilic groups.

For our macrolactamization approach, all fragments were synthesized de novo. The overall strategy had to take into consideration the particular requirements posed by the functionally crowded northern region of **1**. Special attention was given to the sensitive Ser[11]–HyPhe[3] ester bond and to the nonribosomal amino acid HyAsn[10] with its multiple functionalities. In view of our unsuccessful biomimetic cyclization experiments (Scheme 2), we chose to form the ester bond early in the synthesis. This would allow easier differentiation of the alcohols and reduce steric



Scheme 2. Semisynthetic cyclization studies. Biomimetic $C^{1.11}$–$O^{3.3}$ macrolactonization was not observed. Reagents and conditions: a) EDC, DMAP, CH_2Cl_2/DMF; or 2,4,6-trichlorobenzoylchloride, DIPEA, THF; then DMAP. EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DIPEA = diisopropylethylamine; DMAP = 4-dimethylaminopyridine; DMF = *N*,*N*-dimethylformamide.

interactions during the attack on the secondary alcohol of HyPhe[3]. On the other hand, the labile ester would have to be conserved throughout multiple synthetic steps and purification procedures. Three different levels of protecting groups were deemed to be essential (Scheme 3). The situation in the southern region was different. Despite a strongly basic guanidinium group in D-Arg[6], minimal side-chain protection seemed possible, and the unprotected functional groups were envisioned to facilitate preorganization in the cyclization step. For convergence, the cyclic segment of the lysobactin lariat was cut into two equally long subfragments. The depsipeptidic ester bond was concealed in the middle of the linear northern fragment. Two major fragments **11** and **12** were the logical consequence (Scheme 3).

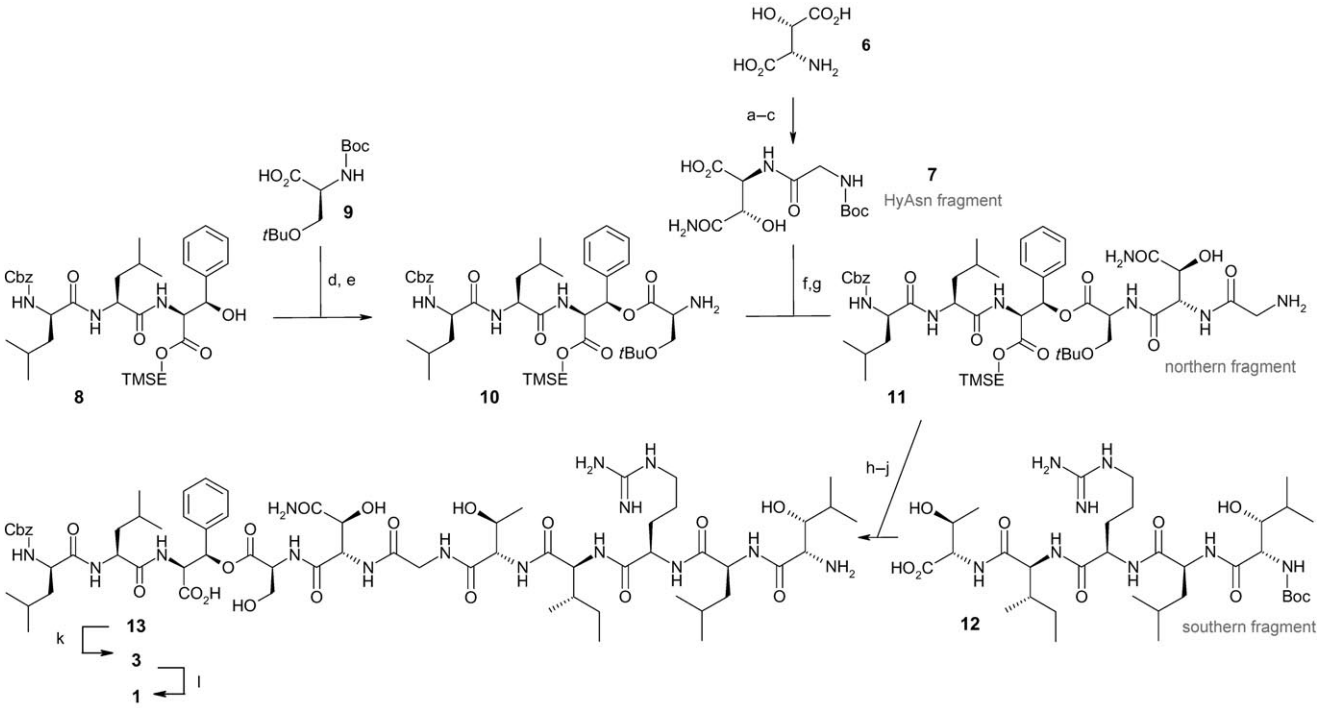
The southern fragment **12** was indeed synthesized with standard liquid-phase peptide chemistry by using Boc-protection and HATU/NMM coupling strategy without protection of the side chains.

For the northern fragment **11**, a 2-(trimethylsilyl)ethyl (TMSE) ester was chosen as the third level of protection (along with Cbz and Boc). The synthesis of a suitable HyAsn precursor turned out to be troublesome until we established the dipeptidic building block Boc-Gly-HyAsn-OH (**7**, Scheme 3). The most efficient method to insert HyAsn[10] into **11** started from **6**[22] by using selective esterification of the side chain. The free amino group of O^4-methyl-HyAsn was captured with an active ester of Boc-Gly followed by aminolysis to yield the primary amide **7**. This sequence avoided using toxic osmium salts, reduced the need for protecting groups, and led to minimal epimerization during aminolysis.

The formation of the depsipeptidic Ser[11]–HyPhe[3] ester bond was most critical. Direct couplings of tripeptide **8** with Boc-Gly-HyAsn-(*t*Bu)Ser-OH or even longer fragments gave unsatisfactory results: low yields and unacceptable levels of epimerization were observed in every case. Hence, **8** was linked by reaction with a single amino acid **9**. The missing dipeptide **7** was subsequently attached to **10**, thereby finishing the northern fragment **11**.

At this stage, we had to connect and cyclize the main fragments **11** and **12**. We chose the sterically less hindered aThr[8]-Gly[9] site for the first step. Coupling of segments **11** and **12** occurred with almost no epimerization. To obtain the full potential of preorganization, all protective groups, with the exception of the Cbz group, were then removed, which liberated the open-chain precursor **13**. Ring closure at a concentration of 1.1 mM occurred smoothly with 72% yield (**13**→**3**). Hydrogenolytic deprotection had to be carried out under strictly controlled conditions to avoid over-reduction (**3**→**1**). We obtained synthetic lysobactin, which was indistinguishable from the natural material according to all analytical and biological data (Table 1).

In summary, we have designed a convergent total synthesis of lysobactin with a high-yielding macrolactamization step. As a result of minimal use of protecting groups, the present approach leaves ample room for postevolutive



Scheme 3. Convergent synthesis of lysobactin through macrolactamization. Reagents and conditions: a) conc. aqueous HCl, MeOH, 130°C, microwave, 3 min, 89%; b) Boc-Gly-OSu, DIPEA, DMAP, quant.; c) aqueous NH_3 (25%), 52%; d) **9**, DCC, DMAP, CH_2Cl_2, molecular sieves (4 Å), 84%; e) 20% TFA in CH_2Cl_2, 10 min, 83%; f) **7**, HATU, NMM, DMF, 79%; g) 20% TFA in CH_2Cl_2, 10 min, 80%; h) **11**, HATU, NMM, DMF, 67%; i) TBAF, Na_2SO_4, molecular sieves (4 Å), THF, 89%; j) iPr_3SiH, TFA, H_2O, 86%; k) HATU, NMM, DMF, 72%; l) H_2, 10% Pd/C, TFA (0.1%), 1,4-dioxane, 88%. DCC = dicyclohexylcarbodiimide, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; OSu = *N*-oxysuccinimido, NMM = *N*-methylmorpholine, TBAF = tetra-*n*-butylammonium fluoride, TFA = trifluoroacetic acid.

Table 1: Minimal inhibitory concentrations (MIC) [$\mu g\,mL^{-1}$] as determined according to CLSI conditions.

	Natural **1**	Synthetic **1**	Vancomycin
Staphylococcus aureus 133[a]	0.125	0.125	0.5
Streptococcus pneumoniae G9A[b]	0.063	0.063	0.5
Enterococcus faecalis 27159[c]	0.5	0.5	2

[a] Müller–Hinton medium. [b] Brain-heart medium + 10% bovine serum. [c] Iso-sensitest medium. CLSI = Clinical and Laboratory Standards Institute.

structural variations, spatially unrestricted modulation of the binding epitope, and biological optimization. New knowledge about the 3D structure of **1** provides a firm basis for a future understanding and rational tuning of the interactions of lysobactin with the biological target. Pharmacological, toxicological, and technical parameters relevant for drug development can now be tackled.

Received: October 16, 2006
Published online: January 9, 2007

Keywords: antibiotics · cyclization · drug resistance · natural products · total synthesis

[1] a) Infectious Diseases Society of America (IDSA): http://www.idsociety.org; b) *46th Interscience Conference on Antimicrobial Agents and Chemotherapy* **2006**, San Francisco, USA: http://www.icaac.org.

[2] For a recent example (platensimycin), see: a) J. Wang et al., *Nature* **2006**, *441*, 358–361; b) D. Häbich, F. von Nussbaum, *ChemMedChem* **2006**, *1*, 951–954; c) K. C. Nicolaou, A. Li, D. J. Edmonds, *Angew. Chem.* **2006**, *118*, 7244–7248; *Angew. Chem. Int. Ed.* **2006**, *45*, 7086–7090.

[3] L. Miesel, J. Greene, T. A. Black, *Nat. Rev. Genet.* **2003**, *4*, 442–456.

[4] F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand, D. Häbich, *Angew. Chem.* **2006**, *118*, 5194–5254; *Angew. Chem. Int. Ed.* **2006**, *45*, 5072–5129.

[5] F. Sarabia, S. Chammaa, A. S. Ruiz, L. M. Ortiz, F. J. López Herrera, *Curr. Med. Chem.* **2004**, *11*, 1309–1332.

[6] a) D. P. Bonner, J. O'Sullivan, S. K. Tanaka, J. M. Clark, R. R. Whitney, *J. Antibiot.* **1988**, *41*, 1745–1751; b) J. O'Sullivan, J. E. McCullough, A. A. Tymiak, D. R. Kirsch, W. H. Trejo, P. A. Prinicipe, *J. Antibiot.* **1988**, *41*, 1740–1744.

[7] J. I. Shoji, H. Hinoo, K. Matsumoto, T. Hattori, T. Yoshida, S. Matsuura, E. Kondo, *J. Antibiot.* **1988**, *41*, 713–718.

[8] β-Amino acids are often-encountered motifs; see: a) P. Spiteller, F. von Nussbaum, β *-Amino Acids in Natural Products* in *Enantioselective Synthesis of* β*-Amino Acids* (Eds.: V. Soloshonok, E. Juaristi), 2nd ed., Wiley, New Jersey, **2005**, pp. 19–91; b) F. von Nussbaum, P. Spiteller, β*-Amino Acids in Nature in Highlights in Bioorganic Chemistry—Methods and Applications* (Eds.: C. Schmuck, H. Wennemers), Wiley-VCH, Weinheim, **2004**, pp. 63–89.

[9] The total synthesis of the ramoplanin aglycon is a recent milestone; see: D. Shin, Y. Rew, D. L. Boger, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11977–11979.

[10] a) C. Palomo, M. Oiarbide, I. Ganboa, J. I. Miranda, *Tetrahedron Lett.* **2001**, *42*, 8955–8957; b) S. Armaroli, G. Cardillo, L. Gentilucci, M. Gianotti, A. Tolomelli, *Org. Lett.* **2000**, *2*, 1105–1107; c) G. Cardillo, S. Fabbroni, L. Gentilucci, R. Perciaccante, A. Tolomelli, *Komppa Centenary Symposium—100 Years of Natural Product Synthesis*, Helsinki, Finland, **2003**, Poster ORGN843; d) A. M. Hafez, T. Dudding, T. R. Wagerle, M. H. Shah, A. E. Taggi, T. Lectka, *J. Org. Chem.* **2003**, *68*, 5819–5825; e) A. Guzman-Martinez, R. B. Lamer, M. S. VanNieuwenhze, *232th ACS National Meeting*, San Francisco, USA, September 10–14, **2006**.

[11] A solid-phase synthesis of a close congener of **1** has been described. To reduce the synthetic complexity, $HyAsn^{10}$ was omitted and substituted by Asn^{10}. Furthermore, $HyLeu^{4}$ and $aThr^{8}$ were both exchanged by Thr (no biological data); see: B. J. Egner, M. Bradley, *Tetrahedron* **1997**, *53*, 14021–14030.

[12] Y. N. Belokon, K. A. Kochetkov, N. S. Ikonnikov, T. V. Strelkova, S. R. Harutyunyan, A. S. Saghiyan, *Tetrahedron: Asymmetry* **2001**, *12*, 481–485.

[13] D. L. Boger, R. J. Lee, P.-Y. Bounaud, P. Meier, *J. Org. Chem.* **2000**, *65*, 6770–6772.

[14] a) H. Maki, K. Miura, Y. Yamano, *Antimicrob. Agents Chemother.* **2001**, *45*, 1823–1827; for an excellent review on lipid II, see: b) E. Breukink, B. de Kruijff, *Nat. Rev. Drug Discovery* **2006**, *5*, 321–332.

[15] a) A. A. Tymiak, D. R. Kirsch, J. O'Sullivan, J. E. McCullough (E. R. Squibb & Sons, Inc.), US 4754018, **1988** [*Chem. Abstr.* **1990**, *112*, 75294]; b) F. von Nussbaum, N. Brunner, S. Anlauf, R. Endermann, C. Fürstner, E. Hartmann, J. Köbberling, J. Ragot, G. Schiffer, J. Schuhmacher, N. Svenstrup, J. Telser, M.-A. Brüning (Bayer HealthCare AG), WO 04099239, **2004** [*Chem. Abstr.* **2004**, *141*, 423388].

[16] A. A. Tymiak, T. J. McCormick, S. E. Unger, *J. Org. Chem.* **1989**, *54*, 1149–1157.

[17] K. C. Nicolaou, S. A. Snyder, *Angew. Chem.* **2005**, *117*, 1036–1069; *Angew. Chem. Int. Ed.* **2005**, *44*, 1012–1044.

[18] CCDC-623590 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[19] J. Blankenstein, J. Zhu, *Eur. J. Org. Chem.* **2005**, 1949–1964.

[20] a) C. T. Walsh, *Science* **2004**, *303*, 1805–1810; b) H. Chen, M. G. Thomas, S. E. O'Connor, B. K. Hubbard, M. D. Burkart, C. T. Walsh, *Biochemistry* **2001**, *40*, 11651–11659.

[21] Compound **2** was obtained from **1** under buffered conditions: Boc_2O, *t*BuOH/H_2O, DIPEA, RT, 44%.

[22] a) G. Cardillo, L. Gentilucci, A. Tolomelli, C. Tomasini, *Synlett* **1999**, 1727–1730; b) P. G. Mattingly, M. J. Miller, *J. Org. Chem.* **1983**, *48*, 3556–3559.

