

Synthesis and Biological Evaluation of Paleo-Soraphens**

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Natural products are exploited as a rich source of biologically active compounds and can be isolated as metabolites from plants, fungi, animals, and microorganisms. In particular, polyketides from fungi and bacteria have been the focus of chemical and biological investigations. Since the biosynthesis of modular polyketides follows a linear assembly, genetic analysis can be used to rationalize the biosynthesis of polyketides and even to predict the stereochemical outcome.^[1] Consequently, these genetic analyses have been used to define the stereochemistry of natural products and thus guide their total synthesis.^[2] So far in natural product synthesis the structures of isolated natural products have served as targets. However, if one compares modular polyketide synthases to the isolated natural products, it becomes apparent that in a large variety of polyketides at least one enzymatic activity is nonproductive. Whether this is the consequence of an evolutionary process or a chemical consequence of the substrate is still unknown. Nevertheless, we were interested in natural products that exhibit the structure predicted by the intact polyketide synthases to see if and how their biological behavior would change as a consequence of the altered structure.

Here, we present the synthesis and biological activity of two soraphen derivatives that contain the structure derived from its polyketide synthases. Soraphen^[3] inhibits the eukaryotic acetyl-coenzyme A carboxylase (ACC)^[4] and consequently was considered a potential antifungal and antitumor compound.^[5] Its biosynthetic origin was described by Ligon et al.,^[6] and Müller and Flosset et al.^[7] and they identified two positions of the isolated natural product that differed from the genetically expected outcome.

Ligon et al. pointed out that the expected trisubstituted double bond (C2–C3), which should be established by module 8, is missing due to the inactivity of the dehydratase activity within that domain.^[6] Additionally, the ketoreductase must have been nonfunctional as well since the expected

secondary alcohol was not produced either. Meanwhile, there is still a double bond between C9 and C10. The presence of this double bond can be rationalized by an isomerization of the preceding α,β -unsaturated ester to the deconjugated β,γ -enoate. Ligon et al. proposed that this double bond is a consequence of postketide transformations. In any case the presence and position of this double bond is believed not to be the direct consequence of the polyketide synthase (Scheme 1).

The synthesis of soraphen A was completed independently by research groups led by Giese^[8] and Trost,^[9] and with partial syntheses put forward by Sinnes and others.^[10] Based on reported difficulties in using the internal double bond as a handle for a convergent synthesis, we decided to use a macrolactonization and a Nozaki–Hiyama–Kishi reaction in the endgame of our syntheses (Scheme 2). As depicted in Scheme 3, our synthesis of the western segment **6** features a strategic olefin cross-metathesis.^[11]

Starting from the known chiral diol **1**,^[12] which in our case was conveniently obtained by proline-catalyzed α -oxidation^[13] of 4-pentenal,^[14] selective protection of the primary hydroxy group and etherification of the remaining secondary alcohol afforded alkene **2** (Scheme 3). This set the stage for the cross-metathesis with the known (*S*)-1-phenyl-3-buten-1-ol (**3**).^[15] In the presence of 1.5 mol % of the Grubbs II catalyst, the cross-metathesis reaction smoothly provided the desired product in good yield. Subsequent reduction of the double bond by either imide reduction^[16] or heterogeneous hydrogenation afforded chiral alcohol **4**, which was then protected as a PMB ether (**5**). Finally, TBS removal with CSA and IBX-mediated oxidation^[17] of the resulting primary alcohol afforded the desired western segment **6**. For the synthesis of the eastern segment (Scheme 4), we envision that the combination of *syn*-Evans aldol reaction^[18] and *anti*-Marshall reaction^[19] with commercially available (*R*)-Roche ester will greatly enhance the efficiency for constructing the five contiguous stereocenters.

Thus, *syn*-Evans aldol reaction of **7** with aldehyde **8**^[20] afforded the corresponding adduct **9**. Subsequent TBS protection of the secondary hydroxy group and reductive removal of the chiral auxiliary afforded alcohol **10**, which was transformed to the corresponding aldehyde **11**.^[21] Finally, an *anti*-Marshall reaction generated two additional desired chiral centers and the vinyl iodide segment **13** was completed through a hydrozirconation–iodination sequence.

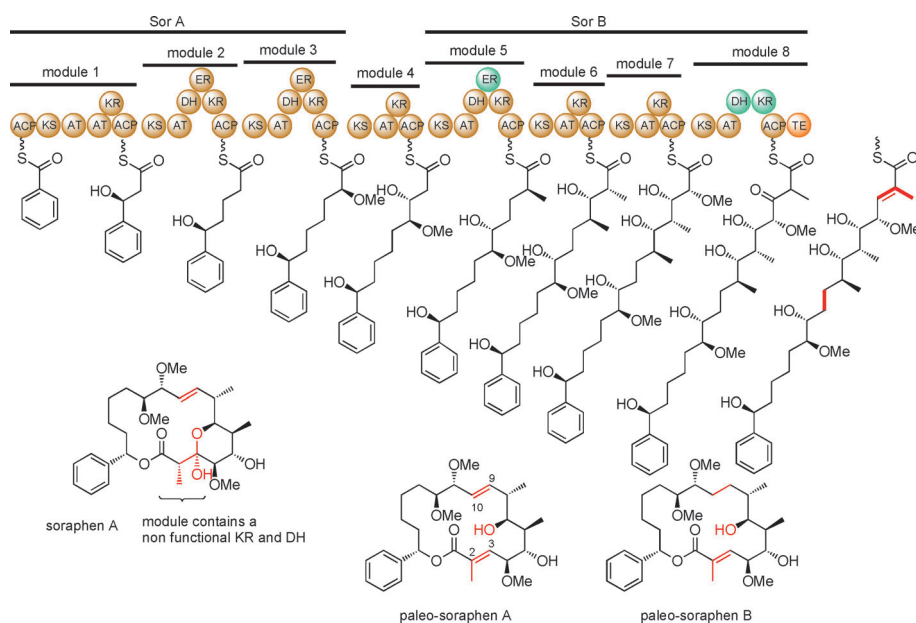
With both segments in hand, we began the endgame of the synthesis with the Nozaki–Hiyama–Kishi coupling (Scheme 5).^[22] The two segments were joined to form the desired adduct, albeit as a 2:1 mixture of the allylic alcohol diastereoisomers. Hence, a sequence of oxidation and chelation-controlled reduction with $\text{Zn}(\text{BH}_4)_2$ ^[23] at low temperature provided the desired alcohol **15** in excellent yield and

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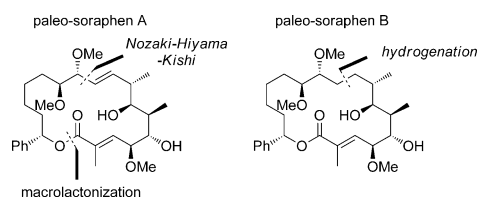
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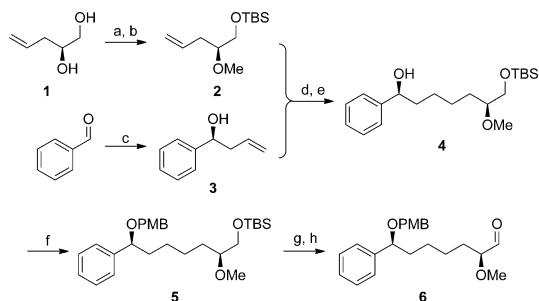
Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201305331>.



Scheme 1. Polyketide synthase of soraphen A and ketides as derived by fully functional domains.



Scheme 2. Retrosynthetic analysis of paleo-soraphens A and B.

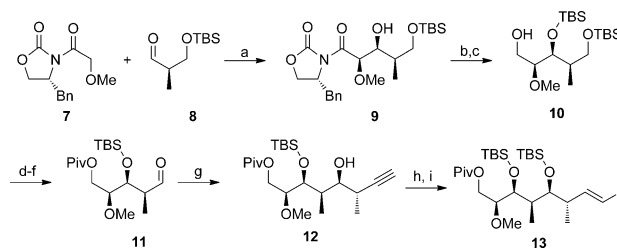


Scheme 3. Synthesis of the western segment. a) TBSCl, imidazole, DMF, RT; b) Me_3OBF_4 , proton sponge, 4 Å MS, CH_2Cl_2 , 80% over 2 steps; c) $(-)\text{-Ipc}_2\text{BOMe}$, allylMgBr, Et_2O , -100°C , 75% (89% ee); d) 1.5 mol% Grubbs II, CH_2Cl_2 , reflux, 80%; e) NBSH, Et_3N , CH_2Cl_2 , 81%, RT or PtO_2 , H_2 (1 atm), EtOAc, RT, 93%; f) KHMDS, TBAI, PMBCl, THF, RT, 73%; g) $(+)\text{-CSA}$, $\text{MeOH-CH}_2\text{Cl}_2$, 0°C to RT, 81%; h) IBX, DMSO, RT, 100%. TBSCl = *tert*-butyldimethylsilyl chloride, Grubbs II = (1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene) dichloro(phenylmethylene) (tricyclohexylphosphine) ruthenium, NBSH = 2-nitrobenzenesulfonylhydrazide, KHMDS = potassium bis(trimethylsilyl)amide, TBAI = tetra-*n*-butylammonium iodide, PMBCl = 4-methoxybenzylchloride, $(+)\text{-CSA}$ = $(+)\text{-camphor-10-sulfonic acid}$, IBX = 2-iodoxybenzoic acid.

selectivity. Methylation and treatment with DIBAL-H afforded primary alcohol **16** and subsequent transformations led to compound **17**. Application of the Shiina protocol^[24]

provided the desired macrolactone **18** in 43% yield. Finally, removal of the TBS groups was achieved with HF-pyridine and furnished paleo-soraphen A (**19**).

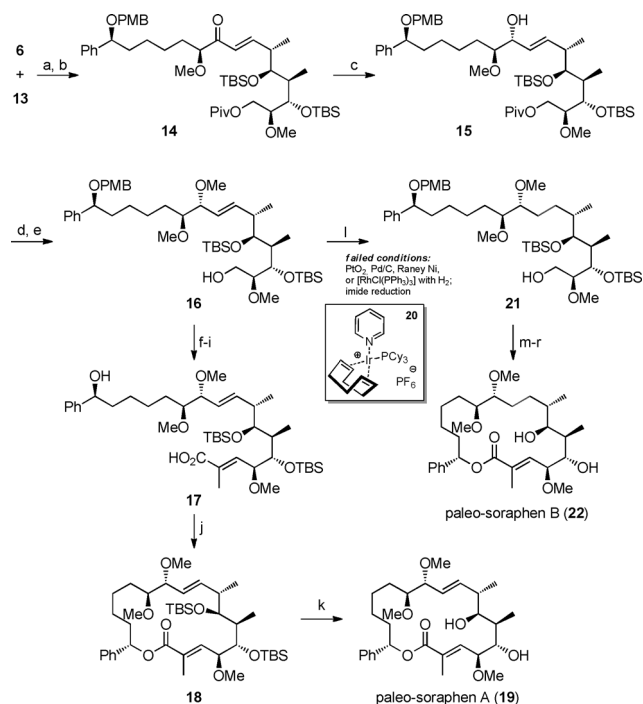
The synthesis of paleo-soraphen B follows the same route, which diverges only at compound **16** (Scheme 5). However, reduction of the double bond of **16** proved nontrivial, and the Crabtree catalyst **20** was finally identified to achieve the desired selective transformation to give **21** in good yield. Other reducing agents either caused no conversion or gave poor selectivity in the reduction of the double bond between C9 and C10 relative to the benzylic position at C17. Then, starting from **21**, we successfully applied the same sequence as that used for the synthesis of paleo-soraphen A to afford paleo-soraphen B (**22**).



Scheme 4. Synthesis of the eastern segment: a) Bu_2BOTf , Et_3N , CH_2Cl_2 , 85%; b) TBSOTf, 2,6-lutidine, CH_2Cl_2 , 90%; c) LiBH_4 , THF, 88%; d) PivCl, Et_3N , DMAP, CH_2Cl_2 , 95%; e) $(+)\text{-CSA}$, $\text{MeOH-CH}_2\text{Cl}_2$, 0°C to RT, 81%; f) $(\text{COCl})_2$, DMSO, Et_3N , CH_2Cl_2 , 61% over 3 steps; g) $(R)\text{-but-3-yn-2-yl methanesulfonate}$, $[\text{Pd}(\text{dppf})\text{Cl}_2]$, InI, THF-HMPA, 75%; h) TBSOTf, 2,6-lutidine, CH_2Cl_2 , 95%; i) $[\text{Cp}_2\text{ZrHCl}]$, I_2 , CH_2Cl_2 . Bu_2BOTf = di-*n*-butylboron triflate, TBSOTf = *tert*-butyldimethylsilyl triflate, PivCl = pivaloyl chloride, $[\text{Cp}_2\text{ZrHCl}]$ = zirconocene hydrochloride.

The biological activity of the paleo-soraphens was tested with three fungal strains and compared with that of soraphen A. Table 1 shows that the paleo-soraphens also have antifungal activity but are much less active than soraphen A. Cytotoxic effects on mammalian cells were tested with a mouse fibroblast (L-929) and a cervical carcinoma cell line (KB-3-1). While soraphen A was found to be active in the nM range ($\text{IC}_{50} = 40 \text{ ng mL}^{-1}$), the paleo-soraphens showed no clear activity up to $1.2 \text{ } \mu\text{g mL}^{-1}$, the highest test concentration.

It has already been established that soraphen A is a eukaryotic ACC inhibitor but we wanted to check the possibility that there was a shift in the mode of action of the soraphens during evolution. We therefore characterized the action of the two paleo-soraphens (at the highest possible concentration of $8.3 \text{ } \mu\text{g mL}^{-1}$) and of soraphen A (83 ng mL^{-1}) by impedance profiling. The basis of this method is that



Scheme 5. Endgame in the synthesis of paleo-soraphens A and B. a) NiCl_2 , CrCl_2 , DMSO, RT; b) DMP, NaHCO_3 , CH_2Cl_2 , 58% over 2 steps; c) $\text{Zn}(\text{BH}_4)_2$, Et_2O , -55°C , 90% (d.r. > 25:1); d) Me_3OBF_4 , proton sponge, CH_2Cl_2 , RT; e) DIBAL, THF, -50°C , 83% over 2 steps; f) DMP, NaHCO_3 , CH_2Cl_2 , 92%; g) $\text{Ph}_3\text{P}=\text{C}(\text{CH}_3)\text{CO}_2\text{Et}$, CH_2Cl_2 , RT, 95%; h) DDQ, pH 7 buffer, CH_2Cl_2 , MeOH, 0°C , 100%; i) LiOH, THF–MeOH– H_2O , RT, 94%; j) MNBA, DMAP, toluene, 4 Å MS, 43%; k) 70% HF, THF, 0°C to RT, 89%; l) 10 mol% Crabtree cat. **20**, CH_2Cl_2 , H_2 (atm), 89%; m) DMP, NaHCO_3 , CH_2Cl_2 , 67%; n) $\text{Ph}_3\text{P}=\text{C}(\text{CH}_3)\text{CO}_2\text{Et}$, CH_2Cl_2 , RT, 85%; o) DDQ, pH 7 buffer, CH_2Cl_2 , MeOH, 0°C , 74%; p) LiOH, THF–MeOH– H_2O , RT, 90%; q) MNBA, DMAP, toluene, 4 Å MS, 26%; r) 70% HF, THF, 0°C to RT, 45%. DMP = Dess–Martin periodinane, DIBAL = diisobutylaluminum hydride, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, MNBA = 2-methyl-6-nitrobenzoic anhydride, DMAP = 4-dimethylaminopyridine.

Table 1: Antifungal activity of paleo-soraphens and soraphen A.

Compound	<i>Pythium debaryanum</i>	<i>Botrytis cinerea</i>	<i>Mucor hiemalis</i>
	IC_{50} [$\mu\text{g mL}^{-1}$] ^[a]		
paleo-soraphen A (19)	0.4	> 40	21
paleo-soraphen B (22)	> 40	> 40	16
soraphen A	0.045	0.0035	0.0006

[a] The IC_{50} was measured using a WST-1 test after incubation with a serial dilution of the compounds for 2 days.

compounds with a similar mode of action induce similar time-dependent impedance curves. L-929 mouse fibroblasts were incubated with soraphen A, **19**, and **22** in wells of a 96-well E-Plate which had gold electrodes integrated in the bottom of the wells. Impedance across the electrodes was measured in real time (Figure S1 in the Supporting Information). The resulting curves for soraphen A, **19**, and **22** were compared to a set of reference compounds covering a broad activity spectrum. We used cubic smoothing splines for the approx-

imation of the impedance data and the basic cubic spline coefficients for statistical analysis (see the Supporting Information). The results of a hierarchical cluster analysis showed paleo-soraphen **19** in close proximity to soraphen A, while compound **22** was found in an unrelated cluster together with camptothecin (Figure 1).^[25] This indicates that **22** unfolds its mode of action through a mechanism that differs from that of the other two soraphens (for details of the camptothecin activity of compound **22** see Supporting Information).

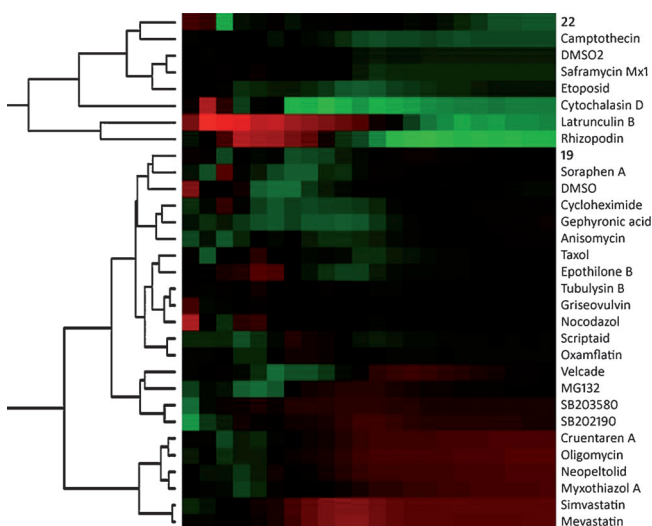


Figure 1. Hierarchical cluster analysis of impedance kinetics. L-929 mouse fibroblasts were incubated with soraphen A, **19**, and **22** and compared with reference compounds. The impedance in each culture was measured over a period of 5 days using an xCelligence system. Basis coefficients of spline approximation curves are used for cluster analysis. The results show compounds with similar modes of action in close proximity.

In conclusion, the synthesis reported herein provides two potential evolutionary predecessors of the natural product soraphen A. Our aim was to see whether the incorporated modifications would lead to altered activities and thus support the concept of evolutionary optimization. Indeed, paleo-soraphen A still inhibits eukaryotic ACC, albeit with reduced activity, which would support the concept of evolutionary optimization. However, paleo-soraphen B shows a completely different mode of action, namely topoisomerase inhibition. At this point this observation might be pure coincidence but it would also be consistent with the concept of horizontal gene transfer (HGT) or biocombinatorial processes^[26] which could lead to different activities and its subsequent fine-tuning by disabling certain PKS activities. The fact that the paleo-soraphens do not undergo Michael addition, which would result in compounds that are structurally more closely related to the parent soraphen, supports the assumption that inactivation of the ketoreductase is probably not due to spontaneous hemiacetal formation. Even though genetic engineering could also provide these products,^[27a] we are convinced that this field would be very attractive for synthetic chemists as they could target “genetic” compounds with potentially new activities rather than synthesizing known

compounds that can be more readily obtained by fermentation or isolated from natural sources. Consequently, these unexpected results propose a strategy as to how natural compounds are fine-tuned with respect to their biological activity and thus might provide an alternative strategy in the search for medicinally active compounds extending the field of paleoenzymology^[27b-e] from its peptidic targets to secondary metabolites.

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- [1] a) R. Reid, M. Piagentini, E. Rodriguez, G. Ashley, N. Viswanathan, J. Carney, D. V. Santi, C. R. Hutchinson, R. McDaniel, *Biochemistry* **2003**, *42*, 72–79; b) P. Caffrey, *ChemBioChem* **2003**, *4*, 654–657; c) D. H. Kwan, Y. Sun, F. Schulz, H. Hong, B. Popovic, J. C. C. Sim-Stark, S. F. Haydock, P. F. Leadlay, *Chem. Biol.* **2008**, *15*, 1231–1240; d) D. H. Kwan, P. F. Leadlay, *Chem. Biol.* **2010**, *17*, 829–838; e) A. T. Keatinge-Clay, R. M. Stroud, *Structure* **2006**, *14*, 737–748; f) A. Kitsche, M. Kalesse, *ChemBioChem* **2013**, *14*, 851–861.
- [2] a) D. Janssen, D. Albert, R. Jansen, R. Müller, M. Kalesse, *Angew. Chem.* **2007**, *119*, 4985–4988; *Angew. Chem. Int. Ed.* **2007**, *46*, 4898–4901; b) D. Menche, F. Arikian, O. Perlova, N. Horstmann, W. Ahlbrecht, S. C. Wenzel, R. Jansen, H. Irschik, R. Müller, *J. Am. Chem. Soc.* **2008**, *130*, 14234–14243; c) C. Jahns, T. Hoffmann, S. Müller, K. Gerth, P. Washausen, G. Höfle, H. Reichenbach, M. Kalesse, R. Müller, *Angew. Chem.* **2012**, *124*, 5330–5334; *Angew. Chem. Int. Ed.* **2012**, *51*, 5239–5243; d) S. Essig, S. Bretzke, R. Müller, D. Menche, *J. Am. Chem. Soc.* **2012**, *134*, 19362–19365.
- [3] a) H. Reichenbach, G. Höfle, H. Augustiniak, N. Bedorf, E. Forche, K. Gerth, H. Irschik, R. Jansen, B. Kurze, F. Sasse, H. Steinmetz, W. Trowitsch-Kienast, EP 282455 [*Chem. Abstr.* **1988**, *111*, 1325871]; b) N. Bedorf, D. Schomburg, K. Gerth, H. Reichenbach, G. Höfle, *Liebigs Ann. Chem.* **1993**, 1017–1021; c) K. Gerth, N. Bedorf, H. Irschik, G. Höfle, H. Reichenbach, *J. Antibiot.* **1994**, *47*, 23–31.
- [4] a) K. Gerth, S. Pradella, O. Perlova, S. Beyer, R. Müller, *J. Biotechnol.* **2003**, *106*, 233–253; b) H. F. Vahlensieck, L. Pridzun, H. Reichenbach, A. Hinnen, *Curr. Genet.* **1994**, *25*, 95–100; c) Y. Shen, S. L. Volrath, S. C. Weatherly, T. D. Elich, L. Tong, *Mol. Cell* **2004**, *16*, 881–891; d) S. C. Weatherly, S. L. Volrath, T. D. Elich, *Biochem. J.* **2004**, *380*, 105–110.
- [5] A. Beckers, S. Organe, L. Tinunermans, K. Scheys, A. Peeters, K. Brusselmans, G. Verhoeven, J. V. Swinnen, *Cancer Res.* **2007**, *67*, 8180–8187.
- [6] a) T. Schupp, C. Toupet, B. Cluzel, S. Neff, S. Hill, J. J. Beck, J. M. Ligon, *J. Bacteriol.* **1995**, *177*, 3673–3679; b) J. Ligon, S. Hill, J. Beck, R. Zirkle, I. Monar, J. Zawodny, S. Money, T. Schupp, *Gene* **2002**, *285*, 257–267.
- [7] S. C. Wenzel, R. M. Williamson, C. Grunanger, J. Xu, K. Gerth, R. A. Martinez, S. J. Moss, B. J. Carroll, S. Grond, C. J. Unkefer, R. Müller, H. G. Floss, *J. Am. Chem. Soc.* **2006**, *128*, 14325–14336.
- [8] a) S. Abel, D. Faber, O. Hüter, B. Giese, *Angew. Chem.* **1994**, *106*, 2522–2523; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2466–2468; b) S. Abel, D. Faber, O. Hüter, B. Giese, *Synthesis* **1999**, 188–197.
- [9] B. M. Trost, J. D. Sieber, W. Qian, R. Dhawan, Z. T. Ball, *Angew. Chem.* **2009**, *121*, 5586–5589; *Angew. Chem. Int. Ed.* **2009**, *48*, 5478–5481.
- [10] a) B. Loubinoux, J. L. Sinnes, A. C. O'Sullivan, T. Winkler, *Helv. Chim. Acta* **1995**, *78*, 122–128; b) B. Loubinoux, J. L. Sinnes, A. C. O'Sullivan, T. Winkler, *J. Org. Chem.* **1995**, *60*, 953–959; c) B. Loubinoux, J. L. Sinnes, A. C. O'Sullivan, T. Winkler, *Tetrahedron* **1995**, *51*, 3549–3558; d) B. Loubinoux, J. L. Sinnes, A. C. O'Sullivan, *J. Chem. Soc. Perkin Trans. 1* **1995**, 521–525; e) H. W. Lee, Y. J. Kim, *Bull. Korean Chem. Soc.* **1996**, *17*, 1107–1108; f) M. K. Gurjar, A. S. Mainkar, P. Srinivas, *Tetrahedron Lett.* **1995**, *36*, 5967–5968; g) G. Vincent, D. J. Mansfield, J.-P. Vors, M. A. Ciufolini, *Org. Lett.* **2006**, *8*, 2791–2794.
- [11] For reviews on olefin cross-metathesis see: a) S. E. Gibson, S. P. Keen, *Top. Organomet. Chem.* **1998**, *1*, 155–181; b) S. J. Connon, S. Blechert, *Angew. Chem.* **2003**, *115*, 1944–1968; *Angew. Chem. Int. Ed.* **2003**, *42*, 1900–1923.
- [12] R. Chênevert, S. Gravel, J. Bolte, *Tetrahedron: Asymmetry* **2005**, *16*, 2081–2086.
- [13] a) S. P. Brown, M. P. Brochu, C. J. Sinz, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2003**, *125*, 10808–10809; b) G. Zhong, *Angew. Chem.* **2003**, *115*, 4379–4382; *Angew. Chem. Int. Ed.* **2003**, *42*, 4247–4250.
- [14] 4-Pentenal is commercially available; alternatively it can be conveniently prepared through a [3,3]-sigmatropic rearrangement of allyl vinyl ether: L. K. Montgomery, J. W. Matt, *J. Am. Chem. Soc.* **1967**, *89*, 6556–6564. See the Supporting Information for the detailed synthesis of diol **1**.
- [15] a) U. S. Racherla, H. C. Brown, *J. Org. Chem.* **1991**, *56*, 401–404; b) B. J. Melancon, N. R. Perl, R. E. Taylor, *Org. Lett.* **2007**, *9*, 1425–1428.
- [16] M. H. Haukaas, G. A. O'Doherty, *Org. Lett.* **2002**, *4*, 1771–1774.
- [17] M. Frigerio, M. Santagostino, *Tetrahedron Lett.* **1994**, *35*, 8019–8022.
- [18] a) D. A. Evans, J. Bartoli, T. L. Shih, *J. Am. Chem. Soc.* **1981**, *103*, 2127–2129; b) G. E. Keck, A. Palani, S. F. McHardy, *J. Org. Chem.* **1994**, *59*, 3113–3122.
- [19] J. A. Marshall, C. M. Grant, *J. Org. Chem.* **1999**, *64*, 8214–8219.
- [20] I. Paterson, G. J. Florence, K. Gerlach, J. P. Scott, N. Sereinig, *J. Am. Chem. Soc.* **2001**, *123*, 9535–9544.
- [21] A. J. Mancuso, D. Swern, *Synthesis* **1981**, 165–185.
- [22] a) K. Takai, K. Kimura, T. Kuroda, T. Hiyama, H. Nozaki, *Tetrahedron Lett.* **1983**, *24*, 5281–5284; b) H. Jin, J. Uenishi, W. J. Christ, Y. Kishi, *J. Am. Chem. Soc.* **1986**, *108*, 5644–5646.
- [23] J. A. Marshall, R. Sedrani, *J. Org. Chem.* **1991**, *56*, 5496–5498.
- [24] I. Shiina, M. Kubota, H. Oshiumi, M. Hashizume, *J. Org. Chem.* **2004**, *69*, 1822–1830.
- [25] E. Elias, N. Lalun, M. Lorenzo, L. A. Blache, P. Chelidze, M. F. O'Donohue, D. Ploton, H. Bobichon, *Exp. Cell Res.* **2003**, *291*, 176–188.
- [26] a) H. Jenke-Kodama, E. Dittmann, *Phytochemistry* **2009**, *70*, 1858–1866; b) H. Jenke-Kodama, A. Sandmann, R. Müller, E. Dittmann, *Mol. Biol. Evol.* **2005**, *22*, 2027–2039.
- [27] a) K. M. Fisch, W. Bakeer, A. A. Yakasai, Z. S. Song, J. Pedrick, Z. Wasil, A. M. Bailey, C. M. Lazarus, T. J. Simpson, R. J. Cox, *J. Am. Chem. Soc.* **2011**, *133*, 16635–16641; b) R. Perez-Jimenez, A. Ingles-Prieto, Z.-M. Zhao, I. Sanchez-Romero, J. Alegre-Cebollada, P. Kosuri, S. Garcia-Manyes, T. J. Kappock, M. Tanokura, A. Holmgren, J. M. Sanchez-Ruiz, E. A. Gaucher, J. M. Fernandez, *Nat. Struct. Mol. Biol.* **2011**, *18*, 592–599; c) E. A. Gaucher, J. M. Thomson, M. F. Burgan, S. A. Benner, *Nature* **2003**, *425*, 285–288; d) J. W. Thornton, E. Need, D. Crews, *Science* **2003**, *301*, 1714–1717; e) J. M. Thomson, E. A. Gaucher, M. F. Burgan, D. W. De Kee, T. Li, J. P. Aris, S. A. Benner, J. M. Thomson, *Nat. Genet.* **2005**, *37*, 630–635.