Natural Product Synthesis

DOI: 10.1002/anie.201006154

Concise Total Syntheses of Variecolortides A and B through an Unusual Hetero-Diels–Alder Reaction**

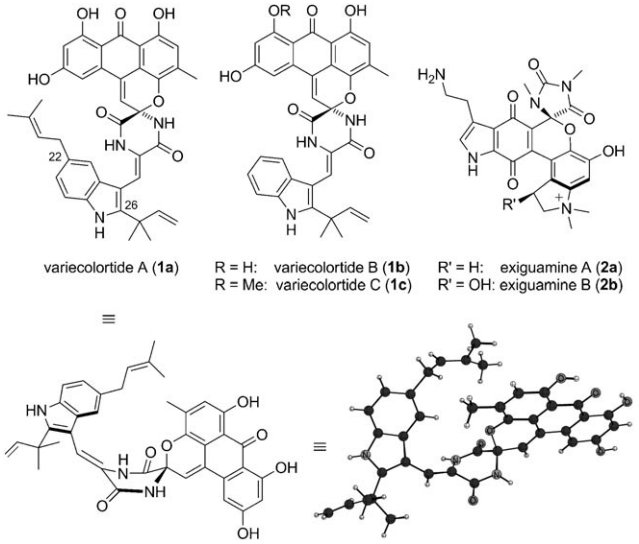
Christian A. Kuttruff, Hendrik Zipse, and Dirk Trauner**

Speculations on the biosynthetic origin of natural products continue to inspire synthetic chemists and have led to many elegant and efficient total syntheses.[1] Many of these involve cascade reactions, where a high-energy substrate is formed and then spontaneously reacts further to create increasingly more complex products.[2] As such, biomimetic strategies do not rely on the use of sophisticated enzymes, which are usually, but not always, required in a "real" biosynthesis.

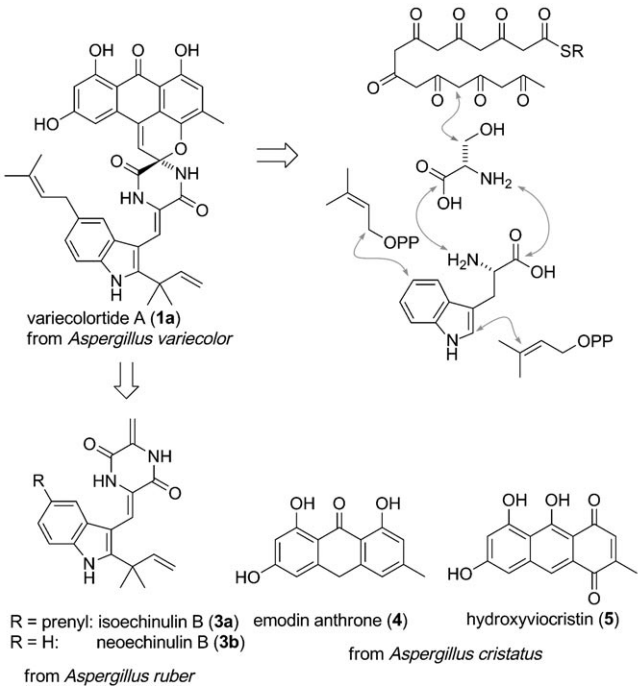
We recently published a synthesis of the dopamine-derived alkaloids exiguamine A and B (**2a, b**) that relied on a biomimetic pericyclic reaction and oxidation cascade (Scheme 1).[3] The exiguamines occur as racemates in nature and feature a spirobicyclic N,O-acetal as a key structural element. It was therefore unsurprising that the variecolortides (**1a–c**), a newly disclosed family of racemic N,O-acetals, piqued our interest.[4] These unusual fungal natural products were isolated from a halotolerant strain of the fungus *Aspergillus variecolor* and were shown to have modest cytotoxic effects.[4]

Structurally, the variecolortides feature an unprecedented 9,10-anthraquinone methide moiety fused to a dihydropyran (Scheme 1). This flat tetracyclic ring system is further linked to a diketopiperazine, generating a spirocyclic N,O-acetal. The central diketopiperazine is also coupled to an indole, which is reverse-prenylated at C26. Variecolortide A (**1a**) is the most complex member of the family, owing to the additional prenyl group at C22 of the indole nucleus, which is lacking in variecolortide B (**1b**) and its methyl ether variecolortide C (**1c**).

Biosynthetically, the variecolortides appear to stem from a C_{16} polyketide, the amino acids serine and tryptophan, and one or two equivalents of dimethylallyl pyrophosphate (Scheme 2). As such, they represent a unique merger of three major streams of biosynthesis: the shikimic acid pathway (for the aromatic amino acid tryptophan), the type II polyketide pathway (for the anthraquinone derivative), and



Scheme 1. Racemic natural products featuring N,O-acetals.



Scheme 2. Biosynthetic analysis of the variecolortides. PP = diphosphate.

[*] C. A. Kuttruff, Prof. Dr. H. Zipse, Prof. D. Trauner
Department of Chemistry and Pharmacology
and Center of Integrated Protein Science (CIPSM)
Ludwig-Maximilians-Universität
Butenandtstrasse 5–13 (F4.086), 81377 Munich (Germany)
Fax: (+49) 89-2180-77972.
E-mail: dirk.trauner@lmu.de
zipse@cup.uni-muenchen.de

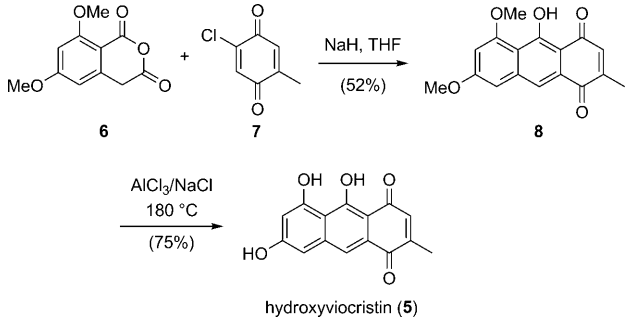
[**] We thank the CIPSM for financial support. C.A.K. is grateful to the Fonds der Chemischen Industrie for a PhD scholarship. We thank undergraduate researchers Simon Geiger and Andreas Fetzer. We also thank Dr. Eddie Myers for proofreading this manuscript and Mesut Cakmak and Anastasia Hager for insightful discussions.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201006154.

the terpenoid pathway (for the prenyl and reverse-prenyl side chains). While many natural compounds are known that integrate three or more different biosynthetic pathways, this particular combination appears to be rare if not unknown.

Natural products that exhibit a subset of the structural features of the variecolortides, however, are well known. Several decades ago, Laatsch and Anke reported the structures of emodin anthrone (**4**) and hydroxyviocristin (**5**), an anthrone and a 1,4-anthraquinone, respectively, that bear a substitution pattern corresponding to the variecolortides (Scheme 2).[5] These compounds were isolated from *Aspergillus cristatus*. The unsaturated diketopiperazine isoechinulin B (**3a**)[6] was obtained from *Aspergillus ruber*, along with several congeners that lack a prenyl moiety or in which the *exo*-methylene moiety is oxidatively cleaved, hydrogenated, or masked as a methanol adduct.[7] At the outset of our studies it was not clear, however, how these natural products, which have not been reported to occur in *Aspergillus variecolor*, would come together to form the variecolortides. We now report a concise total synthesis of **1a** and **1b** that offers a surprisingly simple solution for the linkage of the anthraquinone and diketopiperazine components and incorporates a new type of Diels–Alder reaction[8] that could be biosynthetically relevant.

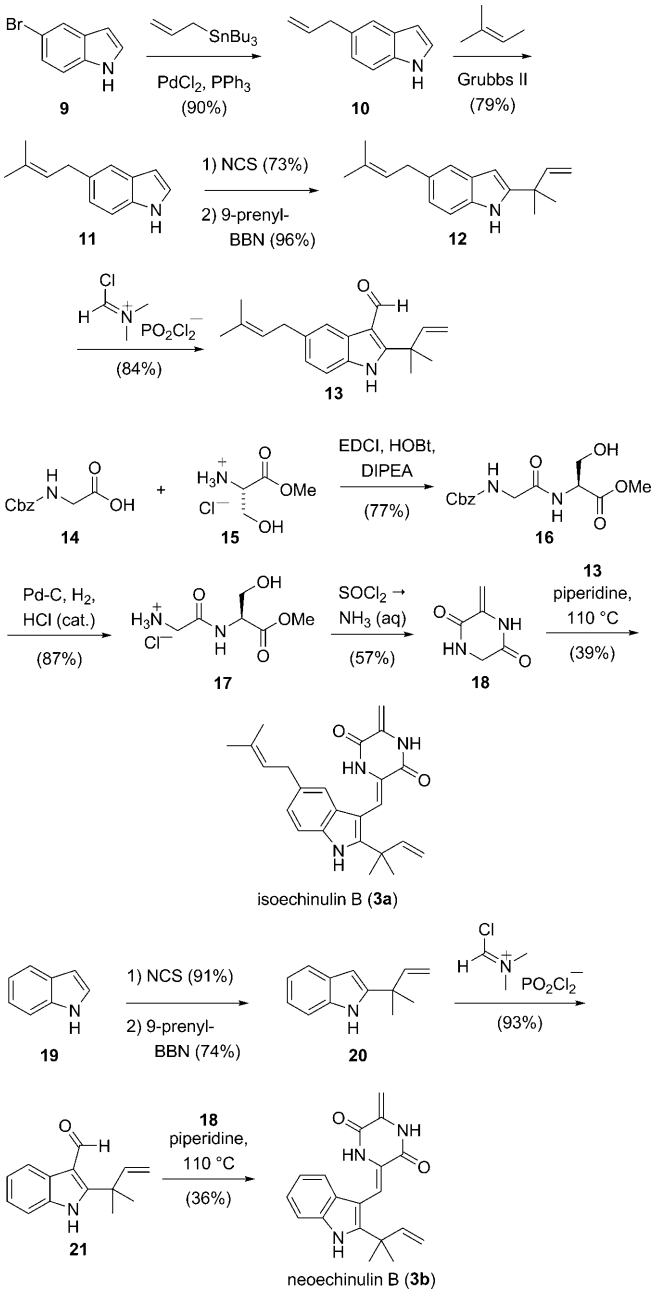
Our total synthesis of the variecolortides started with the preparation of hydroxyviocristin (**5**; Scheme 3). Deprotonation of the known orsellinic acid derived anhydride **6**,[9]



Scheme 3. Total synthesis of hydroxyviocristin (**5**).

followed by addition of the resultant benzylic anion to chloro *para*-benzoquinone **7** resulted in a conjugate addition/decarboxylation sequence to give the known 1,4-anthraquinone **8**.[10] Subsequent demethylation of this material in molten $AlCl_3$/NaCl yielded hydroxyviocristin (**5**).

The synthesis of the diketopiperazine–indole component started with palladium-catalyzed cross-coupling of 5-bromoindole (**9**) with tributylallylstannane, followed by Grubbs olefin cross-metathesis to give prenylated indole **11** (Scheme 4). Installation of the remaining prenyl group in the reverse sense was achieved by subjecting **11** to Danishefsky conditions to give **12**.[11] Intermediate **12** was then formylated with the Vilsmeier reagent to give **13**. In order to incorporate the amino acid portion, carbobenzoxy-protected glycine **14** was condensed with serine methyl ester (**15**) to afford dipeptide **16**, which after removal of the Cbz group, underwent cyclization and formal elimination of water to

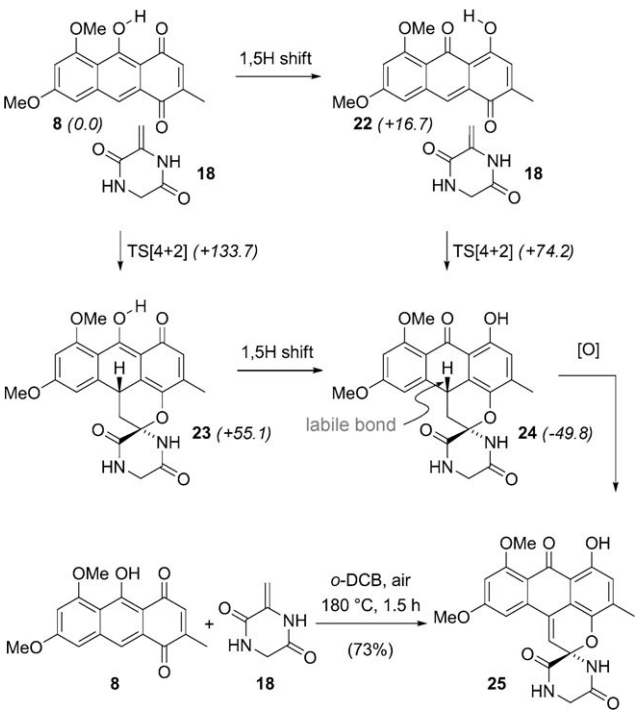


Scheme 4. Total synthesis of isoechinulin B (**3a**) and neoechinulin B (**3b**). BBN = 9-borabicyclo[3.3.1]nonane, Cbz = benzyloxycarbonyl, DIPEA = *N*,*N*-diisopropylethylamine, EDCI = *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide, HOBt = 1-hydroxybenzotriazole, NCS = *N*-chlorosuccinimide.

afford *exo*-methylene diketopiperazine **18**. Following a protocol developed by Kishi et al., we condensed this material with formyl indole **13** to afford isoechinulin B (**3a**) as a single isomer.[12] An analogous sequence starting from indole (**19**→**20**→**21**) provided neoechinulin B (**3b**; Scheme 4).

With both hydroxyviocristin (**5**) and the echinulins (**3a, 3b**) in hand, we were in a position to investigate the critical coupling of these components. Our original plan had called for the use of nucleophilic or radical additions using either **5** or emodin anthrone (**4**) as the polyketide building block.

However, this strategy was eventually abandoned because of the poor reactivity of *exo*-methylene diketopiperazines towards anionic nucleophiles and our inability to control the radical additions of emodin anthrone. We therefore turned our attention to a cycloaddition strategy, which was initially explored experimentally and computationally with a model system (Scheme 5). We reasoned that hydroxyviocristin (**5**)



Scheme 5. A model system for the key cycloaddition. The relative energies of key intermediates and activation barriers are indicated in brackets (see text). *o*-DCB = *ortho*-dichlorobenzene, TS[4+2] = transition state of the [4 + 2] cycloaddition.

and its dimethyl ether **8** could undergo an intramolecular 1,5-hydrogen shift to afford the quinone methide tautomer **22**. This reactive intermediate would be prone to undergo a hetero-Diels–Alder cycloaddition with *exo*-methylene diketopiperazine **18** to afford spiro-N,O-acetal **24**. Being an anthrone, such an intermediate exhibits an extremely labile benzylic C–H bond and would undergo rapid oxidation in the presence of air to yield the 9,10-anthraquinone methide **25**. Indeed, when **8** and **18** were heated together in a sealed tube with an aerobic headspace, **25** was isolated as the only identifiable product (Scheme 5). This compound corresponds to the upper portion of the variecolortides and could even be an intermediate in their synthesis.

Our proposed Diels–Alder/oxidation mechanism is supported by density functional calculations performed at B3LYP/6-31G(d) level (see Scheme 5 and Figure S1 in the Supporting Information). According to these calculations, tautomer **8** reacts with **18** through a concerted, asynchronous pathway to yield cycloaddition product **23**. This process is considerably endothermic (by 55.1 kJ mol^{-1}) and faces a large reaction barrier of + 133.7 kJ mol^{-1}. By contrast, tautomer **22** is slightly less stable than **8** by 16.7 kJ mol^{-1}, but significantly more reactive with respect to the Diels–Alder reaction with dienophile **18**. The reaction barrier now amounts to a mere + 74.2 kJ mol^{-1}, and formation of the cycloaddition product **24** is exothermic by 49.8 kJ mol^{-1}. Owing to the considerable differences in reaction energetics and reaction barriers, it is clear that only cycloaddition through tautomer **22** is relevant under the experimental conditions.

The calculated geometry of the transition state of this reaction is depicted in Figure 1. Our calculations also show that the *exo*-methylene diketopiperazine **18** functions as the nucleophilic component in an asynchronous cycloaddition, wherein the carbon–carbon bond is formed to a larger extent than the carbon–oxygen bond in the transition state.

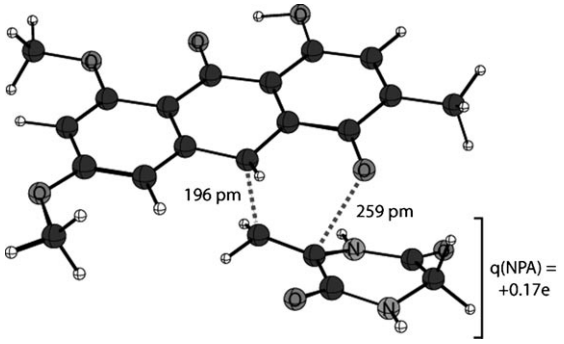
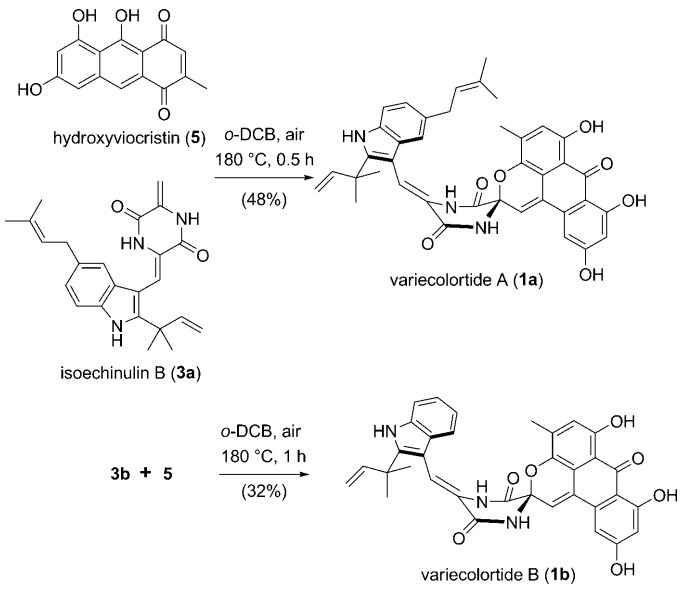


Figure 1. Calculated transition state of the asynchronous hetero-Diels–Alder reaction involving **22** and **18**. The lengths of the forming bonds and the partial charge *q*(NPA) of the heterodienophile are indicated. NPA = natural population analysis.

In order to assess the feasibility of the proposed oxidation of **24** to give the isolated reaction product **25**, the stability of the bisbenzylic C–H bond indicated in Scheme 5 was calculated using a series of reference compounds at the G3B3 level of theory. Using appropriate isodesmic reactions the C–H bond dissociation energy (BDE) for this C–H bond amounts to + 315.9 kJ mol^{-1} (see the Supporting Information for details). This value is even less than that found in common reducing agents such as 1,4-cyclohexadiene [BDE(C–H) = + 318.0 kJ mol^{-1})], $HSnBu_3$ [BDE(Sn–H) = + 328.9 kJ mol^{-1}], and thiophenol [BDE(S–H) = + 335.4 kJ mol^{-1}] and thus in full support of the in situ oxidation pathway proposed above.[13]

Armed with these insights and having optimized our key step with model compounds, we proceeded to complete the syntheses of the variecolortides (Scheme 6). We anticipated that the disubstituted *exo*-methylene moiety in the echinulins would be the most reactive heterodienophile and that the correct regioisomers would be formed. We were pleased to find that heating of hydroxyviocristin (**5**) and isoechinulin B (**3a**) in *ortho*-dichlorobenzene indeed afforded variecolortide A in 48% yield. Similarly, variecolortide B was obtained from building blocks **3b** and **5**. The modest yields of these key reactions probably reflect the known instability of hydroxyviocristin.[5] The variecolortides were the only isomers isolated under these conditions; no regioisomers and no



Scheme 6. The total synthesis of variecolortide A and B.

reaction with other double bonds present in the echinulins were observed.

While the conditions employed in our key reaction can certainly not be deemed biomimetic, the concerted nature of the reaction does raise some interesting biosynthetic questions. Preliminary experiments under more biological conditions (aqueous phosphate buffer at ambient temperature) have failed to yield any identifiable products. Given these results, and the calculated activation barriers, it appears that the variecolortides are not the products of an adventitious, uncatalyzed Diels–Alder reaction occurring in the fungus. This raises the interesting possibility that a "Diels–Alderase" is involved.[14] Also, while it is entirely conceivable that **5** and **3a**/**3b** are the true biosynthetic precursors of the variecolortides, the possibility that the linkage takes place before the polyketide moiety is released from a type II polyketides synthase must also be considered. The exact sequence in which the individual biosynthetic components of the variecolortides come together, and the mechanism of their fusion remain to be determined.

In summary, we have developed a concise total synthesis of variecolortide A and B that provides the natural racemates in seven and five steps, respectively (longest linear sequence). Our synthesis is largely devoid of protecting-group operations[15] and is highly convergent. It incorporates an unprecedented hetero-Diels–Alder reaction of a 1,4-anthraquinone with a didehydrodiketopiperazine to form the central spirocyclic core of the natural products. Density functional theory calculations strongly support our hypothesis that this key reaction proceeds through a concerted cycloaddition. Our synthesis also raises questions about whether a similar step could occur in nature and whether an enzyme is involved. Answering these questions will require detailed biosynthetic studies, which are beyond the scope of the present work. Meanwhile, our laboratory synthesis provides ample access to the variecolortides and allows for a full biological exploration of these fascinating natural products.

Received: October 1, 2010
Revised: October 29, 2010
Published online: December 29, 2010

Keywords: alkaloids · biomimetic synthesis · cascade reactions · cycloaddition · total synthesis

[1] K. C. Nicolaou, D. Vourloumis, N. Winssinger, P. S. Baran, *Angew. Chem.* **2000**, *112*, 46–126; *Angew. Chem. Int. Ed.* **2000**, *39*, 44–122.
[2] a) L. F. Tietze, G. Brasche, K. Gericke, *Domino Reactions in Organic Synthesis*, Wiley-VCH, Weinheim, **2006**; b) K. C. Nicolaou, D. J. Edmonds, P. G. Bulger, *Angew. Chem.* **2006**, *118*, 7292–7344; *Angew. Chem. Int. Ed.* **2006**, *45*, 7134–7186.
[3] M. Volgraf, J.-P. Lumb, H. C. Brastianos, G. Carr, M. K. W. Chung, M. Munzel, A. G. Mauk, R. J. Andersen, D. Trauner, *Nat. Chem. Biol.* **2008**, *4*, 535–537.
[4] W.-L. Wang, T.-J. Zhu, H.-W. Tao, Z.-Y. Lu, Y.-C. Fang, Q.-Q. Gu, W. M. Zhu, *Chem. Biodiversity* **2007**, *4*, 2913–2919.
[5] H. Laatsch, H. Anke, *Liebigs Ann. Chem.* **1982**, 2189–2215.
[6] H. Nagasawa, A. Isogai, A. Suzuki, S. Tamura, *Tetrahedron Lett.* **1976**, *17*, 1601–1604.
[7] W.-L. Wang, Z.-Y. Lu, H.-W. Tao, T.-J. Zhu, Y.-C. Fang, Q.-Q. Gu, W.-M. Zhu, *J. Nat. Prod.* **2007**, *70*, 1558–1564.
[8] K. C. Nicolaou, S. A. Snyder, T. Montagnon, G. Vassilikogiannakis, *Angew. Chem.* **2002**, *114*, 1742–1773; *Angew. Chem. Int. Ed.* **2002**, *41*, 1668–1698.
[9] W. E. Bauta, D. P. Lovett, W. R. Cantrell, B. D. Burke, *J. Org. Chem.* **2003**, *68*, 5967–5973.
[10] E. Caliskan, D. W. Cameron, P. G. Griffiths, *Aust. J. Chem.* **1999**, *52*, 1013–1020.
[11] a) J. M. Schkeryantz, J. C. G. Woo, P. Siliphaivanh, K. M. Depew, S. J. Danishefsky, *J. Am. Chem. Soc.* **1999**, *121*, 11964–11975; b) K. A. Miller, T. R. Welch, T. J. Greshock, Y. Ding, D. H. Sherman, R. M. Williams, *J. Org. Chem.* **2008**, *73*, 3116–3119.
[12] S. Inoue, J. Murata, N. Takamatsu, H. Nagano, Y. Kishi, *Yakugaku Zasshi* **1977**, *97*, 576–581.
[13] J. Hioe, H. Zipse, *Org. Biomol. Chem.* **2010**, *8*, 3609–3617.
[14] E. M. Stocking, R. M. Williams, *Angew. Chem.* **2003**, *115*, 3186–3223; *Angew. Chem. Int. Ed.* **2003**, *42*, 3078–3115.
[15] I. S. Young, P. S. Baran, *Nat. Chem.* **2009**, *1*, 193–205.

