DOI: 10.1002/anie.201107386

Total Synthesis

Merging Chemical Synthesis and Biosynthesis: A New Chapter in the Total Synthesis of Natural Products and Natural Product Libraries

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metabolic engineering · mutasynthesis · natural products · semisynthesis · total synthesis

1. Introduction

> *"The synthetic chemist who is developing a useful drug has two objectives: to make it more efficiently, and to synthesize, by minor structural alterations, analogues which may have more useful properties. I wish to examine here the question whether the chemist can, in principle, manipulate the processes of production of antibiotics by fungi with the same objectives in view."*

About half a century ago, this remarkable statement by A. J. Birch in 1963 was only a vision. Nowadays, however, Birch,[1] who died in 1995, would be most delighted to see how chemical synthesis is being complemented by tools that allow biosynthetic pathways to be manipulated to generate analogues of drugable natural products.

One has to note that natural products have lost much of their attraction to the pharmaceutical industry over the past few decades, although there are a few current examples such as the epothilone derivative ZK-EPO **1**[2] and eribulin (**2**).[3] The former is the result of a total synthesis program by Schering AG (now Bayer AG), which set out to develop a clinical anticancer candidate based on the natural product epothilone, while the latter example is a simplified derivative of halichondrin B, a secondary metabolite from the marine sponge *Halichondria okadai*. Eribulin (**2**) was prepared by total synthesis (developed by Eisai Co., Ltd) and was approved at the end of 2010 in its mesylate form by the Food and Drug Administration (FDA) for the treatment of late-stage breast cancer.[4] The development of both natural product derivatives has demonstrated the strength and superiority of total synthesis when creating new analogues of a natural product lead compound in the most flexible manner, which otherwise is (still) very hard to achieve with biological approaches.

Such examples are, in fact, rare, mainly because the complexity of many natural products is problematic when utilizing total synthesis to either manufacture sufficient amounts for clinical applications or for generating compound libraries for structure–activity relationship studies (SARs). Structurally complex and clinically relevant natural products are commonly obtained either from biological sources or by fermentation, which might be further modified by semisynthesis. A famous and classical example for a clinically widely used natural product is the antibiotic erythromycin A (**3**). It is available from high production strains of *Streptomyces erythreus* (today *Saccharopolyspora erythraea*). The anticancer agent taxotere (**4**) is a semisynthetically accessible derivative of paclitaxel[5] that is obtained by introducing the modified ester side chain at C13 in a suitably protected 10-deacetylbaccatin III. 10-Deacetylbaccatin III is a biosynthetic precursor of paclitaxel (from the pacific yew tree *Taxus brevifolia*) and can be obtained from different sources, including the leaves of *Taxus baccata* (the European yew tree).[6]

The structural complexity of natural products and analogues, such as those depicted in Figure 1, means that chemists commonly prepare them by a highly convergent route. This strategy minimizes the number of linear steps. This is in stark contrast to nature, which has developed highly linear multistep biosyntheses, for example, for peptides, polyketides, and terpenes. One may speculate why that is the case. In short, nature has definitely been hesitant to constantly develop completely new biosynthetic pathways or biotransformations. It relied instead on established processes, often based on iterative concepts which were further elaborated by adding additional ("decorating" or "tailoring") steps. The iteration and addition of steps consequently leads to long linear sequences. Thus, nature relies on a kind of continuous flow process based on steady-state concentrations for all biosynthetic intermediates.[7] These are quickly processed and, therefore, commonly only present in minute amounts, as exemplified by the biosynthesis of ansamitocin P3 (**6**) from 3-amino-5-hydroxybenzoic acid (AHBA, **5**; Scheme 1).[8] In

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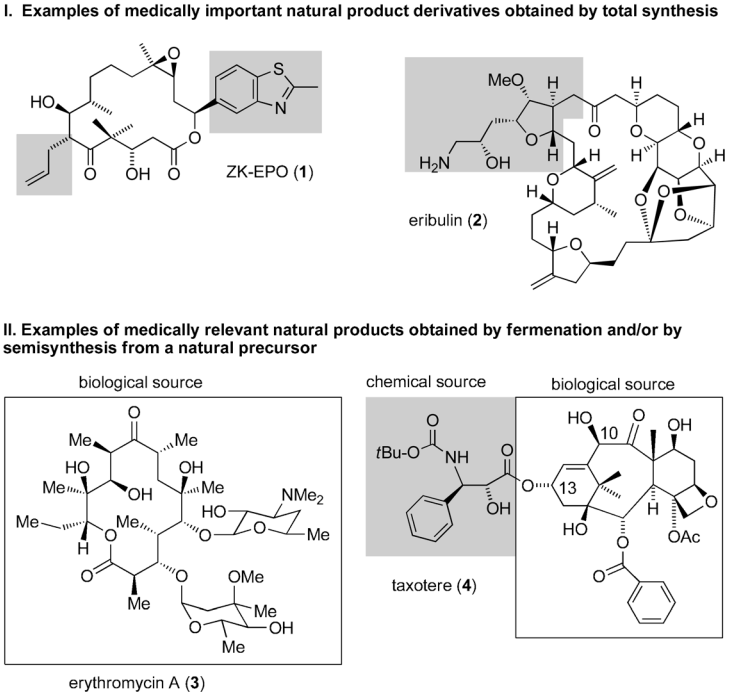

Figure 1. Schering's epothilone derivative **1**[2] (ZK-EPO, synthetic changes with respect to epothilone are marked in gray), eribulin (**2**)[3] prepared on an industrial scale (area marked in gray shows the structural simplification with respect to halichondrin B), erythromycin A (**3**), and taxotere (**4**; semisynthetic change with respect to the parent natural product paclitaxel is marked in gray).

contrast, synthetic chemists still rely on batch-type techniques so that, except for domino reactions, for example, all intermediates are prepared and isolated in stoichiometric amounts. As work-up is often accompanied by loss of material, short, convergent syntheses are highly desired. This is certainly not an issue in biosynthesis. Only recently, the introduction of continuous processes involving minituarized flow reactors to the portfolio of synthetic chemists[9] has meant that multistep synthesis can be carried out in a similar fashion to nature, passing through intermediates that are never isolated stoichiometrically. This approach was elegantly demonstrated by Ley and co-workers in their synthesis of (*rac*)-oxomaritidine (**9**).[10]

With a deeper understanding of biosynthetic pathways of complex secondary metabolites and the development of tools based on molecular biology to manipulate biosynthetic pathways at the genetic level,[11] the total synthesis of natural products and analogues has become more flexible and may return to the agenda of drug development, particularly if engineered biosynthesis is combined with chemical synthesis.[12] In this Minireview we develop a conceptual view on synthetic hybrid strategies, where chemical and biological methods are combined in a flexible way so that a total synthesis like rather than linear access to analogues of complex natural products is achieved.

To more easily grasp the different (possible) options for using hybrid concepts, we suggest the simple classification described in Figure 2. It mainly focuses on synthetic strategies. Thus, we use the two abbreviations CHEM and BIO, where the former refers to a chemical synthesis or partial chemical synthesis, while the latter describes a (partial) biosynthesis or a biotransformation. In principle, there are two ways to accomplish such transformations. One is precursor feeding to an appropriate producer strain or a knockout

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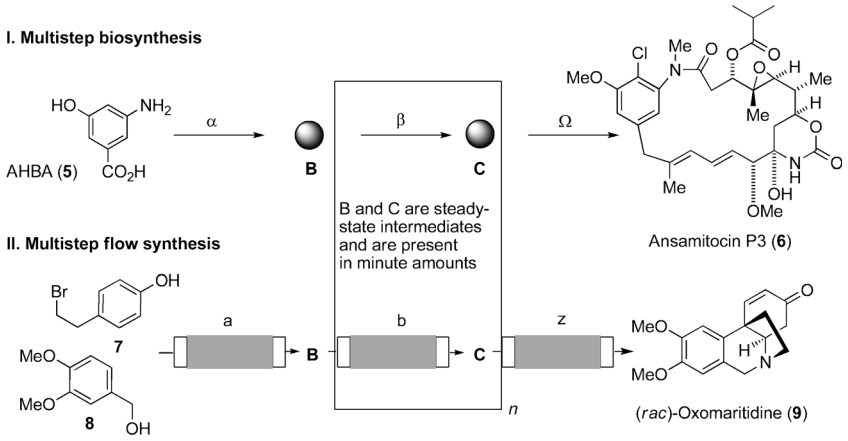
Andreas Kirschning studied chemistry at the University of Hamburg and Southampton University (UK) and completed his PhD in Hamburg with Prof. Ernst Schaumann in 1989. After postdoctoral research at the University of Washington (Seattle, USA) with Prof. Heinz G. Floss, supported by a Feodor-Lynen scholarship of the Alexander-von Humboldt foundation, he started his independent research at the Clausthal University of Technology in 1991, where he obtained his habilitation in 1996. In 2000 he moved to the Leibniz University Hannover and became director of the institute of organic chemistry.



Scheme 1. The biosynthesis of ansamitocin P3 (**6**) and multistep flow synthesis of (*rac*)-oxomaritidine (**9**) are related synthetic concepts (α–Ω=enzymes; a–z = reagents/catalysts; B, C = synthetic or biosynthetic intermediates; *n* = number of steps).

strain that harbors at least parts of the functional biosynthetic machinery. The fermentation product can then be isolated by extraction. Alternatively, the pathway enzymes of interest can be heterologously overexpressed and treated in vitro with precursor molecules.

In this context, a BIO-CHEM synthesis is simply a semisynthetic derivatization of a starting material obtained from a natural source or by fermentation. For example, taxotere (**4**) is obtained by a BIO-CHEM approach. New natural product analogues are accessed by the BIO-CHEM-BIO approach by further modifying the product obtained through semisynthesis by means of an enzymatic biotransformation or by subjecting it to a fermentation. In essence, these total syntheses somewhat resemble the linear strategies that nature has developed through evolution.[13]

2. Key Issues for CHEM-BIO Concepts—A Synthetic Perspective

Before discussing these "total synthesis" hybrid approaches for preparing natural product analogues, three selected key issues that are common to the synthetic chemist but which must also apply to the biosynthetic part need to be stressed:

1. The natural product chosen should either have excellent biological activity in a pharmaceutically relevant field, or alternatively it could serve as a tool for studies of cell biology so as to develop a strategy where one has to solve the problems associated with the interface between synthetic chemistry and biological synthesis. This can be laborious.

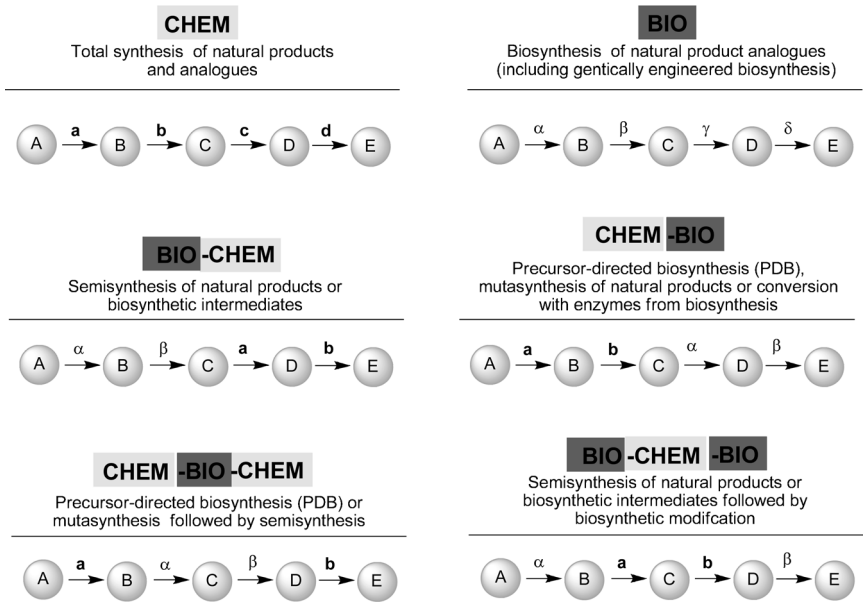


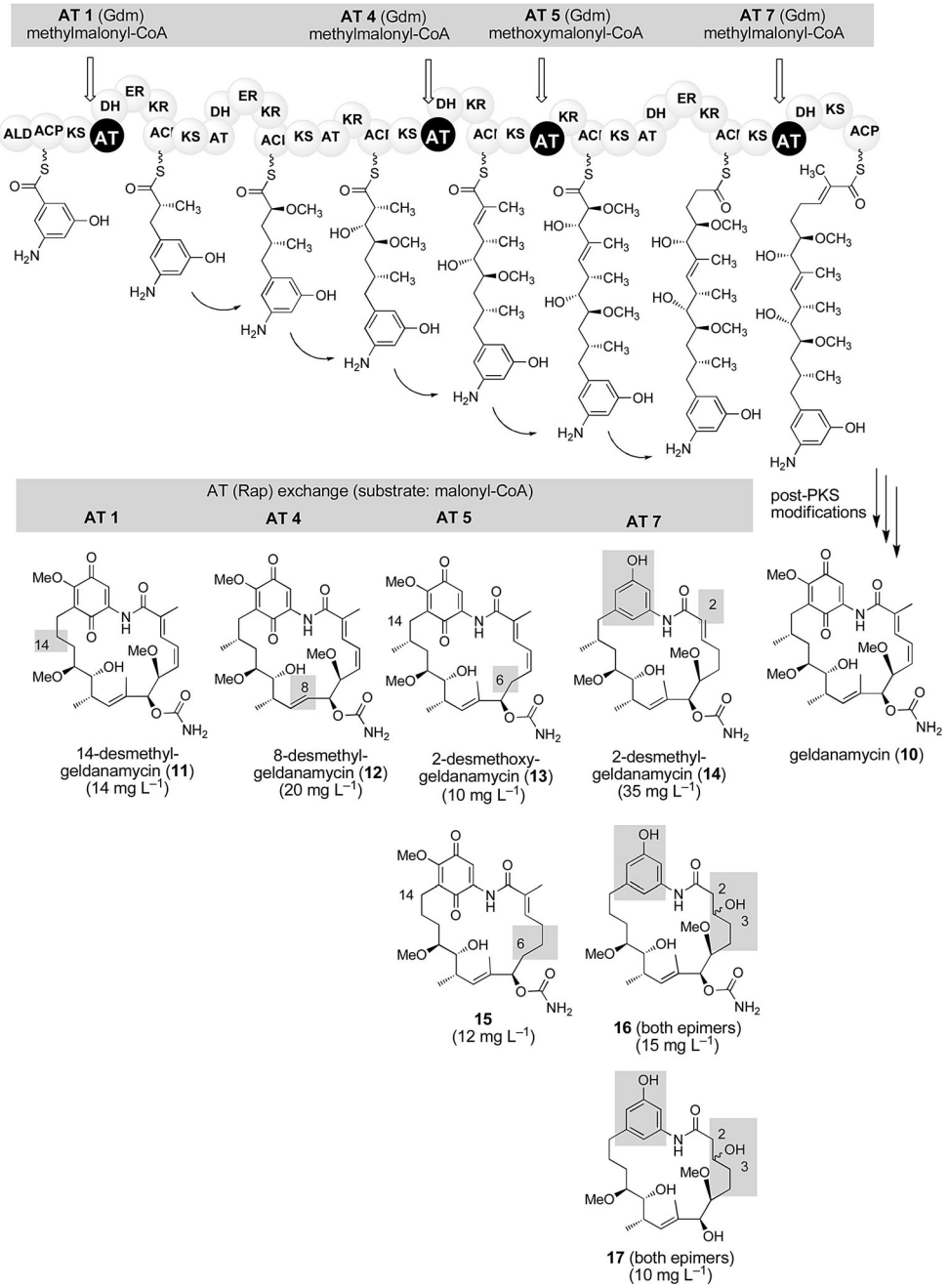
Figure 2. Classification of "total synthesis" approaches towards natural product analogues and libraries based on chemical and biological methods as well as combinations (selected combinations are shown, but other combinations such as BIO-BIO-CHEM can also be envisaged; see also Scheme 9), a–d = chemical reagents or catalysts; α–δ = enzymes; A = starting material; B–D = synthetic and/or biosynthetic intermediates; E = natural product or derivative.[13]

2. The chosen natural product should be structurally so complex that a combined chemical and biological approach is clearly superior to chemical total synthesis for rapidly preparing new derivatives.
3. In all cases, the biological tool, for example, a fermentation or an enzyme, must yield sufficient amounts of the desired metabolite—be it either a final product or an intermediate—for further functionalization. To distinguish it from pure biosynthetic studies, this issue explicitly includes that the spectroscopic characterization of the products must meet the standards for the end products of a successful chemical total synthesis.

In the following, selected and illustrative examples of hybrid techniques are given that fulfil principal synthetic requirements and that demonstrate the power of these synthetic approaches.

3. Manipulation of the Biosynthesis (BIO)

A very elegant study on the genetic manipulation of the geldanamycin polyketide synthase (GdmPKS) was disclosed by Kosan Biosciences, Inc.[14] Geldanamycin (**10**) is a polyketide macrolactam antibiotic that is a lead structure for the



Scheme 2. Geldanamycin derivatives **11**–**17** produced by acyltransferase (AT) substitutions in the Gdm polyketide synthase (numbers refer to the PKS module; areas marked in gray show structural variations with respect to **10**).

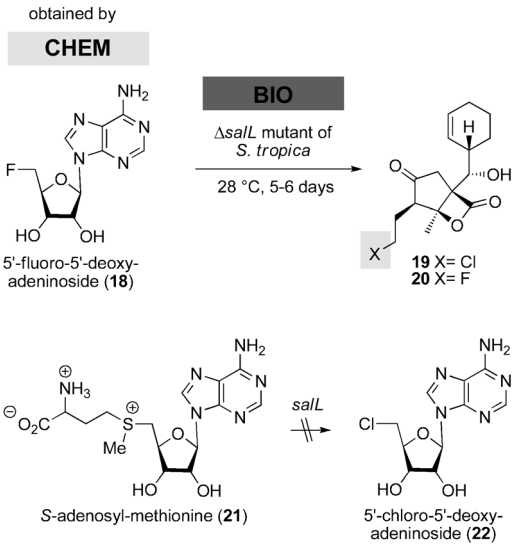
development of new anticancer drugs that target the chaperone Hsp90.[15]

Genetic engineering of the geldanamycin gene cluster in *Streptomyces hygroscopicus*, the producer of geldanamycin, allowed the generation of new geldanamycin derivates that selectively lack methyl or methoxy groups in the ansa chain. These fermentation products were generated by genetically substituting acyltransferase (AT) domains in six different GdmPKS modules that commonly accept methylmalonyl-CoA or methoxymalonyl-CoA by malonyl AT domains from the rapamycin PKS.[16] Four of these manipulations led to the production of 2-desmethyl, 6-desmethoxy-, 8-desmethyl-, and 14-desmethylgeldanamycin derivatives **11**–**14**, the γ,δ-saturated **15**, and the hydratization products **16** and **17** (Scheme 2). The yields were sufficient for full chemical characterization as well as for biological evaluation. Such genetic manipulations are highly attractive for combining them with chemical synthesis.

4. CHEM-BIO (Mutasynthesis)

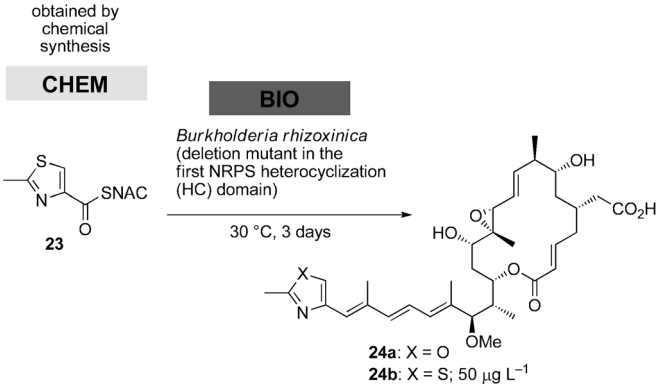
An example of a BIO-CHEM product is taxotere (**4**; Figure 1). The reversed CHEM-BIO case is the classical precursor-directed biosynthesis (PDB) or mutasynthesis, with the latter being a more modern technique. Mutasynthesis requires the generation of mutants of a producer organism blocked in the formation of a biosynthetic building block of the end product. Administration of chemically prepared building blocks, or in short, mutasynthons, to the blocked mutant results in new metabolites.[12]

A recent example of the preparation of the fluoro derivative **20** of the highly potent marine proteasome inhibitor salinosporamide **19** is shown in Scheme 3.[17] Eustáquio and Moore disrupted the biosynthetic chlorination of *S*-adenosylmethionine (SAM), thereby blocking the formation of chloride **22**. Complementation of a 3 L fermentation culture of a Δ*salL* mutant with 30 mg of 5′-fluoro-5′-deoxyadeninoside (**18**) yielded 1.5 mg L^{-1} of the fluoro derivative **20**.



Scheme 3. Disruptants in *salL*, a gene encoding the unusual chlorinase and mutasynthetic formation of fluorosalinosporamide **20**.

Biosynthetically, rhizoxin (**24a**) is a PKS-NRPS-based secondary metabolite (NRPS = nonribosomal peptide synthase) from *Burkholderia rhizoxinica* which exerts pronounced cytotoxic activity. Hertweck and co-workers engineered a mutant disrupted in *rhiA*, a gene that codes for part of the *rhi* PKS-NRPS.[18] It harbors the loading module as well as parts of the oxazole-forming unit. This mutant resulted in the preparation of the thiazole derivative **24b** in a yield of 50 μg L^{-1} after being supplemented with *N*-acetylcysteamine derivative **23**. Surprisingly, this oxygen/sulfur replacement led to an increased incorporation rate (Scheme 4).

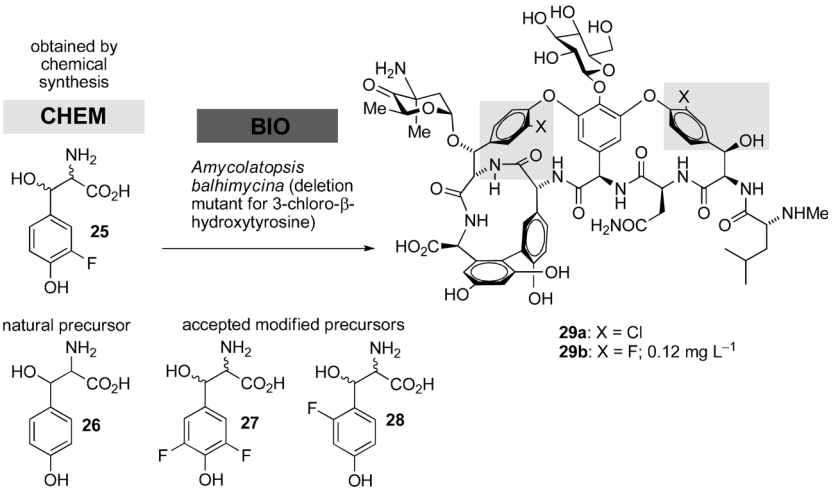


Scheme 4. Disruptants in *rhiA*, a gene encoding the heterocyclization in the loading module and mutasynthetic formation of thiarhizoxin **24b**. SNAC = *N*-acetylcysteamine.

The nonribosomally synthesized glycopeptide balhimycin (**29a**, X = Cl) is a derivative of the clinically important antibiotic vancomycin. Süssmuth, Wohlleben, and co-workers used different block mutants of the actinomycete *Amycolatopsis balhimycina*, the producer of Balhimycin. Besides other deletion mutants,[19] they utilized one that was blocked in the biosynthesis of β-hydroxytyrosine (**26**). Feeding these with advanced fluoro derivatives **25**, **27**, and **28** related to this building block yielded several new vancomycin-type fluoro derivatives modified in the C and E rings (Scheme 5).[20] Noteworthy, semisynthetic approaches towards new derivatives of vancomycin have primarily addressed the peripheral glycon unit. The mutasynthetic preparation of fluorinated balhimycin derivative **29b** clearly demonstrates that mutasynthesis is complementary to semisynthesis, as structural modifications were achieved in the tricyclic glycon backbone of balhimycin that are not easily achieved by semisynthesis.

5. CHEM-BIO (Enzymatic Transformation)

The application of heterologously expressed late-stage enzymes from biosynthetic pathways of the secondary metabolism is an alternative method to access natural products or their derivatives.[21] Synthetically useful enzymatic transformations are chemo- and regioselective cyclizations,

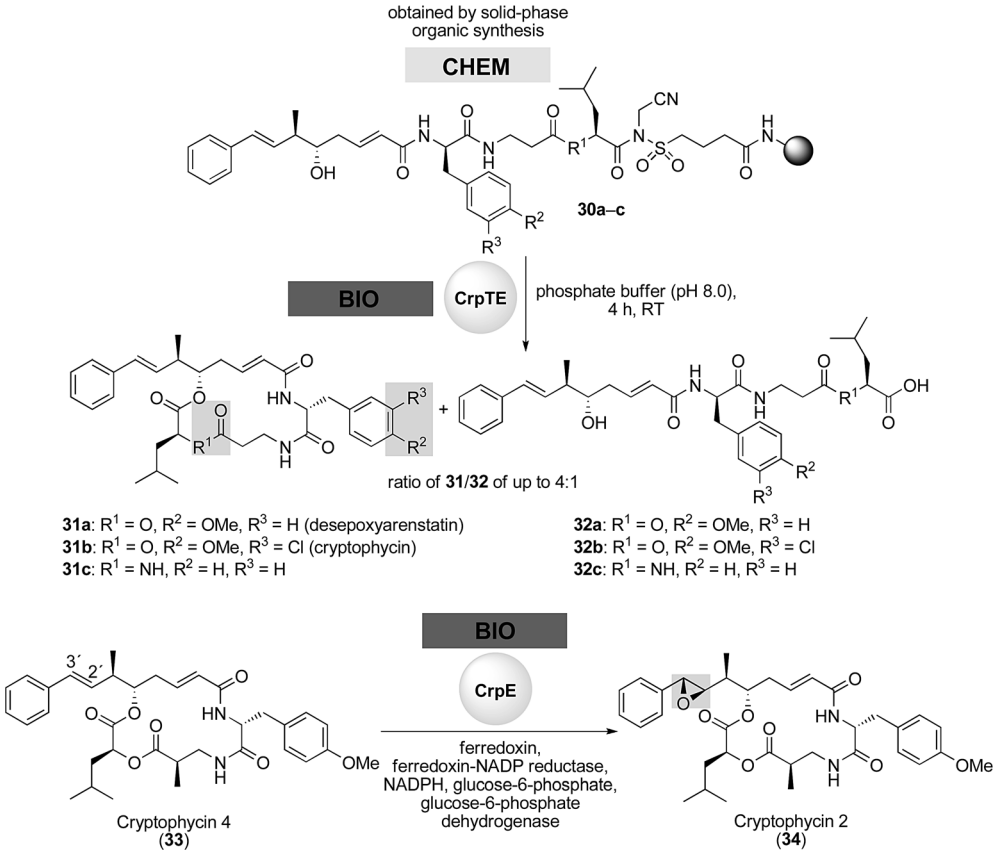


Scheme 5. Mutasynthetic synthesis of new fluorobalhimycin derivatives.[19,20]

including macrocyclizations, glycosylations, redox reactions, halogenations, alkylations, and acylations.

"Stand-alone" enzymes are better suited to synthetic applications than those that are naturally part of assembly lines.[22] In this context, the latter group of enzymes accept substrates that are handed over from upstream modules, so that their catalytic efficiency is usually lower and more dependent on protein–protein interactions. The same is valid for substrate recognition. Nature has evolved this strategy under evolutionary pressure to optimize the catalytic turnover. Nevertheless, both groups of enzymes are potentially valuable tools for the natural product chemist.

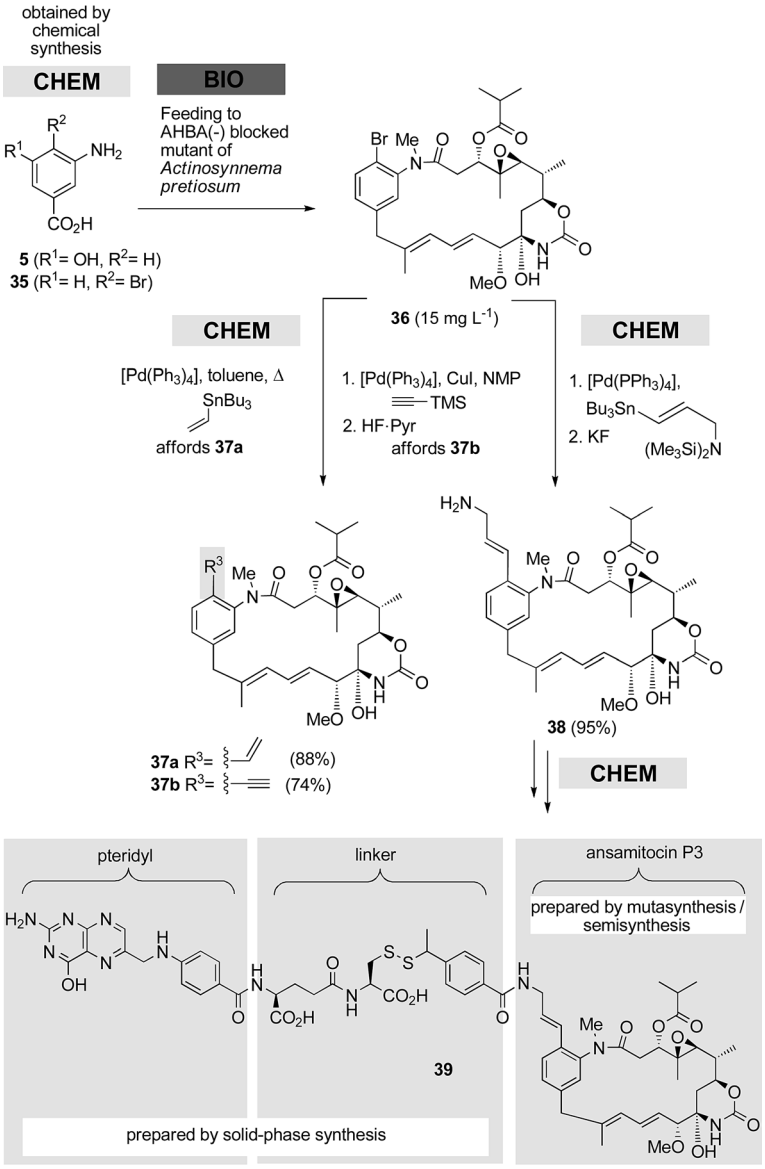
Cryptophycins are potent tubulin polymerization inhibitors with high activity against multidrug-resistant tumor cell lines. Their backbones are biosynthesized by a polyketide synthase-nonribosomal peptide synthase (PKS-NRPS) hybrid



Scheme 6. CrpTE catalyzes the macrocyclization of immobilized polyketide-peptide precursors **30** to desepoxycryptophycins **31**. Epoxidation of the structurally closely related cryptophycin **33** by CrpE leads to cryptophycin **34**.[23]

multienzyme complex, followed by the action of tailoring enzymes to yield the final products. Several enzymes from the pathway have been characterized by in vitro assaying on an analytical scale.[23] In 2007, Seufert et al. reported on the application of the cryptophycin thioesterase (CrpTE) for cyclizing immobilized seco precursors.[24] Three derivatives of desepoxycryptophycin were prepared in milligram quantities (5 mg of **31a**, 6 mg of **31b**, 12 mg of **31c**) by incubating **30** with CrpTE in phosphate buffer, followed by extractive workup and preparative HPLC separation (Scheme 6). Remarkably, CrpTE was able to cyclize substrates that had been immobilized through a sulfonamide linker on a poly(ethylene glycol)-poly(*N*,*N*-dimethylacrylamide) (PEGA) resin to yield 16-membered macrocycles. This highlights the huge potential of macrocyclizing enzymes in the synthesis of natural product analogues. The combination of solid-phase synthesis and biosynthetic transformation could become a versatile and competitive strategy for the generation of PKS-NRPS hybrid libraries.

The biological activity of cryptophycins bearing a β-epoxide group in the 2′,3′-position, is more than 100 times higher than the activity of their α-epoxide- or *trans*-styrene counterparts.[23c] The seemingly crucial epoxidation step within the biosynthesis of cryptophycin is accomplished by the P450-dependent epoxidase CrpE.[23d] Ding et al. showed that CrpE catalyzes the final epoxidation step to cryptophycins with broad substrate tolerance, and also enabled the synthesis of non-natural derivatives, as suggested by HPLC-MS analysis.[23c] In a large-scale experiment, cryptophycin 4 (**33**) was converted into its related β-epoxide cryptophycin 2 (**34**) in >75% conversion.[23d] A tandem in situ reaction of SNAC-seco-cryptophycin 4 with CrpTE and CrpE yielded



Scheme 7. Combined muta-/semisynthesis towards ansamitocin P3 derivatives **37a**, **37b**, and **38** as well as folic acid/ansamitocin conjugate **39** by using an AHBA blocked mutant of *A. pretiosum*. NMP = *N*-methylpyrrolidone, TMS = trimethylsilyl.

cryptophycin 2 (**34**) as a single product without the need to purify the cryptophycin 4 (**33**) intermediate. From a chemical synthesis perspective, the enzymatic macrocyclization and stereoselective epoxidation drastically simplifies the "end game" of the total synthesis of cryptophycin.

6. CHEM-BIO-CHEM

Mutasynthesis can further enhance the opportunities to create natural product analogues by specifically introducing chemical entities that are suited for carrying out semisynthetic modification with the fermentation product. Such a CHEM-BIO-CHEM approach greatly broadens the opportunity to create structural diversity.

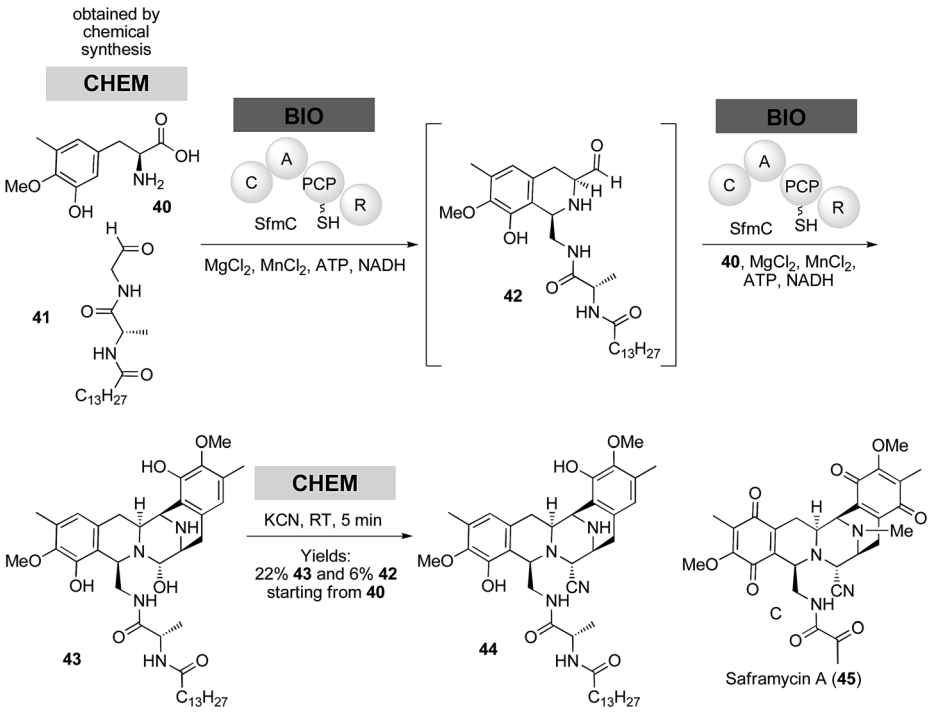
An illustrative example is based on the mutasynthesis with a mutant of the ansamitocin P3 (**6**) producer *Actinosynnema pretiosum* that is blocked in the biosynthesis of 3-amino-5-hydroxybenzoic acid (**5**, AHBA).[25] The ansamitocins are some of the most cytotoxic polyketide-derived natural products known, with IC_{50} values of 10^{-3} to 10^{-7} μg mL^{-1} against different cancer cell lines. They have recently re-attracted attention because of their extraordinary potency and are currently being evaluated in phase I studies for their use as "warheads" in target-directed antibody conjugates.[26]

Supplementing cultures of the *A. pretiosum* mutant with 3-amino-4-bromobenzoic acid **35** leads to bromo-ansamitocin derivative **36**, which has served as the cross-coupling partner in various Pd-catalyzed Stille and Sonogashira reactions (Scheme 7).[27] The power of the combined muta-/semisynthetic strategy becomes evident in the five-step semisynthetic modification of bromide **36** to yield cancer-specific folic acid/ansamitocin conjugate **39**. This and other conjugates obtained by this route showed target specificity for the membrane-bound folic acid receptor of cancer cell lines.[28]

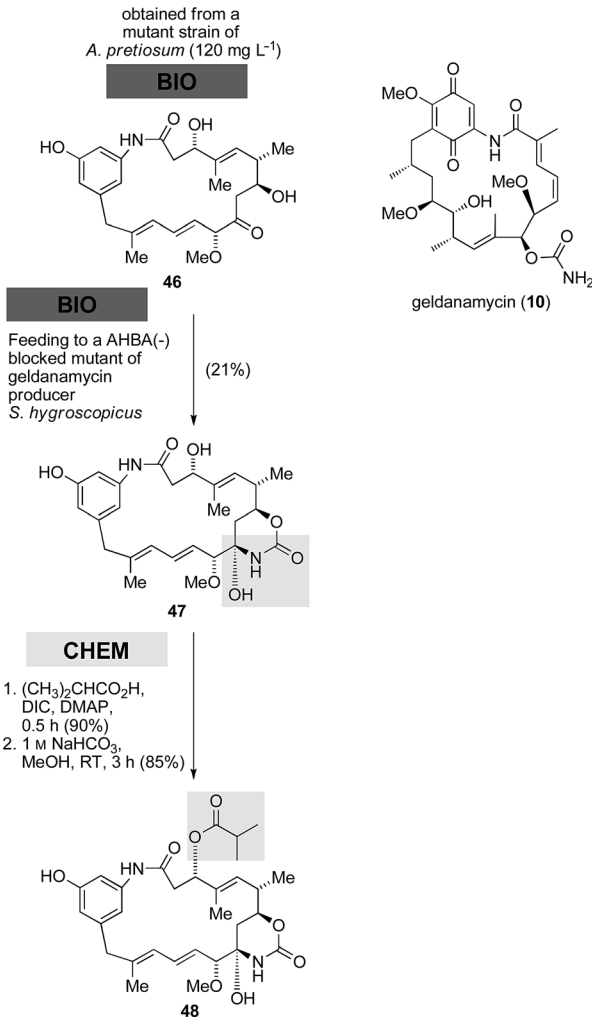
Koketsu et al. studied the function of the unusual NRPS module SfmC within the biosynthesis of saframycin (**45**; Scheme 8).[29] In the presence of ATP and NADH, holo-SfmC was incubated with the tyrosin derivative **40** and the dipeptidyl aldehyde **41**, both obtained by chemical synthesis, to yield the saframycin precursor **43**, which was converted chemically into **44** in sufficient amounts. Compound **44** served as an advanced intermediate in the "end game" synthesis of saframycin (**45**) as well as structurally related alkaloids with similar core structures such as ecteinascidin ET-743 and phthalascidin Pt-650 and derivatives thereof.[30]

7. BIO-BIO-CHEM

The order of chemical synthesis and biosynthesis can also be altered from a CHEM-BIO-CHEM to a BIO-BIO-CHEM synthetic sequence. A notable example is proansamitocin (**46**), the fermentation product of a mutant strain of the ansamitocin producer *A. pretiousum*, whose chloridase and carbamoyl transferase were genetically blocked. This manipulation results in the complete blockage of all post-PKS transformations (Scheme 9).[31] When proansamitocin is fed to a AHBA-blocked mutant of *S. hygroscopicus*, the producer of the Hsp90 inhibitor geldanamycin **10**,[32] carbamoylation at



Scheme 8. The unusual NRPS module SfmC can assemble the core structure of saframycin (**45**) by catalyzing two consecutive Pictet–Spengler reactions; the synthesis is complemented by chemical synthesis (C = condensation domain, A = adenylation domain, PCP = peptidyl carrier protein, R = reduction domain).[29]



Scheme 9. A BIO-BIO-CHEM approach towards ansamitocin P3 derivative **48** by using two different mutant strains of *A. pretiosum* and *S. hygroscopicus* followed by selective introduction of the ester side chain at C3.[31] DIC = diisopropylcarbodiimide, DMAP = 4-dimethylaminopyridine.

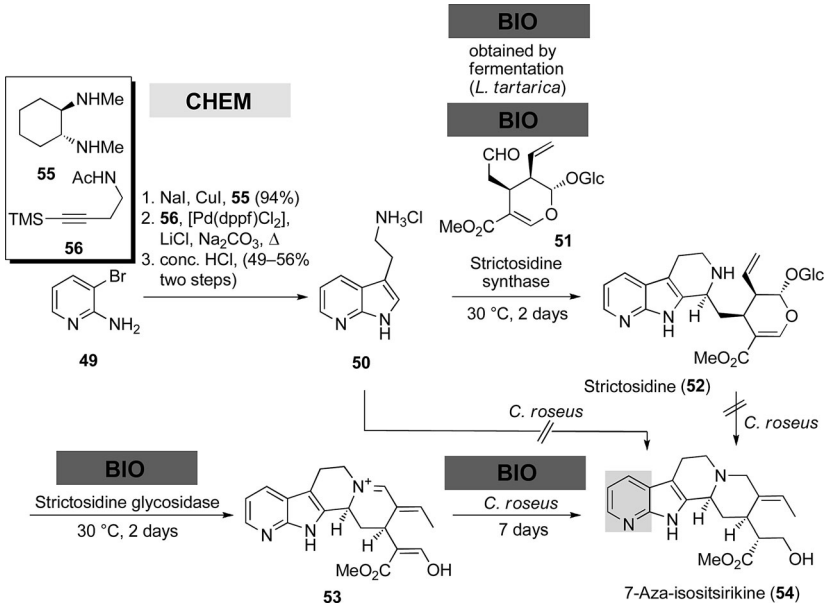
C7 took place to yield proansamtocin derivative **47**. A short semisynthetic sequence allowed selective introduction of the isobutyrate side chain at C3 and formation of cytotoxic ansamitocin derivative **48**. This is an example that shows that blocked mutants of different natural product producers can be included into a synthetic protocol.

8. CHEM-BIO-BIO

An elegant example from the field of mixed terpene-alkaloid synthesis that relies on chemical synthesis followed by a two-step enzymatic transformation protocol and a fermentation is shown in Scheme 10.[33] 7-Aza-tryptamine **50** obtained from pyridyl bromide **49** by chemical synthesis was subjected to a two-step biotransformation based on recombinant stricdosidine synthase and strictosidine glycosidase to yield advanced precursor **53** via intermediate **52**.[34] Stricdosidine synthase requires secologanine (**51**), which was obtained by fermentation.[34a,b] Finally, imminium salt **53** was fed to a plant cell culture of *Catharanthus roseus* to furnish the novel derivative 7-aza-isositsirikine (**54**), which was spectroscopically fully characterized. The HPLC-MS analysis suggested the presence of several aza anlogues of naturally occurring alkaloids in the extract that could, however, not be characterized because of the lack of material. Feeding the synthetic precursor **50** or the glycosylated product **52** alone did not lead to any new fermentation products, thus underlining the appropriateness of the chosen combination of chemical synthesis, enzyme catalysis, and fermentation.

9. Outlook

This short account is far from comprehensive and it is not intended to be so. We chose illustrative examples from the synthetic chemist's perspective, neglecting the huge amount



Scheme 10. Synthesis of **54**, an aza derivative of isositsirikine, by a two-step enzymatic biotransformation and a fermentation of the 7-azatryptamine precursor **53** (Glc = D-glucosyl).[32] dppf = 1,1′-bis(diphenylphosphino)ferrocene.

of molecular biology and protein chemistry necessary to provide the biological tools, being it either blocked mutants or individual enzymes.

Ongoing research in this area will likely make these tools more readily available, thereby lowering the barrier to use them for total syntheses in selected cases. In the future, novel combinations of chemistry with both in vitro and in vivo biotransformations might become possible. By refining the specificity and selectivity of biotransformation tools, their application could also be uncoupled from the biosynthetic pathway they originate from, thereby leading to a box of generally applicable synthetic tools.

With a deeper understanding of biosynthetic pathways of complex secondary metabolites and the development of tools based on molecular biology to manipulate biosynthetic pathways at the genetic level, the total synthesis of natural products and analogues becomes more flexible and may reappear back on the agenda of drug development, particularly when manipulated biosynthesis is "hybridized" with chemical synthesis. It took half a century to reach this sophistication, but Birch's synthetic vision is still young and holds great future prospects.

Received: October 19, 2011
Published online: March 22, 2012

[1] A. J. Birch, *Pure Appl. Chem.* **1963**, *7*, 527 – 538.
[2] U. Klar, B. Buchmann, W. Schwede, W. Skuballa, J. Hoffmann, R. B. Lichtner, *Angew. Chem.* **2006**, *118*, 8110 – 8116; *Angew. Chem. Int. Ed.* **2006**, *45*, 7942 – 7948.
[3] *Anticancer agents from natural products* (Eds.: M. J. Yu, Y. Kishi, B. A. Littlefield in D. J. Newman, D. G. I. Kingston, G. M. Cragg), Washington DC, Taylor & Francis, **2005**, ISBN 0-8493-1863-7.
[4] Total synthesis of halichondrin B: T. D. Aicher, K. R. Buszek, F. G. Fang, C. J. Forsyth, S. H. Jung, Y. Kishi, M. C. Matelich, P. M. Scola, D. M. Spero, S. K. Yoon, *J. Am. Chem. Soc.* **1992**, *114*, 3162 – 3164.
[5] K. C. Nicolaou, W. M. Dai, R. K. Guy, *Angew. Chem.* **1994**, *106*, 38 – 69; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 15 – 44.
[6] J. N. Denis, A. E. Green, D. Guénard, F. Guéritte-Voegelein, L. Mangatal, P. Potier, *J. Am. Chem. Soc.* **1988**, *110*, 5917 – 5919.
[7] P. M. Dewick, *Medicinal Natural Products*: *A Biosynthetic Approach*, Wiley, Chichester, **2009**, ISBN 978-0-470-74167-2.
[8] a) T.-W. Yu, L. Bai, D. Clade, D. Hoffmann, S. Toelzer, K. Q. Trinh, J. Xu, S. J. Moss, E. Leistner, H. G. Floss, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 7968 – 7973; b) P. Spiteller, L. Bai, G. Shang, B. J. Carroll, T.-W. Yu, H. G. Floss, *J. Am. Chem. Soc.* **2003**, *125*, 14236 – 14237.
[9] J. Wegner, S. Ceylan, A. Kirschning, *Chem. Commun.* **2011**, *47*, 4583 – 4592.
[10] I. R. Baxendale, J. Deelay, C. M. Griffiths-Jones, S. V. Ley, S. Saaby, G. K. Tranmer, *Chem. Commun.* **2006**, 2566 – 2568.
[11] a) K. J. Weissman, P. F. Leadlay, *Nat. Rev. Microbiol.* **2005**, *3*, 925 – 936; b) C. T. Walsh, *ChemBioChem* **2002**, *3*, 124 – 134; c) H. G. Menzella, C. D. Reeves, *Curr. Op. Microbiol.* **2007**, *10*, 238 – 245.
[12] a) S. Weist, R. D. Süssmuth, *Appl. Microbiol. Biotechnol.* **2005**, *68*, 141 – 150; b) A. Kirschning, F. Taft, T. Knobloch, *Org. Biomol. Chem.* **2007**, *5*, 3245 – 3259; c) K. Weissman, *Trends Biotechnol.* **2007**, *25*, 139 – 142.
[13] For simplification, the synthetic concepts are depicted in a linear fashion. At least for chemical synthesis, this is not necessarily advantageous, as discussed in the text.
[14] K. Patel, M. Piagentini, A. Rascher, Z. Q. Tian, G. O. Buchanan, R. Regentin, Z. Hu, C. R. Hutchinson, R. McDaniel, *Chem. Biol.* **2004**, *11*, 1625 – 1633.
[15] a) P. Workman, *Curr. Cancer Drug Targets* **2003**, *3*, 297 – 300; b) L. Neckers, K. Neckers, *Expert Opin. Emerging Drugs* **2005**, *10*, 137 – 149; c) L. Whitesell, S. L. Lindquist, *Nat. Rev. Cancer* **2005**, *5*, 761 – 772.
[16] T. Schwecke, J. F. Aparicio, I. Molnar, A. König, L. E. Khaw, S. F. Haydock, M. Oliynyk, P. Caffrey, J. Cortes, J. B. Lester, G. A. Böhm, J. Staunton, P. F. Leadlay, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7839 – 7843.
[17] A. S. Eustáquio, B. S. Moore, *Angew. Chem.* **2008**, *120*, 4000 – 4002; *Angew. Chem. Int. Ed.* **2008**, *47*, 3936 – 3938.
[18] B. Kusebauch, N. Brendel, H. Kirchner, H.-M. Dahse, C. Hertweck, *ChemBioChem* **2011**, *12*, 2284 – 2288.
[19] S. Weist, C. Kittel, D. Bischoff, B. Bister, V. Pfeifer, G. J. Nicholson, W. Wohlleben, R. D. Süssmuth, *J. Am. Chem. Soc.* **2004**, *126*, 5942 – 5943.
[20] a) R. D. Süssmuth, W. Wohlleben, *Appl. Microbiol. Biotechnol.* **2004**, *63*, 344 – 350; b) S. Weist, B. Bister, O. Puk, D. Bischoff, S. Pelzer, G. J. Nicholson, W. Wohlleben, G. Jung, R. D. Süssmuth, *Angew. Chem.* **2002**, *114*, 3531 – 3534; *Angew. Chem. Int. Ed.* **2002**, *41*, 3383 – 3385.
[21] J. D. Mortison, D. H. Sherman, *J. Org. Chem.* **2010**, *75*, 7041 – 7051.
[22] P. Bernhardt, S. E. O'Connor, *Curr. Opin. Chem. Biol.* **2009**, *13*, 35 – 42.
[23] a) Z. Q. Beck, C. C. Aldrich, N. A. Magarvey, G. I. Georg, D. H. Sherman, *Biochemistry* **2005**, *44*, 13457 – 13466; b) Z. Q. Beck, D. A. Burr, D. H. Sherman, *ChemBioChem* **2007**, *8*, 1373 – 1375; c) Y. Ding, W. H. Seufert, Z. Q. Beck, D. H. Sherman, *J. Am. Chem. Soc.* **2008**, *130*, 5492 – 5498; d) N. A. Magarvey, Z. Q. Beck, T. Golakoti, Y. Ding, U. Huber, T. K. Hemscheidt, D. Abelson, R. E. Moore, D. H. Sherman, *ACS Chem. Biol.* **2006**, *1*, 766 – 779.
[24] W. Seufert, Z. Q. Beck, D. H. Sherman, *Angew. Chem.* **2007**, *119*, 9458 – 9460; *Angew. Chem. Int. Ed.* **2007**, *46*, 9298 – 9300.
[25] A. Kirschning, K. Harmrolfs, T. Knobloch, *C. R. Chim.* **2008**, *11*, 1523 – 1543.
[26] a) C. A. Ladino, R. V. Chari, L. A. Bourret, N. L. Kedersha, V. S. Goldmacher, *Int. J. Cancer* **1997**, *73*, 859 – 864; b) I. Niculescu-Duvaz, *Curr. Opin. Mol. Ther.* **2010**, *12*, 350 – 360; c) J. R. Junutula, K. M. Flagella, R. A. Graham, K. L. Parsons, E. Ha, H. Raab, S. Bhakta, T. Nguyen, D. L. Dugger, G. Li, E. Mai, G. D. Lewis Phillips, H. Hiraragi, R. N. Fuji, J. Tibbitts, R. Vandlen, S. D. Spencer, R. H. Scheller, P. Polakis, M. X. Sliwkowski, *Clin. Cancer Res.* **2010**, *16*, 4769 – 4778; d) J. A. Reddy, E. Westrick, H. K. Santhapuram, S. J. Howard, M. L. Miller, M. Vetzel, I. Vlahov, R. V. Chari, V. S. Goldmacher, C. P. Leamon, *Cancer Res.* **2007**, *67*, 6376 – 82.
[27] F. Taft, M. Brünjes, H. G. Floss, N. Czempinski, S. Grond, F. Sasse, A. Kirschning, *ChemBioChem* **2008**, *9*, 1057 – 1060.
[28] F. Taft, K. Harmrolfs, I. Nickeleit, A. Heutling, M. Kiene, N. Malek, F. Sasse, A. Kirschning, *Chem. Eur. J.* **2012**, *18*, 880 – 886.
[29] K. Koketsu, K. Watanabe, H. Suda, H. Oguri, H. Oikawa, *Nat. Chem. Biol.* **2010**, *6*, 408 – 410.
[30] C. Cuevas, M. Perez, M. J. Martín, J. L. Chicharro, C. Fernández-Rivas, M. Flores, A. Francesch, P. Gallego, M. Zarzuelo, F. de La Calle, J. García, C. Polanco, I. Rodríguez, I. Manzanares, *Org. Lett.* **2000**, *2*, 2545 – 2548.
[31] S. Eichner, F. Taft, T. Knobloch, K. Harmrolfs, A. Kirschning, *Angew. Chem.* **2012**, *124*, 776 – 781; *Angew. Chem. Int. Ed.* **2012**, *51*, 752 – 757.

[32] S. Eichner, H. G. Floss, F. Sasse, A. Kirschning, *ChemBioChem* **2009**, *10*, 1801 – 1805.
[33] H. Y. Lee, N. Yerkes, S. E. O'Connor, *Chem. Biol.* **2009**, *16*, 1225 – 1229.
[34] a) E. McCoy, M. C. Galan, S. E. O'Connor, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2475 – 2478; b) G. Kinast, L. F. Tietze, *Chem. Ber.* **1976**, *109*, 3640 – 3645; c) P. Bernhardt, N. Yerkes, S. E. O'Connor, *Org. Biomol. Chem.* **2009**, *7*, 4166 – 4168; d) P. Bernhardt, S. E. O'Connor, *Tetrahedron Lett.* **2009**, *50*, 7118 – 7120; e) P. Bernhardt, A. R. Usera, S. E. O'Connor, *Tetrahedron Lett.* **2010**, *51*, 4400 – 4402; f) N. Yerkes, J. X. Wu, E. McCoy, M. C. Galan, S. Chen, S. E. O'Connor, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3095 – 3098.