## PAPER

CrossMark
← click for updates

# Paleo-soraphens: chemical total syntheses and biological studies†

Hai-Hua Lu,[a] Bettina Hinkelmann,[b] Thomas Tautz,[a] Jun Li,[a] Florenz Sasse,[b] Raimo Franke[b] and Markus Kalesse*[a,b]

The soraphens are natural products that exhibit a molecular structure different from what would have been expected by following its polyketidal assembly line. The most significant differences are the presence of a hemiketal instead of a trisubstituted double bond and a double bond at C9 and C10 where a saturated carbon chain was expected. We were interested in the biological activity of the soraphens with architectures as described by the polyketide synthase since we hypothesized that these modifications reflect the evolutionary optimization of the soraphens. Herein we describe four additional derivatives of the so-called paleo-soraphens and their biological profiling to provide a picture of the hypothetical evolutionary optimization of this family of natural products. The syntheses required a unified and convergent strategy and their biological profiling was performed with the aid of impedance measurements. The results of these biological experiments are consistent with the proposed evolutionary optimization of the soraphens.

## Introduction

Natural products are the source of biologically active compounds and in particular secondary metabolites isolated from microorganisms have been in the focus of natural product chemistry for quite some time. One of the most fundamental questions associated with natural products is the question on the origin of their biological activity and the ultimate purpose of their existence. While questions on the genuine relevance of natural products are answered by working groups concerned with the ecological aspects of natural products, the question of how the activity was generated in the first place is still unknown. With this state of knowledge, we started a program that aimed at providing natural products as if they were derived by the precise specification of polyketide synthases.[1] Based on this concept and previous contributions on the biosynthesis of soraphens[2] we recently described the synthesis and biological evaluation of two so-called paleo-soraphens of which one exhibited a biological activity completely different from the natural soraphen A (7).[3] This result supported the

hypothesis that biological activities can be altered by turning off distinct enzymatic activities within polyketide synthases. In order to shed more light on this topic we completed the syntheses of four additional paleo-soraphens, which exhibit modifications associated with the hypothetical evolutionary optimization (Fig. 1a). The paleo-soraphens **1** and **2** exhibit the structure as defined by the ketoreductase (KR) and dehydratase (DH) in module 8. Therefore, the hemiacetal moiety is transformed to a trisubstituted double bond. Further in paleo-soraphen **2** the double bond between C9 and C10 becomes a saturated alkyl chain as defined by the enoyl reductase (ER) in module 5. One post-ketidal modification that could potentially also provide altered biological activities is the methylation at the hydroxyl group at C11. In contrast to the methoxy group at C12, which is incorporated during the polyketidal synthesis, installation of the methoxy group at this position takes place by a post-ketidal methylation. This sets the background for the syntheses of the corresponding paleo-soraphens **3** and **4** that incorporate the modifications of compounds **1** and **2** but were exhibiting the hydroxyl group at C11. These compounds would be structurally even more distant from soraphen A (7) compared to compounds **1** and **2**. An additional set of modifications in this particular area was inversion of configuration at C11, paleo-soraphen **5** has the opposite configuration at C11 compared to soraphen A (7).

Finally, we completed the synthesis of 17-*epi*-paleo-soraphen F (**46**), which contains the reduced carbonyl group at C3 but still lacks the elimination step. However, we were not able to complete the synthesis of paleo-soraphen F (**6**) without

[a]*Institute for Organic Chemistry and Centre for Biomolecular Drug Research (BMWZ), Leibniz Universität Hannover, Schneiderberg 1B, 30167 Hannover, Germany. E-mail: Markus.Kalesse@oci.uni-hannover.de*
[b]*Department of Chemical Biology, Helmholtz Centre for Infection Research (HZI), Inhoffenstr. 7, Braunschweig, Germany*

This journal is © The Royal Society of Chemistry 2015

*Org. Biomol. Chem.*, 2015, **13**, 8029–8036 | **8029**
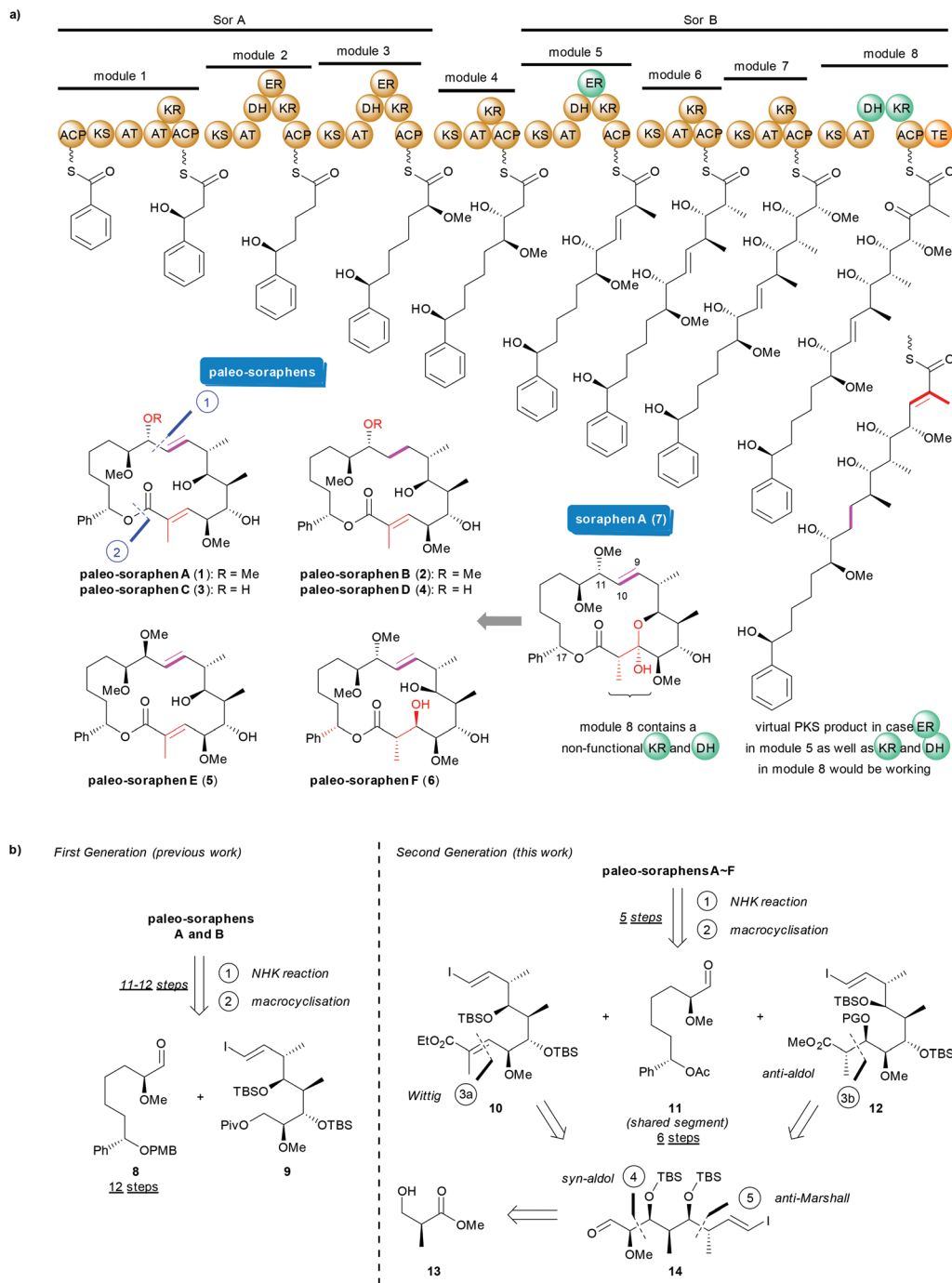
**Fig. 1** (a) Biosynthesis of the soraphen A and paleo-soraphens; (b) retrosynthetic analysis of the paleo-soraphens.

inversion of configuration at the ester linkage, despite considerable effort.

## Results and discussion

### Synthetic considerations and completion of segments

In order to provide rapid access to these modifications we first worked on an optimized route to the required building blocks

compared to our 1st generation synthesis (Fig. 1b). For this, the synthesis of a more advanced eastern segment rather than **9** should minimize late-stage manipulations to the paleo-soraphens. These strategic considerations lead to evaluate a more convergent and unified strategy to all soraphens with either the trisubstituted double bond or the aldol fragment in place. The essential segments **10** and **12** could be accessed from the same aldehyde **14** through either a Wittig or an *anti*-aldol reaction, while the key aldehyde **14** could be conveniently obtained
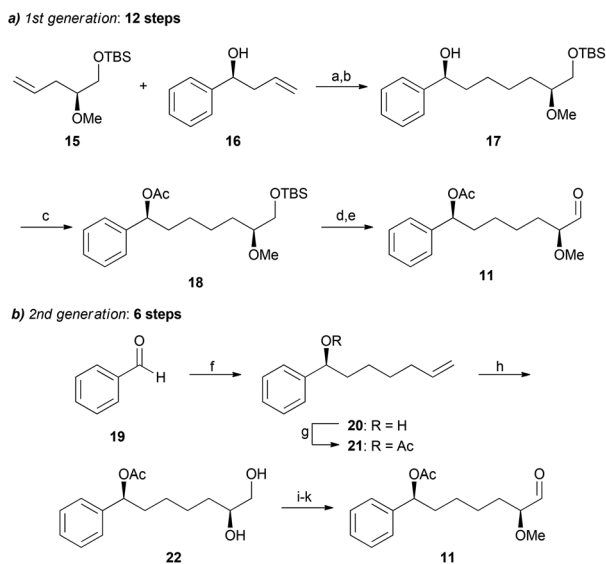
**Scheme 1** First- and second-generation approaches to the shared western segment **11**. Reagents and conditions: (a) 1.5 mol% Grubbs 2nd generation catalyst, $CH_2Cl_2$, 65%; (b) $PtO_2$, $H_2$ (1 atm.), EtOAc, 81%; (c) DMAP, $Ac_2O$, $Et_3N$, $CH_2Cl_2$, 96%; (d) CSA, MeOH; (e) IBX, DMSO, 85%, 2 steps; (f) 5-hexen-1-ylmagnesium bromide, $Ti(O^iPr)_4$, (S)-BINOL, BDMAEE, 54%, 88% ee; (g) DMAP, $Ac_2O$, $Et_3N$, $CH_2Cl_2$, 100%; (h) AD-mix-$\alpha$, $^tBuOH$–$H_2O$, 91%; (i) TESCl, imidazole, DMAP, DMF; (j) $Ag_2O$, MeI, $Et_2O$; (k) IBX, DMSO, 74%, 3 steps. DMAP = 4-dimethyl-aminopyridine; CSA = camphorsulfonic acid; IBX = 2-iodoxybenzoic acid; (S)-BINOL = (S)-(−)-1,1'-bis(2-naphthol); BDMAEE = bis(2-di-methylaminoethyl)ether; TESCl = chlorotriethylsilane.

from (S)-Roche ester (**13**) by a combination of *anti*-Marshall and *syn*-Evans aldol reaction. The new western segment **11** was initially prepared by our established route from compounds **15** and **16** in totally 12 steps from commercially available materials (Scheme 1a). Later, we developed a concise 6-step route by using an asymmetric alkylation and a Sharpless dihydroxylation as the key steps (Scheme 1b). The advantage of using **11** instead of **8** was the fact that in the endgame of the synthesis, both the hydroxyl group and the acid required for the macrolactonization could be liberated in one step.

Thus, under chiral titanium catalysis by a modification of Wang's deactivating strategy,[4] the first desired stereocenter was secured and the desired alcohol **20** was obtained in good yield with high enantioselectivity from 5-hexen-1-ylmagnesium bromide and benzaldehyde (**19**). Subsequent hydroxyl protection and terminal alkene asymmetric dihydroxylation provided the diol **22** in excellent yield. Finally, three more simple manipulations (primary hydroxyl group protection with TES, secondary hydroxyl group methylation and IBX-mediated TES deprotection/oxidation) completed the western segment **11** in good overall yield (Scheme 1b).

Meanwhile, for the eastern segments, we changed our previous synthetic sequence, as shown in Scheme 2. The synthesis starts from a Marshall reaction with aldehyde **23**,[5] generated by an established two-step sequence from Roche ester,[6] to provide the chiral alcohol **24** which was TBS-protected and

then subjected to a hydrozirconation. Treatment of the thus-generated zirconocene species with iodine produced iodide **26**. The primary TBS group could be selectively removed by treating the crude iodide **26** with $NaIO_4$ to afford alcohol **27** in good yields.[7]

Next, Dess–Martin oxidation of **27** followed by an Evans aldol reaction provided intermediate **29**. With compound **29** in hand, we were able to obtain lactone **30**, which could be used to independently confirm the established configurations with the aid of X-ray analysis. In the synthetic direction, the aldol product **29** was subsequently TBS-protected, the Evans auxiliary reductively removed, and the corresponding alcohol **31** was oxidized by the Dess–Martin reagent to provide aldehyde **14**. From **14**, the eastern segment **10**, required for the paleo-soraphens A–E, was obtained through a Wittig olefination. Meanwhile, an *anti*-selective Masamune aldol reaction between aldehyde **14** and chiral ester **33**[8] afforded the *anti*-aldol product **34**, which is required for the synthesis of paleo-soraphen F. For further manipulations, **34** could be transferred to its methyl ester and subsequently protected either as the TBS-(**12a**) or MOM-ether (**12b**).

## Completion of paleo-soraphens A–E

Our 1st generation synthesis required a variety of late-stage manipulations after the NHK coupling (Scheme 3a). With aldehyde **11** and vinyl iodide **10** in hand, we then commenced on the 2nd generation synthesis. The NHK coupling between **11** and **10** provided the desired product albeit with moderate selectivity. Fortunately, the diastereoisomers **36** and **37** could be easily separated from each other. Continuing with compound **36**, hydroxyl acid **38** was obtained through a two-step sequence involving methylation and basic saponification. Macrolactonization under Shiina conditions[9] and the removal of the TBS groups completed a 23-step synthesis of paleo-soraphen A (**1**) compared to 32 steps for our 1st generation route. For the synthesis of paleo-soraphen C (**3**), the C-11 hydroxyl group in compound **36** was protected as the TBS ether and the subsequent three same transformations completed the synthesis of this paleo-soraphen. For the completion of the paleo-soraphens B (**2**) and D (**4**), the selective reduction of the C9, C10-double bond was required and proved to be non-trivial. This reduction was finally achieved with Adam's catalyst and provided the desired product **39** in 72% yield along with ketone **40** in 21% yield. Ketone **40** could be converted to **39** by reduction with $Zn(BH_4)_2$.[10] Finally, the syntheses of the paleo-soraphens B and D were completed in four further steps from **39** respectively. While continuing with isomer **37**, paleo-soraphen E (**5**) could be easily completed by the established sequence of methylation, saponification, macrocyclization and final deprotection.

## Towards paleo-soraphen F (6) and completion of 17-*epi*-paleo-soraphen F (46)

For the synthesis of paleo-soraphen F, we first started from aldehyde **11** and vinyl iodide **12a**. Following our established 3-step sequence,[3] the NHK coupling product **41** was obtained
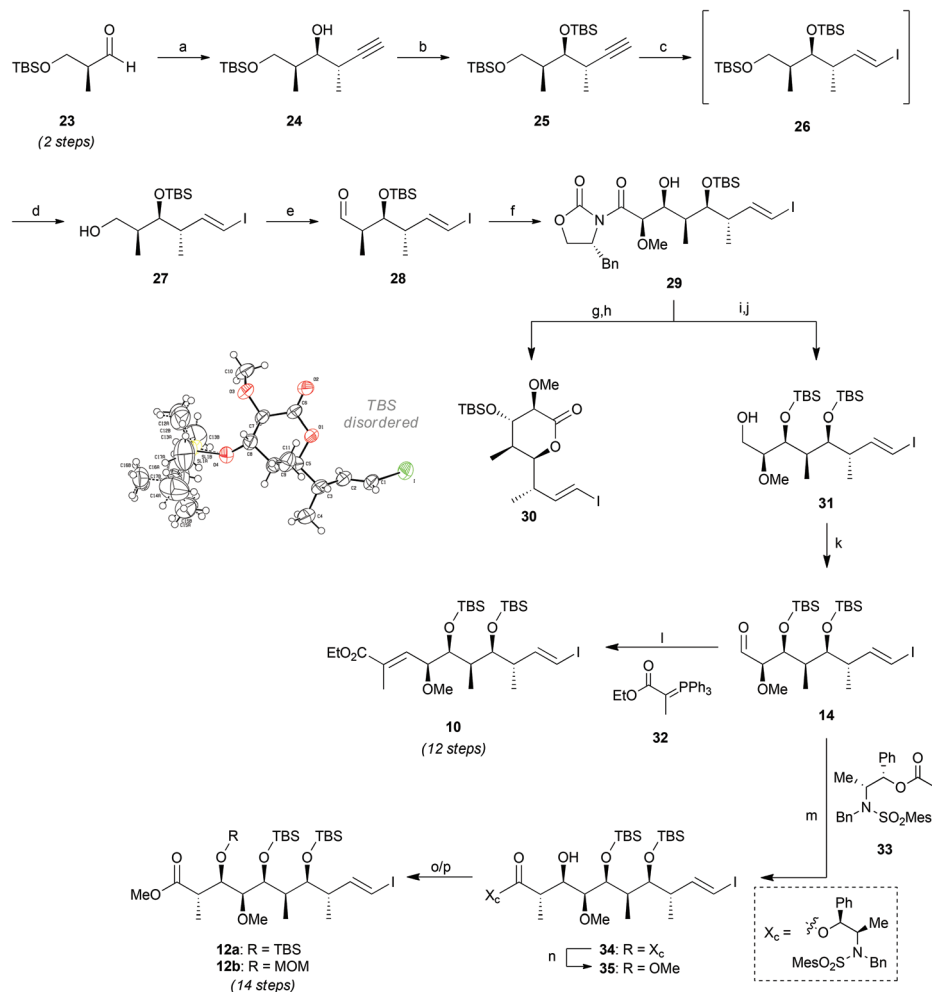
**Scheme 2** Syntheses of the eastern segments **10** and **12**. Reagents and conditions: (a) (*R*)-but-3-yn-2-yl-methanesulfonate, Pd(OAc)$_2$, PPh$_3$, Et$_2$Zn, THF, 80%; (b) 2,6-lutidine, TBSOTf, THF, −78 °C, 94%; (c) Cp$_2$ZrCl$_2$, LiHBEt$_3$, THF, then I$_2$; (d) NaIO$_4$, THF/H$_2$O, 78%, 2 steps; (e) DMP, NaHCO$_3$, CH$_2$Cl$_2$, 92%; (f) (*R*)-4-benzyl-3-(2-methoxyacetyl)oxazolidin-2-one, Bu$_2$BOTf, Et$_3$N, CH$_2$Cl$_2$, 91%; (g) 70% HF in pyridine, THF; (h) 2,6-lutidine, TBSOTf, CH$_2$Cl$_2$, 68%, 2 steps; (i) 2,6-lutidine, TBSOTf, CH$_2$Cl$_2$; (j) LiBH$_4$, THF, 87%, 2 steps; (k) DMP, NaHCO$_3$, CH$_2$Cl$_2$, 95%; (l) **32**, CH$_2$Cl$_2$, 95%, 2 steps from **31**; (m) **33**, Cy$_2$BOTf, Et$_3$N, CH$_2$Cl$_2$, 88%; (n) NaOMe, MeOH/THF, 88%; (o) 2,6-lutidine, TBSOTf, CH$_2$Cl$_2$, 91%; (p) MOMCl, DIPEA, CH$_2$Cl$_2$, 90%. TBSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate; DMP = Dess−Martin periodinane; MOMCl = chloromethyl methyl ether.

as a single diastereoisomer. Subsequent methylation provided **42** and basic saponification liberated hydroxyl acid **43** albeit with concomitant loss of the C3 protecting group. Unfortunately, macrolactonizations by acid activation protocols (Shiina, Yamaguchi, Mukaiyama methods, *etc.*) were unsuccessful to get **44**. Thus, we decided to enter from the sterically hindered carboxylic acid moiety, and to our delight, the expected macrolactone **45** was obtained in good yield under Mitsunobu reaction conditions. Subsequent removal of both TBS groups afforded a new soraphen (17-*epi*-paleo-soraphen E, **46**) (Scheme 4).

Encouraged by these results, we employed 17-*epi*-**11** in the NHK coupling and obtained compound **47** by the same strategy. To our surprise, its Mitsunobu reaction produced only a trace of the desired product **48** but the eliminated product **49** always dominated significantly under various conditions

[Scheme 5, eqn (1)]. Obviously, the inversion of configuration at C-17 caused steric effects resulting in very slow macrocyclization compared to elimination. Thus, we used the MOM-protected vinyl iodide **12b**, and repeated this sequence again. Unfortunately, this time basic hydrolysis only provided the undesired elimination product 17-*epi*-**38** [Scheme 5, eqn (2)].

Finally, a strategy based on a ring-closing metathesis reaction (RCM) was also conceived and evaluated (Scheme 6). From the known compound **51**,[11] aldehyde **52** was conveniently obtained and following our established 3-step route, coupling product **53** was obtained in good overall yield. Subsequent methylation and ester saponification gave us the desired corresponding acid. Then, an intermolecular esterification under Mitsunobu conditions cleanly provided the metathesis precursor **54** in good yield, which to some extent was evidence that steric hindrance was the cause of the failure of
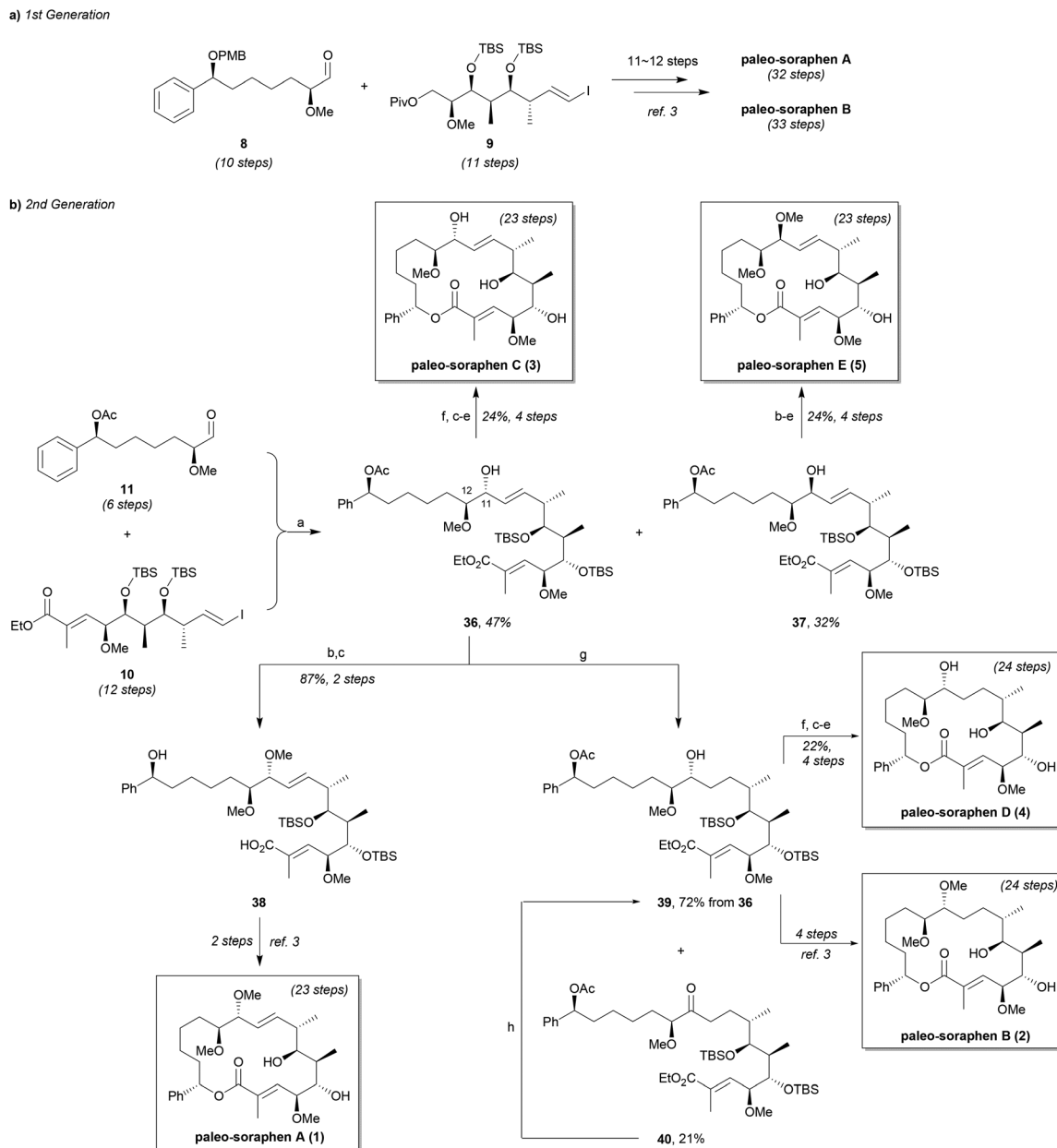
**Scheme 3** Synthesis of paleo-soraphens A–E. Reagents and conditions: (a) NiCl₂, CrCl₂, DMSO; (b) Ag₂O, MeI, 4 Å MS, Et₂O; (c) LiOH, THF/MeOH/H₂O; (d) MNBA, DMAP, 4 Å MS, toluene; (e) 70% HF in pyridine, THF; (f) 2,6-lutidine, TBSOTf, CH₂Cl₂; (g) PtO₂, H₂ (1 atm.), EtOAc; (h) Zn(BH₄)₂, Et₂O. MNBA = 2-methyl-6-nitrobenzoic anhydride.

the macrolactonization of **47**. Nevertheless, the key RCM to provide compound **55** failed under various conditions utilizing ruthenium catalysts.

### Biological studies of the paleo-soraphens

To compare biological activities of the paleo-soraphens, we characterized them *via* impedance profiling. Time-dependent cellular response profiles (TCRP) were recorded by using the xCELLigence system (ACEA Biosciences, RTCA SP Instrument). Abassi *et al.* showed that compounds with a similar mode of action often induce similar time-dependent response pro-

files.[12] The method is therefore suited to get first hints of the mechanisms of biological activities of substances. Soraphen A is known to inhibit the eukaryotic acetyl CoA carboxylase (ACC), which plays an essential role in fatty acid synthesis and degradation. To monitor this effect in cell culture we used L-929 mouse fibroblasts that were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% FBS for 24 h. Experiments on the kinetics of the soraphen A action showed that the inhibitory effect in cell cultures is delayed, and this delay is dependent on the serum concentration. Despite the fact that soraphen A blocks fatty acid synthesis, the cells obviously feed
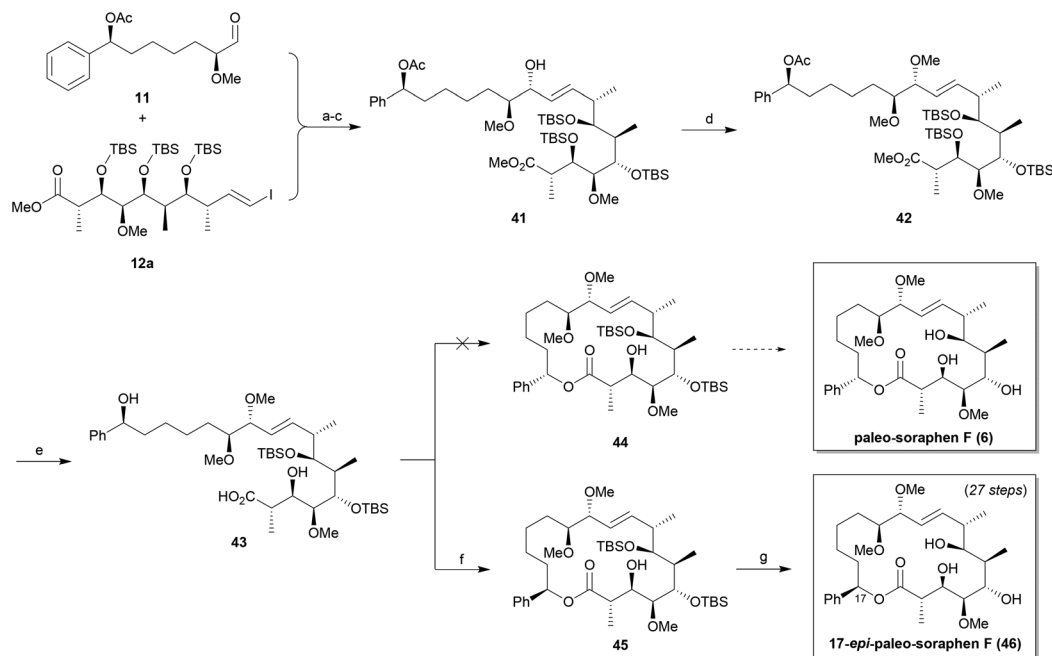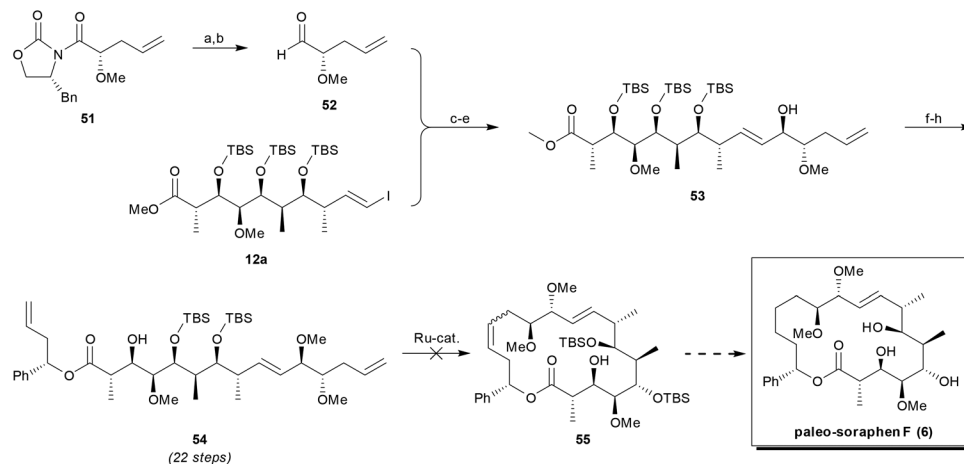
**Scheme 4** Towards paleo-soraphen F and completion of 17-*epi*-paleo-soraphen F (**46**) by an intramolecular Mitsunobu approach. Reagents and conditions: (a) NiCl₂, CrCl₂, DMSO; (b) DMP, NaHCO₃, CH₂Cl₂; (c) Zn(BH₄)₂, Et₂O, 58%, 3 steps; (d) Ag₂O, MeI, 4 Å MS, Et₂O, 96%; (e) LiOH, THF/MeOH/H₂O, 79%; (f) PBu₃, DIAD, toluene/THF, 76%; (g) 70% HF in pyridine, THF, 67%. DIAD = diisopropyl azodicarboxylate.
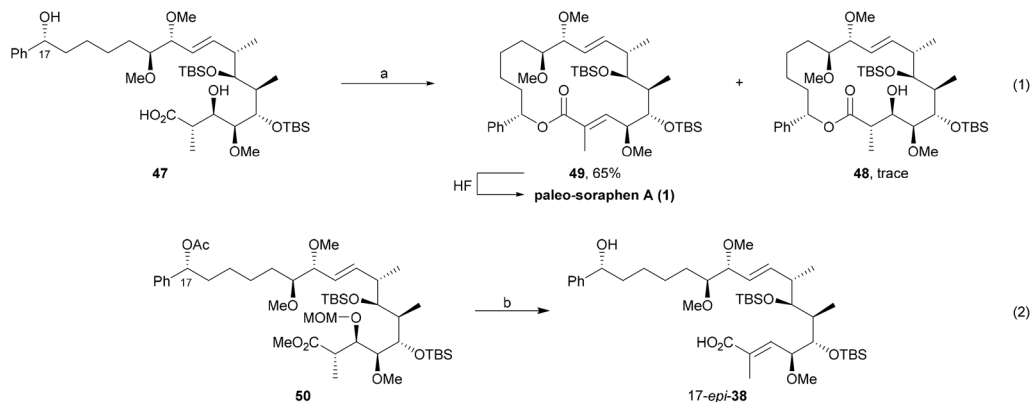


**Scheme 5** Towards paleo-soraphen F by the intramolecular–Mitsunobu approach. Reagents and conditions: (a) PBu₃, DIAD, toluene/THF; (b) LiOH, THF/MeOH/H₂O, 49%.

on the fatty acids provided by the serum for a certain time. To monitor the effect of the fatty acid synthesis inhibitors the medium was therefore changed to DMEM with 2% FBS to reduce the fatty acids supply by the culture medium before compound addition. As reference compounds we included soraphen A, TOFA – also a known inhibitor of fatty acid synthesis that blocks the synthesis of malonyl-CoA by ACC[13] – and cerulenin, a natural inhibitor of fatty acid synthase (FAS).[14] The impedance measurements were carried out as described before[3,15] with slight alterations. The L-929 cells were incubated with paleo-soraphens A, C, D, E and 17-*epi*-paleo-soraphen F (25 µg mL⁻¹), soraphen A (25 µg mL⁻¹), TOFA (10 µg

mL⁻¹) and cerulenin (2.7 µg mL⁻¹) 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 triplicates for each compound over 5 days, and cell indices were recorded by the RTCA Software (Version 1.2). Further data processing was carried out using the statistical programming language R.[16] After importing the raw data, the TCRPs were normalized by dividing each measured cell index after compound addition by the last cell index measured before compound addition (reference time point). For subsequent analysis, only the measurements starting at the reference time point (with normalized cell index = 1) and later were con-

**8034** | *Org. Biomol. Chem.*, 2015, **13**, 8029–8036

This journal is © The Royal Society of Chemistry 2015

**Scheme 6** Towards paleo-soraphen F by RCM-approach. Reagents and conditions: (a) NaBH$_4$, THF/H$_2$O, 83%; (b) SO$_3$·pyridine, Et$_3$N, DMSO, CH$_2$Cl$_2$, 85%; (c) NiCl$_2$, CrCl$_2$, DMSO; (d) DMP, NaHCO$_3$, CH$_2$Cl$_2$; (e) Zn(BH$_4$)$_2$, Et$_2$O, 67%, 3 steps; (f) Ag$_2$O, MeI, 4 Å MS, Et$_2$O, 97%; (g) LiOH, THF/MeOH/H$_2$O, 81%; (h) (R)-1-phenylbut-3-en-1-ol, PBu$_3$, DIAD, toluene/THF, 80%.

sidered. From the triplicate measurements median TCRPs were calculated. The median TCRPs were then normalized using median cell indices from the appropriate DMSO control (cells incubated with the same amount of DMSO used for compound treatment). To achieve dimension reduction we used cubic smoothing splines to fit the median TCRPs and used the basis spline coefficients as descriptors to construct a distance matrix using the Euclidean distance function. We carried out hierarchical cluster analysis and constructed a heatmap displaying the Z-transformed values of the 22 descriptors of each TCRP (Fig. 2). The hierarchical cluster analysis shows that soraphen A and paleo-soraphen A are found within one cluster with TOFA, in agreement with previous results.[3] The phenotype induced by paleo-soraphen A in the xCELLigence assay is thus most closely related to the one induced by soraphen A. Paleo-soraphens C and D are found within one cluster that is more distant to soraphen A. Their closely related phenotype in this assay could be explained by the absence of a methyl-group (hydroxy instead of methoxy) at C11. This feature is unique in paleo-soraphens C and D. Since these methylations are done as post-ketidal transformations the larger distance in

the dendrogram is consistent with an optimization of the biological activity starting from the unmodified polyketide to soraphen A. Paleo-soraphen E on the other hand, that exhibits only the opposite configuration at C11 is much more closely related to paleo-soraphen A. Although paleo-soraphen E is found within one compact cluster together with the FAS inhibitor cerulenin, its profile shows also similarities with paleo-soraphen A, especially the first three descriptors corresponding to the early events after compound addition are quite similar. 17-epi-Paleo-soraphen F, which is biosynthetically between soraphen A and paleo-soraphen A takes a more distant position in the hierarchical cluster analysis.

## Conclusions

In conclusion, we were able to provide additional paleo-soraphens. The design of these additional variations of the soraphen family was based on hypothetical biosynthetic precursors that could provide evidence for the evolutionary optimization of these compounds in retrospect. The paleo-soraphens C and D are lacking the methylation at C11 which was shown to be a post-ketidal transformation. The hierarchical cluster analysis shows that these soraphens are more distant from soraphen A than the paleo-soraphens A and B which from an evolutionary point of view is consistent with an optimization of its biological activity. The hierarchical positioning of 17-epi-paleo-soraphen F on the other hand, is not paralleled by its synthetical positioning between soraphen A and paleo-soraphen A. However, it has to be mentioned that the results from 17-epi-paleo-soraphen F are not clear-cut since the configuration at C17 was changed as well. Changing the configuration at C11 also seems to have influenced the activity.

Taking all these results together, we have now gathered more evidence that at least in the case of the soraphens an optimization and maybe even introduction of biological



**Fig. 2** Hierarchical cluster analysis of impedance kinetics. The heatmap displays Z-transformed descriptors derived from basis spline coefficients.

activity has emerged from a non-related activity through altering the polyketide assembly line and post-ketidal modification. Tracing back the origin of biological activities in natural products might help us to understand the origin of natural products and their biological activity as well as provide hints for new targets in the realm of medicinal chemistry.

## Acknowledgements

## Notes and references

1 (a) D. Janssen, D. Albert, R. Jansen, R. Müller and M. Kalesse, *Angew. Chem.*, 2007, **119**, 4985–4988, (*Angew. Chem., Int. Ed.*, 2007, **46**, 4898–4901); (b) D. Menche, F. Arikan, O. Perlova, N. Horstmann, W. Ahlbrecht, S. C. Wenzel, R. Jansen, H. Irschik and 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 and R. Müller, *Angew. Chem.*, 2012, **124**, 5330–5334, (*Angew. Chem., Int. Ed.*, 2012, **51**, 5239–5243); (d) S. Essig, S. Bretzke, R. Müller and D. Menche, *J. Am. Chem. Soc.*, 2012, **134**, 19362–19365; (e) O. Hartmann and M. Kalesse, *Angew. Chem.*, 2014, **126**, 7463–7466, (*Angew. Chem., Int. Ed.*, 2014, **53**, 7335–7338).

2 (a) T. Schupp, C. Toupet, B. Cluzel, S. Neff, S. Hill, J. J. Beck and J. M. Ligon, *J. Bacteriol.*, 1995, **177**, 3673–3679; (b) J. Ligon, S. Hill, J. Beck, R. Zirkle, I. Monar, J. Zawodny, S. Money and T. Schupp, *Gene*, 2002, **285**, 257–267;

3 H.-H. Lu, A. Raja, R. Franke, D. Landsberg, F. Sasse and M. Kalesse, *Angew. Chem.*, 2013, **125**, 13791–13795, (*Angew. Chem., Int. Ed.*, 2013, **52**, 13549–13552).

4 Y. Liu, C.-S. Da, S.-L. Yu, X.-G. Yin, J.-R. Wang, X.-Y. Fan, W.-P. Li and R. Wang, *J. Org. Chem.*, 2010, **75**, 6869–6878.

5 (a) J. A. Marshall and E. Keith, *Tetrahedron Lett.*, 2004, **45**, 1351–1353; (b) J. A. Marshall and M. P. Bourbeau, *Org. Lett.*, 2003, **5**, 3197–3199.

6 R. A. Urbanek, S. F. Sabes and C. J. Forsyth, *J. Am. Chem. Soc.*, 1998, **120**, 2523–2533.

7 J. Li and D. Menche, *Synthesis*, 2009, 1904–1908.

8 A. Abiko, *Org. Synth.*, 2002, **79**, 109–115.

9 I. Shiina, M. Kubota, H. Oshiumi and M. Hashizume, *J. Org. Chem.*, 2004, **69**, 1822–1830.

10 J. A. Marshall and R. Sedrani, *J. Org. Chem.*, 1991, **56**, 5496–5498.

11 M. T. Crimmins, K. A. Emmitte and J. D. Katz, *Org. Lett.*, 2000, **2**, 2165–2167.

12 Y. A. Abassi, B. Xi, W. Zhang, P. Ye, S. L. Kirstein, M. R. Gaylord, S. C. Feinstein, X. Wang and X. Xu, *Chem. Biol.*, 2009, **31**, 712–723.

13 D. L. Halvorson and S. A. McCune, *Lipids*, 1984, **19**, 851–856.

14 S. Omura, *Bacteriol. Rev.*, 1976, **40**, 681–697.

15 R. Frei, D. Staedler, A. Raja, R. Franke, F. Sasse, S. Gerber-Lemaire and J. Waser, *Angew. Chem.*, 2013, **125**, 13615–13618, (*Angew. Chem., Int. Ed.*, 2013, **52**, 13373–13376).

16 R Core Team, *R: A language and environment for statistical computing*, R Foundation for Statistical Computing, Vienna, Austria, 2014, URL http://www.R-project.org/.

**8036** | *Org. Biomol. Chem.*, 2015, **13**, 8029–8036

This journal is © The Royal Society of Chemistry 2015