CHEMISTRY
A EUROPEAN JOURNAL

ChemPubSoc
Europe

DOI: 10.1002/chem.201202553

Total Synthesis of (−)-Zampanolide and Structure–Activity Relationship Studies on (−)-Dactylolide Derivatives

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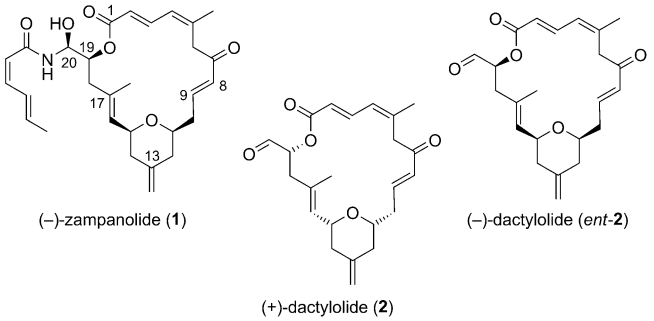
Abstract: A new total synthesis of the marine macrolide (−)-zampanolide (**1**) and the structurally *and* stereochemically related non-natural levorotatory enantiomer of (+)-dactylolide (**2**), that is, *ent*-**2**, has been developed. The synthesis features a high-yielding, selective intramolecular Horner–Wadsworth–Emmons (HWE) reaction to close the 20-membered macrolactone ring of **1** and *ent*-**2**. The β-keto phosphonate/aldehyde precursor for the ring-closure reaction was obtained by esterification of a ω-diethylphosphono carboxylic acid fragment and a secondary alcohol fragment incorporating the THP ring that is embedded in the macrocyclic core structure of **1** and *ent*-**2**. THP ring formation was accomplished through a segment coupling Prins-type cyclization. Employing the same overall strategy, 13-desmethylene-*ent*-**2** as well as the monocyclic desTHP derivatives of **1** and *ent*-**2** were prepared. Synthetic **1** inhibited human cancer cell growth in vitro with nM IC_{50} values, while *ent*-**2**, which lacks the diene-containing hemiaminal-linked side chain of **1**, is 25- to 260-fold less active. 13-Desmethylene-*ent*-**2** as well as the reduced versions of *ent*-**2** and 13-desmethylene-*ent*-**2** all showed similar cellular activity as *ent*-**2** itself. The same activity level was attained by the monocyclic desTHP derivative of **1**. Oxidation of the aldehyde functionality of *ent*-**2** gave a carboxylic acid that was converted into the corresponding N-hexyl amide. The latter showed only μM antiproliferative activity, thus being several hundred-fold less potent than **1**.

Keywords: cancer · dactylolide · natural product · total synthesis · tubulin · zampanolide

Introduction

Marine organisms are the source of a vast array of natural products with diverse structural architectures, many of which exhibit potent biological activity.[1] As such, marine natural products have gained increasing importance as lead structures for drug discovery research or as tool compounds for chemical biology studies. So far, three such compounds have been developed into FDA-approved drugs, either directly or through appropriate structural modifications,[2] and many other drug candidates derived from marine natural products are currently at different stages of clinical development. Among other modes of action, several marine natural products have been shown recently to be microtubule-stabilizing agents (MSA)[3] and thus to inhibit cancer cell proliferation through the same mechanism as the taxane-based anticancer drugs taxol, docetaxel, and cabazitaxel or the epothilone derivative ixabepilone. MSA of marine origin include discodermolide, laulimalide and isolaulimalide, pelorusides A and B, dictyostatin, and (−)-zampanolide (**1**),[4] as the latest addition to this group; with the exception of discodermolide all of these compounds are macrolides of different ring sizes.



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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201202553.

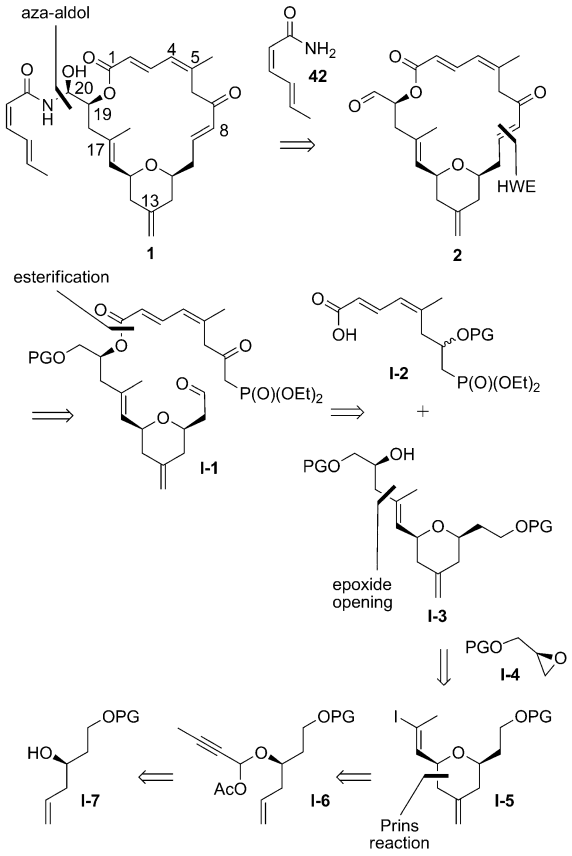
While the microtubule-stabilizing properties of zampanolide were discovered only very recently,[4] this compound had been reported to be a potent antiproliferative agent already several years prior. Thus, (−)-zampanolide (**1**) was originally isolated by Higa and Tanaka from the marine sponge *Fasciospongia rimosa* near Okinawa in 1996[5] and found to inhibit human cancer cell growth in vitro with IC_{50} values in the low nanomolar range (2–10 nM). More recently, **1** was also isolated from the Togan sponge *Cacospongia mycofijiensis* by Northcote, Miller, and co-workers, who confirmed the in vitro antiproliferative activity of the compound and, most importantly, revealed its microtubule-stabilizing and tubulin-polymerizing activity.[4] In the meantime it has been demonstrated that the binding of **1** to tubulin occurs at the taxol binding site on β-tubulin and leads to the formation of a covalent complex (by addition of His229 (and, to a lesser extent, also Asn228) to the enone system in the eastern part of the macrocycle).[6] Retrospectively, it appears somewhat surprising that zampanolide was not recognized as a MSA earlier, as material for biochemical testing would have been available from the total synthesis work by Smith[7] or Hoye[8] (see below) before re-isolation of the compound by Northcote and co-workers in 2009; in fact, our own synthetic work on zampanolide was partly driven by the hypothesis that it might be an MSA, given its structural resemblance to other MSA of marine origin.

Structurally, zampanolide is characterized by a highly unsaturated 20-membered macrolactone core, which includes a *syn*-2,6-disubstituted tetrahydropyran (THP) ring with an exocyclic methylene group and an unusual hemiaminal-linked side chain, a structural motif that is found only in a limited number of other secondary metabolites.[9] Interestingly, in 2001, a macrolactone structurally related to zampanolide, that is, (+)-dactylolide (**2**), was isolated from the sponge *Dactylospongia sp.* at Vanuatu Island by Cutignano and co-workers.[10] In contrast to **1**, **2** was reported to be only a moderately potent inhibitor of human cancer cell growth with IC_{50} values in the low μM range. While Cutignano and co-workers did not establish the absolute configuration of **2**, it was initially assumed that it would be identical with the configuration of the macrolactone core of zampanolide. However, this premonition has been disproven by Smith and co-workers as part of their elegant synthetic work on zampanolide/dactylolide,[7] which revealed the absolute configuration of the macrolactone core in **1** to be opposite to that of natural **2**; in fact, the levorotatory *ent*-**2**, the configuration of which corresponds with that of the macrolactone core in (−)-zampanolide (**1**), has not been isolated from natural sources so far.[11] The discovery of the opposite configuration of (+)-dactylolide and the macrolactone core of (−)-zampanolide immediately raised the question, if the difference in biological activity between **1** and **2** was related to the difference in the absolute stereochemistry of the macrolide ring or to the presence/absence of the hemiaminal-linked side chain (or perhaps both), a question that had provided additional impetus on the work reported in this paper. While this work was in progress, Ding and Jennings reported synthetic *ent*-**2** to be slightly more active than natural **2**, although a direct comparison of GI_{50} values is available only for the SK-OV-3 cell line (GI_{50} of 1.8 μg mL^{-1} for *ent*-**2** vs 3.2 μg mL^{-1} for **2**).[12b] These findings clearly indicate that the profound activity difference between **1** and **2** derives from the presence of the hemiaminal side chain in the former rather than the different configurations of their macrolactone rings.

A number of stereoselective syntheses of **1**[8,11,13]/*ent*-**1**[7a,b] and of **2**[7c,14,15]/*ent*-**2**[12,16,17] have been reported in the literature, together with approaches towards **1** that have not (yet) been carried through to the natural product.[18] This work has included different strategies for the closure of the macrolide ring, such as intramolecular Horner–Wadsworth–Emmons (HWE) reaction at C2/C3,[7,14,15] ring-closing metathesis (RCM) at C8/C9[8,12,16] or C16/C17,[17] or ester formation by metal-mediated epoxy-acid coupling[8] or Kita–Trost macrolactonization.[11] In contrast, little work has been reported so far on analogue structures and their biological activity.[19] Intrigued by the divergent stereochemistry of natural **1** and **2** and in light of the distinct lack of SAR data for these structures, we had embarked on the total synthesis of natural **1** and non-natural *ent*-**2**, and of analogue structures for SAR studies, even before the recent discovery of the tubulin-polymerizing activity of **1**.[4] We have recently communicated some initial results from this work in relation to the synthesis and biological evaluation of *ent*-**2** and its C13-desmethylene derivative.[20] In this paper we provide full details on our total synthesis of (−)-zampanolide (**1**) and (−)-dactylolide (*ent*-**2**); in addition, we describe the synthesis of 13-desmethylene-*ent*-**2** and a number of analogues of **1**/*ent*-**2** and the assessment of their antiproliferative activity.

Results and Discussion

Retrosynthetic analysis: When reflecting upon conceivable strategies for the closure of the 20-membered macrocycle in zampanolide/dactylolide, we recognized that one attractive, novel option for this key step would be the formation of the C8=C9 double bond through an intramolecular HWE reaction. Quite surprisingly, this particular ring-closure had not been part of any of the previous syntheses of dactylolide/zampanolide, despite the fact that HWE-based macrocyclizations involving the formation of the C=C double bond in α,β-unsaturated ketone units are well precedented in natural product synthesis (even if they are not used extensively).[21] Our retrosynthesis for **1**/*ent*-**2** was thus developed around a ring-opening disconnection between C8 and C9 (Scheme 1); **1** was to be obtained from *ent*-**2** and amide **42** employing an aza-aldol reaction as had been reported by Hoye and coworkers.[8] The requisite β-keto phosphonate/aldehyde precursor for the HWE-based macrocyclization (**I-1**) would be obtained by esterification of acid **I-2** with an appropriately protected alcohol **I-3**, followed by protecting group manipulations and oxidation. **I-3** was envisioned to be accessible from protected (*R*)-glycidol **I-4** through regioselective epox-

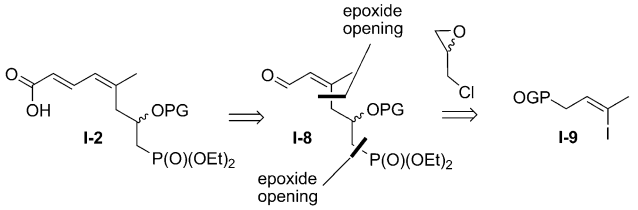


Scheme 1. Retrosynthesis of (−)-zampanolide (**1**) and (−)-dactylolide (*ent*-**2**). Only key disconnections are highlighted explicitly. PG = protecting group; protecting groups may vary independently.

ide opening with lithiated vinyl iodide **I-5**. The latter could be obtained by Prins-type reaction of alkyne **I-6** to deliver a 4-iodo tetrahydropyran derivative; the iodo substituent would then be elaborated into the desired methylene group through displacement with an oxygen nucleophile, oxidation to the ketone and methylenation. Finally, **I-6** would be derived from homoallylic alcohol **I-7**, which, in turn, was planned to be accessed from D-aspartic acid. The segment coupling approach (to produce **I-6**) that we envisioned for the construction of the THP-subring had not been employed in any of the previous syntheses of **1**/*ent*-**1** or **2**/*ent*-**2**.

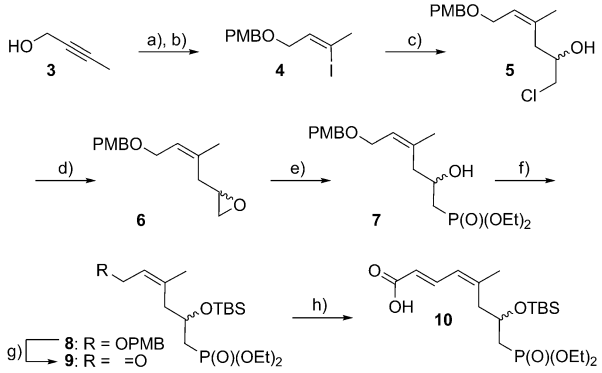
As illustrated in Scheme 2, acid **I-2** was envisaged to be elaborated from aldehyde **I-8** through HWE elongation. Aldehyde **I-8** was to be obtained from vinyl iodide **I-9** via two epoxide opening reactions; this was to include the reaction of **I-9** with epichlorohydrin, conversion of the resulting chlorohydrin to a new oxirane, and opening of the latter with lithiated diethylphosphite to produce the desired β-hydroxy phosphonate.

Synthesis of acid 10: The synthesis of building block **10** (corresponding to retron **I-2** with PG = TBS; Schemes 1 and 2) departed from 2-butynol (**3**), which was submitted to Corey's reductive alumination/iodination[22] procedure, followed by PMB-protection of the free hydroxyl group by re-



Scheme 2. Retrosynthesis of building block **I-2**. PG = protecting group.

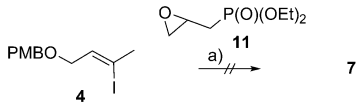
action with PMB-trichloroacetimidate (Scheme 3).[23] Conversion of the resulting *Z* vinyl iodide **4** into the corresponding vinyl lithium species with *n*BuLi followed by treatment



Scheme 3. a) $NaAlH_2(OCH_2CH_2OMe)_2$, Et_2O, 0 °C, then EtOAc, then I_2 in THF, −78 °C, 88 %; b) $PMBO(C{=}NH)CCl_3$, PPTS (cat.), CH_2Cl_2/cyclohexane 2:1, RT, 90 %; c) *n*BuLi, epichlorohydrin, $BF_3{\cdot}OEt_2$, toluene, −85 °C, 50–70 %; d) KOH, EtOH, 0 °C, 89 %; e) $HP(O)(OEt)_2$, *n*BuLi, $BF_3{\cdot}OEt_2$, THF, −78 °C, 80 %; f) TBSCl, ImH, DMAP, DMF, RT, 84 %; g) 1) DDQ, CH_2Cl_2/H_2O 20:1, 0 °C; 2) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, −78 °C→RT, 88 % (2 steps); h) 1) $(EtO)_2P(O)CH_2COOEt$, *n*BuLi, THF, 0 °C, 2) NaOH, EtOH, 0 °C, 94 %.

with racemic epichlorohydrin and $BF_3{\cdot}OEt_2$ then afforded chlorohydrin **5** in 50–70 % yield. Surprisingly, however, **5** was obtained only in toluene as the solvent;[24] no conversion was observed in THF, although epoxide opening reactions with lithiated vinyl species have been reported to proceed smoothly in THF or Et_2O solution.[25] In addition, although TLC analysis generally indicated clean conversion of **4** into **5** (in toluene), the reaction never went to completion and yields for this transformation could not be improved beyond 70 %, neither by varying the temperature (−95 °C up to RT) nor by the use of apolar solvent mixtures (such as toluene/cyclohexane, toluene/Et_2O, toluene/hexane, cyclohexane/hexane, CH_2Cl_2, Et_2O and THF). Excess epichlorohydrin led to higher conversion of **4**, while the use of significantly larger than stoichiometric amounts of $BF_3{\cdot}OEt_2$ did not produce any improvement in yield. Thus, the reaction was best carried out with an excess of epichlorohydrin (3 equiv) in the presence of 1.3 equiv of $BF_3{\cdot}OEt_2$, which gave **5** in at least 50 % yield even on a 55 mmol scale. Treatment of **5** with KOH/EtOH gave the epoxide **6**, which could be opened regioselectively with lithiated diethylphosphite in THF,[26] to afford β-hydroxy phosphonate **7** in 71 % overall

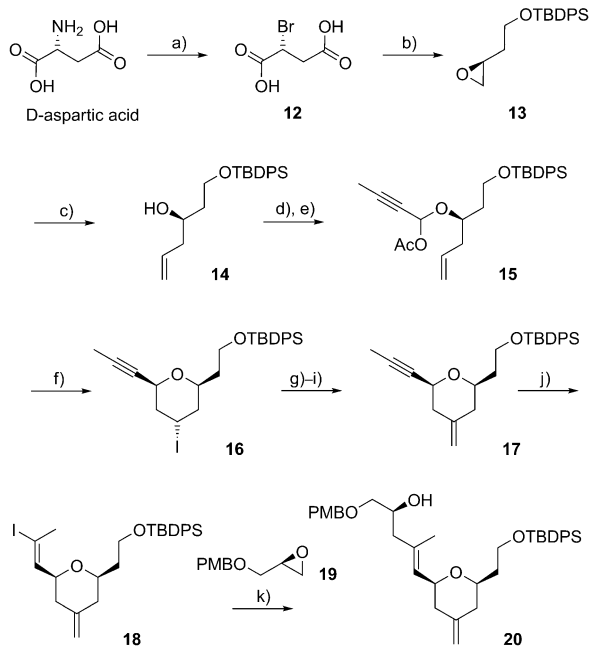
yield (from **5**). Attempts to prepare **7** directly from **4** through reaction with epoxide **11** did not yield any of the desired product, either with THF or toluene as the solvent (Scheme 4).



Scheme 4. a) *n*BuLi, THF or toluene, $BF_3{\cdot}OEt_2$, −78 °C.

TBS protection of **7** with TBSCl/imidazole in DMF required catalysis by DMAP (1 equiv), in order to achieve complete conversion. Subsequent PMB removal under oxidative conditions (DDQ) afforded a mixture of the unsaturated aldehyde **9** and the corresponding primary alcohol. This mixture was submitted to Swern oxidation, which provided the desired **9** in 74% overall yield from **7**. HWE reaction of **9** with triethyl phosphonoacetate then gave the entire C1−C8 carbon fragment of zampanolide/dactylolide as a carboxylic ester, which could be hydrolyzed to the desired acid **10** with NaOH/EtOH in excellent yield (94% for the two-step sequence from **9**). The whole sequence leading from **3** to **10** was amenable to scale-up and provided **10** in multigram quantities.

Synthesis of alcohol 20: After having established a scalable route to acid **10** we next addressed the synthesis of alcohol **20** (corresponding to retron **I-3** with PG = PMB, TBDPS; Scheme 1), which would be joined with **10** to produce the fully elaborated linear precursor for the projected HWE-based macrocyclization (after conversion of the terminal TBDPS-ether into the requisite aldehyde functionality). As discussed above, a key strategic element in the synthesis of **20** was to be the construction of the 2,6-*syn*-substituted THP subring through a Prins-type cyclization reaction with an acyl acetal of a chiral homoallylic alcohol as an oxonium ion precursor. The implementation of this strategy is summarized in Scheme 5; in the initial series of steps this involved the elaboration of D-(−)-aspartic acid into epoxide **13** through conversion of the former into α-bromo acid **12**, followed by borane reduction and consecutive in situ treatment of the ensuing diol with NaH and TBDPSCl, thus directly producing the TBDPS-protected epoxy alcohol **13**.[27] A regioselective Cu-mediated epoxide opening with CH_2=CHMgBr[28] then afforded the desired homoallylic alcohol **14** in excellent yield (98%) and in multigram quantities. In light of its reliance on readily available and cheap starting materials, the synthesis of **14** from D-aspartic acid is an attractive alternative to approaches based on asymmetric allylation chemistry. Alcohol **14** was esterified with 2-butynoic acid[29] and the ester was submitted to reductive acylation, to provide the acid-sensitive acetylated acetal **15**. The Prins-type cyclization of **15** was effectively promoted by TMSI as the Lewis acid (2.5 equiv),[30] to afford **16** in 85% yield, with the substituents at positions 2 and 6 of the THP ring in a

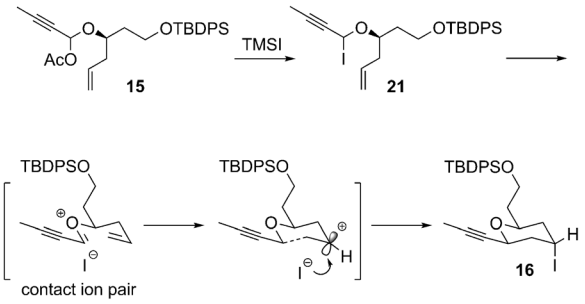


Scheme 5. a) KBr, H_2SO_4, $NaNO_2$, H_2O, 0 °C, 90%; b) 1) $BH_3{\cdot}THF$ or $BH_3{\cdot}DMS$, THF, 0 °C→RT, 96%, 2) NaH, THF, then TBDPSCl, THF, −10 °C, 90%; c) CH_2=CHMgBr, CuI (cat.), THF, −55→−30 °C, 98%; d) 2-butynoic acid, DCC, DMAP, CH_2Cl_2, 0 °C→RT, 85%; e) DIBAL-H, then Ac_2O, pyridine, DMAP, CH_2Cl_2, −78 °C, 92%; f) TMSI, 2,6-dimethylpyridine (0.2 equiv), CH_2Cl_2, −19 °C, 85%; g) CsOAc, [18]c-6, toluene, 60 °C, 4 d, 72%; h) 1) K_2CO_3, MeOH/H_2O 20:1, RT, 2) DMP, CH_2Cl_2, RT, 85%; i) CH_3Ph_3PBr, *n*BuLi, THF, 0 °C→50 °C, 94%; j) 1) Bu_3SnH, *n*BuLi, CuCN, THF, MeOH, −78 °C, 2) NIS, THF, −17 °C, 97%; k) *t*BuLi, **19**, $BF_3{\cdot}OEt_2$, toluene, −85→−78 °C, 61%.

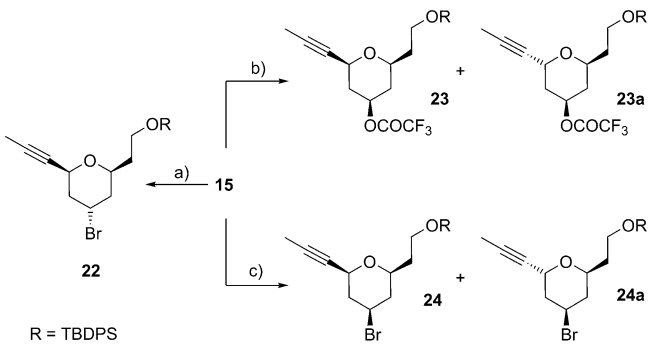
syn orientation and the iodo substituent at position 4 occupying an axial position (i.e., being *anti* to the 2- and 6-substituents). Thus, the configuration of the two stereocenters formed in the course of the cyclization reaction was fully controlled by the configuration of the chiral center originating from homoallylic alcohol **14**.

The particular stereochemical outcome of the cyclization reaction with **15** may be rationalized by a model originally proposed by Rychnovsky and co-workers for TMSBr-induced Prins-type cyclizations of α-acetoxy ethers that assumes a least motion pathway for the attacking nucleophile.[30] According to this model, **15** would be initially converted into iodo ether **21** which would then undergo solvolysis to a contact ion pair (Scheme 6); subsequent cyclization to a chair intermediate (still as a contact ion pair) followed by proximal attack of iodide ion at C4 would then result in the axial product **16**.

Cyclization of **15** could also be affected with TMSBr, which gave axial bromide **22** in 69% yield (Scheme 7). Compared to TMSI, a significantly larger excess of TMSBr had to be employed (ca. 24-fold) and longer reaction times were required to achieve full consumption of starting material. In contrast to TMSI or TMSBr, the use of either TFA or $SnBr_4$ to induce the cyclization of **15** gave only mixtures of 2,6-*syn* and -*anti* isomers (Scheme 7).[31]

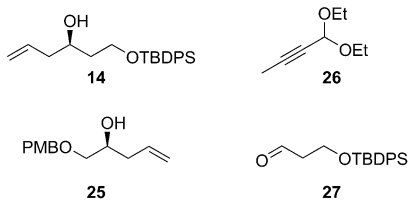


Scheme 6. Model to explain the axial selectivity in the segment-coupling Prins-type cyclization of acyl acetal **15**.[30]



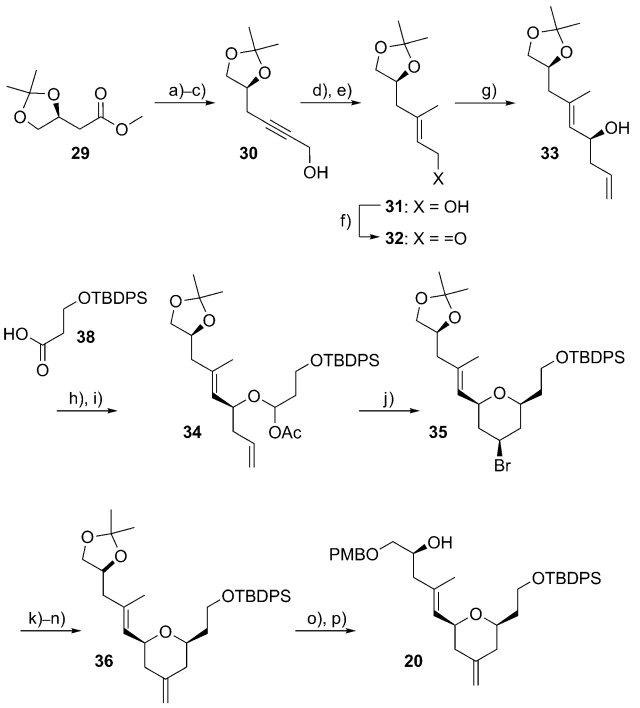
Scheme 7. a) TMSBr (24 equiv), 2,6-dimethylpyridine (0.2 equiv), CH_2Cl_2, 0 °C→RT, 4 h, 69 %; b) TFA, CH_2Cl_2, 40 %, about 1:2 mixture of **23**/**23a**; c) $SnBr_4$, CH_2Cl_2, −78 °C, 69 %, 1:1.7 mixture of **24**/**24a**.

It may be speculated that the dependence of the stereochemical outcome of the cyclization reaction on the exact reaction conditions reflects the small *A*-value of an alkyne group (ca. 0.4 kcal mol^{-1}),[32] which leads only to a low preference for a pseudo-equatorial orientation in the transition state depicted in Scheme 6. However, care should be exercised when applying the *A*-value concept outside of a cyclohexane structural framework (for an excellent review on the concept of *A*-values see ref. [33]). All attempts to affect intermolecular Prins reactions with homoallylic alcohols **14** or **25** and diethoxy acetal **26** or aldehyde **27**, respectively, met with failure and did not provide any of the desired cyclization products.



Elaboration of the iodo substituent in **16** into the required C13 *exo*-methylene group began with the conversion of **16** into the corresponding acetate by reaction with CsOAc in the presence of [18]crown-6.[34] This reaction was best conducted at 55–60 °C; these conditions offered the best compromise between a practical reaction rate and the suppression of elimination side products, thus furnishing the desired acetate in yields of about 70 % after reaction times of 3–4 days. At higher temperature (initial experiments were carried out at 90 °C), the displacement reaction was accompanied by substantial elimination to form both possible cyclohexene isomers in about a 1:1 ratio (see Scheme 10 for a later discussion). Among alternative sources of oxygen nucleophiles investigated, $AgOCOCF_3$[35] or $AgClO_4$[36] gave only elimination products, while no conversion was observed with $PhI(OCOCF_3)_2$.[37] Aqueous $CuSO_4$ in DMSO[38] afforded a multitude of products that were not further characterized. The acetate was then transformed into the desired olefin **17** in 80 % overall yield by base-mediated hydrolysis of the ester group, oxidation of the ensuing free hydroxyl group with DMP and finally Wittig methylenation (Scheme 5). The subsequent conversion of **17** into vinyl iodide **18** was accomplished by stannylcupration/iodination with $Bu_3Sn(Bu)CuCNLi_2$[39] and iodine (or NIS) in THF/CH_2Cl_2; these conditions provided **18** in good yields as a single isomer. In contrast, attempted hydrozirconation with Schwartz reagent[40] followed by treatment with iodine afforded only unchanged starting material. Similar to the reaction of epichlorohydrin with lithiated vinyl iodide **4**, the elaboration of **18** into alcohol **20** via lithiation and subsequent reaction with PMB-protected (*R*)-glycidol (**19**) proved to be a significant challenge, in spite of literature precedence for a related transformation.[41] Thus, initial experiments with the vinyl lithium species derived from iodide **18** either in THF, Et_2O or mixtures thereof did not deliver any of the desired product. In marked contrast, the extended alcohol **20** was obtained from vinyl iodide **18** in 61 % yield when the epoxide opening was performed in toluene as the reaction solvent instead of THF or Et_2O (with $BF_3{\cdot}OEt_2$ as the Lewis acid); while the efficiency of this transformation is relatively moderate, the approach proved to be amenable to reasonable scale-up and has allowed the preparation of multigram quantities of the desired secondary alcohol **20**.

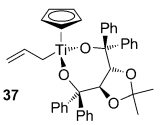
Alternative synthesis of alcohol 20: In light of the difficulties encountered in the reaction of epoxide **19** with metalated derivatives of vinyl iodide **18** and before discovering the pronounced solvent dependence of the reaction (or the one between lithiated vinyl iodide **4** and epichlorohydrin) we had started to investigate an alternative approach to alcohol **20** that would not rely on epoxide opening by any metal-vinyl intermediates. While these efforts finally did not come to bear on the total synthesis of **1**, as we were able eventually to overcome the initial problems in the synthesis of **20** from **18** and **19**, they still led to a viable approach to **20**, whose most important features are outlined in this paragraph (Scheme 8). Departing from L-malic acid, ester **29** was prepared in three steps and 51 % overall yield according to literature procedures.[42,43] Reduction of **29** with DIBAL-H followed by conversion of the ensuing aldehyde into the corresponding dibromoolefin according to the Corey–Fuchs protocol,[44] treatment of the latter with *n*BuLi and finally



Scheme 8. a) DIBAL-H, CH_2Cl_2, −70 °C, 82 %; b) CBr_4, PPh_3, 2,6-dimethylpyridine, THF, 0 °C, 86 %; c) *n*BuLi, THF, −78 °C then $(CHO)_n$, 64 %; d) $NaAlH_2(OCH_2CH_2OMe)_2$, THF, 0 °C→RT, then EtOAc, I_2, THF, −78 °C, 75 %; e) 1) TMSCl, NEt_3, 2) Me_2Zn, [Pd(dppf)Cl_2], THF, 80 °C, 3) K_2CO_3, MeOH, RT, 81 % (3 steps); f) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, −78 °C, 84 %; g) **37**, Et_2O, −78 °C, 15 min, then NH_4F, −78 °C→RT, 80–97 %; h) **38**, EDCI, DMAP, CH_2Cl_2, 0 °C, 94 %; i) DIBAL-H, then Ac_2O, pyridine, DMAP, CH_2Cl_2, −78 °C, 91 %; j) 1) $SnBr_4$, CH_2Cl_2, −78 °C, 2) $Me_2C(OMe)_2$, pTsOH·H_2O, RT, 62 % (2 steps); k) CsOAc, [18]c-6, toluene, 130 °C, 20 h, 88 %; l) K_2CO_3, MeOH/H_2O 10:1, RT; m) DMP, CH_2Cl_2, RT, 94 % (2 steps); n) $MePh_3PBr$, *n*BuLi, THF, 0→45 °C, 92 %; o) $CuCl_2$·$2H_2O$, MeOH, 60 °C, 84 %; p) Bu_2SnO, toluene, Dean–Stark, 140 °C, 1.5 d, then PMBCl, TBAI, 120 °C, 1.5 h, 57 %.

quenching of the resulting acetylide anion with paraformaldehyde gave propargylic alcohol **30** in 45 % overall yield (from **29**).

Reductive iodination of **30** and subsequent Negishi cross-coupling with Me_2Zn and [Pd(dppf)Cl_2] gave the trisubstituted olefin **31** in high yield as a single isomer. In order to avoid reduction of the vinyl iodide moiety in the cross-coupling step, temporary protection of the allylic alcohol moiety as a TMS ether was required.[45] In contrast to acetonide **30**, reductive iodination of the corresponding bis-TBS ether gave the desired vinyl iodide only in 34 % yield (vs. 75 % for **30**); similar results were obtained when the primary and secondary hydroxyl groups were protected as PMB and TBS ethers, respectively. Cross-coupling was also possible with Me_2CuLi[46] (64–77 % yield), but this was accompanied by partial reduction of the vinyl iodide moiety. Swern oxidation of allylic alcohol **31** then provided unsaturated aldehyde **32**, which was transformed into homoallylic alcohol **33** by asymmetric allyltitanation with the tartrate-derived Duthaler–Hafner reagent **37**,[47] thus setting the stereocenter at C15 (zampanolide numbering). Based on ^1H and ^{13}C NMR analysis **33** was obtained as a single isomer in yields between 80 and 97 % after reaction times of only 15 min at −78 °C. The use of the Duthaler–Hafner reagent



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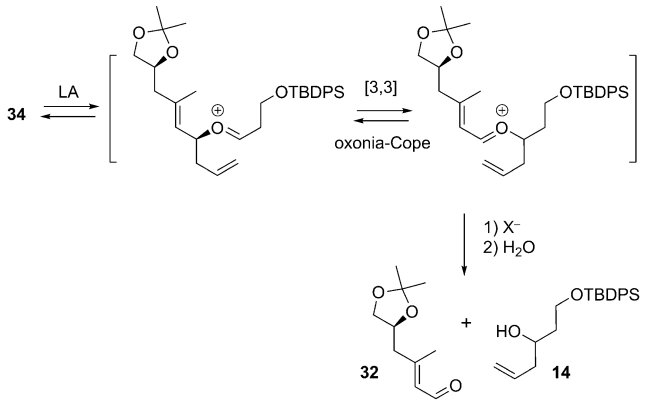
was clearly superior to other asymmetric allylation methods investigated, with Brown allylation of **32** ((−)-DIPCl/allylMgBr)[48] affording **33** with selectivities of up to 10:1, but in highly variable yields (0–70 %), while attempted Keck allylation ((*S*)-BINOL/Ti(O*i*Pr)$_4$)[49] never led to complete conversion of starting material. The Duthaler–Hafner allylation protocol was amenable to scale-up (gram scale) and NH_4F-work-up allowed recovery of the (*R*)-taddol ligand.

Secondary alcohol **33** was then esterified with acid **38** (obtained in three steps from propane-1,3-diol by mono-TBDPS-protection followed by two-step oxidation to O-TBDPS-3-hydroxy propanal (**27**) and finally **38**), which was followed by reductive acylation with Ac_2O and DIBAL-H to give acyl acetal **34** in good yield (85 % from **33**). Rather surprisingly, the Prins-type cyclization of **34** worked only with $SnBr_4$ as the Lewis acid; unfortunately, under these conditions THP ring formation was accompanied by cleavage of the acetonide moiety, which had to be re-installed after the cyclization step, thus providing the desired cyclization product in 62 % total yield. It is noteworthy that the THP ring in **35** exhibits an all-*syn* configuration of the three substituents at positions 2, 4, and 6 (based on NOE measurements), that is the bromo substituent occupies an equatorial position. The stereochemical outcome of the $SnBr_4$-mediated cyclization of **34**, thus, is distinctly different from the one observed for the TMSI-induced cyclization of **15** (Schemes 5 and 6); this is in accordance with the results of previous studies on the stereochemical course of segment coupling Prins-type cyclizations by Rychnovsky and coworkers.[30]

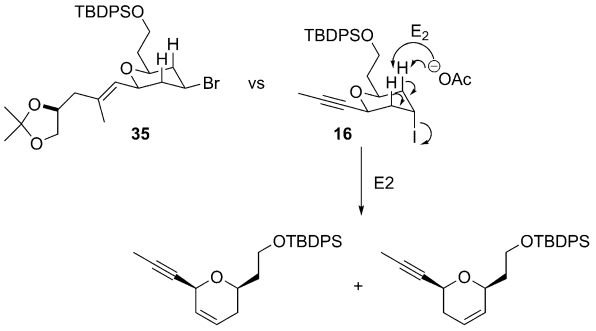
No THP ring formation was observed with TMSI, instead the major products formed in the reaction were homoallylic alcohol **14** (stereochemistry unknown) and aldehyde **32** (Scheme 9), based on NMR and TLC analysis of crude reaction products after extractive work-up and comparison with authentic reference samples. The formation of **14** and **32** may be rationalized by an oxonia-Cope rearrangement[50] of the oxonium ion initially formed from **34** as illustrated in Scheme 9. Likewise, attempts to induce cyclization with TMSBr, TMSOTf, BF_3·OEt_2/AcOH, TFA, or TFA/NaO$COCF_3$ did not produce any of the desired cyclization product.

The elaboration of bromide **35** into olefin **36** was based on the same sequence of transformations that had been followed for the conversion of **16** into **17** (see Scheme 5); thus, displacement of the bromo substituent in **35** with CsOAc followed by acetate hydrolysis and oxidation of the resulting secondary alcohol gave a ketone that was converted into **36** by Wittig olefination (Scheme 8). Olefin **36** was obtained in 76 % overall yield for the four-step sequence from **35**. It is worth pointing out that acetate formation from bromide **35**



Scheme 9. Formation of **32** and **14** from **34** via oxonia-Cope rearrangement. LA = Lewis acid.

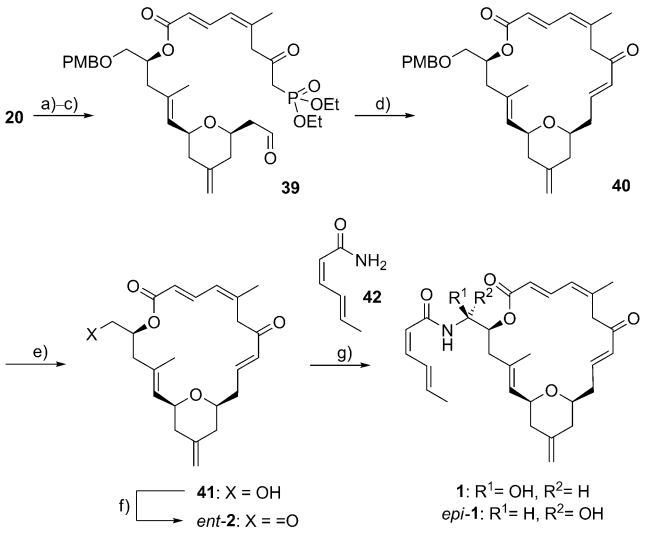
was less problematic (no elimination side products) and thus higher yielding than from iodide **16** (Scheme 5). As illustrated in Scheme 10, this is a direct consequence of the equatorial orientation of the bromo substituent in **35**, which effectively precludes elimination of HBr by an E2 mechanism, due to the gauche arrangement of the bromine atom and the axial hydrogens on carbon atoms 3 and 5 of the THP ring. In contrast, the axial orientation of the iodo substituent in **16** leads to an antiperiplanar arrangement with the axial hydrogens on C3 and C5, which allows for facile elimination of HI (which is what is observed experimentally; see above).



Scheme 10. Unfavorable configuration of bromide **35** for dehydrohalogenation and dehydrohalogenation of iodide **16**.

Cleavage of the acetonide moiety in **36** with $CuCl_2 \cdot 2H_2O$[51] followed by regioselective PMB protection of the primary hydroxyl group in the resulting diol via a cyclic Sn-acetal[52] then concluded the alternative synthesis of secondary alcohol **20**. In comparison to the D-aspartic acid-based route depicted in Scheme 5, the malic acid-based approach to **20** involved more steps (22 steps for the longest linear sequence vs 14 from D-aspartic acid) and provided the target alcohol in lower overall yield (2.1 vs 17%). Material for the progression of the total synthesis was thus generally produced via the D-aspartic acid route. Samples of **20** obtained from both approaches displayed fully identical spectral and chiro-optical properties.

Assembly of building blocks and completion of the total synthesis: The esterification of alcohol **20** with acid **10**, as the first step in the elaboration of the macrocyclic core structure of (−)-dactylolide (*ent*-**2**) and (−)-zampanolide (**1**), was best accomplished under Yamaguchi conditions,[53] which provided the desired ester in 85% yield (Scheme 11). In contrast, the use of DCC or EDCI as condensing agents gave only trace amounts of product. Esterification was followed by simultaneous cleavage of both silyl ethers with HF·py and oxidation of the resulting diol to the β-keto phosphonate/aldehyde **39** in 63% overall yield.



Scheme 11. a) 2,4,6-Trichlorobenzoyl chloride, NEt_3, DMAP, **10**, toluene, RT, 85%; b) HF·py, THF, 0°C→RT, 85%; c) DMP, CH_2Cl_2, RT, 74%; d) $Ba(OH)_2 \cdot 0.8H_2O$, THF/H_2O 40:1, 0°C→RT, 81%; e) DDQ, CH_2Cl_2/H_2O 5:1, RT, 82%; f) DMP, CH_2Cl_2, RT, 78%; g) **42**, DIBAL-H then *ent*-**2**, THF, RT, for (−)-**1**: 18%; (−)-*epi*-**1**: 12% (see text).

With **39** in hand, the stage was set for the exploration of the intramolecular HWE reaction as a means to affect macrocyclization to the dactylolide and zampanolide core structure (Scheme 1). Initial experiments to this end involved the use of NaHMDS as a base in THF; ring-closure to the desired macrocycle **40** (as single isomer) was indeed observed under these conditions, but extended reaction times were required (up to 4 d for complete consumption of starting material) and product yields were highly variable. Gratifyingly, these problems could be eliminated by the use of $Ba(OH)_2$,[54] which led to significantly reduced reaction times of 0.5–1 h and afforded macrocycle **40** in very good yields (ca. 80%) in a reproducible fashion. In the largest single preparation performed so far, 430 mg of **39** were successfully converted into **40** in 78% yield. Oxidative PMB removal with DDQ and oxidation of the resulting free alcohol **41** with DMP[55] then provided (−)-dactylolide (*ent*-**2**) in 64% overall yield.

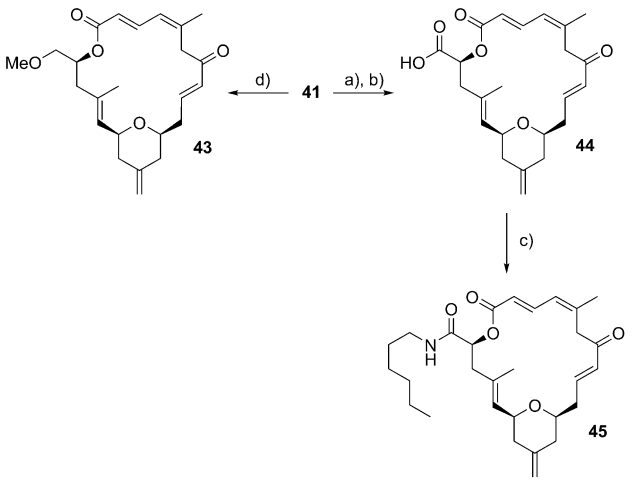
The elaboration of *ent*-**2** into (−)-zampanolide (**1**) was accomplished by aza-aldol reaction with (*Z*,*E*)-sorbamide (**42**) (obtained in two steps from crotonaldehyde; see Supple-

mentary Information) as had been reported by Hoye and co-workers[8] (and also based on the more general work on aza-aldol reactions by Maier and co-workers).[56] Thus, treatment of **42** with DIBAL-H in CH_2Cl_2 followed by reaction with *ent*-**2** furnished **1** as a 1.1:1 mixture with its C20 epimer (*epi*-**1**) in 46% yield after flash chromatography on deactivated silica gel.[57] The isomers could only be separated by normal phase HPLC; both **1** and *epi*-**1** were subsequently submitted to final purification by RP-HPLC, which gave both compounds in analytically pure form in 18 and 12% yield, respectively (based on *ent*-**2**). While these final yields are clearly unsatisfactory, they have to be ascribed largely to the lack of selectivity in the aza-aldol step and the resulting need for isomer separation by HPLC. After completion of our own work Ghosh and co-workers have shown that the reaction of *ent*-**2** and **42** in the presence of a matched chiral phosphoric acid catalyst ((*S*)-TRIP) proceeds with about 3:1 selectivity, thus providing **1** in 51% yield after isomer separation by HPLC.[13] However, no fully asymmetric synthesis of **1** has been reported to date. Overall, our synthesis proved to be highly reliable and notwithstanding the modest overall yield in the aza-aldol step, it has allowed us to produce sufficient amounts of material for extensive biological and biochemical profiling of **1**;[6] in addition, this material has been used to prepare a tubulin-bound complex for X-ray crystallographic studies.[58]

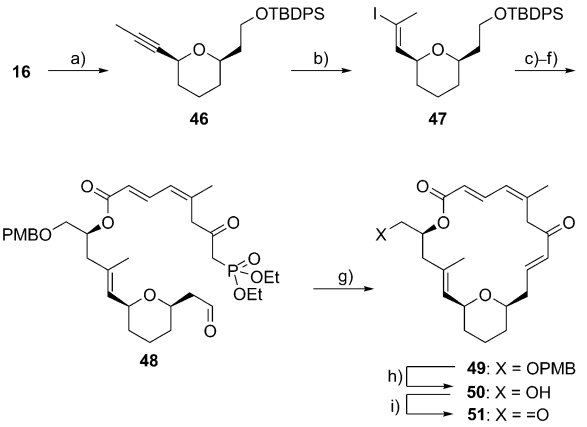
Synthesis of analogues: As indicated in the introductory section, the SAR of dactylolide/zampanolide to this date has remained largely unexplored. Building on the chemistry that we had developed for the synthesis of natural **1** and non-natural *ent*-**2** we have thus started to explore the importance of individual structural features of **1**/*ent*-**2** for microtubule stabilization and antiproliferative activity.

In a first step these SAR inquiries involved the investigation of side chain-modified zampanolide analogue **45**, of alcohol **41**, which was an intermediate in the synthesis of **1** and *ent*-**2**, and of methyl ether **43** (Scheme 12); the latter was obtained from **41** by reaction with Meerwein salt in 83% yield (Scheme 12). Amide-based zampanolide analogue **45** was obtained from *ent*-**2** by Pinnick–Kraus oxidation[59] (to give acid **44** in almost quantitative yield) followed by HATU-mediated coupling with *n*-hexylamine. For reasons unknown, the efficiency of the coupling reaction was low and provided **45** only in 13% yield (from **44**); however, no attempts were made to optimize this transformation.

As part of our efforts to identify highly active, but structurally simplified analogues of zampanolide/dactylolide with improved synthetic accessibility, we have also assessed the significance of the *exo*-methylene group on the THP ring for biological activity. The corresponding analogue of *ent*-**2**, that is 13-desmethylene-(−)-dactylolide (**51**), was accessed from iodide **16**, which could be converted into the 4-unsubstituted THP derivative **46** by radical reduction with Bu_3SnH/AIBN in excellent yield (88%; Scheme 13) and without affecting the alkyne moiety.[39a] Attempts to transform **16** into **46** by iodide/lithium exchange with *t*BuLi followed by protonolysis



Scheme 12. a) DMP, CH_2Cl_2, RT, 78%; b) $NaClO_2$, $NaH_2PO_4{\cdot}H_2O$, *t*BuOH/H_2O, 2-methyl-2-butene, RT, 97%; c) *n*-hexylamine, HATU, DIPEA, DMF, RT, 13%; d) Me_3OBF_4, Proton Sponge, CH_2Cl_2, RT, 83%.



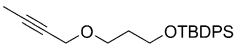
Scheme 13. a) Bu_3SnH, AIBN (cat.), toluene, 60°C, 88%; b) 1) Bu_3SnH, *n*BuLi, CuCN, THF, MeOH, −78→−10°C, 2) NIS, THF, −78°C, 73%; c) *t*BuLi, **19**, $BF_3{\cdot}OEt_2$, toluene, −78°C, 31%; d) 2,4,6-trichlorobenzoyl chloride, NEt_3, DMAP, **10**, toluene, RT, 74%; e) HF·py, THF, 0°C→RT, 80%; f) DMP, CH_2Cl_2, RT, 72%; g) NaHMDS, THF, −78°C→RT, 2 d, 49%; h) DDQ, CH_2Cl_2/H_2O 5:1, RT, 72%; i) DMP, CH_2Cl_2, RT, 77%.

resulted in significantly lower yields that did not exceed 35%.

As for alkyne **17**, reductive iodination of **46** was achieved by stannylcupration/iodination with $Bu_3Sn(Bu)CuCNLi_2$[39a] and NIS to provide the trisubstituted vinyl iodide **47** in 73% yield. Other methods investigated for the conversion of **46** into **47**, such as hydrozirconation/iodination,[40] silylcupration/iodination[60] or Pd-mediated hydrostannylation[39] either gave lower yields or were (additionally) plagued by the formation of the regioisomeric iodination product. Subsequent lithiation of **47** and reaction with epoxide **19** in toluene followed by esterification of the resulting secondary alcohol with acid **10**, simultaneous cleavage of TBS and TBDPS ethers, and oxidation of the free hydroxyl groups with DMP gave aldehyde-phosphonate **48** in 13% overall yield (based on **47**); macrocyclization with NaHMDS as the

base then gave the desired protected macrolactone **49** in 49% yield. This experiment was carried out before the investigation of $Ba(OH)_2$-mediated macrocyclizations (see above); as it provided sufficient material of the corresponding free alcohol **50** and of 13-desmethylene-*ent*-**2** (**51**) for biological testing, no attempts were made to improve the yield of the cyclization, although we assume that the use of $Ba(OH)_2$ in place of NaHMDS would have provided the cyclization product in superior yield. In analogy to the synthesis of *ent*-**2**, **51** was obtained from **49** by oxidative PMB removal and DMP oxidation in 55% overall yield.

Encouraged by the cellular data obtained for dactylolide analogues **50** and **51** (see below), we have also followed a more radical approach to simplified zampanolide/dactylolide analogues that involved complete removal of the THP subring from the macrolactone core. As illustrated in Scheme 14, the synthesis of the corresponding desTHP derivatives **60** and **59**, respectively, was based on the same overall strategy that had led to the successful synthesis of **1**, *ent*-**2**, and THP ring-containing analogues thereof, with secondary alcohol **55** substituting for intermediate **20** (or its desmethylene variant) in the esterification with acid **10**. The synthesis of **55** departed from propargylic alcohol (**3**), which was converted into vinyl iodide **52** according to literature procedures.[39] The latter was then elaborated into ether **54** by reaction with allyl bromide in the presence of NaH (to produce **53**) followed by hydroboration and TBDPS protection of the resulting terminal hydroxyl group in 21% overall yield. In contrast to allylation with allyl bromide, all attempts at the direct alkylation of **52** with TBDPS-protected 3-bromo-1-propanol proved to be unsuccessful. The latter could be used to alkylate **3** in 45% yield, but we were unable to convert the resulting propargylic ether **61** into the



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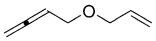

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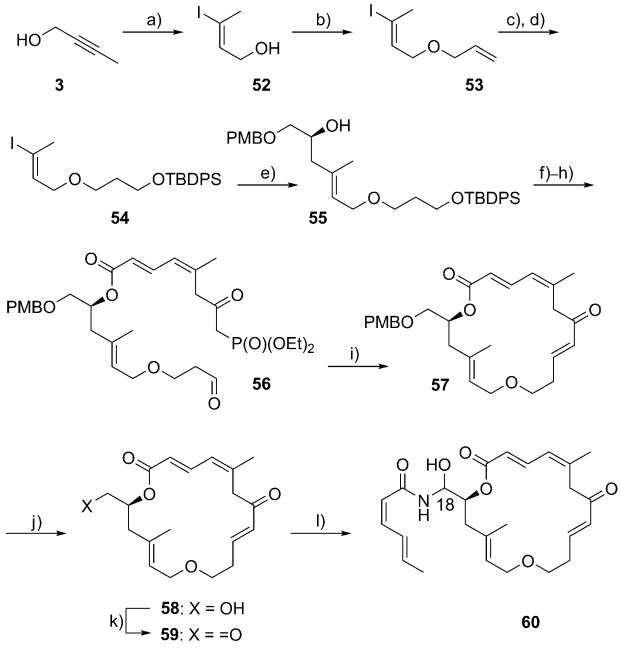
required vinyl iodide **54** under the conditions previously established for the conversion of **17** into **18** and **46** into **47**, respectively. The moderate yield in the allylation step (42%) is the result of the formation of a side product we assume to be allene **62** and that was difficult to separate from **53**;[61] while the formation of this side product could be minimized under optimized conditions (1.7 equiv NaH), it could not be suppressed completely.

Hydroboration of **53** was best performed with BH_3·THF, while neither $(Sia)_2BH$ nor 9-BBN gave any of the desired alcohol. Reaction of lithiated **54** with epoxide **19** in toluene in the presence of BF_3·OEt_2 then afforded secondary alcohol **55** in 61% yield, that is with similar efficiency as for the conversion of **18** into **20** (Scheme 5). Esterification of **55** with acid **10** under Yamaguchi conditions followed by global desilylation and subsequent oxidation of the liberated hydroxyl groups with DMP provided β-keto phosphonate/aldehyde **56**; the latter underwent smooth macrocyclization with



Scheme 14. a) 1) Bu_3SnH, *n*BuLi, CuCN, THF, MeOH, −78→−15°C, 74%, 2) I_2, THF, −17°C, 94%; b) CH_2=$CHCH_2Br$, NaH, THF, 0°C, 42%; c) BH_3·THF, THF, 0°C, NaOH/H_2O_2, 0°C, 52%; d) TBDPSCl, DMAP (cat.), NEt_3, CH_2Cl_2, RT, 94%; e) *t*BuLi, **19**, BF_3·OEt_2, toluene, −78°C, 61%; f) 2,4,6-trichlorobenzoyl chloride, NEt_3, DMAP, **10**, toluene, RT, 81%; g) HF·py, THF, 0°C→RT, 86%; h) DMP, CH_2Cl_2, RT, 73%; i) $Ba(OH)_2$·$0.8H_2O$, THF/H_2O 40:1, 0°C→RT, 85%; j) DDQ, CH_2Cl_2/H_2O 5:1, RT, 77%; k) DMP, CH_2Cl_2, RT, 75%; l) **42**, DIBAL-H, then **59**, THF, RT, 72% for the mixture of epimers (ca. 1.6:1).

$Ba(OH)_2$ to furnish macrolactone **57** as a single isomer in 85% yield. PMB removal from **57** under oxidative conditions (DDQ) followed by DMP oxidation then gave (−)-dactylolide analogue **59** (77%). Finally, aza-aldol reaction between **59** and **42** gave desTHP-zampanolide (**60**) in 28% yield as a ca. 1.6:1 mixture of isomers at C18 after HPLC purification (see Scheme 14 for atom numbering).[62] As the isomers were very difficult to separate, initial biological testing was performed with the isomeric mixture.

Antiproliferative activity: As illustrated by the data summarized in Table 1, synthetic (−)-zampanolide (**1**) was found to inhibit human cancer cell proliferation with nM IC_{50} values, which is in perfect agreement with previous literature data for natural[4,5] as well as synthetic[11] material; likewise, our data confirm the previous observation by Uenishi et al. that *epi*-**1** is about one order of magnitude less active than the natural product **1**.[11] (−)-Dactylolide (*ent*-**2**) was found to be even less active than *epi*-**1**, with IC_{50} values being similar to those reported for natural **2**, as had been observed previously by Ding and Jennings.[12b] These findings identify the hemiaminal-linked side chain of **1** as the major determinant of the activity difference between **1** and **2**, rather than the absolute configuration of the macrocycle.

Analogues **41**, **50**, and **51** showed comparable activity with *ent*-**2**; in all three cases a slight trend towards higher potency relative to *ent*-**2** was observed, but the true signifi-

Table 1. Antiproliferative activity of (−)-zampanolide (**1**), *epi*-**1**, *ent*-**2**, and derivatives of **1** and *ent*-**2** in four human cancer cell lines (IC_{50} values [nM]).[a]

Compound	A549 (lung)	MCF-7 (breast)	HCT116 (colon)	PC-3 (prostate)
1	3.2 ± 0.4	6.5 ± 0.7	7.2 ± 0.8	2.9 ± 0.4
epi-**1**	53 ± 5.9	42 ± 9.3	88 ± 5.1	50 ± 11.7
ent-**2**[b]	301 ± 4.3	247 ± 2.6	210 ± 4.7	751 ± 69
41	127 ± 2.9	106 ± 3.6	155 ± 2.1	320 ± 26
50	189 ± 19.3	114 ± 10.2	74 ± 1.5	104 ± 4.1
51	149 ± 12.8	68 ± 5.6	249 ± 28	n.d.
43	1072 ± 103	1489 ± 83	1603 ± 122	1274 ± 117
44	9732 ± 260	7624 ± 303	12733 ± 379	9338 ± 242
45	973 ± 90	1138 ± 72	1204 ± 63	829 ± 27
58	2378 ± 70	3891 ± 102	1846 ± 92	3051 ± 178
59	3921 ± 216	2894 ± 144	2653 ± 68	4021 ± 102
60[c]	n.d.	165 ± 13	309 ± 47	218 ± 7

[a] Cells were exposed to compounds for 72 h. n.d. = not determined. [b] IC_{50} values of 198–346 nM have been reported for *ent*-**2** in ref. [12b]. [c] Mixture of diastereoisomers at C18.

cance of these differences remains to be determined. Independent of this, the data clearly suggest that neither the aldehyde functionality as such nor the 13-methylene group are required for the antiproliferative activity of *ent*-**2**. However, methylation of the C20-hydroxyl group in **41** leads to a clear loss in antiproliferative potency (up to 14-fold in the MCF-7 cell line; methyl ether **43**). Analogues **41**, **50**, and **51** (like **1**,[4,6] and *ent*-**2**[6,20]) all promote tubulin polymerization.[20] Details on the interactions of these and other compounds described in this paper with the tubulin/microtubule system will be reported elsewhere; for (−)-zampanolide (**1**) and *ent*-**2** a detailed study on their binding to dimeric tubulin and microtubules has been published recently.[6]

Compared to alcohol **41**, the corresponding carboxylic acid **44** exhibits significantly reduced cellular activity. This may be a consequence of poor cell penetration, due to the negatively charged carboxyl group, although this conclusion is purely hypothetical at this point. Interestingly, the activity of amide **45** is about 10-fold enhanced over that of **44**; while this makes the compound several hundred-fold less potent than **1**, the activity of **45** is still encouraging, as it is well conceivable that fine-tuning of the substituent moiety on the amide nitrogen could lead to improved potency. The synthesis of such analogues is currently ongoing in our laboratory.

Perhaps the most intriguing finding that has emerged from the cellular profiling experiments is the clearly sub-μM activity of desTHP-zampanolide (**60**). Although the compound is 25- to 80-fold less active than the parent zampanolide (for the cell lines investigated), the retention of significant antiproliferative activity appears truly surprising, in light of the removal of two (out of four) chiral centers and a rigidifying structural element. As for **1** and *ent*-**2**, desTHP-(−)-dactylolide (**59**) is less active than desTHP-(−)-zampanolide (**60**). It is important to emphasize that **60** is a ca. 1.6:1 mixture of diastereoisomers at C18; in light of the results obtained for **1** and *epi*-**1** it is tempting to speculate that the IC_{50} values for 18*S*-**60** could in fact be lower than those observed for the mixture. Overall, the activity of **60** makes it an interesting lead structure in its own right. We are now investigating whether improvements in the activity of **60** are possible without any undue (re)increase in structural complexity. The results of these efforts will be reported in due course.

Conclusion

We have established a new total synthesis of the marine natural product (−)-zampanolide (**1**) which is based on the construction of its 20-membered macrolide core structure by means of a high-yielding intramolecular HWE reaction and the equally effective formation of the embedded THP ring in a Prins-type cyclization. This strategy gave efficient access to the non-natural, levorotatory enantiomer of (+)-dactylolide (**2**), that is *ent*-**2**, which served as the immediate precursor for **1**. The same HWE-based macrocyclization approach was then followed to prepare 13-desmethylene-(−)-dactylolide (**51**), based on THP-derivative **16** as a common advanced intermediate; likewise, this strategy has also allowed the synthesis of monocyclic desTHP-**1** (**60**) and desTHP-*ent*-**2** (**59**). Simple manipulations of the aldehyde moiety in *ent*-**2** gave access to amide **45**, which may be considered a simplified variant of (−)-zampanolide (**1**).

Natural **1** was confirmed to be a highly potent cancer cell growth inhibitor, while *ent*-**2** was 30- to 260-fold less potent than **1**; these findings highlight the importance of the zampanolide side chain for nanomolar antiproliferative activity. Removal of the C13-methylene group and/or the reduction of the C20-aldehyde moiety in *ent*-**2** did not significantly affect cellular potency. Intriguingly, analogue **60**, which lacks the entire THP ring and is thus based on a monocyclic scaffold, retains significant antiproliferative activity. It will be interesting to investigate whether modification of this compound can lead to even more potent analogues with similarly reduced structural complexity (relative to the natural product lead **1**). Amide **45** was 3- to 6-fold less potent than *ent*-**2** and several hundred-fold less active than (−)-zampanolide (**1**); nevertheless, given the micromolar activity of **45**, amide-based analogues of (−)-zampanolide (**1**) may still be an interesting group of analogues for further exploration.

Experimental Section

General: All solvents used for reactions were purchased as anhydrous grade from Fluka (puriss.; dried over molecular sieves; $H_2O < 0.005\%$) and used without further purification. Solvents for extractions, FC and thin-layer chromatography (TLC) were purchased as commercial grade and distilled prior to use. All non-aqueous reactions were performed under an argon atmosphere using flame-dried glassware and standard syringe/septa techniques. All other commercially available reagents were used without further purification, unless otherwise noted. In general, reactions were magnetically stirred and monitored by TLC on Merck TLC aluminum sheets (silica gel 60 F_{254}). Spots were visualized with UV light ($\lambda = 254$ nm) or through staining with $Ce_2(SO_4)_3$/phosphomolybdic acid/H_2SO_4 (CPS) or $KMnO_4/K_2CO_3$. Purification of products by flash chro-

matography (FC) was performed using Fluka silica gel 60 for preparative column chromatography (particle size 40–63 μm).

Melting points were obtained in open capillary tubes using a Büchi melting point apparatus B-540 and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or [D$_4$]MeOH (unless otherwise noted) on Bruker AV-400 400 MHz and AV-500 500 MHz instruments at room temperature. Chemical shifts (δ) are reported in ppm and are referenced to the solvent signal as an internal standard (chloroform $\delta = 7.26$ ppm for ^{1}H, $\delta = 77.16$ ppm for ^{13}C and [D$_4$]MeOH $\delta = 3.34$ ppm for ^{1}H, $\delta = 49.00$ ppm for ^{13}C). All ^{13}C NMR spectra were measured with complete proton decoupling. Data for NMR spectra are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, m = multiplet, br = broad signal, J = coupling constant in Hz. Infrared spectra (IR) were recorded on a Jasco FT/IR-6200 instrument as thin film. Resonance frequencies are given as wavenumbers in cm^{-1}. Optical rotations were measured on a Jasco P-1020 polarimeter operating at the sodium D line with a 10 mm or 100 mm path length cell and are reported as follows: $[\alpha]_D^T$, concentration (g per 100 mL), and solvent. Mass spectra were recorded by the ETH Zürich MS service; HRMS (ESI) spectra were obtained on a Bruker Daltonics maxis (UHR-TOF) and HRMS (EI) on a Waters Micromass AutoSpec Ultima instrument.

1-((*R*)-1-(*tert*-Butyldiphenylsilyloxy)hex-5-en-3-yloxy)but-2-ynyl acetate (15): To a solution of homoallylic alcohol **14** (1.84 g, 5.2 mmol, 1.00 equiv) in dry CH_2Cl_2 (15 mL) were added sequentially DMAP (65 mg, 0.52 mmol, 0.10 equiv), 2-butynoic acid (0.49 g, 5.70 mmol, 1.10 equiv), and a solution of DCC (1.30 g, 6.30 mmol, 1.20 equiv) in CH_2Cl_2 (15 mL) at 0 °C. The suspension formed was allowed to warm to RT and stirring was continued for 16 h. Et_2O (100 mL) was then added, the mixture was filtered, and the filter cake was washed with Et_2O (50 mL). The filtrate was concentrated under reduced pressure to give a brown-red oil that was again treated with Et_2O (100 mL) followed by re-filtration of the mixture and washing of the precipitate with Et_2O (50 mL). The combined filtrates and washings were concentrated under reduced pressure and the residue was purified by FC (EtOAc/Hex 1:30→1:20), to give the desired ester as a colorless oil (1.87 g, 4.44 mmol, 85 %). $R_f = 0.44$ (EtOAc/Hex 1:10, UV, CPS); $[\alpha]_D^{24} = -18.04°$ (c = 0.93, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.67–7.65 (m, 4H), 7.46–7.37 (m, 6H), 5.77 (ddt, J = 17.4, 9.7; 7.0, 1H), 5.29–5.23 (m, 1H), 5.13–5.07 (m, 2H), 3.77–3.68 (m, 2H), 2.46–2.34 (m, 2H), 1.98 (s, 3H), 1.89–1.83 (m, 2H), 1.07 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.3, 135.7, 135.6, 133.8, 133.6, 133.2, 129.7, 127.8, 118.2, 85.3, 72.8, 72.6, 60.0, 38.6, 36.1, 26.9, 19.2, 3.9 ppm; IR (thin film): $\tilde{\nu}$ = 3071, 3050, 2957, 2359, 2342, 2243, 1706, 1472, 1428, 1389, 1249, 1110, 1063, 700 cm^{-1}. HRMS (ESI): m/z: calcd for $C_{23}H_{27}O_3Si$ [$M-CH_3H_5^+$] 379.1730, found 379.1724.

To a solution of the above ester (1.77 g, 4.20 mmol, 1.00 equiv) in CH_2Cl_2 (40 mL) at −78 °C was added slowly DIBAL-H (1 M in toluene, 8.40 mL, 8.40 mmol, 2.00 equiv) such that the temperature did not exceed −70 °C; after 30 min pyridine (1 mL, 12.60 mmol, 3.00 equiv), DMAP (1.54 g, 12.60 mmol, 3.00 equiv), and Ac_2O (2.37 mL, 25.20 mmol, 6.00 equiv) were added sequentially at −78 °C and the mixture was stirred at this temperature for 22 h. Sat aq NH_4Cl (20 mL) and sat aq Rochelle salt (40 mL) were added at −78 °C and the mixture was allowed to warm to RT. Vigorous stirring was continued for 90 min in a beaker, resulting in the formation of two clear phases that were readily separable. The aqueous phase was extracted with CH_2Cl_2 (3×40 mL) and the combined organic phases were washed with sat aq $NaHCO_3$ (2×20 mL) and brine (10 mL), and then dried over $MgSO_4$. Concentration of the solution under reduced pressure and purification of the residue by FC (EtOAc/Hex 1:30→1:20, 2 % NEt_3 v/v) afforded **15** (1.80 g, 3.87 mmol, 92 %) as a 1.6:1 mixture of diastereomers as a colorless, viscous oil. Spectroscopic data are for the diastereomeric mixture. $R_f = 0.40$ (EtOAc/Hex 1:10, UV, CPS); $[\alpha]_D^{24} = -19.24°$ (c = 0.99, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.72–7.66 (m, 4H), 7.45–7.36 (m, 6H), 6.45 (q, J = 1.8; 0.37H), 6.44 (q, J = 1.8; 0.63H), 5.87–5.75 (m, 1H), 5.12–5.02 (m, 2H), 4.17–4.06 (m, 1H), 3.85–3.71 (m, 2H), 2.41–2.30 (m, 2H), 2.05 (s, 1.12H), 1.99 (s, 1.88H), 1.86 (d, J = 1.8; 1.15H), 1.84 (d, J = 1.8; 1.85H), 1.83–1.71 (m, 2H), 1.07 (s, 3.38H), 1.06 ppm (s, 5.62H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9, 169.7, 135.7, 135.7, 135.6, 135.6, 134.5, 134.1, 134.0, 133.9, 133.9, 129.7, 129.7, 129.7, 127.8, 127.8, 118.0, 117.2, 87.2, 86.2, 83.1, 82.9, 76.4, 74.9, 74.4, 74.1, 60.6, 60.3, 39.7, 39.2, 37.4, 37.4, 27.0, 26.9, 21.2, 21.2, 19.3, 19.3, 3.7, 3.7 ppm; IR (thin film): $\tilde{\nu}$ = 3072, 2956, 2857, 2259, 1740, 1472, 1370, 1228, 1082, 903, 822, 701 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{28}H_{36}NaO_4Si$ [$M+Na^+$], 487.2281, found 487.2265.

***tert*-Butyl-(2-((2*S*,4*S*,6*S*)-4-iodo-6-(prop-1-ynyl)tetrahydro-2*H*-pyran-2-yl)ethoxy)diphenylsilane (16)**: To a solution of **15** (1.79 g, 3.85 mmol, 1.00 equiv) in CH_2Cl_2 (55 mL) at −19 °C (NaCl/ice) was added 2,6-dimethylpyridine (0.09 mL, 0.77 mmol, 0.20 equiv) followed by slow addition of TMSI (1.37 mL, 9.62 mmol, 2.50 equiv). The cooling bath was removed after 10 min and the yellow solution was allowed to warm to RT. After a total of 45 min sat aq $NaHCO_3$ (20 mL) was carefully added, the phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3× 10 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the residue by FC (EtOAc/Hex 1:30→1:20) afforded **16** (1.78 g, 3.34 mmol, 85 %) as a pale-yellow, viscous oil. Material obtained in this way was generally contaminated by 2–3 % of aldehyde **27**. Spectroscopic data for **16** were acquired with a pure sample. $R_f = 0.45$ (EtOAc/Hex 1:10, UV, CPS); $[\alpha]_D^{24} = -3.30°$ (c = 1.00, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.71–7.67 (m, 4H), 7.44–7.36 (m, 6H), 4.83 (quin, J = 3.1; 1H), 4.57 (br dquin, J = 10.8; 2.1, 1H), 4.21–4.15 (m, 1H), 3.86 (ddd, J = 10.5, 8.2, 4.9; 1H), 3.73 (dt, J = 10.3, 5.4; 1H), 2.16 (dq, J = 14.8, 2.3; 1H), 1.99 (ddd, J = 14.7, 2.4, 2.1; 1H), 1.93–1.82 (m, 2H), 1.87 (d, J = 2.1; 3H), 1.69 (ddt, J = 13.7, 8.3, 5.3; 1H), 1.57–1.50 (m, 1H), 1.07 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 135.7, 135.7, 134.1, 133.9, 129.7, 127.8, 127.8, 81.5, 77.9, 71.1, 65.1, 60.1, 41.8, 40.3, 38.3, 29.3, 27.0, 19.4, 3.8 ppm; IR (thin film): $\tilde{\nu}$ = 3070, 2953, 2856, 2360, 2341, 1472, 1427, 1389, 1232, 1107, 1095, 1049, 822, 737, 702 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{22}H_{24}IO_2Si$ [$M-C_4H_9^+$], 475.0590, found 475.0585.

***tert*-Butyl-(2-((2*R*,6*S*)-6-((*E*)-2-iodoprop-1-enyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)ethoxy) diphenylsilane (18)**: To a suspension of CuCN (668 mg, 7.42 mmol, 5.00 equiv) in THF (16 mL) at −78 °C was added a solution of *n*BuLi (1.6 M in hexane, 9.30 mL, 14.82 mmol, 10.00 equiv). After 5 min the flask was immersed in a cooling bath at −40 °C, resulting in the formation of a pale-yellow, almost clear solution. The mixture was cooled back to −78 °C after 10 min, which made it become slightly heterogenous. Neat Bu_3SnH (4.00 mL, 14.82 mmol, 10.00 equiv) was then added dropwise, immediately leading to a turbid yellow solution with liberation of gas. After 20 min at −78 °C the mixture was stirred for 5 min at −40 °C, giving an almost clear golden-yellow solution. After 10 min at −40 °C the solution was cooled back to −78 °C followed by addition of MeOH (6.60 mL, 163.00 mmol, 110.00 equiv) under vigorous stirring. After 10 min at −78 °C the flask was immersed in a cooling bath at −40 °C; the reaction mixture now was a clear red solution. After 10 min at −40 °C this solution was cooled back to −78 °C and a solution of **17** (0.62 g, 1.48 mmol, 1.00 equiv) in THF (10 mL) was added. The mixture was stirred for 15 h, during which period the temperature was allowed to rise to −15 °C. Sat aq NH_4Cl (30 mL) and 25 % aq NH_4OH (6 mL) were then added together with EtOAc (20 mL). Stirring was continued for 30 min, the two almost clear phases were separated, and the aqueous phase was extracted with EtOAc (3×10 mL). The combined organic extracts were dried over $MgSO_4$ and the solution was concentrated under reduced pressure. Purification of the residue by FC (Hex→EtOAc/Hex 1:100→1:50, 1 % (v/v) NEt_3) gave the vinylstannane (1.02 g, 1.43 mmol, 97 %) as a pale-yellow oil that was used immediately in the next step.

A solution of the above vinylstannane in THF (11 mL) was cooled to −17 °C (NaCl/ice) followed by addition of *N*-iodosuccinimide (0.49 g, 2.10 mmol, 1.50 equiv) in THF (2 mL), to give an almost clear yellow solution. After 20 min a mixture of sat aq $Na_2S_2O_3$ (5 mL) and sat aq $NaHCO_3$ (5 mL) was added followed by EtOAc (5 mL). Stirring was continued for 2 min when two clear, colorless phases had formed. The phases were separated and the aqueous phase was extracted with EtOAc (3×5 mL). The combined organic extracts were dried over $MgSO_4$ and then concentrated under reduced pressure. The residue was purified by FC (Hex/EtOAc 1:100) to afford the desired product **18** (0.79 g, 1.44 mmol, quant.) as a pale yellow oil. $R_f = 0.64$ (EtOAc/Hex 1:20, UV, CPS); $[\alpha]_D^{24} = +4.90°$ (c = 1.81, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ =

7.70–7.67 (m, 4H), 7.47–7.38 (m, 6H), 6.24 (dq, $J=7.7$, 1.5; 1H), 4.81–4.77 (m, 2H), 3.99 (ddd, $J=10.8$, 7.7, 2.6; 1H), 3.87 (ddd, $J=10.1$, 8.1, 5.4; 1H), 3.76 (dt, $J=10.1$, 5.6; 1H), 3.64–3.57 (m, 1H), 2.44 (d, $J=1.5$; 3H), 2.27–2.20 (m, 2H), 2.13–2.06 (m, 1H), 2.00–1.94 (m, 1H), 1.87–1.73 (m, 2H), 1.08 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=141.6$, 135.6, 135.6, 134.0, 133.9, 129.7, 127.7, 109.3, 98.5, 76.3, 75.3, 60.2, 40.6, 40.4, 39.0, 28.8, 26.9, 19.3 ppm; IR (thin film): $\tilde{\nu}=3070$, 2931, 2890, 2856, 1651, 1472, 1427, 1360, 1105, 1087, 998, 858, 700 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{27}H_{36}IO_2Si$ $[M+H^+]$, 547.1524, found 547.1503. The ^{1}H NMR spectrum indicated the presence of ca. 3% of the undesired regioisomer.

(*S*,*E*)-5-((2*S*,6*R*)-6-(2-(*tert*-Butyldiphenylsilyloxy)ethyl)-4-methylenetetrahydro-2*H*-pyran-2-yl)-1-(4-methoxybenzyloxy)-4-methylpent-4-en-2-ol (20): Vinyl iodide **18** (385 mg, 0.70 mmol, 1.00 equiv, azeotropically dried once with 2 mL of acetonitrile or toluene immediately before use) was dissolved in dry toluene (7 mL) and the solution was cooled to −78 °C. *t*BuLi (1.6 M in pentane, 0.88 mL, 1.41 mmol, 2.00 equiv) was then added and the near colorless solution was stirred for 30 min; it was then cooled to around −85–−90 °C with liquid nitrogen and a solution of **19** (342 mg, 1.76 mmol, 2.50 equiv, azeotropically dried once with 2 mL of acetonitrile or toluene immediately before use) in dry toluene (2 mL) was added followed by $BF_3{\cdot}OEt_2$ (0.22 mL, 1.76 mmol, 2.50 equiv; addition of $BF_3{\cdot}OEt_2$ about 1 min after the addition of **19**) giving a pale yellow solution. Stirring was continued at −78 °C for 1 h; then the cooling bath was removed and sat aq $NaHCO_3$ (10 mL) and 10 mL of EtOAc were added. After the mixture had reached RT, the phases were separated and the aqueous phase was extracted with EtOAc (3×5 mL). The combined organic extracts were dried over $MgSO_4$, concentrated under reduced pressure, and the residue purified by FC (EtOAc/Hex 1:5→1:4) to give **20** (264.2 mg, 0.43 mmol, 61%) as a colorless oil. Note: Chromatographic separation was difficult and two FC runs were needed in order to remove the iodohydrin derived from competing epoxide opening by iodide. $R_f=0.17$ (EtOAc/Hex 1:5, UV, CPS); $[\alpha]_D^{24}=+5.97°$ (c = 0.88, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.68$–7.65 (m, 4H), 7.44–7.34 (m, 6H), 7.28–7.24 (m, 2H), 6.90–6.87 (m, 2H), 5.29 (dq, $J=7.7$, 1.2; 1H), 4.75–4.73 (m, 2H), 4.49 (s, 2H), 3.99 (ddd, $J=10.9$, 7.7, 2.7; 1H), 3.98–3.91 (m, 1H), 3.84 (ddd, $J=10.1$, 8.0, 5.5; 1H), 3.80 (s, 3H), 3.74 (dt, $J=10.1$, 5.7; 1H), 3.60–3.54 (m, 1H), 3.46 (dd, $J=9.5$, 3.5; 1H), 3.33 (dd, $J=9.5$, 7.1; 1H), 2.31 (d, $J=3.5$; 1H), 2.25–2.22 (m, 1H), 2.20 (d, $J=6.8$; 2H), 2.16–2.12 (m, 1H), 2.04–2.00 (m, 1H), 1.97–1.90 (m, 1H), 1.89–1.80 (m, 1H), 1.77–1.71 (m, 1H), 1.69 (d, $J=1.2$; 3H), 1.05 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=159.4$, 144.7, 135.7, 135.7, 135.4, 134.1, 134.0, 130.2, 129.7, 129.5, 129.0, 127.7, 127.7, 114.0, 108.7, 75.5, 75.3, 73.7, 73.2, 68.6, 60.4, 55.4, 43.7, 41.0, 40.7, 39.2, 27.0, 19.4, 17.3 ppm; IR (thin film): $\tilde{\nu}=3070$, 2932, 2857, 1612, 1513, 1471, 1427, 1247, 1106, 1087, 1058, 1036, 998, 821, 702 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{38}H_{50}NaO_5Si$ $[M+Na^+]$, 637.3320, found 637.3322.

(1*S*,2*E*,5*S*,8*E*,10*Z*,14*E*,17*S*)-5-((4-Methoxybenzyloxy)methyl)-3,11-dimethyl-19-methylene-6,21-dioxabicyclo[15.3.1]henicosa-2,8,10,14-tetraene-7,13-dione (40): To a stirred solution of **39** (62.2 mg, 0.094 mmol, 1.00 equiv, co-evaporated with 3 mL of toluene immediately before use) in THF (31 mL) was added H_2O (0.8 mL) followed by freshly activated $Ba(OH)_2{\cdot}0.8H_2O$ at 0 °C. (Commercial $Ba(OH)_2$ was activated by heating to 100–140 °C for 1–2 h before use)).[54d] The cooling bath was removed after 30 min and stirring was continued at RT for additional 30 min; 30 mL of Et_2O were then added and the solution was washed first with sat aq $NaHCO_3$ (2×10 mL) and then with brine (1×10 mL). The clear organic phase was dried over $MgSO_4$ and concentrated. The resulting yellow oil was purified by FC (EtOAc/Hex 1:3) to afford **40** (38.6 mg, 0.076 mmol, 81%) as a colorless oil. $R_f=0.40$ (EtOAc/Hex 1:3, UV, $KMnO_4$, CPS); $[\alpha]_D^{24}=-158.79°$ (c = 0.25, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.62$ (dd, $J=15.1$, 11.6; 1H), 7.27–7.24 (m, 2H), 6.90–6.86 (m, 2H), 6.83 (ddd, $J=16.2$, 9.8, 4.4; 1H), 6.10 (d, $J=11.6$; 1H), 5.94 (d, $J=15.1$; 1H), 5.92 (d, $J=16.4$; 1H), 5.40–5.34 (m, 1H), 5.17 (dd, $J=8.1$, 0.9; 1H), 4.74–4.70 (m, 2H), 4.52 (d, $J=11.8$; 1H), 4.48 (d, $J=11.8$; 1H), 4.17 (d, $J=13.6$; 1H), 3.96 (ddd, $J=11.3$, 8.1, 2.5; 1H), 3.81 (s, 3H), 3.58 (dd, $J=10.4$, 6.0; 1H), 3.51 (dd, $J=10.4$, 4.9; 1H), 3.30–3.24 (m, 1H), 3.00 (d, $J=13.5$; 1H), 2.37 (dddd, $J=15.0$, 10.1, 4.4, 2.0; 1H), 2.26–2.20 (m, 1H), 2.20 (d, $J=6.7$; 2H), 2.16–2.11 (m, 1H), 2.11–2.05 (m, 1H), 1.97–1.89 (m, 2H), 1.79 (s, 3H), 1.70 ppm (d, $J=1.1$; 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=198.3$, 166.9, 159.4, 146.5, 143.9, 142.9, 139.4, 132.6, 131.6, 130.2, 129.5, 129.5, 125.6, 121.3, 114.0, 109.1, 76.7, 76.1, 73.1, 71.6, 69.6, 55.4, 45.2, 42.8, 41.1, 40.9, 40.4, 23.6, 16.8 ppm; IR (thin film): $\tilde{\nu}=3016$, 2923, 2852, 1713, 1668, 1635, 1614, 1513, 1463, 1360, 1281, 1249, 1215, 1176, 1152, 1086, 1035, 978 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{31}H_{38}NaO_6$ $[M+Na^+]$, 529.2561, found 529.2571.

(1*S*,2*E*,5*S*,8*E*,10*Z*,14*E*,17*S*)-5-(Hydroxymethyl)-3,11-dimethyl-19-methylene-6,21-dioxabicyclo[15.3.1]henicosa-2,8,10,14-tetraene-7,13-dione (41): To a solution of PMB ether **40** (4 mg, 0.008 mmol, 1.00 equiv) in CH_2Cl_2 (0.5 mL) was added H_2O (0.1 mL) followed by DDQ (5.4 mg, 0.024 mmol, 3.50 equiv) at RT. The mixture was vigorously stirred for 3 h. Then sat aq $NaHCO_3$ (5 mL) and CH_2Cl_2 (5 mL) were added and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (3×5 mL) and the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by FC (EtOAc/Hex 1:3→1:2) gave **41** (2.49 mg, 0.0064 mmol, 82%) as a colorless solid. $R_f=0.30$ (EtOAc/Hex 1:1, UV, CPS); $[\alpha]_D^{24}=-136.26°$ (c 0.11, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.64$ (dd, $J=15.1$, 11.6; 1H), 6.84 (ddd, $J=16.2$, 9.6, 4.6; 1H), 6.11 (d, $J=11.7$; 1H), 5.94 (d, $J=15.1$; 1H), 5.93 (d, $J=16.5$; 1H), 5.28 (dddd, $J=10.8$, 5.9, 4.1, 2.1; 1H), 5.19 (d, $J=8.0$, 1H), 4.73 (d, $J=1.6$; 1H), 4.73 (d, $J=1.6$; 1H), 4.14 (d, $J=13.7$; 1H), 3.97 (ddd, $J=11.2$, 8.2, 2.7, 1H), 3.77–3.70 (m, 2H), 3.29 (ddt, $J=11.8$, 9.5, 2.1, 1H), 3.04 (d, $J=13.7$, 1H), 2.38 (dddd, $J=15.1$, 10.1, 4.6, 2.0, 1H), 2.30–2.08 (m, 5H), 1.98–1.91 (m, 2H), 1.81 (s, 3H), 1.73 ppm (d, $J=1.2$, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=198.1$, 167.1, 146.5, 143.9, 143.3, 139.8, 132.6, 131.6, 129.6, 125.6, 121.0, 109.2, 76.7, 76.1, 71.9, 65.4, 45.2, 42.1, 41.1, 40.8, 40.3, 23.7, 16.8 ppm; IR (thin film): $\tilde{\nu}=3389$, 2925, 2853, 1715, 1669, 1634, 1553, 1449, 1436, 1357, 1280, 1259, 1148, 1086, 1049, 1019, 976, 799 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{23}H_{30}NaO_5$ $[M+Na^+]$: 409.1985; found: 409.1983.

(−)-Dactylolide (*ent*-2): To a solution of alcohol **41** (2.33 mg, 0.006 mmol, 1.00 equiv) in CH_2Cl_2 (0.5 mL) was added DMP (15 mg, 0.036 mmol, 6.00 equiv; added in 3 equal portions in 20 min intervals) and stirring was continued for 60 min. A mixture of sat aq $NaHCO_3$ (5 mL) and sat aq $Na_2S_2O_3$ (5 mL) was then added and stirring was continued for 10 min, when two clear phases had formed. The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried over $MgSO_4$, concentrated under reduced pressure and the residue was purified by FC (EtOAc/Hex 1:3) to provide *ent*-**2** (1.8 mg, 0.0048 mmol, 78%) as a colorless solid. $R_f=0.57$ (EtOAc/Hex 1:1, UV, CPS or $KMnO_4$); $[\alpha]_D^{24}=-258.33°$ (c = 0.11, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=9.67$ (s, 1H), 7.63 (dd, $J=15.1$, 11.6; 1H), 6.85 (ddd, $J=16.2$, 8.6, 6.0; 1H), 6.16 (d, $J=11.7$; 1H), 6.03–5.94 (m, 2H), 5.32 (dd, $J=11.3$, 2.5; 1H), 5.24 (d, $J=8.0$; 1H), 4.75 (d, $J=1.6$; 1H), 4.75 (d, $J=1.6$; 1H), 3.97 (ddd, $J=11.5$, 8.1, 2.7; 1H), 3.94 (d, $J=14.3$; 1H), 3.33 (ddt, $J=11.1$, 8.7, 2.7; 1H), 3.24 (d, $J=14.5$; 1H), 2.55 (d, $J=14.3$; 1H), 2.36–2.28 (m, 3H), 2.19–2.15 (m, 1H), 2.14–2.09 (m, 1H), 1.99–1.93 (m, 2H), 1.87 (s, 3H), 1.72 ppm (d, $J=0.9$; 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=199.2$, 197.6, 166.4, 146.1, 144.2, 143.6, 140.6, 131.6, 131.1, 130.7, 125.7, 119.9, 109.5, 76.6, 75.9, 75.5, 45.0, 40.9, 40.6, 39.9, 39.8, 24.3, 16.2 ppm; IR (thin film): $\tilde{\nu}=2936$, 2858, 1733, 1716, 1706, 1670, 1635, 1438, 1355, 1278, 1256, 1144, 1086, 1050, 978, 890 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{23}H_{28}NaO_5$ $[M+Na^+]$: 407.1829; found: 407.1820.

(−)-Zampanolide (1): To a solution of amide **42** (36.6 mg, 0.33 mmol, 4.6 equiv) in THF (2 mL) was added DIBAL-H (1 M in CH_2Cl_2, 0.27 mL, 0.27 mmol, 3.76 equiv) and the mixture was stirred at RT for 45 min. After that time a solution of *ent*-**2** (27.6 mg, 0.072 mmol, 1 equiv) in THF (1 mL, flask rinsed twice with 0.5 mL of THF) was added and stirring was continued for a total of 3 h. Sat aq Rochelle salt (10 mL) was then added together with EtOAc (10 mL) and the mixture was stirred for 15 min. After addition of brine (10 mL) the phases were separated and the aqueous phase was further extracted with EtOAc (3×5 mL). The combined organic extracts were dried over $MgSO_4$, the solvent was evaporated and the residue was purified by FC (EtOAc/Hex 1:3→1:1, 2% NEt_3 v/v) to give a 1.1:1 mixture of **1** and *epi*-**1** (16.4 mg, 0.033 mmol, 46%) as a pale-yellow foam. The epimers were separated by semipreparative normal phase HPLC (Phenomenex Luna 5 μm NH_2 100 Å, 150×

10 mm, EtOH/Hex 1:9) followed by the purification of the individual isomers by reverse phase HPLC (Waters Symmetry C18, 5 μ, 100×7.8 mm, CH_3CN/H_2O 1:1). After lyophilization, 6.4 mg (0.013 mmol, 18%) of (−)-zampanolide (**1**) and 4.42 mg (0.0089 mmol, 12%) of *epi*-(−)-**1** were obtained. R_f=0.40 (EtOAc/Hex 1:1, UV, CPS); $[\alpha]_D^{24}=-241.33°$ (c = 0.18, $CHCl_3$, deactivated over basic Alox); ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.35 (d, J=8.9; 1H). 7.51 (dd, J=14.9, 11.8; 1H). 7.45 (dd, J=14.9, 11.8; 1H), 6.75 (ddd, J=16.3, 8.6, 5.7; 1H), 6.36 (t, J=11.3; 1H), 6.20 (d, J=11.9; 1H), 6.18 (brs, 1H), 6.00–5.94 (m, 1H), 5.95 (d, J=15.9; 1H), 5.93 (d, J=15.1; 1H), 5.65 (d, J=11.4; 1H), 5.32 (dd, J=8.4, 6.4; 1H), 5.10 (d, J=7.7; 1H), 4.96 (dd, J=10.2, 6.2; 1H), 4.73 (brs, 2H), 4.13 (d, J=14.2; 1H), 3.86 (ddd, J=11.4, 7.7, 1.8; 1H), 3.26 (t, J=10.1, 1H), 3.00 (d, J=14.3; 1H), 2.35–2.26 (m, 3H), 2.17 (d, J=12.7; 1H), 2.11–2.05 (m, 2H), 1.89–1.86 (m, 1H), 1.85–1.82 (m, 1H), 1.79 (d, J=6.7; 3H), 1.74 (s, 3H), 1.61 ppm (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=197.3, 165.6, 165.3, 145.9, 143.8, 143.0, 140.6, 139.5, 137.2, 132.5, 130.9, 129.0, 128.6, 125.1, 120.7, 119.2, 109.0, 76.0, 75.1, 72.9, 72.0, 44.9, 40.9, 40.3, 40.3, 39.3, 23.6, 18.3, 16.7 ppm; IR (thin film): $\tilde{\nu}$=3325, 3015, 2960, 2924, 2853, 1708, 1664, 1634, 1604, 1520, 1431, 1355, 1281, 1259, 1213, 1147, 1085, 1050, 1034, 1025, 802 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{29}H_{38}NO_6$ [M+H^+]: 496.2694; found: 496.2681.

(−)-*epi*-Zampanolide (*epi*-1): R_f=0.40 (EtOAc/Hex 1:1, UV, CPS); $[\alpha]_D^{24}=-172.92°$ (c = 0.65, $CHCl_3$, deactivated over Alox); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.39 (d, J=9.0; 1H), 7.48 (dd, J=15.1, 11.5; 1H), 7.47–7.42 (m, 1H), 6.74 (ddd, J=16.1, 8.3, 5.7; 1H), 6.41 (t, J=11.3; 1H), 6.22 (d, J=11.5; 1H), 6.03–6.01 (m, 1H), 6.01–5.97 (m, 1H), 5.98 (d, J=14.8; 1H), 5.93 (d, J=16.2; 1H), 5.65 (d, J=11.4; 1H), 5.33 (dd, J=8.9, 6.0; 1H), 5.07 (d, J=7.9; 1H), 5.02 (ddd, J=9.8, 5.9, 2.9; 1H), 4.72 (brs, 2H), 4.16 (d, J=14.1; 1H), 3.87 (ddd, J=11.0, 8.2, 2.4; 1H), 2.91 (d, J=14.2; 1H), 2.33–2.27 (m, 2H), 2.18–2.09 (m, 3H), 2.07–2.03 (m, 1H), 1.87–1.79 (m, 2H), 1.80 (dd, J=6.8, 1.1; 3H), 1.75 (s, 3H), 1.62 ppm (s, 3H) (one signal overlapping with the solvent peak); ^{13}C NMR (125 MHz, $CDCl_3$): δ=197.4, 165.9, 165.2, 146.0, 143.7, 142.7, 140.9, 138.9, 137.4, 132.0, 130.9, 129.1, 128.6, 125.2, 121.3, 119.1, 109.0, 75.9, 75.2, 72.8, 71.8, 44.9, 40.6, 40.3, 40.3, 39.3, 23.5, 18.4, 16.4 ppm; IR (thin film): $\tilde{\nu}$=3325, 2962, 2927, 2853, 1714, 1654, 1634, 1520, 1431, 1355, 1280, 1259, 1213, 1147, 1085, 1048, 1034, 1024 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{29}H_{37}NNaO_6$ [M+Na^+]: 518.2513; found: 518.2518.

(1*S*,2*E*,5*S*,8*E*,10*Z*,14*E*,17*S*)-3,11-Dimethyl-19-methylene-7,13-dioxo-6,21-dioxabicyclo[15.3.1]henicosa-2,8,10,14-tetraene-5-carboxylic acid (44): To a solution of *ent*-**2** (9.5 mg, 0.0247 mmol, 1 equiv) in *t*BuOH (3 mL) and 2-methyl-2-butene (2 mL, 18.88 mmol, 764 equiv) was added a solution of $NaClO_2$ (22.3 mg, 0.247 mmol, 10 equiv) and $NaH_2PO_4{\cdot}H_2O$ (27.3 mg, 0.198 mmol, 8 equiv) dissolved in H_2O (1.2 mL) slowly at RT. After 40 min stirring the reaction mixture was diluted with brine (10 mL) and the phases were separated. The aqueous phase was extracted with EtOAc (3×10 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. Purification of the residue by FC (EtOAc, 0.5% AcOH) gave **44** (9.6 mg, 0.024 mmol, 97%; after coevaporation with toluene (1×2 mL)). R_f=0.31 (EtOAc, 0.5% AcOH, UV, CPS or $KMnO_4$); $[\alpha]_D^{24}=-68.82°$ (c = 0.49, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.56 (dd, J=15.1, 11.7; 1H), 6.85 (dt, J=16.2, 7.2; 1H), 6.13 (d, J=11.8; 1H), 6.04 (d, J=16.1; 1H), 5.91 (d, J=15.5; 1H), 5.43 (dd, J=11.3, 2.6; 1H), 5.30 (d, J=7.9; 1H), 4.75 (s, 2H), 3.96 (ddd, J=11.1, 7.9, 2.5; 1H), 3.73 (d, J=14.8; 1H), 3.41 (d, J=14.8; 1H), 3.39–3.32 (m, 1H), 2.63 (brd, J=13.5; 1H), 2.53 (dd, J=14.1, 11.3; 1H), 2.35–2.30 (m, 2H), 2.19–2.15 (m, 1H), 2.14–2.09 (m, 1H), 2.00–1.93 (m, 2H), 1.87 (s, 3H), 1.71 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=197.4, 174.6, 166.2, 145.8, 143.8, 143.7, 140.3, 131.7, 131.2, 130.7, 126.0, 120.2, 109.5, 76.5, 75.8, 69.1, 45.0, 41.8, 40.8, 40.5, 39.4, 24.5, 16.1 ppm; IR (thin film): $\tilde{\nu}$=3020, 2936, 1714, 1711, 1635, 1436, 1355, 1258, 976, 889, 752 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{23}H_{28}NaO_8$ [M+Na^+]: 423.1778; found: 423.1767.

(1*S*,2*E*,5*S*,8*E*,10*Z*,14*E*,17*S*)-*N*-Hexyl-3,11-dimethyl-19-methylene-7,13-dioxo-6,21-dioxabicyclo[15.3.1]henicosa-2,8,10,14-tetraene-5-carboxamide (45): To a solution of **44** (26 mg, 0.065 mmol, 1 equiv) in dry DMF (2 mL) was added HATU (27.4 mg, 0.072 mmol, 1.1 equiv) and DIEA (0.023 mL, 0.13 mmol, 2 equiv), producing a yellow solution. After 10 min hexylamine (0.026 mL, 0.195 mmol, 3 equiv; freshly distilled immediately before use) was added and the mixture was stirred for a total of 16 h. Water (5 mL) was then added followed by Et_2O (5 mL). The phases were separated and the aqueous phase was further extracted with Et_2O (3×5 mL). The combined organic extracts were washed with H_2O (2×5 mL) and the washing solutions were re-extracted with CH_2Cl_2 (2×5 mL). The combined organic extracts were dried over $MgSO_4$, concentrated under reduced pressure and the residue was purified by FC (EtOAc/Hex 1:5→1:3) to give amide **45** (4.1 mg, 0.0085 mmol, 13%) as a yellow oil. R_f=0.15 (EtOAc/Hex 1:3, UV, CPS); $[\alpha]_D^{24}=-166.22°$ (c = 0.82 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.70 (dd, J=15.1, 11.6; 1H), 6.82 (ddd, J=16.4, 9.5, 4.7; 1H), 6.23–6.19 (m, 1H), 6.14 (d, J=11.5; 1H), 5.97 (d, J=14.9; 1H), 5.93 (d, J=16.0; 1H), 5.56 (dd, J=11.2, 2.1; 1H), 5.18 (d, J=8.1; 1H), 4.75–4.71 (m, 2H), 4.19 (d, J=13.8; 1H), 3.97 (ddd, J=11.2, 8.1, 2.6; 1H), 3.32–3.24 (m, 3H), 3.01 (d, J=13.7; 1H), 2.74 (d, J=13.7; 1H), 2.34 (dddd, J=14.8, 10.0, 4.8, 1.8; 1H), 2.28–2.25 (m, 1H), 2.24–2.21 (m, 1H), 2.17–2.13 (m, 1H), 2.12–2.07 (m, 1H), 1.99–1.88 (m, 2H), 1.83 (s, 3H), 1.74 (d, J=0.9; 3H), 1.55–1.48 (m, 3H), 1.35–1.28 ppm (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.8, 169.6, 165.5, 146.6, 144.6, 143.7, 140.9, 132.6, 131.7, 130.1, 125.3, 119.7, 109.3, 76.7, 76.0, 71.1, 45.1, 43.7, 41.1, 40.8, 40.3, 39.5, 31.6, 29.7, 26.7, 23.9, 22.7, 16.4, 14.1 ppm; IR (thin film): $\tilde{\nu}$=3336, 2929, 2859, 1717, 1668, 1635, 1533, 1436, 1355, 1277, 1256, 1206, 1176, 1141, 1117, 1086, 1053, 977, 888 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{29}H_{42}NO_5$ [M+H^+]: 484.3057; found: 484.3057.

(*S*,3*E*,9*E*,11*Z*,15*E*)-6-((4-Methoxybenzyloxy)methyl)-4,12-dimethyl-1,7-dioxacyclooctadeca-3,9,11,15-tetraene-8,14-dione (57): To a solution of phosphonate **56** (216.1 mg, 0.355 mmol, 1.0 equiv; co-evaporated once with 2 mL of toluene immediately before use) in THF (300 mL) and H_2O (7.5 mL) was added freshly activated $Ba(OH)_2{\cdot}0.8H_2O$[53d] (53 mg, 0.284 mmol, 0.8 equiv) at 0°C. After 30 min the cooling bath was removed and stirring of the orange mixture was continued for a total of 3 h. Et_2O (50 mL) was then added followed by sat aq $NaHCO_3$ (50 mL), the phases were separated, and the organic phase was washed with sat aq $NaHCO_3$ (50 mL) and with brine (50 mL). The combined aqueous extracts were washed once with Et_2O (20 mL) and the combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The remaining yellow oil was purified by FC (EtOAc/Hex 1:3→1:1) to afford 136.9 mg of macrolactone **57** (0.30 mmol, 85%) as a pale-yellow oil. R_f=0.50 (EtOAc/Hex 1:1, UV, CPS); $[\alpha]_D^{24}=-76.05°$ (c = 0.61, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.60 (dd, J=15.2, 11.6; 1H), 7.26–7.24 (m, 2H), 6.89–6.80 (m, 3H), 6.12 (d, J=11.6; 1H), 6.04 (d, J=16.2; 1H), 5.90 (d, J=15.2; 1H), 5.41–5.35 (m, 1H), 5.30–5.27 (m, 1H), 4.53 (d, J=11.8; 1H), 4.47 (d, J=11.8; 1H), 4.01 (dd, J=12.1, 8.0; 1H), 3.88 (dd, J=12.1, 4.7; 1H), 3.80 (s, 3H), 3.76 (d, J=12.8; 1H), 3.59–3.37 (m, 4H), 3.26 (d, J=12.8; 1H), 2.48–2.38 (m, 2H), 2.35–2.24 (m, 2H), 1.83 (s, 3H), 1.69 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=197.1, 166.7, 159.4, 146.8, 142.4, 139.5, 134.6, 130.3, 130.2, 129.5, 125.9, 124.9, 121.3, 114.0, 73.0, 71.6, 69.7, 67.8, 67.8, 55.4, 45.9, 42.0, 33.0, 24.1, 16.7 ppm; IR (thin film): $\tilde{\nu}$=3009, 2999, 2959, 2916, 2857, 1708, 1667, 1633, 1613, 1586, 1513, 1456, 1441, 1360, 1301, 1279, 1247, 1208, 1173, 1148, 1089, 1033, 976, 890, 846, 819 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{27}H_{34}NaO_6$ [M+Na^+]: 477.2248; found: 477.2230.

(*S*,3*E*,9*E*,11*Z*,15*E*)-6-(Hydroxymethyl)-4,12-dimethyl-1,7-dioxacyclooctadeca-3,9,11,15-tetraene-8,14-dione (58): To a solution of macrolactone **57** (72 mg, 0.158 mmol, 1.0 equiv) in CH_2Cl_2 (4 mL) was added H_2O (0.8 mL) followed by DDQ (72 mg, 0.32 mmol, 2.0 equiv) and the mixture was stirred vigorously at room temperature. After 60 min the reaction mixture was added to sat aq $NaHCO_3$ (10 mL) and CH_2Cl_2 (5 mL), the phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3×10 mL). The combined organic extracts were dried over $MgSO_4$, filtered and the filtrate concentrated under reduced pressure. Purification of the residue by FC (EtOAc/Hex 1:1) gave alcohol **58** (40.7 mg, 0.122 mmol, 77%) as a pale-yellow oil. R_f=0.19 (EtOAc/Hex 1:1, UV, CPS); $[\alpha]_D^{24}=-74.67°$ (c= 0.29, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.62 (dd, J=15.1, 11.6; 1H), 6.85 (dt, J=16.2, 6.6; 1H), 6.13 (d, J=11.5; 1H), 6.06 (dt, J=16.2, 1.5; 1H), 5.91 (d, J=15.2; 1H), 5.32–5.25 (m, 2H), 4.02 (dd, J=12.0, 7.9; 1H), 3.91–3.86 (m, 1H), 3.79–3.70 (m, 3H), 3.51–3.38 (m, 2H), 3.29 (d, J=12.9; 1H), 2.49–2.33 (m, 3H),

2.22 (d, $J=13.8$; 1H), 1.84 (s, 3H), 1.71 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=197.0$, 167.1, 146.8, 142.8, 139.8, 134.3, 130.3, 125.9, 125.0, 121.0, 72.2, 68.0, 67.8, 65.5, 46.0, 41.5, 33.0, 24.1, 16.8 ppm; IR (thin film): $\tilde{\nu}=3442$, 2929, 2855, 1703, 1693, 1667, 1631, 1437, 1380, 1359, 1279, 1258, 1208, 1174, 1148, 1113, 1088, 1059, 1038, 976, 936, 891 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{19}H_{26}NaO_5$ $[M+Na^+]$: 357.1672; found: 357.1666.

desTHP-(−)-dactylolide (59): To a solution of alcohol **58** (40.7 mg, 0.122 mmol, 1.0 equiv) in CH_2Cl_2 (1.5 mL) was added solid DMP (156 mg, 0.37 mmol, 3.0 equiv; added in two equal portions, with the second portion added after 10 min). The mixture was stirred for a total of 30 min at room temperature. Sat aq $NaHCO_3$ (5 mL) and sat aq $Na_2S_2O_3$ (5 mL) were then added and stirring was continued for 15 min, leading to a clear organic phase and a turbid aqueous phase. The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3×5 mL). The combined organic extracts were washed with sat aq $NaHCO_3$ (2×5 mL), dried over $MgSO_4$, and concentrated under reduced pressure. Purification of the residue by FC (EtOAc/Hex 1:2→1:1) gave alcohol **59** (30.5 mg, 0.092 mmol, 75%) as a pale-yellow semisolid. $R_f=0.37$ (EtOAc/Hex 1:1, UV, CPS); $[\alpha]_D^{24}=-50.49°$ (c = 0.44, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=9.65$ (s, 1H), 7.66 (dd, $J=15.2$, 11.6; 1H), 6.86 (dt, $J=16.2$, 6.7; 1H), 6.19–6.15 (m, 1H), 6.10 (dt, $J=16.2$, 1.5; 1H), 5.94 (d, $J=15.2$; 1H), 5.43–5.37 (m, 1H), 5.33–5.30 (m, 1H), 4.01 (dd, $J=12.0$, 7.8; 1H), 3.93–3.89 (m, 1H), 3.58–3.44 (m, 4H), 2.61–2.58 (m, 1H), 2.47–2.37 (m, 3H), 1.89 (s, 3H), 1.71 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 199.0, 196.6, 166.3, 146.8, 143.8, 140.5, 133.1, 130.5, 126.0, 125.9, 120.0, 75.8, 67.9, 67.5, 45.7, 39.2, 32.9, 24.5, 16.4 ppm; IR (thin film): $\tilde{\nu}=3424$, 2957, 2921, 2853, 1732, 1706, 1668, 1632, 1456, 1437, 1377, 1356, 1317, 1258, 1206, 1174, 1143, 1112, 1080, 1026, 976, 888, 800 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{19}H_{24}NaO_5$ $[M+Na^+]$: 355.1516; found: 355.1523.

desTHP-zampanolide (60): To a solution of amide **42** (6.0 mg, 54.2 μmol, 6.0 equiv; co-evaporated with 0.5 mL toluene immediately before use) in THF (0.5 mL) was added dropwise DIBAL-H (1.2 M in toluene, 38 μL, 45.1 μmol, 5.0 equiv) at 0°C under argon. The colorless solution was stirred at 0°C for 30 min before a solution of **59** (3.0 mg, 9.0 μmol, 1.0 equiv; vial flushed with argon) in THF (0.3 mL) was added dropwise. After 30 min the cooling bath was removed and the reaction was stirred at RT overnight. Sat aq Rochelle salt (3 mL) and EtOAc (5 mL) were then added. The phases were separated and the aqueous layer was extracted with EtOAc (3×5 mL). The combined organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was filtered through a short plug of silica (hexane/EtOAc 2:3, 1% NEt_3 v/v) and then further purified by preparative HPLC to give **60** (1.1 mg, 28%) as a ca. 1.6:1 mixture of C18 isomers. Preparative HPLC was carried out on a Gilson system equipped with a photodiode array detector and employing a Waters SymmetryPrepTM C-18 column (5 μm, 19×100 mm). $\lambda=215$ nm and 230 nm. Flow rate 25 mL min^{-1}. Eluent: 0–2 min: 10% acetonitrile in water, 2–17 min: gradient from 10% to 100% acetonitrile; t_R = 9.3–9.9 min). $R_f=0.24$ (EtOAc/Hex 1:1, UV, CPS); ^{1}H NMR (500 MHz, [D$_6$]DMSO): $\delta=8.40$ (d, $J=9.0$; 1H (isomer 1)), 8.29 (d, $J=9.2$; 1H (isomer2)), 7.58–7.52 (m, 1H), 7.48–7.43 (m, 1H), 6.83–6.73 (m, 1H), 6.42–6.35 (m, 1H), 6.25–6.18 (m, 1H+1H (isomer 1)), 6.14 (d, $J=5.2$; 1H (isomer 2)), 6.05–5.93 (m, 2H+1H (isomer 1)), 5.92 (d, $J=15.0$; 1H (isomer 2)), 5.67–5.64 (m, 1H), 5.39–5.33 (m, 1H), 5.23–5.18 (m, 1H), 5.08–5.04 (m, 1H (isomer 1)), 5.00–4.96 (m, 1H (isomer 2)), 3.97–3.92 (m, 1H), 3.90–3.87 (m, 1H (isomer 1)), 3.83–3.77 (m, 1H+1H (isomer 2)), 3.42–3.29 (m, 2H), 3.23 (d, $J=12.9$; 1H (isomer 2)), 3.12 (d, $J=12.7$; 1H (isomer 1)), 2.42–2.32 (m, 3H), 2.22–2.16 (m, 1H), 1.80–1.76 (m, 6H), 1.63 (s, 3H (isomer 2)), 1.62 ppm (s, 3H (isomer 1)); ^{13}C NMR (125 MHz, [D$_6$]DMSO): $\delta=196.4$, 196.4, 165.8, 165.6, 165.4, 165.2, 146.5, 146.4, 142.4, 142.0, 140.9, 140.8, 139.8, 139.4, 137.5, 137.3, 134.4, 134.1, 130.0, 129.8, 128.6, 125.6, 125.6, 124.6, 124.5, 121.2, 120.7, 119.1, 119.1, 72.9, 72.9, 72.2, 71.8, 67.1, 67.0, 66.7, 66.6, 45.4, 45.1, 32.3, 32.2, 24.0, 23.9, 18.4, 16.7, 16.4 ppm (one set of signals is hidden underneath the DMSO signal); IR (thin film): $\tilde{\nu}=3326$, 2960, 2854, 1691, 1661, 1633, 1604, 1518, 1434, 1358, 1278, 1259, 1208, 1147, 1085, 1047, 1037, 1019, 977, 928, 835, 828, 801 cm^{-1}; HRMS (ESI): m/z: calcd for $C_{25}H_{34}NO_6$ $[M+H^+]$: 444.2381; found: 444.2380.

Cytotoxicity: Inhibition of cell proliferation was determined in the MCF-7, (breast), A549 (lung), HCT-116 (colon), and PC-3 (prostate) cell lines, which were obtained as a kind gift from Markus Wartmann (Novartis Institute for Biomedical Research (NIBR) Basel, Switzerland). Cells were maintained in a 5% CO_2 humidified atmosphere at 37°C in RPMI medium 1640 (Gibco BRL) containing 10% fetal bovine serum, penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}) (Gibco BRL). Cells were seeded at 1.5×10^3 per well into 96-well microtiter plates and incubated overnight. Compounds were added in serial dilutions on day 1. Subsequently, the plates were incubated for two population doublings (72 h) and then fixed with 3.3% v/v glutaraldehyde, washed with water and stained with 0.05% methylene blue. After washing, the dye was eluted with 3% v/v HCl and the optical density (OD) measured at 665 nm with a TECAN GeniosPro (Switzerland). IC_{50} values were determined with Graphpad Prism 4 using the formula $(OD_{treated}-OD_{start})/(OD_{control}-OD_{start})\times100$. The IC_{50} is the drug concentration for which the total cell number per well corresponds to 50% of the cell number in untreated control cultures (100%) at the end of the incubation period. Data shown in Table 1 represent the mean of three independent experiments.

Experimental details for all other compounds can be found in the Supporting Information, including synthetic procedures, full analytical data, and ^{1}H/^{13}C NMR spectra.

Acknowledgements

We thank the Roche Research Foundation for a PhD fellowship to D.Z. We are indebted to Dr. Bernhard Pfeiffer and Fabienne Gaugaz (ETHZ) for NMR support, to Kurt Hauenstein (ETHZ) for technical advice and to Louis Bertschi from the ETHZ-LOC MS-Service for HRMS spectra acquisition. This work was conducted within the framework of COST Action CM0804 "Chemical Biology with Natural Product".

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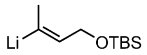

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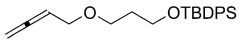

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[62] In a different experiment **60** was obtained in 45% yield and as a ca. 1:1 isomeric mixture after preparative HPLC purification. The differences in yield and isomer ratio are likely due to the more conservative pooling of product fractions in the purification step for the lower yielding experiment. The latter material (ca. 1.6:1 mixture of isomers) was used in the biological experiments.

Received: July 17, 2012
Published online: November 7, 2012

