Pure Appl. Chem., Vol. 85, No. 6, pp. 1103–1120, 2013.
http://dx.doi.org/10.1351/PAC-CON-12-09-12

Stereochemistry and total synthesis of complex myxobacterial macrolides*

Sebastian Essig[1] and Dirk Menche[2,‡]

[1]Institut für Organische Chemie, Universität Heidelberg, INF 270, D-69120 Heidelberg, Germany; [2]Kekulé-Institut für Organische Chemie und Biochemie der Universität Bonn, Gerhard-Domagk-Str. 1, D-53121 Bonn, Germany

Abstract: Polyketides are a very diverse family of natural products with an extremely broad range of biological activities and pharmacological properties, including antiproliferative, antibiotic, antifungal, or antiplasmodial activities, and in many cases specific targets are addressed at the molecular level. Their structures are characterized by diverse assemblies of methyl- and hydroxyl-bearing stereogenic centers enabling large numbers of stereochemical permutations, which are often embedded into macrolide rings. This complexity renders the stereochemical assignment and directed total synthesis challenging tasks. Within this review, we will detail practicable approaches for the stereochemical determination of diverse complex polyketides of myxobacterial origin by using computational and NMR methods in combination with novel procedures based on bioinformatics. Furthermore, we have developed efficient preparative strategies for the synthesis of these compounds, which have culminated in several first total syntheses. Key aspects of these various endeavors, which will also focus on the importance of conformational bias in complex polyketide analysis and synthesis, will be discussed within this review in the realm of the potent macrolide antibiotics etnangien and rhizopodin. Along these lines, we will also summarize novel methods for the rapid assembly of key structural elements of polyketides including a novel domino concept relying on a combination of a nucleophilic addition and a Tsuji–Trost reaction.

Keywords: macrolides; natural products; polyketides; stereochemistry; total synthesis.

INTRODUCTION

Polyketides are structurally a diverse class of natural metabolites that are characterized by a broad range of biological activities [1], and polyketide antibiotics, antifungals, cytostatics, antiparasitics, and natural insecticides are in commercial use [2]. Biosynthetically, they are derived from iterative condensations of acetyl and propionyl subunits giving rise to diverse assemblies of methyl- and hydroxyl-bearing stereogenic centers. This enables large numbers of stereochemical permutations [3], which are often embedded into a macrolide substructure. The resulting diversity together with a high degree of spectral complexity and conformational flexibility renders the stereochemical assignment of polyketides a challenging task [4]. In addition to various marine organisms, fungi and plants myxobacteria are a particularly rich source of novel polyketides. Over the last three decades, owing to the pioneering work of the groups of Höfle and Reichenbach at the Helmholtz-Zentrum für Infektionsforschung (Center for

Pure Appl. Chem.* **85, 1079–1239 (2013). A collection of invited papers based on presentations at the 19th International Conference on Organic Synthesis (ICOS-19), Melbourne, Australia, 1–6 July 2012.
‡Corresponding author

Infection Research in Braunschweig, HZI), an impressive number of structurally unique and biosynthetically diverse polyketides [5] have been reported from these soil-living organisms [6]. In total, they span a range of approximately 80 structurally new polyketide classes and many structural variants thereof. Most prominently, the epothilones (**1**) [7] are natural antiproliferative agents from the myxobacterium *Sorangium cellulosum* (Fig. 1), which have been developed and approved as anticancer drugs for the treatment of metastatic breast cancer [8]. In contrast, many other polyketides are much less advanced, despite similarly promising biological profiles, including antiproliferative, antibiotic, antifungal, or antiplasmodial activities. Also, in many cases specific targets are selectively addressed on a molecular level, ranging from the cytoskeleton, nucleic acid polymerases, the respiratory chain, the nuclear transport, microfilaments, protein or fatty acid synthesis, which adds to the attractiveness for further development of these bioactive agents [1]. Prominent examples of such compounds, which were investigated in our group, include rhizopodin (**2**) [9], archazolid (**3**) [10], etnangien (**4**) [11], and the ajudazols (**5**) [12]. Rhizopodin (**2**) and archazolid (**3**) are powerful antiproliferative agents that inhibit the growth of various cancer cell lines with IC_{50} values in low nanomolar or subnanomolar range, by selectively addressing and G-actin [13] and vacuolar type ATPases (V-ATPases) [14], respectively. Etnangien (**4**), in turn, presents an effective antibiotic, which interacts with bacterial RNA-polymerase [11,15], one of the few validated targets in antibiotic research, while the fungicide ajudazol A (**5**) is a highly effective inhibitor of the mitochondrial respiratory chain by binding selectively to complex I (NADH-dehydrogenase) [16].

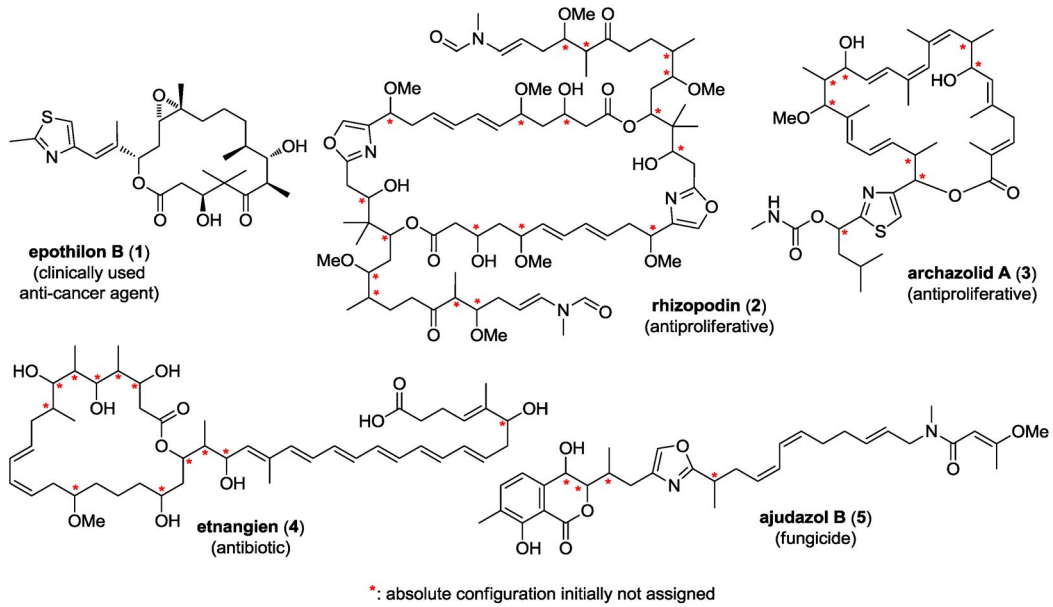


Fig. 1 Potent polyketide macrolides of myxobacterial origin.

Surprisingly, the stereochemistry of these and many other polyketides has originally not been assigned, despite the importance of configuration for biological activity and the general significance of stereochemical knowledge to many other fields ranging from chemical physics, biochemistry to synthetic organic chemistry or catalysis, and, consequently, also no total synthesis of these compounds has initially been developed. With this background, we initiated a program directed towards the stereochemical determination and total synthesis of complex myxobacterial polyketides [4]. Herein, we report key aspects of these studies that implied the development and application of generally useful and prac-

tical methods for the stereochemical determination and total synthesis of complex polyketide macrolides. Within this review, etnangien (**4**) and rhizopodin (**2**) will be discussed as representative examples for the various aspects involved.

STEREOCHEMICAL DETERMINATION OF ETNANGIEN

The polyketide natural product etnangien (**4**, Fig. 2) from the myxobacterium *S. cellulosum* constitutes a structurally novel type of a particularly efficient RNA polymerase inhibitor in vitro and in vivo [11]. It is effective against a broad panel of Gram-positive bacteria, especially those belonging to the actinomycetes. In addition, etnangien (**4**) appears to exhibit no cross-resistance to rifampicin, a clinically validated RNA-polymerase inhibitor. The increase of bacterial resistance against different types of antibiotics renders the development of structurally new types of antibiotics an important research goal [17] and raised our interest in the stereochemical determination, total synthesis, and further biological evaluation of etnangien (**4**). The planar constitution of etnangien (Fig. 1) was elucidated by Schummer and Jansen on the basis of NMR data [^{1}H and ^{13}C NMR, correlation spectroscopy (COSY), and heteronuclear multiple-quantum coherence (HMQC)] [11] and consists of a 22-membered macrolactone with two alkenes (30*Z*,32*E*) and a polyunsaturated side chain with 7 *trans*-configured alkenes. In summary, 12 stereogenic centers were determined, which have not been assigned, leaving 2^{12} = 4096 possible diastereomers. These stereogenic centers may be clustered into a northern (C35 to C40) and central (C20 to C24) subunit of vicinal and proximal stereogenic centers.

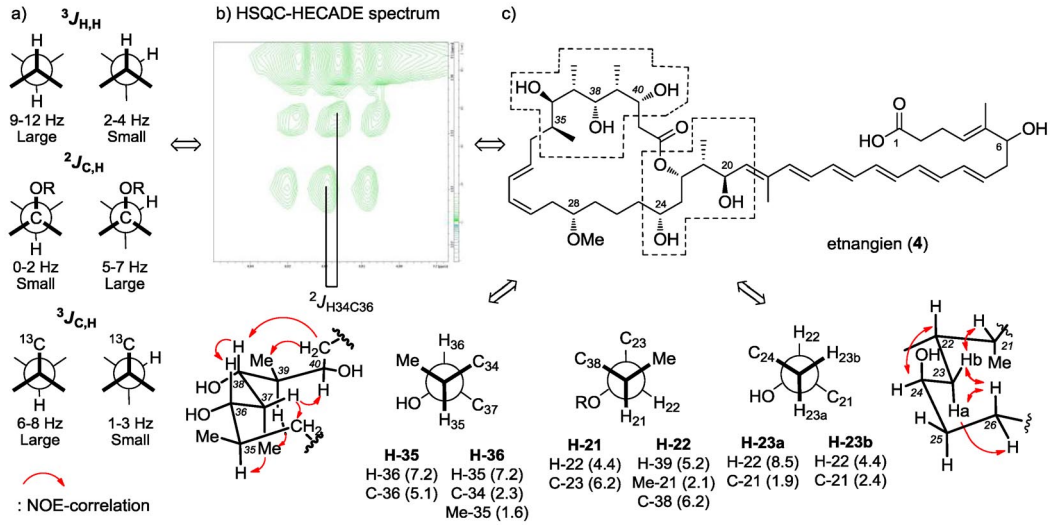
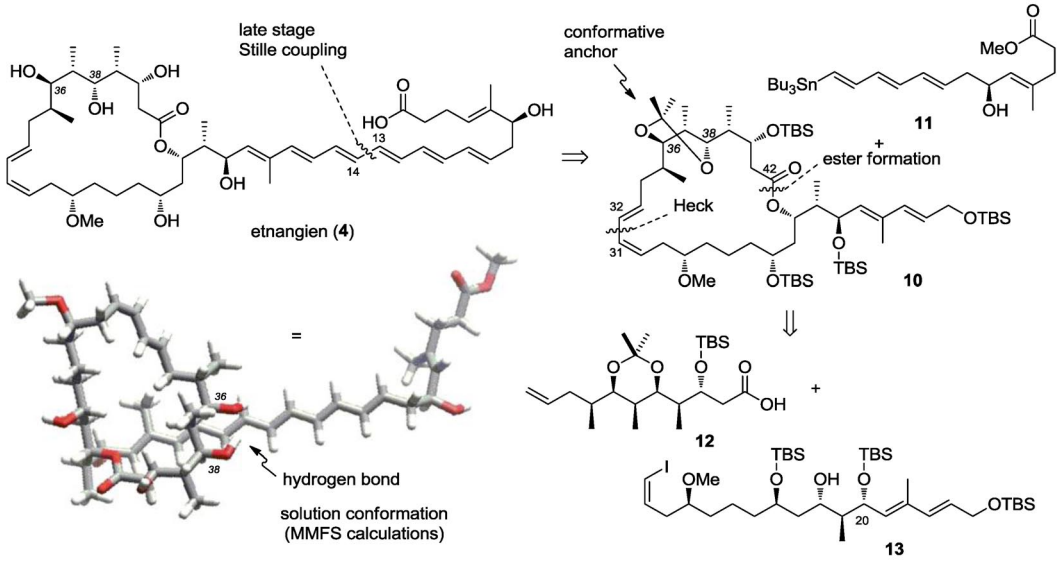


Fig. 2 NMR-based configurational assignment of etnangien (**4**).

The first step for the determination of the correct configuration was the assignment of the relative configuration within these fragments [18]. For this purpose, we chose a *J*-based approach, which is also known as Murata's method [19]. The general background of the method is outlined in Fig. 2a. It relies on a detailed conformational analysis on the basis of the respective $^{2/3}J_{C,H}$ and $^{3}J_{H,H}$ coupling constants. In contrast to $^{3}J_{H,H}$ coupling constants, detection of proton-carbon coupling constants, which is necessary for the application of this technique, is more challenging. As schematically shown in Fig. 2b, they could be extracted from an heteronuclear single-quantum coherence (HSQC)-HECADE [20] experiment, where all C,H-correlations are split into two separate resonances. The horizontal distance

represents the $^{2/3}J_{C,H}$ coupling constants between the corresponding proton and carbon signal. As shown in Fig. 2c, these data in combination with nuclear Overhauser effect (NOE) correlations can then be utilized for assigning the relative configuration of vicinal stereogenic centers, as represented for parts of the isolated stereogenic clusters of etnangien. For determination of the relationship between these stereoclusters (C20–C24 vs. C35–C40) and the isolated stereogenic center at C28, molecular modeling studies were performed on the possible stereochemical permutations, using Macromodel (Version 8.5) [21] involving 20 000-step Monte Carlo searches and the generalized Born/surface area (CB/SA) water solvent model [22]. These studies revealed a series of discrete families of low energy conformations for the various stereoisomers within 10.00 kcal mol^{-1} of the global minimum. The comparison of the calculated dihedral angles for the lowest energy conformation of **4** (see Scheme 1) to the corresponding series of $^{3}J_{H,H}$ coupling constants, as determined by NMR, resulted in a close match. For other stereochemical permutations, including the C28 epimer, lower degrees of resemblance between spectral and calculated data were obtained.



Scheme 1 Retrosynthetic analysis of etnangien (**4**), involving a conformative anchor for fixation of the 3D conformation.

In a complementary fashion, an innovative bioinformatics approach for stereochemical determination was chosen. As shown in Fig. 3b, hydroxyl-bearing stereogenic centers are derived from β-ketoesters **5** by NADH-dependent ketoreductases (KRs) to furnish the respective β-hydroxyketones **6** or **7**, with either D- or L-configuration. The groups of McDaniel [23] and Caffrey [24] have analyzed in detail the core region of these KRs and proposed a mechanism for their stereospecificity. According to their model, the substrate can coordinate in two ways. These only differ in the orientation of the reducible carbonyl bond axis, which arranges the substrate differently as shown in Fig. 3b. On the basis of homology modeling to the short-chain dehydrogenase/reductase (SDR) superfamily of enzymes in combination with mutational studies on the 6-deoxyerythronolide B synthase (DEBS), the authors propose that from the various amino acids involved, the presence of only one amino acid, an aspartate residue (Y100, in the case of the homolog tropinone reductase II from *Datura stramonium*, Fig. 3b) is critical for the orientation of the substrate and thus for the facial bias of the reduction. When aspartate is present, this amino acid is believed to align with catalytic lysine (K163) and tyrosine (Y159) residues, thus leading to the D-configured product. In the absence, however, the orientation of the substrates is

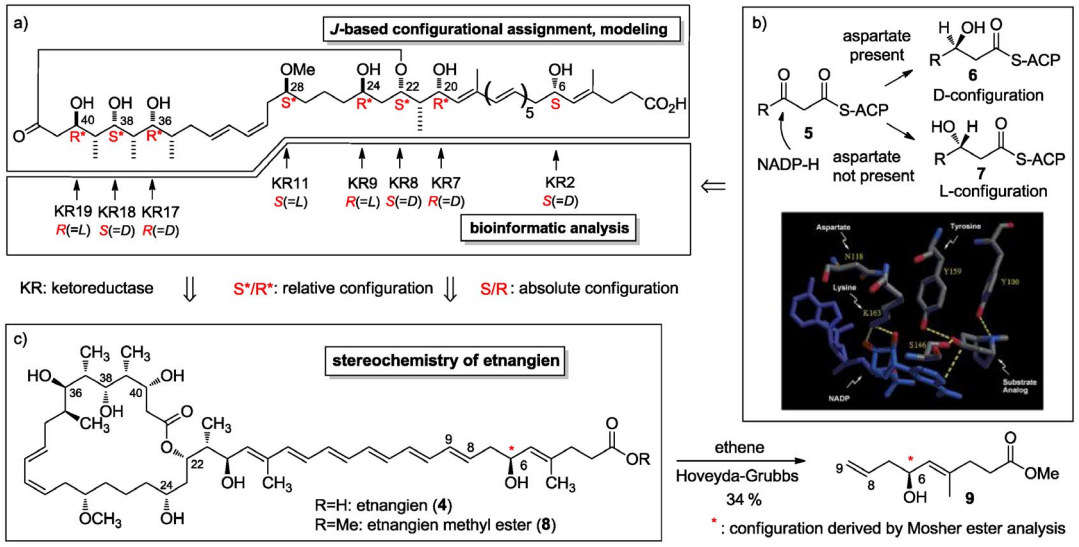


Fig. 3 Bioinformatics-based configurational assignment of etnangien (**4**).

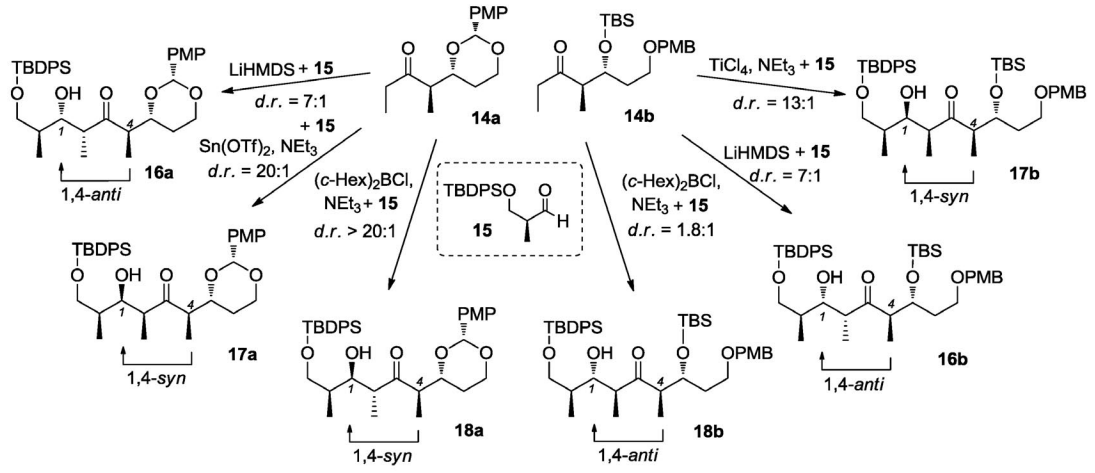
rotated, thus giving the L-diastereomer. At the same time as our NMR studies on etnangien (**4**), Müller's group elucidated the biosynthetic gene cluster of etnangien (**4**). This enabled an application of the method of McDaniel and Caffrey. Accordingly, analysis of the respective KR core regions revealed aspartic acid residues in the KR core region for alcohols at C6, C20, C22, C36, and C38. Correspondingly, the absence of this amino acid in modules 11, 13, and 19 suggested these hydroxyl-bearing stereogenic centers to be L-configured. As shown in Fig. 3a, comparing these data to the configurations independently derived by our more NMR-based approach as discussed above, resulted in a perfect match, which validates our previous assignment and vice versa. These data were further corroborated by the configuration at C6 in the side chain, which was determined by Mosher ester analysis of a truncated product (**9**) obtained by cleavage of **8** using a reverse cross-metathesis. With these results in hand, we felt confident enough to propose the full stereochemistry (Fig. 3c) of etnangien (**4**) and to devise a strategy for the synthesis of this macrolide.

TOTAL SYNTHESIS OF ETNAGIEN

In general, one of the advantages of this computational NMR approach for stereochemical determination is the valuable information gained on the solution conformation of these metabolites. This information will not only be valuable in the rational development of analogs, but also in the design of effective synthetic strategies. The Merck molecular force field (MMFF)-derived solution conformation of etnangien (**4**), for example, is characterized by a strong hydrogen bond between OH-36 and OH-38 (Scheme 1). In order to mimic this conformation in our synthetic approach, an acetonide protection group was chosen for these two hydroxyls in a rational to potentially facilitate the macrocyclization between the C31 and C32 bond [25]. Along these lines, we studied different macrocyclization strategies including Stille, Suzuki, Heck, and metathesis-based approaches. Finally, we focused on an advantageous intramolecular Heck reaction [26] with the hope of beneficial *E*/*Z*-selectivity, also in comparison to our experiences during our archazolid synthesis [27]. Notably, the full potential of Heck reactions in complex target synthesis has not been fully exploited, which made it even more attractive to devise such a disconnection for our route. Accordingly, the macrocyclic core **10** can be dissected into terminal alkene **12** and vinyl-iodide **13**. Furthermore, a late-stage introduction of the side chain **11** by usage of

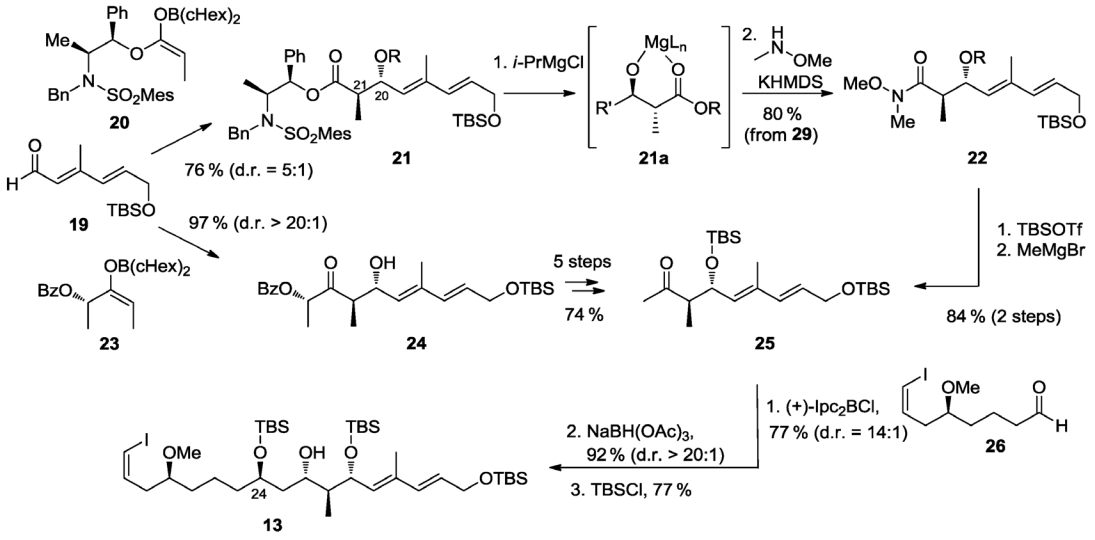
a Stille coupling [28] was planned, in order to study the biological importance of this labile element for biological activity [15b,29].

Due to initial insecurities with the configurational assignment of the northern polypropionate fragment **12**, a modular and stereodivergent route to this segment was developed [30]. As shown in Scheme 2, all possible diastereomeric aldol products **16–18** may be obtained by coupling of ketone **14** and aldehyde **15** with good levels of asymmetric induction, purely based on substrate control. To obtain useful stereoselectivities in all cases, careful choice of reaction conditions was critical [25b]. Presumably, the stereochemical induction is mainly derived from the ketone. Furthermore, the protective group on the β-oxygen was shown to impart a crucial influence on the stereochemical outcome in these reactions. The correctly configured propionate fragment **17b** was then further homologated to the desired building blocks **14** and **27** in a straightforward manner [25a]. In the course of this endeavor, effective protocols for the 1,3-*syn* reduction of sterically hindered β-hydroxyketones under chelation control with $(c\text{Hex})_2\text{BCl}$ and LiBH_4 and a selective method for oxidative cleavage of primary *tert*-butyldimethylsilyl (TBS) ethers using NaIO_4 were developed in our group [31] (not shown).



Scheme 2 Stereodivergent aldol approach to the polypropionate fragment of etnangien (**4**).

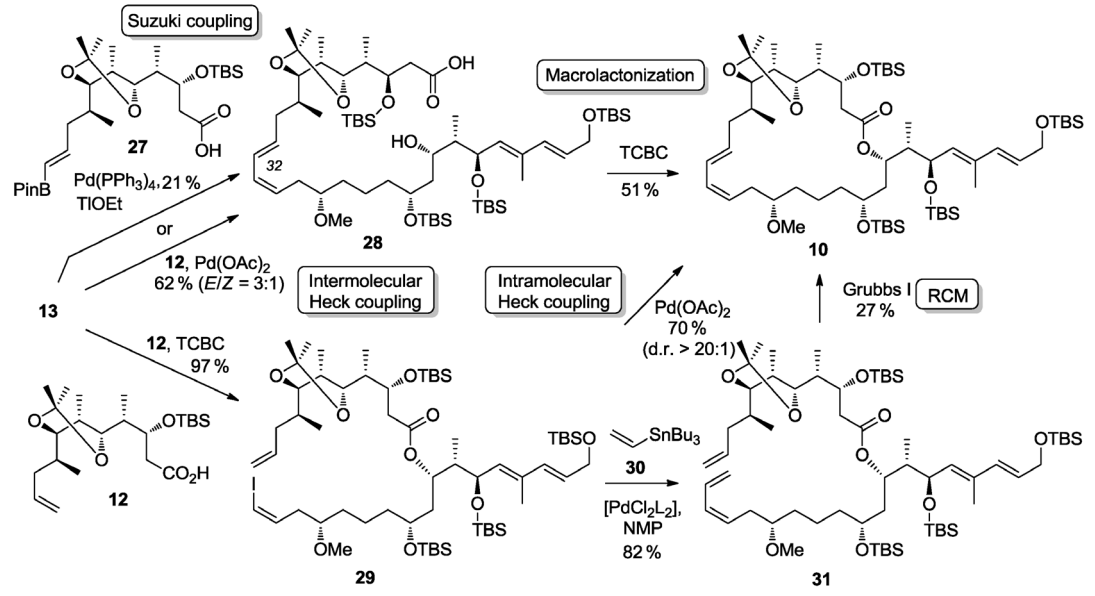
As shown in Scheme 3, two main strategies for construction of the central fragment **13** were evaluated. Both routes were based on a boron-mediated aldol reaction. Unexpectedly, the envisioned Abiko–Masamune *anti*-aldol reaction [32] with ephedrine-derived ketone **19** proceeded in only moderate selectivity and yield (76 %, d.r. = 5:1). Additionally, another drawback of this method was subsequent difficulties in cleaving the norephedrin-derived auxiliary of **21** with nucleophiles other than hydrides, which appears to be a general difficulty of this method. To solve this problem, efforts were directed to enable a more facile direct displacement of the Abiko–Masamune auxiliary [33]. As shown in Scheme 3, our concept to promote such a direct substitution was based on an ester activation by suitable additives. For this reason, a variety of Lewis acids were evaluated to substitute **21** under mild reaction conditions. Best results were obtained with *i*-PrMgCl. The proposed intermediate **21a**, generated in situ, is then treated with metallated Weinreb amine, giving the desired amide **22** in a direct manner. Finally, TBS protection of the free hydroxyl and introduction of the methyl group gave methyl ketone **25** in good yields in a concise fashion. Alternatively, also a lactate-derived Paterson *anti*-aldol reaction with **23** was evaluated [34]. In this process, the aldol product **24** was obtained in excellent selectivity and yield (97 %, d.r. > 20:1). The subsequent transformation into methyl ketone **25** by a diol-cleavage sequence and MeLi-mediated chain elongation succeeded well with good yields (74 %, 5 steps).



Scheme 3 Synthesis of the C15–C31 subunit **13**: a novel method for cleavage of the Abiko–Masamune auxiliary.

Finally, the pivotal aldol coupling of **25** with Jacobsen HKR-derived [35] vinyl iodide **26** to install the center at C24 proceeded with high diastereoselectivity and yield using an IPC-boron-mediated aldol coupling (d.r. = 14:1, 77 %). Subsequent 1,3-*anti* reduction of the derived hydroxyl ketone with the Evans–Saksena protocol [36] and TBS protection of the less hindered 24-OH gave building block **13** in good yields.

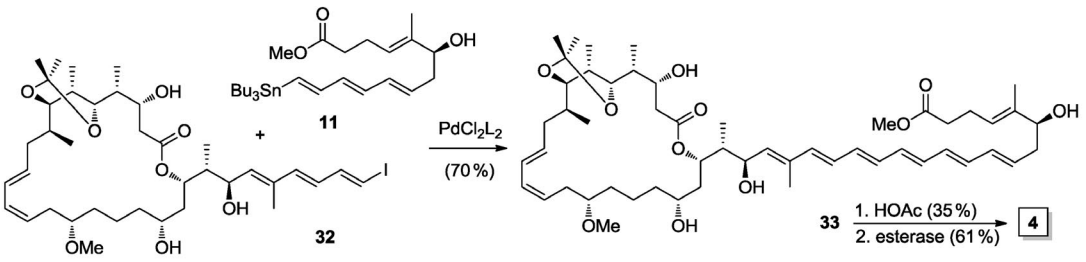
With these building blocks in hand, efforts were then directed towards a macrocyclization of etnangien (**4**). A particular focus was placed on the Heck reaction. As shown in Scheme 4, after esterification [37] of **12** and **13**, the intramolecular Heck coupling of **29** proceeded after careful optimiza-



Scheme 4 Conformational bias in complex macrolide synthesis: Diastereoselective intramolecular Heck coupling.

tion with excellent yield and diastereoselectivity (70 %, *E*/*Z* > 20:1). Presumably, this remarkable diastereoselectivity may be caused by a favored conformational control exerted by the rigid acetonide protection group. In contrast, an intermolecular Heck coupling resulted in (*E*/*Z*)-mixtures of **28**, which clearly validated our macrocyclization strategy. Likewise, tested alternatives to the Heck coupling were much less effective. An intermolecular Stille coupling failed on a test system with various catalysts, and an intramolecular Suzuki coupling proved challenged due to difficulties in the required hydroboration (not shown), while an intermolecular variant (**13** + **27**), followed by a Yamaguchi macrolactonization (**28** to **10**) resulted in only low yields. Also, a ring-closing metathesis (RCM) of **31**, after esterification and Stille coupling with **30**, proved to be difficult. In detail, the main product of the RCM under various conditions was a contracted 20-membered ring (formal expulsion of ethylene).

Homologation of **10** and introduction of the terminal vinyl iodide could then be effected in a straightforward sequence. However, global TBS deprotection proved extremely challenging. In detail, we were faced with a pronounced tendency for translactonization, δ-lactone formation with the 38-OH and steric hindrance of the 20-OTBS group. In particular, removal of the acetonide, which had served us so well in the macrocyclization step, was difficult to cleave due to the occurrence of translactonization [25b]. In contrast, attachment of the side chain **11** proceeded smoothly under conventional Stille coupling conditions [28a] giving **33** in good yields (Scheme 5). Finally, removal of the acetonide could be effected under mildly acidic conditions (35 %, AcOH). At last, enzyme-catalyzed ester cleavage provided the labile synthetic etnangien (**4**) in 61 % yield.



Scheme 5 Completion of the first total synthesis of etnangien.

CONFIGURATIONAL ASSIGNMENT OF RHIZOPODIN

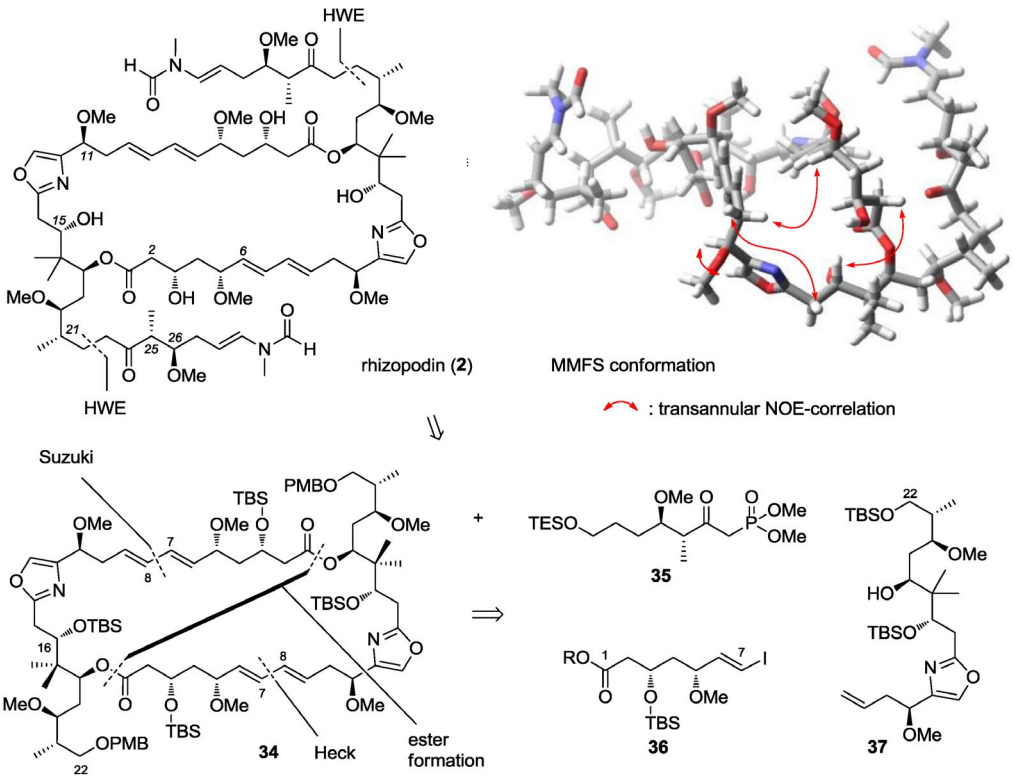
The other example of a myxobacterial macrolide synthesis discussed within this review is rhizopodin (**2**, Fig. 1), an architecturally unique polyketide from the myxobacterium *Myxococcus stipitatus* [9]. In addition to its complex and fascinating structure, we became interested in this compound due to its impressive biological properties, including antifungal activity and potent cytotoxicity against a broad range of tumor cell lines in the low nanomolar range [9,13]. Its cytotoxicity has been attributed to its ability to interact with actin and disrupt the actin cytoskeleton by binding specifically to few critical sites of G-actin [13b]. It has also been shown that incubation of yeast cells with rhizopodin (**2**) reduces their phagocytosis efficiency [38]. The planar structure of rhizopodin, as elucidated by Höfle and Steinmetz, was originally considered to be monomeric [9a]. However, in the course of our studies, it became apparent to be a C_2-symmetric dimer, which is characterized by a 38-membered macrolide ring with two conjugated diene systems in combination with two disubstituted oxazol systems and two enamide side chains [9b]. In total, 18 stereogenic centers are present in the carbon backbone of **2**. Besides its impressive number of stereogenic centers and structural complexity, the stereochemical assignment of this macrolide posed several additional challenges [39]. These included a limited amount of material (3 mg) that was available for our studies, thus also disabling the use of HSQC-HECADE [20] measurements for determination of proton-carbon coupling constants. Consequently, NMR studies

had to rely exclusively on ^{1}H NMR data. Furthermore, several hydroxylated centers bear a methyl group and, therefore, are not accessible for a chemical derivatization. Finally, the impressive size and structural complexity in combination with the C_2 symmetry presented additional challenges to be addressed within this program. After the evaluation of a range of NMR solvents, optimal resolution of ^{1}H signals was obtained in CD_3OD at 600 MHz, allowing complete assignment of all resonances. Importantly, also a set of highly specific transannular NOE correlations were observed under these conditions, which were critical in defining the stereochemical relationships within the C15 to C21, the C25 to C26 and the C2 to C6 stereoclusters. This also allowed the relative stereochemical assignment of the C15 to C21 to the C2 to C6 fragment. For proposing a stereochemistry of the C11 stereogenic center, Monte Carlo searches within the MMFFs [21] and the CB/SA water solvent model [22] were again successfully applied. As shown for the low-energy conformations for **2**, this conformer accounted for a number of key long-range rotating frame Overhauser effect spectroscopy (ROESY) correlations (i.e., H5–H10, H16, H16–H2b) and resulted in an acceptable match between the calculated dihedral angles and corresponding series of $^3J_{H,H}$ coupling constants. This assignment was further corroborated by Mosher ester analysis [40] and a remarkable structural homology of the side chain of rhizopodin (**2**) with similar actin binding macrolides like jaspisamide A [41], halichondramide [42], mycalolide [43], and ulapualide A [44]. Finally, the stereochemistry was independently confirmed by an X-ray structure analysis [13b] of rhizopodin (**2**) in a joint complex of this macrolide with G-actin and later on by bioinformatic studies [45] using the techniques described above. This agreement again demonstrates the usefulness and reliability of our in silico/NMR approach, even for the assignment of polyketides as complex as rhizopodin (**2**), which are only available in limited amounts.

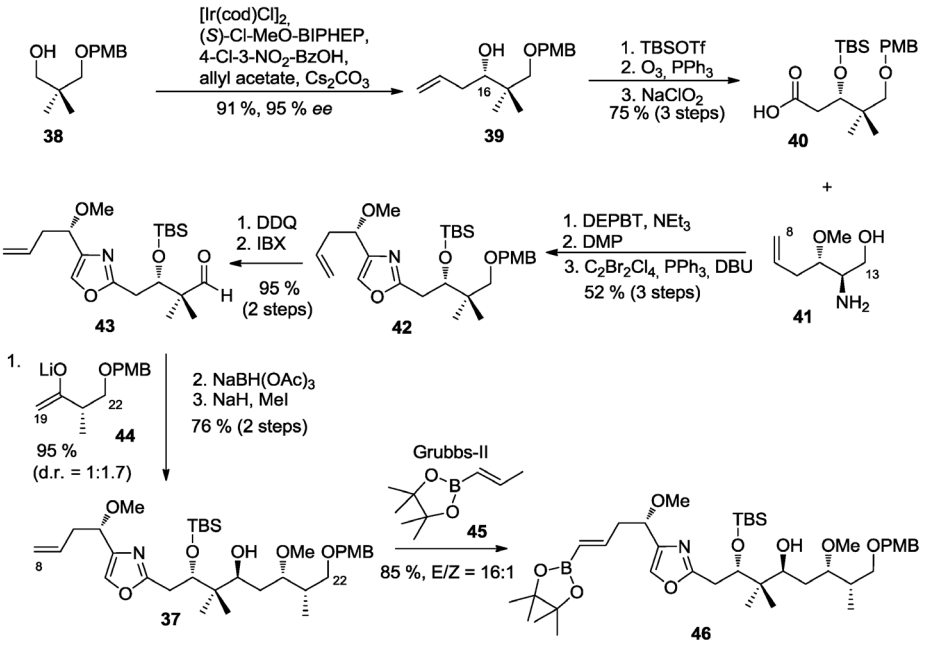
TOTAL SYNTHESIS OF RHIZOPODIN

With this independently confirmed assignment in hand, we were planning a synthetic approach towards this macrolide. As shown in Scheme 6, the symmetry of the molecule allows the double disconnections indicated and the adoption of a highly convergent plan using three building blocks of similar complexity, i.e., the macrocyclic fragments **36**, **37**, and the side chain **35**. In detail, sequential disconnection at the C6–C8 diene linkages defines a conventional cross-coupling and a macrocyclization cross-coupling as late steps in the synthesis. The corresponding starting material for these couplings could be formed by two site-selective esterifications. Finally, introduction of the C23–C30 (vide infra) side-chain segment **35** was finally realized through a suitable Horner–Wadsworth–Emmons (HWE)-coupling/hydrogenation sequence. In principle, several methodologies may be employed for ring closure, thus offering considerable flexibility in the synthesis. Importantly, the modular synthetic approach is highly convergent and thus offers the potential to provide a range of structural derivatives for structure–activity relationship (SAR) studies for further exploration of the biological potential of this promising macrolide antibiotic.

With regard to an efficient synthesis of the single fragments, considerable efforts had to be invested for the development of a practicable and concise route to the central C8–C22 building block **37** [46]. After various strategies, it was realized that an effective synthesis would be based on a Krische allylation [57]. As depicted in Scheme 7, iridium-catalyzed allylation of *p*-methoxybenzyl (PMB)-protected **38** gave an easy and scalable access to homoallylic alcohol **39** in excellent yield and optical purity. TBS protection of the secondary alcohol, ozonolysis, and subsequent Pinnick oxidation [47] of the terminal double bond furnished acid **40** in good yield. Amide-coupling with known amine **41** [48] proceeded best with 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT) [49] as coupling reagent, which was superior to dimethyl carbonate (DCC) or HATU. The respective β-hydroxyamide was then oxidized and subsequently transformed into oxazole **42** applying Wipf's conditions [50] in good yield and without further protecting-group manipulations. PMB deprotection and oxidation led to the primary aldehyde **43**, which could be elaborated to **37** in an aldol reaction with ketone **44**.



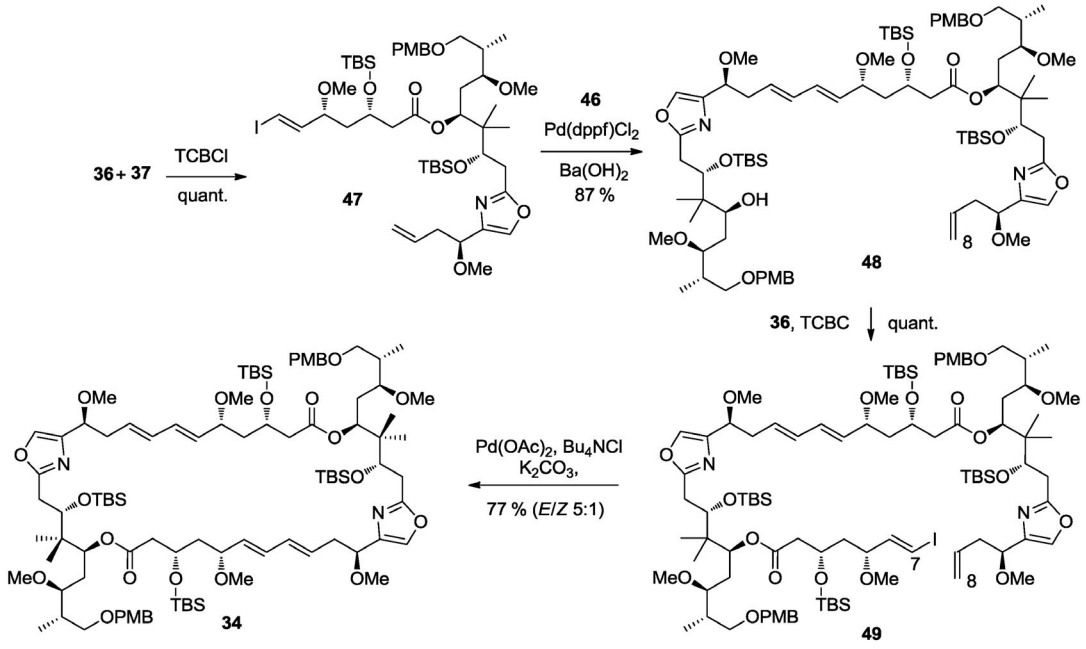
Scheme 6 3D structure and retrosynthetic analysis of rhizopodin (**2**).



Scheme 7 Concise synthesis of the C8–C22 fragment **36** of rhizopodin.

Although we achieved satisfying yields, all attempts to install the stereocenter selectively were unsuccessful. After considerable experimentation, we obtained a mixture of the chromatographically separable diastereomeric alcohols in high yields (d.r. = 1:1.7, 95 %). Both products could be transformed into building block **37** in a stereoconvergent manner: the desired aldol product by a 1,3-*anti*-reduction and methylation and the undesired one by a 1,3-*syn*-reduction, methylation, and inversion of the C18 configuration (not shown) [51]. This sequence delivered building block **37** in only 13 linear steps and 19 % overall yield based on a scalable and reproducible route. Notably, this represents the shortest route to this fragment reported so far [51,52]. Subsequent cross-metathesis using Grubbs-II catalyst in the presence of boronate **45** gave access to vinylboronate **46** in good yield with an *E*/*Z*-selectivity of 16:1 [53].

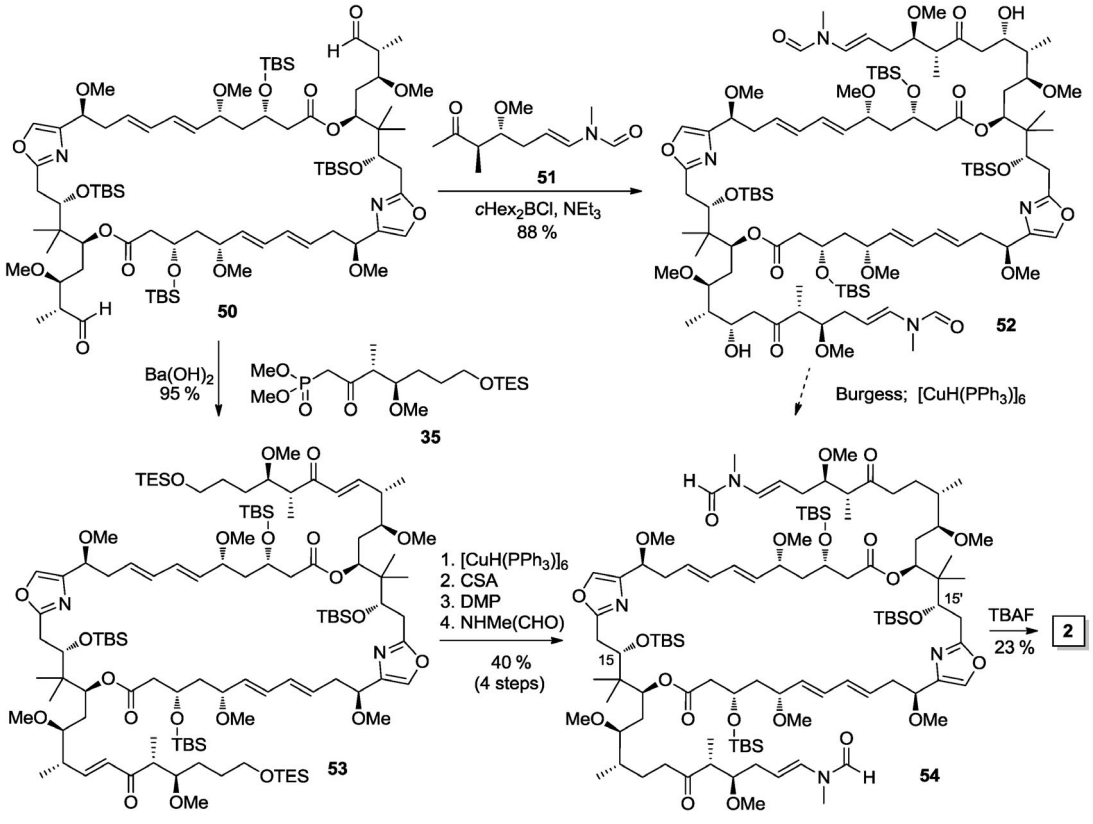
Based on our experiences in the total synthesis of archazolid (**3**) and etnangien (**4**), we envisioned two possibilities for a successful synthesis of the macrocyclic core fragment **34**. One possibility included a Suzuki coupling, which would be followed by a Heck macrocyclization. The other would construct the macrocyclic core by a double Suzuki coupling strategy. A stepwise coupling strategy seems to be very crucial, as efforts towards a Suzuki dimerization appear not to have been successful and only allowed formation of the monomeric core [52e]. Due to the efficiency of the etnangien Heck macrocyclization strategy, we initially turned our attention to the formation of the corresponding coupling precursor. Accordingly, acid **36** was connected with the sterically hindered secondary C18 alcohol of fragment **37** applying Yamaguchi's reagent [37] in excellent yield (Scheme 8). With building block **47** in hand, bearing both, a terminal olefin and a vinyl-iodine moiety, the stage was set for a chemoselective cross-coupling strategy. Firstly, an intermolecular Suzuki coupling with boronate **46** proceeded smoothly at the vinyl-iodine terminus and afforded only the *E*-isomer as judged from ^{1}H NMR [54]. Secondly, another Yamaguchi esterification with acid **36** gave rise to the macrocyclization precursor **49** in outstanding yield. While these steps required essentially no optimization, the key element of our strategy proved more challenging. Finally, more effective conditions were based on those described by Jeffrey [25f] in analogy to our etnangien (**4**) synthesis allowing the formation of **34** in 77 % yield with an *E*/*Z*-selectivity of 5:1. Importantly, this represents one further complex example for



Scheme 8 Concise macrocyclization based on a sequential Suzuki–Heck cross-coupling strategy.

the successful implementation of an intramolecular Heck macrocyclization in complex natural product synthesis [26].

The final endgame strategy proved extremely difficult, and extensive efforts had to be invested before a successful completion of the total synthesis could be realized. Difficulties included both the effective removal of protective groups and introduction of the side chain. Firstly, it proved impossible to remove the terminal PMB group of **34**, leading to concommitant cleavage of the allylic OMe ethers. Therefore, a reprotection sequence (change to a terminal TBS ether) had to be applied as an earlier stage of the synthesis, before aldehyde **50** could be obtained. For coupling of the side chain, firstly an aldol sequence with **51** to give **52** followed by dehydration [27a,55] was envisioned (Scheme 9). However, this route proved to be extremely unreliable, despite first very positive results. Finally, the successful strategy involved a base-mediated HWE coupling of phosphonate **35** and **50** giving **53** in 95 % yield. This was followed by a 1,4-reduction of the resulting enone with the Stryker reagent [56] and selective cleavage of the primary triethylsilyl (TES) ethers. For introduction of the sensitive *N*-vinylformamide units, we eventually found that **53** was best oxidized to the respective aldehyde with DMP before a reaction with HNMe(CHO) and P_2O_5 performed TBS-protected rhizopodin **54** [57]. The final global deprotection proved extremely difficult. In particular, cleavage of the TBS ethers at 15-OH and 15'-OH at this stage was challenging, and it was only after extensive experimentation that the target product **2** could be detected in low yields together with various side products. In agreement with the solution structure of rhizopodin (**2**) together with the observation that a deprotection is much more facile before macrocyclization, suggest that the TBS groups may be hidden in the macrocyclic ring and therefore be very difficult to access. In conclusion, a first total synthesis of rhizopodin (**2**) was accomplished in 31 steps

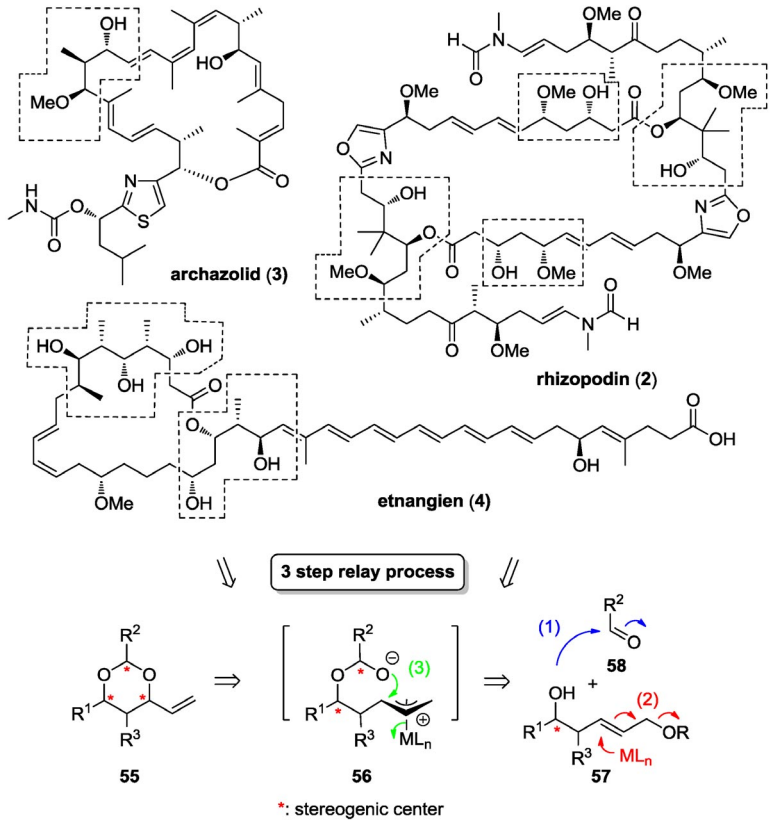


Scheme 9 Completion of the total synthesis of rhizopodin (**2**).

(longest linear sequence) and confirms the relative and absolute configuration, in agreement with our earlier stereochemical analysis [39] and the X-ray structure of the actin complex [13b]. In combination with the available structural data on actin-bound rhizopodin (**2**) and related macrolides, the design of simplified analogues may now be pursued.

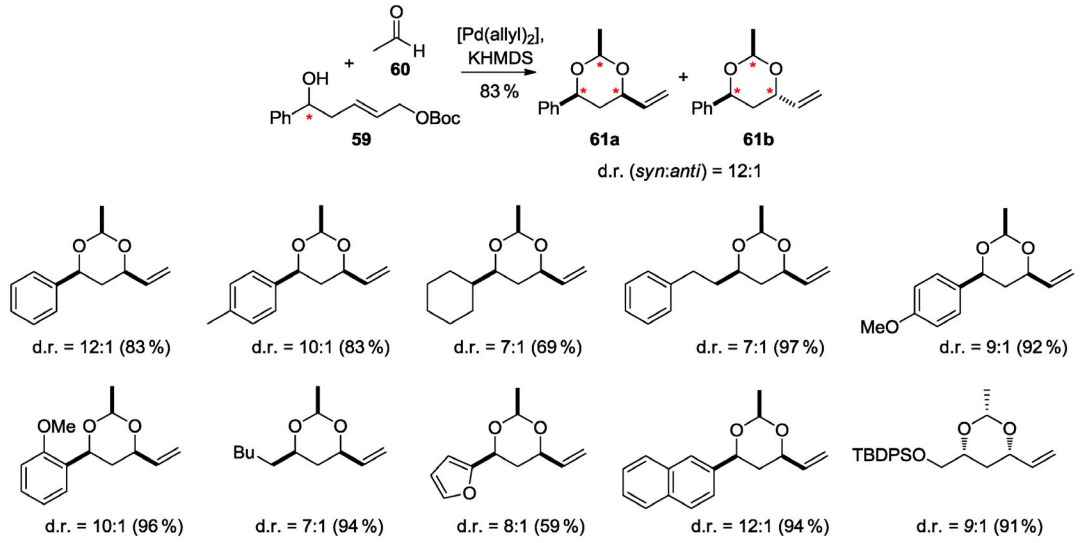
DOMINO REACTIONS FOR THE CONSTRUCTION OF 1,3-DIOLS

As exemplified by archazolid (**3**), rhizopodin (**2**), and etnangien (**4**), proximal 1,3-arrays of hydroxyl-bearing stereogenic centers present prevalent structural phenotypes in polyketides and a wide variety of natural products, pharmaceuticals and bioactive agents in general, rendering the development of effective synthetic approaches for this structure motif an important research goal [1,58]. Motivated by present targets in our group, in combination with certain limitations of existing methods, we desired a more direct and concise sequence for stereoselective 1,3-diol synthesis. Inspired by previous developed cascade concepts [59], our synthetic approach relied on a three-step relay process [60]. As shown in Scheme 10, this involves the addition of a homo-allylic alcohol **57** to a suitable carbonyl compound **58**, giving the corresponding hemiacetal alkoxide (step 1). Formation of an electrophilic π-allyl complex (step 2) then results in the generation of intermediate **56**, which finally undergoes an intramolecular allylic substitution reaction to the desired 1,3-allylic alcohols **55** in a suitably protected form (step 3). Notably, the synthetic design of this two-component coupling is highly convergent and enables a considerable increase in structural complexity by assembling two new stereogenic centers from simple and readily available starting materials.



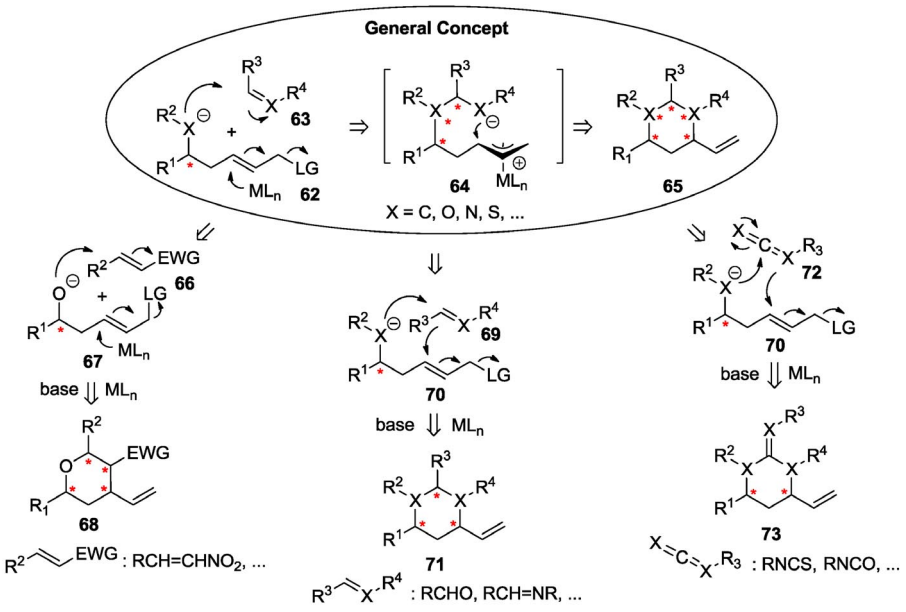
Scheme 10 Three-step tandem concept for the synthesis of 1,3-diol motifs in polyketides.

As shown in Scheme 11, this novel domino sequence could indeed be realized [61]. It was found that optimized conditions for the selective generation of the 1,3-*syn*-dioxane products involved treatment of homoallylic alcohol **59** in acetaldehyde **60** as cosolvent with a slight excess of KHMDS (1.5 equiv), catalytic amounts (10 %) of $[Pd(allyl)Cl]_2$ and PPh_3 (30 %) and conducting the reaction in toluene at room temperature. This enabled an efficient access to a broad range of suitably protected 1,3-*syn*-diols with good stereoselectivities and excellent yields (Scheme 11). The substrate scope ranged from simple phenolic or benzylic over aliphatic to TBS-protected compounds. Importantly, the starting homoallylic alcohols are readily available in enantiopure form (e.g., asymmetric allylation) and cross-metathesis [62], and the assembled products bear a terminal allylic alkene, which may be directly used as a functional basis for further elaboration. This process represents one of the few examples of hemiacetal nucleophiles in allylic substitutions [63]. Furthermore, the effectiveness of this method for convergent polyketide synthesis has been demonstrated [61].



Scheme 11 Realization and substrate scope of the 1,3-domino reaction concept.

In addition to the application of this concept for the formation of 1,3-diols, this novel domino sequence was shown to be a generally useful method for rapid construction of molecular complexity. As shown in Scheme 12, it could be implemented into a sequential process for the highly concise synthesis of polysubstituted tetrahydropyrans from simple starting materials (left part). Mechanistically, this procedure is based on a sequential oxa-Michael–Tsuji–Trost reaction [60b,c,64] and generates up to three new stereogenic centers in a one-pot fashion. It also was successfully applied for the stereoselective synthesis of quaternary carbon centers bearing a nitro group [59b]. Furthermore, this concept was also applicable to sp-type electrophiles (right part of Scheme 12) and an efficient protocol for the stereoselective synthesis of 1,3-*syn* and 1,3-*anti* tetrahydropyrimidinones (*syn*- and *anti*-**73** for X=N) has been developed [59a]. The modular procedure is based on a stereodivergent cyclization, which proceeds with excellent yield (up to 99 %) and selectivity (up to d.r. > 20:1), purely based on substrate control. Importantly, the product pyrimidines can be readily transformed into the corresponding free *syn*- and *anti*-amines [65]. It is expected that this novel domino concept will be further explored and applied to the synthesis of functional molecules.



Scheme 12 A novel domino concept based on the combination of nucleophilic addition reactions and Tsuji–Trost couplings.

CONCLUSION

Within this review we have outlined our studies on structure elucidation and total synthesis of complex myxobacterial macrolides. A combination of computational and NMR methods have been developed as highly valuable and reliable tool for the stereochemical determination of complex polyketide structures. NMR-based methods are particularly effective for determination of vicinal and proximal stereogenic centers by Murata's *J*-based configurational method, while computational methods are critical for assigning the relationship of remote stereogenic centers. Furthermore, as an alternative to these more conventional methods, bioinformatic approaches are becoming increasingly important as an independent and highly valuable method for stereochemical determination. The powerfulness of these approaches has been demonstrated in the full stereochemical determination of etnangien (**4**), a labile macrolide antibiotic with 12 initially unassigned stereogenic centers and rhizopodin (**2**), a C_2 symmetric polyketide with 18 stereogenic centers of inititally unknown configuration [18,39]. Taken the solution conformation into account modular and flexible preparative routes to these synthetically challenging targets were developed, which culminated in concise first total syntheses [25a,51]. Along these lines, novel synthetic methodologies were implemented and developed, most importantly a novel domino concept involving concomitant cyclizations on the basis of nucleophilic additions and Tsuj–Trost couplings [59,61]. Furthermore, the importance of conformational bias in complex synthetic designs was demonstrated. It is expected that these results will be beneficial in further advancing and potentially exploiting the promising biological potential of complex polyketides in general.

ACKNOWLEDGMENTS

Financial support by the Deutsche Forschungsgemeinschaft, the Volkswagenstiftung, the Fonds der Chemischen Industrie (fellowship to S.E.) and the Wild-Stiftung is gratefully acknowledged. We greatly appreciate the dedicated work of those members of our group who have developed this field of research

and the stimulating cooperations with our scientific partners—their names can be seen from the literature cited.

REFERENCES

1. K. J. Weissman, R. Müller. *Nat. Prod. Rep.* **27**, 1276 (2010).
2. (a) A. L. Demain. *Biotechnol. Adv.* **18**, 499 (2000); (b) G. M. Cragg, D. J. Newman. *Cancer Invest.* **17**, 153 (1999).
3. (a) J. Staunton, K. J. Weissman. *Nat. Prod. Rep.* **18**, 380 (2001); (b) C. Hertweck. *Angew. Chem., Int. Ed.* **48**, 4688 (2009); (c) K. J. Weissman. *Methods Enzymol.* **459**, 3 (2009).
4. D. Menche. *Nat. Prod. Rep.* **25**, 905 (2008).
5. C. Hertweck. *Angew. Chem., Int. Ed.* **48**, 4688 (2009).
6. (a) H. Reichenbach, G. Höfle. *Drug Discovery from Nature*, Springer, Heidelberg (1999); (b) H. Reichenbach. *J. Ind. Microbiol. Biotechnol.* **27**, 149 (2001).
7. (a) G. Höfle, N. Bedorf, H. Steinmetz, D. Schomburg, K. Gerth, H. Reichenbach. *Angew. Chem., Int. Ed. Engl.* **35**, 1567 (1996); (b) D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, C. M. Woods. *Cancer Res.* **55**, 2325 (1995); (c) K. C. Nicolaou, F. Roschangar, D. Vourloumis. *Angew. Chem., Int. Ed. Engl.* **37**, 2014 (1998).
8. (a) A. Conlin, M. Fornier, C. Hudis, S. Kar, P. Kirkpatrick. *Nat. Rev. Drug Discov.* **6**, 953 (2007); (b) J. Cortes, J. Baselga. *Oncologist* **12**, 271 (2007).
9. (a) F. Sasse, H. Steinmetz, G. Hoefle, H. Reichenbach. *J. Antibiot.* **46**, 741 (1993); (b) R. Jansen, H. Steinmetz, F. Sasse, W.-D. Schubert, G. Hagelüken, S. C. Albrecht, R. Müller. *Tetrahedron Lett.* **49**, 5796 (2008).
10. (a) G. Höfle, H. Reichenbach, F. Sasse, H. Steinmetz. German Patent DE 41 42 951 C1 (1993); (b) F. Sasse, H. Steinmetz, G. Hofle, H. Reichenbach. *J. Antibiot.* **56**, 520 (2003); (c) J. Hassfeld, C. Farès, H. Steinmetz, T. Carlomagno, D. Menche. *Org. Lett.* **8**, 4751 (2006); (d) D. Menche, J. Hassfeld, H. Steinmetz, M. Huss, H. Wieczorek, F. Sasse. *Eur. J. Org. Chem.* 1196 (2007); (e) C. Farès, J. Hassfeld, D. Menche, T. Carlomagno. *Angew. Chem.* **120**, 3782 (2008); (f) N. Horstmann, S. Essig, S. Bockelmann, H. Wieczorek, M. Huss, F. Sasse, D. Menche. *J. Nat. Prod.* **74**, 1100 (2011); (g) D. Menche, J. Hassfeld, H. Steinmetz, M. Huss, H. Wieczorek, F. Sasse. *J. Antibiot.* **60**, 328 (2007).
11. (a) G. Höfle, H. Reichenbach, H. Irschik, D. Schummer. German Patent DE 196 30 980 A1 (1998); (b) H. Irschik, D. Schummer, G. Hofle, H. Reichenbach, H. Steinmetz, R. Jansen. *J. Nat. Prod.* **70**, 1060 (2007).
12. R. Jansen, B. Kunze, H. Reichenbach, G. Höfle. *Eur. J. Org. Chem.* 917 (2002).
13. (a) T. M. A. Gronewold, F. Sasse, H. Liinsdorf, H. Reichenbach. *Cell Tissue Res.* **295**, 121 (1999); (b) G. Hagelueken, S. C. Albrecht, H. Steinmetz, R. Jansen, D. W. Heinz, M. Kalesse, W.-D. Schubert. *Angew. Chem., Int. Ed.* **48**, 595 (2009).
14. (a) M. Huss, F. Sasse, B. Kunze, R. Jansen, H. Steinmetz, G. Ingenhorst, A. Zeeck, H. Wieczorek. *BMC Biochem.* **6**, 13 (2005); (b) S. Bockelmann, D. Menche, S. Rudolph, T. Bender, S. Grond, P. von Zezschwitz, S. P. Muench, H. Wieczorek, M. Huss. *J. Biol. Chem.* **285**, 38304 (2010); (c) S. Dreisigacker, D. Latek, S. Bockelmann, M. Huss, H. Wieczorek, S. Filipek, H. Gohlke, D. Menche, T. Carlomagno. *J. Chem. Inf. Model.* **52**, 2265 (2012).
15. (a) D. Menche, P. Li, H. Irschik. *Bioorg. Med. Chem. Lett.* **20**, 939 (2010); (b) M. Altendorfer, H. Irschik, D. Menche. *Bioorg. Med. Chem. Lett.* **22**, 5731 (2012).
16. B. Kunze, R. Jansen, G. Höfle, H. Reichenbach. *J. Antibiot.* **57**, 151 (2004).
17. (a) F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand, D. Häbich. *Angew. Chem., Int. Ed.* **45**, 5072 (2006); (b) K. C. Nicolaou, J. S. Chen, D. J. Edmonds, A. A. Estrada. *Angew. Chem., Int. Ed.* **48**, 660 (2009).

18. D. Menche, F. Arikan, O. Perlova, N. Horstmann, W. Ahlbrecht, S. C. Wenzel, R. Jansen, H. Irschik, R. Müller. *J. Am. Chem. Soc.* **130**, 14234 (2008).
19. (a) N. Matsumori, D. Kaneno, M. Murata, H. Nakamura, K. Tachibana. *J. Org. Chem.* **64**, 866 (1999); (b) G. Bifulco, P. Dambruoso, L. Gomez-Paloma, R. Riccio. *Chem. Rev.* **107**, 3744 (2007).
20. (a) W. Koźmiński, D. Nanz. *J. Magn. Reson.* **142**, 294 (2000); (b) B. L. Marquez, W. H. Gerwick, R. T. Williamson. *Magn. Reson. Chem.* **39**, 499 (2001).
21. F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still. *J. Comput. Chem.* **11**, 440 (1990).
22. W. C. Still, A. Tempczyk, R. C. Hawley, T. Hendrickson. *J. Am. Chem. Soc.* **112**, 6127 (1990).
23. R. Reid, M. Piagentini, E. Rodriguez, G. Ashley, N. Viswanathan, J. Carney, D. V. Santi, C. R. Hutchinson, R. McDaniel. *Biochemistry* **42**, 72 (2003).
24. P. Caffrey. *ChemBioChem* **4**, 654 (2003).
25. (a) P. Li, J. Li, F. Arikan, W. Ahlbrecht, M. Dieckmann, D. Menche. *J. Am. Chem. Soc.* **131**, 11678 (2009); (b) P. Li, J. Li, F. Arikan, W. Ahlbrecht, M. Dieckmann, D. Menche. *J. Org. Chem.* **75**, 2429 (2010).
26. (a) H. Yokoyama, T. Satoh, T. Furuhata, M. Miyazawa, Y. Hirai. *Synlett* 2649 (2006); (b) M. J. Stocks, R. P. Harrison, S. J. Teague. *Tetrahedron Lett.* **36**, 6555 (1995); (c) X. Geng, M. L. Miller, S. Lin, I. Ojima. *Org. Lett.* **5**, 3733 (2003); (d) A. B. Dounay, L. E. Overman. *Chem. Rev.* **103**, 2945 (2003); (e) M. Shibasaki, C. D. J. Boden, A. Kojima. *Tetrahedron* **53**, 7371 (1997); (f) T. Jeffery. *Tetrahedron* **52**, 10113 (1996).
27. (a) D. Menche, J. Hassfeld, J. Li, S. Rudolph. *J. Am. Chem. Soc.* **129**, 6100 (2007); (b) D. Menche, J. Hassfeld, J. Li, K. Mayer, S. Rudolph. *J. Org. Chem.* **74**, 7220 (2009).
28. (a) J. K. Stille. *Angew. Chem., Int. Ed. Engl.* **25**, 508 (1986); (b) P. Espinet, A. M. Echavarren. *Angew. Chem., Int. Ed.* **43**, 4704 (2004).
29. M. Altendorfer, D. Menche. *Chem. Commun.* **48**, 8267 (2012).
30. J. Li, D. Menche. *Synthesis* 2293 (2009).
31. J. Li, D. Menche. *Synthesis* 1904 (2009).
32. (a) A. Abiko, J.-F. Liu, S. Masamune. *J. Am. Chem. Soc.* **119**, 2586 (1997); (b) J.-F. Liu, A. Abiko, Z. Pei, D. C. Buske, S. Masamune. *Tetrahedron Lett.* **39**, 1873 (1998); (c) T. Inoue, J.-F. Liu, D. C. Buske, A. Abiko. *J. Org. Chem.* **67**, 5250 (2002).
33. J. Li, P. Li, D. Menche. *Synlett* 2417 (2009).
34. (a) I. Paterson, K. R. Gibson, R. M. Oballa. *Tetrahedron Lett.* **37**, 8585 (1996); (b) I. Paterson, D. J. Wallace, C. J. Cowden. *Synthesis* 639 (1998).
35. (a) M. Tokunaga, J. F. Larrow, F. Kakiuchi, E. N. Jacobsen. *Science* **277**, 936 (1997); (b) A. G. Myers, B. A. Lanman. *J. Am. Chem. Soc.* **124**, 12969 (2002).
36. A. K. Saksena, P. Mangiaracina. *Tetrahedron Lett.* **24**, 273 (1983).
37. J. Inanaga, K. Hirata, H. Saeki, T. Katsuki, M. Yamaguchi. *Bull. Chem. Soc. Jpn.* **52**, 1989 (1979).
38. N. Klippel, U. Bilitewski. *Anal. Lett.* **40**, 1400 (2007).
39. N. Horstmann, D. Menche. *Chem. Commun.* 5173 (2008).
40. (a) J. A. Dale, D. L. Dull, H. S. Mosher. *J. Org. Chem.* **34**, 2543 (1969); (b) J. M. Seco, E. Quiñoá, R. Riguera. *Chem. Rev.* **104**, 17 (2004).
41. J. Kobayashi, O. Murata, H. Shigemori. *J. Nat. Prod.* **56**, 787 (1993).
42. M. R. Kernan, T. F. Molinski, D. J. Faulkner. *J. Org. Chem.* **53**, 5014 (1988).
43. N. Fusetani, K. Yasumuro, S. Matsunaga, K. Hashimoto. *Tetrahedron Lett.* **30**, 2809 (1989).
44. (a) S. K. Chattopadhyay, G. Pattenden. *Perkin Trans. 1* 2429 (2000); (b) G. Pattenden, N. J. Ashweek, C. A. G. Baker-Glenn, G. M. Walker, J. G. K. Yee. *Angew. Chem., Int. Ed.* **46**, 4359 (2007).
45. D. Pistorius, R. Müller. *ChemBioChem* **13**, 416 (2012).
46. M. Dieckmann, S. Rudolph, S. Dreisigacker, D. Menche. *J. Org. Chem.* **77**, 10782 (2012).

47. B. S. Bal, W. E. Childers Jr., H. W. Pinnick. *Tetrahedron* **37**, 2091 (1981).
48. M. Toumi, F. Couty, G. Evano. *Angew. Chem., Int. Ed.* **46**, 572 (2007).
49. H. Li, X. Jiang, Y.-h. Ye, C. Fan, T. Romoff, M. Goodman. *Org. Lett.* **1**, 91 (1999).
50. (a) P. Wipf, S. Lim. *J. Am. Chem. Soc.* **117**, 558 (1995); (b) P. Wipf, C. P. Miller. *J. Org. Chem.* **58**, 3604 (1993).
51. M. Dieckmann, M. Kretschmer, P. Li, S. Rudolph, D. Herkommer, D. Menche. *Angew. Chem., Int. Ed.* **51**, 5667 (2012).
52. (a) Z. Chen, L. Song, Z. Xu, T. Ye. *Org. Lett.* **12**, 2036 (2010); (b) T. K. Chakraborty, M. Sreekanth, K. K. Pulukuri. *Tetrahedron Lett.* **52**, 59 (2011); (c) T. K. Chakraborty, K. K. Pulukuri, M. Sreekanth. *Tetrahedron Lett.* **51**, 6444 (2010); (d) K. K. Pulukuri, T. K. Chakraborty. *Org. Lett.* **14**, 2858 (2012); (e) K. C. Nicolaou, X. Jiang, P. J. Lindsay-Scott, A. Corbu, S. Yamashiro, A. Bacconi, V. M. Fowler. *Angew. Chem., Int. Ed.* **50**, 1139 (2011); (f) M. Kretschmer, D. Menche. *Org. Lett.* **14**, 382 (2012).
53. C. Morrill, R. H. Grubbs. *J. Org. Chem.* **68**, 6031 (2003).
54. A. Gopalarathnam, S. G. Nelson. *Org. Lett.* **8**, 7 (2005).
55. (a) I. Paterson, K. Ashton, R. Britton, G. Cecere, G. Chouraqui, G. J. Florence, J. Stafford. *Angew. Chem., Int. Ed.* **46**, 6167 (2007); (b) I. Paterson, K. Ashton, R. Britton, G. Cecere, G. Chouraqui, G. J. Florence, H. Knust, J. Stafford. *Chem. Asian J.* **3**, 367 (2008).
56. W. S. Mahoney, D. M. Brestensky, J. M. Stryker. *J. Am. Chem. Soc.* **110**, 291 (1988).
57. (a) I. Paterson, C. Cowden, C. Watson. *Synlett* 209 (1996); (b) I. Paterson, C. Watson, K.-S. Yeung, P. A. Wallace, R. A. Ward. *J. Org. Chem.* **62**, 452 (1997).
58. (a) D. O. Hagan. *The Polyketide Metabolites*, Ellis Horwood, Chichester, (1991); (b) D. J. Newman, G. M. Cragg. *J. Nat. Prod.* **67**, 1216 (2004).
59. (a) M. Morgen, S. Bretzke, P. Li, D. Menche. *Org. Lett.* **12**, 4494 (2010); (b) L. Wang, P. Li, D. Menche. *Angew. Chem., Int. Ed.* **49**, 9270 (2010).
60. (a) L. F. Tietze. *Chem. Rev.* **96**, 115 (1996); (b) P. J. Parsons, C. S. Penkett, A. J. Shell. *Chem. Rev.* **96**, 195 (1996); (c) I. Ryu, N. Sonoda, D. P. Curran. *Chem. Rev.* **96**, 177 (1996).
61. L. Wang, D. Menche. *Angew. Chem., Int. Ed.* **51**, 9425 (2012).
62. (a) D. J. Hart, K. Kanai. *J. Org. Chem.* **47**, 1555 (1982); (b) A. K. Chatterjee, F. D. Toste, T.-L. Choi, R. H. Grubbs. *Adv. Synth. Catal.* **344**, 634 (2002); (c) D. L. Comins, J. M. Dinsmore, L. R. Marks. *Chem. Commun.* 4170 (2007).
63. (a) R. Lakhmiri, P. Lhoste, B. Kryczka, D. Sinou. *J. Carbohydr. Chem.* **12**, 223 (1993); (b) I. Frappa, B. Kryczka, P. Lhoste, S. Porwanski, D. Sinou. *J. Carbohydr. Chem.* **16**, 891 (1997); (c) I. Frappa, B. Kryczka, P. Lhoste, S. Porwanski, D. Sinou, A. Zawisza. *J. Carbohydr. Chem.* **17**, 1117 (1998).
64. (a) C. F. Nising, S. Bräse. *Chem. Soc. Rev.* **37**, 1218 (2008); (b) B. M. Trost, M. L. Crawley. *Chem. Rev.* **103**, 2921 (2003).
65. (a) P. J. Dunn, R. Haener, H. Rapoport. *J. Org. Chem.* **55**, 5017 (1990); (b) V. Kumar, N. G. Ramesh. *Tetrahedron* **62**, 1877 (2006).

