Published in final edited form as:
European J Org Chem. 2012 July 1; 2012(22): 4130–4139. doi:10.1002/ejoc.201200286.

Total Synthesis of Potent Antitumor Macrolide, (-)-Zampanolide: An Oxidative Intramolecular Cyclization-Based Strategy

Arun K. Ghosh[a], **Xu Cheng**[a], **Ruoli Bai**[b], and **Ernest Hamel**[b]

Arun K. Ghosh: akghosh@purdue.edu

[a]Department of Chemistry and Department of Medicinal Chemistry, Purdue University 560 Oval Drive, West Lafayette, IN 47907 (USA), Fax: (+1) 765-496-1612

[b]Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, MD 21702 (USA)

Abstract

A detailed account of the enantioselective total synthesis of (-)-zampanolide, a macrolide marine natural product with high anti-cancer activity is described. For synthesis of the 4-methylene tetrahydropyran unit of (-)-zampanolide, initially, we relied upon an oxidative C-H activation of an alkenyl ether and intramolecular cyclization to provide the substituted tetrahydropyran ring. However, this strategy was unsuccessful. Subsequently, we found that a cinnamyl ether is critical for the successful oxidative intramolecular cyclization reaction. The synthesis also features a cross metathesis reaction to construct a tri-substituted olefin, a ring-closing metathesis to form a highly functionalized macrolactone and a chiral phosphoric acid promoted *N*-acyl aminal formation to furnish (-)-zampanolide stereoselectively and in good yield. The synthetic (-)-zampanolide had effects on cultured cells and on tubulin assembly consistent with properties reported for the natural product.

Keywords

Natural product; Total Synthesis; C-H bond activation; Metathesis; Stereoselective catalysis

Introduction

Zampanolide (**1**, Figure 1), an unsaturated 20-membered macrolide with an *N*-acyl hemiaminal side chain, was initially isolated by Tanakai and Higa in 1996 from the marine sponge *Fasciospongia rimosa* in Okinawa, Japan.[1] More recently, (-)-zampanolide was isolated from the Tongan marine sponge, *Cacospongia mycofijiensis* by Northcote, Miller, and co-workers. [2] Zampanolide exhibited potent cytotoxicity against a variety of cell lines. It has shown IC_{50} values of 1.1 and 2.9 nM, against SKM-1 and U937 cell lines, respectively. Moreover, zampanolide is very active against HL-60, 1A9, and particularly A2780AD cells, which showed resistance to paclitaxel based on overexpression of P-glycoprotein.[2] Its biological mechanism of action involves the stabilization of microtubules with enhancement of microtubule assembly and blocking cell division in the G2/M phase of the cell cycle.[2, 3] Macrolide (+)-dactylolide (**2**, Figure 1) was isolated from the sponge *Dactylospongia* sp.[4] Interestingly, it displayed only modest cytotoxicity. However, it

Correspondence to: Arun K. Ghosh, akghosh@purdue.edu.

Supplementary Materials: ^{1}H NMR and ^{13}C NMR spectra for all new compounds and HPLC traces for compounds **1**, **16**, **39** associated with this article can be found in the online version.

contains opposite configurations of the macrolide core of (-)-zampanolide (**1**). The biological relevance of the macrolide core of (+)-dactylolide and (-)-zampanolide is not clear.

Both zampanolide and dactylolide have attracted much interest in synthesis and in biological studies of structural variants.[3] Smith and co-workers first reported the total synthesis of (+)-zampanolide, the unnatural antipode, and provided tentative assignment of the absolute configurations of natural (-)-zampanolide.[5] Since then, Hoye et al in 2003[6a] and Uenishi et al in 2009[6b] reported the total synthesis of natural (-)-zampanolide. Recently, we reported an enantioselective synthesis of (-)-zampanolide.[7] Since the isolation of (+)-dactylolide by Riccio and co-workers in 2001,[4] a number of total syntheses and various synthetic approaches to (+)-dactylolide have been reported.[8] The *N*-acyl side chain of (-)-zampanolide is critical to its potent cytotoxic properties.[1,2] Previous syntheses have furnished zampanolide through direct *N*-acylaminal formation from unnatural (-)-dactylolide, albeit in only 12% yield. Other major products include zampanolide epimer and *bis*-acylated products.[6] This direct transformation was achieved through an Al reagent promoted[6a] or a CSA[6b] catalyzed strategy, although in both cases no stereoselectivity was observed. Herein, we report the details of our synthetic efforts that have led to a convergent total synthesis of (-)-zampanolide. The synthesis featured a novel intramolecular oxidative cyclization reaction and an organocatalyic *N*-acylaminal formation of dactylolide that stereoselectively furnished (-)-zampanolide and no *bis*-amide byproduct.

Results and Discussion

Our initial retrosynthetic analysis of (-)-zampanolide (**1**) is outlined in Figure 2. Our synthetic strategy was to synthesize (-)-dactylolide (**2**) and carry out a stereo selective amidation to provide (-)-zampanolide. Strategic disconnection of (-)-dactylolide at the C8-C9 and the C1-C19 ester results in the 4-methylenetetrahydropyran derivative **4** and the unsaturated carboxylic acid. Our plan was to form an ester with the C-19 alcohol and carboxylic acid **3** and then carry out ring closing metathesis to construct the C-20 macrolactone core of (-)-dactylolide. Functionalized 4-methylenetetrahydropyran **4** was planned to be derived from an intramolecular oxidative cyclization of allyl ether **5**. This substrate can be synthesized from allylic alcohol **6** and optically active β-hydroxy ester **7**.

We first examined the feasibility of oxidative cyclization of allylsilane derivative **5** to yield tetrahydropyran **4**. The synthesis of **5** is shown in Scheme 1. Optically active methyl ketone **8** was prepared in 5 steps from L-malic acid, as described previously.[9] Horner-Wadsworth-Emmons reaction of ketone **8** with NaH and *t*-butylphosphonoacetate at 23 °C afforded α,β-unsaturated ester **9** as a mixture (4:1) of *E/Z* isomers. In contrast, triethyl phosphonoacetate provided **10** with lower *E/Z* selectivity (2:1). The ester was reduced by exposure to excess DIBAL-H to provide allyl alcohol **11**. Treatment of **11** with NaH and trichloroacetonitrile in ether furnished trichloroimidate **12**. Initially, we attempted etherification of known alcohol **7**[10] with imidate **12** in the presence of a number of acids and Lewis acids such as TfOH, $BF_3{\cdot}Et_2O$, and TMSOTf. However, these conditions resulted in decomposition of starting materials. The use of TBSOTf resulted in a considerable amount of ether **13**. The coupling reaction with TIPSOTf proceeded well, providing **13** in 79% yield as a 2:1 mixture of *E/Z* isomers (by ^1H NMR analysis). The ester functionality of **13** was converted to allylsilane derivative **5** using the procedure developed by Benelle and co-workers.[11] With this allylsilane substrate, we have attempted an oxidative cyclization reaction with DDQ under a variety of reaction conditions.[12] However, we could not obtain any desired cyclization. At this point, we speculated that a cinnamyl ether may be more convenient for such an oxidative cyclization reaction. Therefore, we modified our synthetic strategy.

Our alternative retrosynthetic analysis is illustrated in Figure 3. Disconnection of the *N*-acyl aminal side chain would provide protected macrolactone core **14**. Further disassembly of the macrolactone would give rise to tetrahydropyran derivative **15** and trisubstituted olefin **3**. Functionalized 4-methylenedihydropyran unit **15** was planned to be synthesized from dihydropyran **16** by a cross metathesis reaction. The dihydropyran **16** with a cinnamyl side chain would be assembled from cinnamyl ether **17** by an oxidative cyclization reaction. We presumed that oxidative activation of a cinnamyl group with DDQ would proceed favorably compared to allylic ether **5**. The oxidative cyclization substrate **17** can be derived from β-hydroxy ester **18**. This β-hydroxy ester would be prepared in optically active form by an enantioselective hydrogenation process. The polyene unit **3** could be obtained by a Reformatsky reaction of bromo olefin **19** and acrolein, followed by a Wittig olefination.

Based upon our revised plan, optically active β-hydroxy ester **20** was synthesized based upon Noyori hydrogenation.[13] This was converted to cinnamyl ether **22** as shown in Scheme 2. Catalytic hydrogenation of **20** over 10% Pd-C, followed by selective protection of the primary alcohol with TBDPSCl and imidazole in THF at 0 °C afforded the corresponding silyl ether. The free secondary hydroxyl was then protected with as a cinnamyl ether using a Tsuji-Trost reaction with a catalytic amount of $Pd(PPh_3)_4$ and *t*-butyl cinnamyl carbonate **21** to provide **22** in 71% yield.[14] The ester group in **22** was converted to allylsilane **17** by treatment with excess trimethylsilylmethylmagnesium bromide in the presence of $CeCl_3$, as described by Bunnelle and co-workers. [11] We then investigated a variety of reaction conditions for effective oxidative cyclization of key substrate **17**. The results are shown in Table 1. As can be seen, our initial attempt of using DDQ in the presence of $InCl_3$ as a promoter resulted in the desired dihydropyran cyclization product **16** in 57% yield (entry 1) as a single diastereoisomer (by ^{1}HNMR analysis). The *cis* stereochemical identity of dihydropyran **16** was established by ^{1}H NMR NOESY experiments. The oxidative cyclization presumably proceeded through the Zimmerman-Traxler transition state shown in stereochemical model **23**. The presence of 2,6-dichloropyridine did not improve reaction yields (entry 2). Other Lewis acids did not offer any advantage (entries 3-6).[12] The cyclization reaction in the presence of CSA, however, proved to be the most effective in both CH_2Cl_2 as well as CH_3CN (entries 7 and 8). The catalytic version of this reaction in the presence of PPTS marginally improved the yield to 71% (entry 9). The reaction was then carried out on a 4 gram scale at −38 °C in CH_3CN to provide **16** in 81% yield.

For elaboration of the C17-C20 chain of (-)-zampanolide with an *E*-trisubstituted olefin, we initially relied upon a cross metathesis of **16** with the corresponding olefin substrate **26**. However, our many attempts under a variety of conditions and catalysts resulted in no desired cross metathesis product. This is possibly due to the lack of reactivity of the styrene side chain. Even our attempted cross metathesis with ethylene resulted in no cross metathesis product **24**. We therefore devised an alternative strategy to install this side chain. As shown in Scheme 3, we carried out selective cleavage of the styrenyl double bond using dihydroxylation with AD-mix-α, followed by diol cleavage with $NaIO_4$ as described previously.[15] The resulting unstable aldehyde was subjected to Wittig reaction with methylenetriphenylphosphorane to provide alkene **24** in 78% yield for the three steps. For the planned cross metathesis with **24**, we prepared **26** by ring opening of a PMB-protected glycidyl derivative **25** with isopropenylmagnesium bromide, followed by protection of the resulting alcohol as a TES ether. Cross metathesis of **24** and **26** was carried out with second generation Grubbs catalyst [16] in CH_2Cl_2 at reflux for 9 h to furnish trisubstituted olefin as a *E*/*Z* mixture of isomers (ratio 1.7:1) in 57% combined yield. The mixture of olefin was treated with HF.Py to remove both silyl groups, and the resulting diols were separated by

silica gel chromatography. Olefin ***Z*-27** was subjected to photochemical isomerization to provide the ***E*-27** isomer in 51% yield (brsm 68%).

Synthesis of the C1-C9 triene carboxylic acid **3** was carried out using a Reformatsky reaction as the key step. As outlined in Scheme 4, allyl bromide **19** was prepared as an *E/Z* mixture (1:1) according to the procedure of Fallis and Lei.[17] Treatment of this *E/Z* mixture with Zn dust, followed by reaction with acrolein, afforded *E*-alcohol **28** (47%) and α, β-unsaturated δ-lactone **29** (36%) resulting from concomitant lactonization of the *Z*-alcohol. These products were separated by silica gel chromatography. Treatment of lactone **29** with DIBAL-H, followed by reaction of the resulting lactol with (carbethoxymethylene)-triphenylphosphorane, afforded unsaturated ester **30** in 33% yield for the two steps. The free allylic alcohol was protected as a PMB-ether and saponification of the resulting ester furnished the C1-C9 carboxylic acid fragment **3** in 62% yield in two steps.

For elaboration of the macrolactone core of (-)-dactylolide or (-)-zampanolide, as shown in Scheme 5, the primary alcohol in **27** was first selectively oxidized with TEMPO and $PhI(OAc)_2$.[18] Wittig olefination of the resulting aldehyde with methylenetriphenyl phosphorane afforded olefin **31** in 60% overall yield. The secondary alcohol in **31** was esterified under Yamaguchi conditions[19] with carboxylic acid **3** using 2,4,6-trichlorobenzoyl chloride in the presence of DMAP to provide a mixture (1:1) of diastereomeric ester **32** in excellent yield. Subjection of this mixture of diastereomers to Grubbs' second generation catalyst (12 mol %) in benzene at 60 °C for 20 h furnished the corresponding 20-member macrolactone. Exposure of the macrolactone mixture to DDQ in aqueous CH_2Cl_2 removed the PMP group, providing a mixture (1:1 at C7) of alcohols **14** in 65% yield in two steps. Oxidation of **14** with Dess-Martin reagent[20] furnished (-)-dactylolide **2** in 80% yield. The spectral data (^{1}H-NMR and ^{13}C-NMR) of (-)-**2** is in full agreement with natural (+)-dactylolide except optical rotation of (-)-**2** ($[\alpha]_D^{24}$ -148, *c* 0.09, MeOH).

Previous direct *N*-acylaminal formation proceeded only with limited success, providing (-)-zampanolide (**1)** along with a mixture of C-20 diastereomers in poor yield (~12%).[6] To improve this transformation, we first selected cyclohexane carboxaldehyde **33** and aldehyde **34** as model substrates. We investigated *N*-acylaminal formation using the reaction conditions reported by Williams and co-workers.[21] As shown in Scheme 6, our initial attempts with both aldehyde substrates **33** and **34** and isobutyramide **35** proceeded well, providing *N*-acylamides **36** (90% yield) and **37** (56% yield) as a 1:1 mixture of diastereomers. However, our attempts to carry out this reaction with (-)-dactylolide **2** and (2*Z*,4*E*)-hexa-2,4-dienamide **38** were unsuccessful.

We subsequently planned to optimize direct amidation conditions reported by Uenishi and co-workers.[6b] Reaction of (-)-dactylolide and amide **38** with the *p*-TsOH was reported to provide (-)-zampanolide **1** (12%), C-20 epimeric product (12%) and the corresponding *bis*-amide (23%).[6b] In this transformation, the lack of stereoselectivity and the formation of *bis*-amide limited the yield of desired (-)-zampanolide. In an effort to improve the reaction yield and minimize conversion to the *bis*-amide byproduct, we investigated reaction of (-)-**2** and amide **38** in the presence of Brφnsted acid diphenylphosphoric acid (10 mol-%) in CH_2Cl_2 at 23 °C for 14 h, as depicted in Scheme 7 As indicated by HPLC analysis, the above reaction condition provided (-)-zampanolide **1**, its epimer **39**, and *bis*-amide **40** in better ratio than reported for the *p*-TsOH catalyzed reaction.[6b]

Encouraged by this result, we investigated the effect of chiral phosphoric acid TRIP **41**[22] in the *N*-acyl aminal formation of (-)-**2** and amide **38**. As shown in Scheme 8, reaction of (-)-**2** in the presence of (*S*)-TRIP (20 mol %) at 23 °C for 12 h furnished *N*-acyl aminal

derivatives diastereoselecivity in good yields and, most significantly, the formation of the byproduct, *bis*-amide **40** was not observed. The reaction provided (-)-zampanolide (**1**) in 53% yield and *epi*-zampanolide **39** in 18% yield after separation by HPLC. In contrast, the corresponding reaction with **38** and (-)-**2** in the presence of mismatched (*R*)-TRIP **42** resulted in (-)-**1** and **39** as a 1:1 mixture of diastereomers. Interestingly, the formation of *bis*-amide byproduct **40** was not observed. The spectral data (^{1}H NMR and ^{13}C NMR) of synthetic (-)-zampanolide (**1**, $[\alpha]_D$ - 94, *c* 0.08, CH_2Cl_2) is in full agreement with that of natural zampanolide (Lit.[1] $[\alpha]_D$ - 101, *c* 0.12, CH_2Cl_2).

Biological Activities

The biological properties of compounds **1**, **39** and **40** were evaluated with purified tubulin combined with heat-treated microtubule-associated proteins, and cytotoxic effects were examined with several cell lines. Paclitaxel was used as a control in all experiments. With tubulin, **1** strongly induced assembly, as was reported previously,[2] while compounds **39** and **40** had only slight activity (data not shown). The cytotoxic activities of the three agents against three human cancer cell lines are summarized in Table 2. We used MCF-7 breast cancer cells and two ovarian lines, OVCAR 8 and its isogenic derivative NCI/ADR-RES, which, like A2780AD,[2] overexpresses P-glycoprotein, an important cellular drug efflux pump. Overexpression of P-glycoprotein produces the major multidrug resistance phenotype. P-glycoprotein overexpressing cell lines are of particular interest in evaluating agents with a paclitaxel-like mechanism of action, since such cell lines are highly resistant to paclitaxel, and multidrug resistance may cause clinical loss of sensitivity to paclitaxel.

Compound **1** was similar in its cytotoxic activity to paclitaxel in both the MCF-7 and OVCAR 8 cell lines, but it was much more active than paclitaxel in the multidrug resistant NCI/ADR-RES cell line. Compounds **39** and **40** were substantially less active than **1** in all cell lines examined. However, **39** and **40** were more active than paclitaxel in the NCI/ADR-RES cells, indicating that they, too, are not good substrates for P-glycoprotein.

We also did a limited study with **1**, **39** and **40** in human CA46 Burkitt lymphoma cells, since this cell line yields a high proportion of cells arrested in the G2/M phase of the cell cycle when treated with antitubulin agents at higher concentrations, such as 10-fold higher than the IC_{50}. With such high concentrations, all three compounds and paclitaxel caused over 90% G2/M arrest in the Burkitt cells. With compound **1** and paclitaxel, the agents were used at 100 nM, while with **39** and **40**, the concentration used was 5 μM (data not presented).

Conclusions

In summary, a detailed account of the enantioselective total synthesis of (-)-zampanolide is described. Our initial synthesis route to the zampanolide tetrahydropyran core involved oxidative C-H activation of an allylic ether derivative. However, this strategy did not provide the functionalized tetrahydropyran core for zampanolide. We subsequently devised an alternative oxidative C-H activation strategy where a cinnamyl ether was used in place of the allyl ether. This cyclization method provided the 4-methylene tetrahydropyran derivative in a highly stereoselective manner. The C1-C9 triene carboxylic acid unit **3** was synthesized using a Reformatsky reaction as the key step. Yamaguchi esterification and ring closing olefin metathesis provided the zampanolide core. An organocatalytic *N*-acyl aminal formation with a chiral phosphoric acid proceeded stereoselectively and in good yield without the formation of the *bis*-amide byproduct. The current synthesis is amenable to a variety of structural analogs of zampanolide. The synthetic zampanolide had biological properties equivalent to those previously described for the natural product.[1,2]

Experimental Section

General Methods—All moisture sensitive reactions were carried out in an oven dried flask under an argon atmosphere. Anhydrous solvents were obtained as follows: THF, diethyl ether and benzene, distilled from sodium and benzophenone; dichloromethane, pyridine, triethylamine, and diisopropylethylamine, distilled from CaH_2. All other solvents were HPLC grade. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian INOVA300-1 and Bruker Avance ARX-400 spectrometers. NMR data were resolved with Mestrec software. IR spectra were recorded on a Perkin Elmer spectrometer. Optical rotations were recorded on a Perkin Elmer 341 polarimeter. Mass spectra were obtained at the Purdue University Campus-wide Mass Spectrometry Center. Column chromatography was peRformed with Whatman 240-400 mesh silica gel under a low pressure of 3-5 psi. TLC was carried out with E. Merck silica gel 60-F-254 plates. HPLC was peRformed on an Agilent 1100 instrument.

Compound 9—To a suspension of NaH (1.35 g, 60% on silcon oil, 33.7 mmol) on THF (40 mL) at 0 °C was added a solution of *t*-butyl 2-(dimethoxyphosphoryl) acetate (8.7 g, 38.8 mmol) in THF (10 mL). The mixture was stirred at 0 °C for 30 min and at room temperature for 30 min. After cooling to 0 °C, to the solution was added ketone 8 (1.69 g, 10.7 mmol) in THF (10 mL). After stirring at 0 °C for 10 min and at room temperature overnight, the reaction was quenched with saturated NH_4Cl. The aqueous layer was extracted with ether twice. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography with hexanes/ethyl acetate = 4:1 to give a mixture of *E*/*Z* isomers, and pure ethyl acetate was used to recover excess *t*-butyl 2-(dimethoxyphosphoryl) acetate. The mixture was bound to a silica gel column and eluted with hexanes/ethyl acetate = 9:1 to yield the *Z*-isomer (0.4 g) and *E*-isomer (1.7 g), overall yield 77%. *Z*-isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69 (s, 1H),4.36-4.27 (m, 1H), 4.07 (dd, J = 6.0, 8.1 Hz, 1H), 3.62 (dd, J = 7.1, 8.0 Hz, 1H), 3.07 (dd, J = 4.7, 12.7 Hz, 1H), 2.68 (dd, J = 7.8, 12.7 Hz, 1H), 1.95 (d, J = 1.3 Hz, 3H), 1.46 (s, 9H), 1.42 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 154.5, 119.5, 108.8, 79.6, 75.3, 69.1, 36.7, 28.1, 28.0, 26.8, 26.3, 25.6. IR (thin film, cm^{-1}) 2981, 1706, 1646, 1500, 1368, 1254, 1221, 1072, 968, 860, 844. $[\delta]_D^{23}$ = −43° (c 2.3, ethyl acetate). Rf = 0.29, hexanes/ethyl acetate = 90:10, $KMnO_4$ stain. MS (ESI) m/z 279 ($M+Na^+$). *E*-isomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 (d, J = 1.2 Hz, 1H), 4.32-4.24 (m, 1H), 4.06 (dd, J = 5.9, 8.1 Hz, 1H), 3.57 (dd, J = 6.8, 8.1 Hz, 1H), 2.46 (dd, J = 6.9, 13.4 Hz, 1H), 2.27 (dd, J = 7.0, 14.1 Hz, 1H), 2.17 (d, J = 1.3 Hz, 3H), 1.48 (s, 9H), 1.42 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.9, 153.4, 119.5, 109.1, 79.7, 73.8, 69.1, 44.8, 28.1, 26.9, 25.5, 18.6. IR (thin film, cm^{-1}) 2982, 1714, 1651, 1455, 1380, 1184, 1110, 1073, 862, 829. $[\delta]_D^{23}$ = −11.7° (c 2.2, ethyl acetate). Rf = 0.28, hexanes/ethyl acetate = 90:10, $KMnO_4$ stain. MS (ESI) *m/z* 279 ($M+Na^+$).

Compound 11—To a solution of the *E*-isomer of **9** (0.85 g, 3.3 mmol) in CH_2Cl_2 (20 mL) at −15 °C was added DIBAL-H (1.18 g, 1.47 mL, 8.3 mmol). The mixture was stirred at −15 °C for 5 min and quenched with saturated NH_4Cl. After stirring at room temperature for 5 min, the resulting gel was diluted with CH_2Cl_2 and filtered through celite. The aqueous layer was extracted with CH_2Cl_2 once. The combined organic layer was dried (Na_2SO_4) and concentrated, followed by silica gel chromatography with hexanes/ether (2:3) as eluent to yield **11** as a colorless oil (0.56 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.63 (t, J = 6.8 Hz, 1H), 4.27-4.18 (m, 1H), 4.12 (d, J = 6.6 Hz, 2H), 4.01 (dd, J = 6.0, 8.0 Hz, 1H), 3.53 (t, J = 7.5 Hz, 1H), 2.34 (dd, J = 7.0, 13.9 Hz, 1H), 2.17 (dd, J = 6.2, 14.0 Hz, 1H), 1.98 (brs, 1H), 1.69 (s, 3H), 1.39 (s, 3H), 1.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.0, 126.1, 108.9, 76.7, 69.1, 58.9, 43.5, 26.8, 25.5, 16.7. IR (thin film, cm^{-1}) 3413, 2985, 2935, 1668, 1455,

1371, 1216, 1157, 1062, 999, 831, 790. $[\delta]_D^{23}$ = +1.5° (c 2.6, ethyl acetate). Rf = 0.27, hexanes/ethyl ether (2:3), $KMnO_4$ stain. MS (ESI) *m/z* 209 ($M+Na^+$).

Compound 12—To a suspension of NaH (60% in silicon oil, 10 mol-%, 8.6 mg, 0.22 mmol) in diethyl ether (1.5 mL) at 0 °C was added a solution of **11** (400 mg, 2.15 mmol) in ether (1 mL) dropwise. Then the solution was stirred at room temperature for 20 min and cooled to −40 °C. Next, CCl_3CN (215 μL, 2.15 mmol) was added. Then the mixture was stirred sequentially at −40 °C for 1 h and room temperature for 1 h, followed by addition of MeOH (9 μL). The solution was concentrated and suspended in hexanes. The suspension was filtered through celite. The filtrate was concentrated and checked by ^1H NMR. The resulting yellow oil **12** (697 mg, 98%) was pure enough and used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 5.54 (t, J = 6.8 Hz, 1H), 4.82 (d, J = 6.8 Hz, 2H), 4.30-4.21 (m, 1H), 4.02 (dd, J = 5.9, 8.1 Hz, 1H), 3.57 (t, J = 7.5 Hz, 1H), 2.44 (dd, J = 6.7, 13.4 Hz, 1H), 2.25 (dd, J = 6.9, 14.0 Hz, 1H), 1.79 (s, 3H), 1.42 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 139.1, 120.3, 108.9, 74.2, 69.1, 65.8, 43.5, 26.9, 25.6, 17.2. IR (thin film, cm^{-1}) 3470, 3344, 2986, 1738, 1662, 1455, 1370, 1291, 1229, 1157, 1068, 984, 827, 799, 650. $[\delta]_D^{23}$ = −1.9° (c 2.0, ethyl acetate). Rf = 0.28, hexanes/ethyl acetate = 90:10, $KMnO_4$ stain. MS (ESI) *m/z* 352 ($M+Na^+$).

Compound 13—To a suspension of **7** (40 mg, 0.25 mmol), **12** (90 mg, 0.27 mmol) and 4 Å MS (40 mg) in CH_2Cl_2 (1 mL) was added TIPSOTf (25 μL,) at −78 °C and, the mixture was stirred at room temperature overnight. The reddish suspension was quenched with $_{Et3N}$ (0.1 mL) and concentrated. The residue was purified by silica gel chromatography with hexanes/ethyl ether = 4:1 as eluent to give the target product **13** as a colorless oil (32 mg, *E/Z* = 2:1) containing residual **7** (20 mg). The yield was 79% brsm. ^1H NMR (300 MHz, $CDCl_3$) δ 5.87-5.73 (m, 2H), 5.57-5.35 (m, 2H), 5.12-5.06 (m, 4H), 4.27-3.95 (m, 12H), 3.88-3.79 (m, 2H), 3.57-3.43 (m, 2H), 2.55-2.15 (m, 12H), 1.78 (s, 1.7H), 1.69 (s, 4.3H), 1.41 (s, 1.7H), 1.40 (s, 4.3H), 1.34 (s, 6H), 1.25 (t, J = 7.2 Hz, 6H). Rf = 0.40 (hexanes/ethyl acetate = 4:1, $KMnO_4$ stain).

Compound 5—Finely powdered $CeCl_3 \cdot 7H_2O$ (0.228 g, 0.61 mmol) was heated at 160 °C under high vacuum for 2.5 h and cooled to room temperature under argon. The white powder was suspended in 1 mL of THF and stirred at room temperature for 2 h. Then the white suspension was cooled to −78 °C and to it was added $TMSCH_2MgCl$ (0.615 mL, 1 M in diethyl ether, 0.615 mmol). The yellow suspension was stirred at −78 °C for 1 h, and to it was added **13** (40 mg, 0.12 mmol) in THF (0.4 mL). Then the mixture was stirred sequentially at −78 °C for 2 h and room temperature overnight. The white suspension was cooled to 0 °C and diluted with anhydrous ether (5 mL), followed by addition of HCl (1 mL, 2 M). Then the reaction mixture was diluted with water/ether. The aqueous layer was extracted with ether twice. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by silca gel chromatography with hexanes/ethyl acetate (93:7) as eluent to give the product as a colorless oil, the *E/Z* mixture (ca. 2:1) of **5** (32 mg, 73%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.90-5.80 (m, 2H), 5.48 (t, J = 6.6 Hz, 0.66H), 5.40 (t, J = 6.6 Hz, 1.38H), 5.13-5.07 (m, 4H), 5.10-5.04 (m, 4H), 4.65 (s, 2H), 4.59 (s, 2H), 4.27-4.14 (m, 2H), 4.05-3.98 (m, 6H), 3.57-3.46 (m, 4H), 2.46-2.37 (m, 2H), 2.30-2.17 (m, 8H), 2.08 (dd, J = 6.3, 14.1 Hz, 2H), 1.78 (s, 2H), 1.70 (s, 4H), 1.58 (s, 1.4H), 1.55 (s, 2.6H), 1.42 (s, 2H), 1.41 (4H), 1.35 (s, 6H), 0.02 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.5, 135.6, 135.2, 135.1, 124.8, 124.1, 116.8, 109.7, 109.6, 108.9, 108.8, 108.7, 74.6, 74.5, 69.3, 65.6, 65.4, 65.3, 43.7, 42.9, 38.5, 36.5, 27.1, 27.0, 26.9, 25.7, 25.6, 24.1, 23.2, 17.0, -1.3. IR (thin film, cm^{-1}) 3075, 2953, 1633, 1435, 1370, 1248, 1157, 1068, 857, 695. Rf = 0.41 (hexanes/ethyl acetate =4:1, PMA stain). MS (ESI) *m/z* 389 ($M+Na^+$).

Optimization of oxidative cyclization—To a reaction vial charged with **17** (56 mg, 0.1 mmol) and 4 Å MS (0.1 g) was added solvent (4 mL) at −38 °C under argon and DDQ (34 mg, 0.15 mmol) and acid (1.5 equiv.). Then the mixture was stirred until full conversion as monitored by TLC (hexanes/ethyl acetate = 98:2, developed four times, ca. 6-8 h to reach completion). Then the reaction was quenched with Et3N (0.2 mL) and filtered under pressure through a silica gel pad. The concentrated residue was purified by silica gel chromatography with hexanes/ethyl acetate (95:5) as eluent to give **16** as a colorless oil.

Compound 16—Cyclization reaction with stiochiometric amount of DDQ (Table 1, entry 10): To a flask charged with DDQ (1.56 g, 6.87 mmol), PPTS (1.78 g, 6.87 mmol) and 4 Å MS (9.0 g) under argon at −38 °C was added anhydrous acetonitrile (100 mL). A solution of (*R*)-tert-butyl(3-(cinnamyloxy)-5-((trimethylsilyl)methyl)hex-5-enyloxy)diphenylsilane 17 (2.63 g, 4.72 mmol, 97% ee) in acetonitrile (31 mL) was added dropwise for 5 min. The mixture was stirred at −38 °C for 3 h. The reaction was quenched with triethylamine (3 mL) and filtered through a pad of Celite. The filtrate was concentrated and then dissolved in ethyl acetate, washed with brine, and dried (Na_2SO_4). Evaporation of the solvent gave a residue which was purified by silica gel chromatography with hexanes/ethyl acetate (98:2) to provide compound **16** as a pale yellow oil (1.81g, 81%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.69-7.66 (m, 4H), 7.45-7.21 (m, 11H), 6.59 (d, J = 16.0 Hz, 1H), 6.24 (dd, J = 16.0, 5.7 Hz, 1H), 4.78 (s, 2H), 3.99-3.86 (m, 2H), 3.81-3.74 (m, 1H), 3.70-3.62 (m, 1H), 2.36 (d, J = 14.8 Hz, 1H), 2.28 (d, J = 14.4, 1H), 1.92-1.76 (m, 2H), 1.05 (s, 9H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ 144.3, 136.8, 135.5, 133.9, 133.8, 130.1, 129.5, 128.4, 127.6, 126.4, 108.9, 78.5, 75.1, 60.1, 41.0, 40.7, 39.0, 26.8, 19.2. IR (thin film, cm^{-1}) 3070, 2857, 1959, 1823, 1651, 1590, 1427, 1359, 1110, 966, 892, 741, 703, 614. Rf = 0.36 (hexanes/ethyl acetate = 95:5, UV) The Rf is almost the same as that of the starting material, but development with hexanes/ethyl acetate = 99:1 three times gave a slightly higher Rf than that of the starting material. $[\alpha]_d^{20}$ = +3.5 (c 1.2, ethyl acetate). MS (ESI) m/z 505 ($M+Na^+$). HRMS (ESI) calculated for ($C_{32}H_{38}O_2SiNa$) 505.2539, found 505.2536.

Determination of ee—To a solution of 16 (30 mg, 60.4 μmol) in THF (1 mL) was added TBAF (0.4 mL, 1 M in THF, 0.4 mmol). Then the solution was stirred at room temperature for 4 h and concentrated. The residue was purified by silica gel chromatography with hexanes/ethyl aceate (v/v = 3:2) as eluent to give the free alcohol as a colorless oil (8.0 mg, 60%), 97% ee. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.23 (m, 5H), 6.23 (dd, J = 6.1, 16.0 Hz, 1H), 6.58 (d, J = 16.0 Hz, 1H), 4.79 (s, 2H), 4.03-3.99 (m, 1H), 3.86-3.82 (m, 2H), 3.68-3.62 (m, 1H), 2.64 (s, 1H), 2.36 (d, J = 13.3 Hz, 1H), 2.23 (d, J = 13.3 Hz, 1H), 2.20-2.08 (m, 2H), 1.94-1.84 (m, 1H), 1.83-1.76 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ143.4, 130.6, 129.5, 128.4, 127.6, 126.4, 109.3, 79.0, 78.7, 61.2, 40.7, 40.4, 37.9. IR (thin film, cm^{-1}) 3401, 2942, 2900, 1719, 1650, 1450, 1421, 1315, 1062, 966, 746, 693. Rf = 0.35, hexanes/ethyl acetate = 7:3 UV. $[\alpha]_d^{20}$ = +6.8 (c 0.73, ethyl acetate). HPLC, Daicel IC column, hexanes/IPA = 9:1, 0.5 mL/min, 254 nm, t_1 = 14.1 min, t_2 = 14.9 min. Peak at t_2 is the major enantiomer.

Compound 16—Catalytic version (Table 1, entry 9): To a suspension of DDQ (0.147 g, 20 mol-%), PPTS (1.63 g, 6.48 mmol), CAN (3.51 g, 6.48 mmol), and 4 Å MS (3.6 g) on CH_3CN (120 mL) was added a solution of **17** (1.80 g, 3.24 mmol) in CH_3CN (10 mL). The suspension was stirred at -38 °C for 16 h and quenched with Et3N (1 mL). The mixture was filtered through a silica gel column with hexanes/ethyl acetate (v/v =95:5) as eluent. The concentrated product was purified by silica gel chromatography with hexanes/ethyl acetate (v/v = 95:5) as eluent to give methylenetetrahydropyran **16** as a reddish oil (1.17 g, 71%). The analytical data comply with the reported value.

Compound 36—To a reaction vial charged with with isobutyramide (20.9 mg, 0.24 mmol) in diethyl ether (1.2 mL) was added Et3N (56 μL, 0.4 mmol) and Cy_2BCl (0.24 mL, 1 M in hexanes, 0.24 mmol) in sequence to yield a white suspension. The mixture was stirred at 0 °C for 15 min, and to it was added cyclohexanecarbaldehyde (24 μL, 0.2 mmol). After being stirred at 0 °C for 1 h, the reaction mixture was loaded onto a silica gel column with 0.5 mL of CH_2Cl_2 to dissolve the white precipitate. The column was eluted with hexanes/ethyl acetate = 3:2 to give the product **36** as a white solid (40 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.12 (d, J = 7.1 Hz, 1H), 5.08-5.04 (m, 1H), 3.95 (d, J = 3.7 Hz, 1H), 2.39-2.32 (m, 1H), 1.78-1.65 (m, 4H), 1.54-1.44 (m, 1H), 1.21-0.99 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.2, 77.6, 42.1, 35.6, 28.13, 27.9, 26.2, 25.7, 25.6, 19.5, 19.2. IR (thin film, cm^{-1}) 1693, 1639, 1512, 1366, 1249, 1171, 778. Rf = 0.20 (hexanes/ethyl acetate = 3:2, PMA stain). MS (ESI) *m/z* 222 ($M+Na^+$).

Compound 34—To a solution of TBS glycidol (2 g, 10.6 mmol) in THF (20 mL) was added Li_2CuCl_4 (5.3 mL, 0.1 M in THF) and isoprenyl magnesium bromide (0.5 M in THF, 26 mL, 13 mmol) at −78 °C. After stirring at −78 °C for 15 min and at room temperature for 2 h, crotonyl chloride (1.22 mL, 12.8 mmol) was added, and the reaction mixture was stirred at 0 °C for 5 min. Then the reaction was quenched with water and extracted with ethyl acetate twice. The combined organic layer was washed with sat. $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography with hexanes/ethyl acetate = 97:3 as eluent to give TBS ether as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98-6.91 (m, 1H), 5.81 (dd, J = 1.6, 15.5 Hz, 1H), 5.23-5.16 (m, 1H), 4.77 (s, 1H), 4.71 (s, 1H), 3.66 (d, J = 5.2 Hz, 2H), 2.36 (dd, J = 7.3, 13.9 Hz, 1H), 2.24 (dd, J = 6.0, 13.8 Hz, 1H), 1.86 (dd, J = 1.5, 6.9 Hz, 3H), 1.75 (s, 3H), 0.88 (s, 9H), 0.02 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 144.4, 141.5, 122.8, 113.1, 72.2, 64.0, 38.9, 25.7, 22.5, 18.1, 17.9, -5.4. IR (thin film, cm^{-1}) 2930, 1722, 1657, 1258, 1182, 1101, 836, 777. Rf = 0.31 (hexanes/ethyl acetate = 97:3, PMA stain). MS (ESI) *m/z* 321 ($M+Na^+$).

To a solution of TBS ether prepared as described above (50 mg, 0.168 mmol) in MeOH/CH_2Cl_2 (v/v 4:1, 5 mL) was added HCl (170 μL, 2 M) and water (170 μL). Then the reaction was quenched with sat. $NaHCO_3$ and diluted with water. The aqueous layer was extracted with ethyl acetate three times. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography with hexanes/ethyl acetate (7:3) as eluent to give the alcohol as a colorless oil (25 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05-6.93 (m, 1H), 5.85 (dd, J = 1.7, 15.5 Hz, 1H), 5.15-5.10 (m, 1H), 4.81 (s, 1H), 4.76 (s, 1H), 3.78-3.72 (m, 1H), 3.68-3.62 (m, 1H), 2.37 (dd, J = 7.5, 14.1 Hz, 1H), 2.30 (dd, J = 6.0, 14.2 Hz, 1H), 2.06 (t, J = 6.0 Hz, 1H), 1.88 (d, J = 5.9 Hz, 3H), 1.77 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.5, 145.3, 141.0, 122.4, 113.5, 73.1, 64.5, 39.0, 22.4, 17.9. IR (thin film, cm^{-1}) 3441, 2918, 1719, 1655, 1445, 1310, 1293, 1184, 1103, 1048, 969, 894, 838. Rf = 0.28 (hexanes/ethyl acetate = 7:3, I_2 or $KMnO_4$ stain). MS (ESI) *m/z* 207 ($M+Na^+$).

To a suspension of the above alcohol (25 mg, 0.136 mmol) and $NaHCO_3$ (0.1 g, 1.2 mmol) in CH_2Cl_2 (5 mL) was added Dess-Martin reagent (0.68 mL, 0.3 M in CH_2Cl_2, 0.204 mmol). The mixture was vigorously stirred for 2 h and quenched with a $Na_2S_2O_3$/$NaHCO_3$ solution. The aqueous layer was extracted with CH_2Cl_2 twice. The combined organic layer was dried (Na_2SO_4) and concentrated. The residue was filtered through a silica gel plug with hexanes/ethyl acetate (7:3) as eluent to give **34** as a colorless oil (21 mg, 83%). The compound was unstable and used immediately in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.56 (s, 1H), 7.13-7.01 (m, 1H), 5.92 (dd, J = 1.7, 15.6 Hz, 1H), 5.20 (dd, J = 5.7, 9.0 Hz, 1H), 4.85 (s, 1H), 4.78 (s, 1H), 2.56 (dd, J = 4.6, 14.9 Hz, 1H), 2.45 (dd, J = 8.9, 15.8 Hz, 1H), 1.91 (dd, J = 1.6, 6.9 Hz, 3H), 1.76 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.3, 165.6, 146.6, 139.4, 121.4, 114.3, 76.1, 36.9, 22.3, 18.0. IR (thin film, cm^{-1}) 2943, 2851,

1722, 1656, 1444, 1377, 1294, 1263, 1178, 1105, 969, 898, 837, 688. Rf = 0.52 (hexanes/ethyl acetate = 7:3, I_2 stain or $KMnO_4$). MS (ESI) *m/z* 205 ($M+Na^+$).

Compound 37—To a solution of amide **35** (10.4 mg, 0.12 mmol) and Et3N (28μL), 0.2 mmol) in diethyl ether (0.4 mL) was added Cy_2BCl solution (0.20 mL, 0.6 M in hexanes, 0.12 mmol). The resulting white suspension was stirred at 0 °C for 15 min, and to it was added aldehyde **34** (28.4 mg, 0.1 mmol) in diethyl ether (0.2 mL). After being stirred at 0 °C for 1 h, the mixture was quenched with THF/phosphate buffer (pH = 7.4)/30% H_2O_2 (v/v/v = 1:1:1, 2 mL). Then the aqueous layer was extracted with ethyl acetate three times. The combined organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography with hexanes/ethyl acetate (v/v = 1:1) as eluent to yield **37** as a mixture of diastereomixtures in the ratio of 1:1, (15 mg, 56%). ^1H NMR (300 MHz, $CDCl_3$) δ 10.2 (s, 1H), 9.9 (s, 1H), 7.06-6.93 (m, 2H), 6.70 (d, J = 7.6 Hz, 1H), 6.60 (d, J = 8.3 Hz, 1H), 5.86-5.84 (m, 1H), 5.81-5.79 (m, 1H), 5.16-5.07 (m, 2H), 4.82-8.81 (m, 2H), 4.75-4.74 (m, 2H), 2.48-2.34 (m, 4H), 2.15-2.02 (m, 2H), 1.90-1.87 (m, 6H), 1.75 (s, 3H), 1.73 (s, 3H). 1.20-1.16 (m, 12H). IR (thin film, cm-1) 3339, 1711, 1659, 1531, 1444, 1293, 1183, 1103, 1045, 969, 893. Rf = 0.35 (hexanes/ethyl acetate = 1:1, $KMnO_4$). MS (ESI) *m/z* 292 (M+Na +).

Compounds 1, 39 and 40—Amidation using diphenyl phosphate as catalyst. The reaction mixture containing dactylolide **2** (0.5 mg, 1.31 μmol), amide **38** (0.30 mg, 2.76 μmol) and diphenyl phosphate (0.03 mg, 0.13 μmol) in CH_2Cl_2 (30 μL) was stirred at 23 °C for 14 h. The mixture was loaded onto a silica gel column with hexanes/ethyl acetate (1:1) as eluent to get a mixture of **1**, **38**, **39** and **40** that was further separated on HPLC (IC, hexanes/i-PrOH = 1:1, 0.5 mL/min, 254 nM). The ratio of **1** to **39** was close to 1:1.

Bisamide 40—^1H-NMR (500 MHz, $CDCl_3$) δ 7.66 (dd, J = 12.1, 14.7 Hz, 1H), 7.48-7.41 (m, 2H), 6.85-6.80 (m, 1H), 6.45-6.39 (m, 3H), 6.33 (d, J = 7.3 Hz, 1H), 6.10 (d, J = 11.6 Hz, 1H), 6.06-5.98 (m, 2H), 5.93 (d, J = 14.7 Hz, 1H), 5.92 (d, J = 16.2 Hz, 1H), 5.68-5.61 (m, 2H), 5.41 (d, J = 11.0 Hz, 1H), 5.40 (d, J = 11.0 Hz, 1H), 5.16 (d, J = 8.0 Hz, 1H), 4.72 (s, 2H), 4.17 (d, J = 13.6 Hz, 1H), 3.97-3.93 (m, 1H), 3.26 (t, J = 10.4 Hz, 1H), 3.00 (d, J = 13.4 Hz, 1H), 2.39-2.33 (m, 2H), 2.24-2.19 (m, 2H), 2.13 (d, J = 12.7 Hz, 1H), 2.06 (d, J = 13.6 Hz, 1H), 1.93 (d, J = 12.4 Hz, 2H), 1.86 (d, J = 3.9 Hz, 6H), 1.79 (s, 3H), 1.71 (s, 3H). MS (ESI) m/z 611 ($M+Na^+$).

(-)-Zampanolide 1 and 39—To a flask containing (-)-**2** (7 mg, 18.3 μmol), and (2*Z*, 4*E*)-hexa-2,4-dienamide **38** (6.1 mg, 55 μmol), (*S*)-TRIP **41** (2.7 mg, 20 mol-%) in CH_2Cl_2 (0.7 mL) was added. The resulting mixture was stirred at 23 °C for 12 h. After this period, the reaction was loaded on a short silica gel column and eluted with hexanes/ethyl acetate (3:2) to provide a crude mixture of (*S*)-TRIP **41**, **1** and **39**. The mixture was separated in HPLC to give (-)-zampanolide **1** (4.6 mg, 51%) and **39** (1.6 mg, 18%). HPLC condition: hexanes/*i*-PrOH = 1:1, 0.5 mL/min, 254 nm, $t_{(S)\text{-TRIP}}$ = 7.4 min, $t_{\text{amide}\mathbf{38}}$ = 12.5 min, $t_{\text{zampanolide }\mathbf{1}}$= 15.3 min, $t_{\mathbf{39}}$ = 23.3 min.

Zampanolide 1—Rf = 0.35 (hexanes/ethyl acetate = 1:1, UV, PMA). $[\alpha]_D^{20}$ = -94 (*c* 0.08, CH_2Cl_2). ^1H-NMR (500 MHz, $CDCl_3$) δ 7.65 (dd, *J* = 11.6, 14.9 Hz, 1H), 7.45-7.40 (m, 1H), 6.85-6.79 (m, 1H), 6.46 (t, *J* = 11.3 Hz, 1H), 6.31 (d, *J* = 7.8 Hz, 1H), 6.11 (d, *J* = 11.9 Hz, 1H), 6.08-6.02 (m, 1H), 5.95 (d, *J* = 15.0 Hz, 1H), 5.94 (d, *J* = 16.4 Hz, 1H), 5.46-5.43 (m, 2H), 5.31-5.27 (m, 1H), 5.20 (d, *J* = 7.9 Hz, 1H), 4.73 (s, 2H), 4.13 (d, *J* = 13.0 Hz, 1H), 3.98-3.94 (m, 1H), 3.66 (brs, 1H), 3.29 (t, *J* = 10.5 Hz, 1H), 3.04 (d, *J* = 13.6 Hz, 1H), 2.41 (d, *J* = 13.6 Hz, 1H), 2.38-2.29 (m, 1H), 2.29-2.20 (m, 2H), 2.14 (d, *J* = 13.4 Hz, 1H), 2.09 (d, *J* = 13.4 Hz, 1H), 1,97-1.91 (m, 2H), 1.87 (d, *J* = 7.0 Hz, 3H), 1.81 (s, 3H), 1.72 (s,

3H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ197.9, 167.6, 166.8, 146.3, 143.8, 143.6, 143.5, 140.2, 140.1, 132.0, 131.4, 129.9, 128.1, 125.2, 120.2, 116.8, 109.1, 76.5, 75.8, 75.3, 71.4, 45.1, 41.8, 40.9, 40.6, 40.1, 23.6, 18.6, 16.6. IR (thin film, cm^{-1}) 3368, 2924, 2854, 2360, 1636, 1539, 1456, 1357, 1281, 1260, 1210, 1149, 1086, 979, 889, 803, 666. MS (ESI) *m/z* 518 (M $+Na^+$). HRMS(ESI) calculated for ($C_{29}H_{37}NO_6Na$) 518.2519, found 518.2520.

Zampanolide diastereomer 39—Rf = 0.35 (hexanes/ethyl acetate = 1:1, UV, PMA). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.66 (dd, *J* = 11.6, 15.0 Hz, 1H), 7.46-7.39 (m, 1H), 6.86-6.80 (m, 1H), 6.46 (t, *J* = 11.3 Hz, 1H), 6.35 (d, *J* = 7.5 Hz, 1H), 6.12 (d, *J* = 11.6 Hz, 1H), 6.06 (dd, *J* = 6.9, 15.0 Hz, 1H), 6.00 (d, *J* = 15.0 Hz, 1H), 5.94 (d, *J* = 16.3 Hz, 1H), 5.54-5.51(m, 1H), 5.45 (d, *J* = 11.2 Hz, 1H), 5.37-5.31 (m, 1H), 5.24 (d, *J* = 8.6 Hz, 1H), 4.73 (brs, 2H), 4.11 (d, *J* = 13.7 Hz, 1H), 3.98-3.94 (m, 1H), 3.60 (br, 1H), 3.34-3.27 (m, 1H), 3.05 (d, *J* = 13.7 Hz, 1H), 2.51 (dd, *J* = 11.0, 13.8 Hz, 1H), 2.39-2.23 (m, 3H), 2.15 (d, *J* = 13.7 Hz, 1H), 2.10 (d, *J* = 13.1 Hz, 1H), 2.00-1.93 (m, 2H), 1.87 (d, *J* = 6.6 Hz, 3H), 1.82 (s, 3H), 1.71 (s, 3H).

Biological Methods—Tubulin and heat-treated microtubule-associated proteins were prepared as described previously.[23] Evaluation of compound-induced microtubule assembly by turbidimetry at 350 nm was performed as described previously.[24] Evaluation of cytotoxicity of agents against the MCF-7, OVCAR-8, and NCI/ADR-RES human cancer cell lines, generously provided by the National Cancer Institute drug screening group, was performed by the rhodamine B method,[25] in which cellular protein is the parameter quantitated. Paclitaxel was generously provided by the Drug Chemistry and Synthesis Branch of the National Cancer Institute.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support for this work was provided in part by the National Institutes of Health and Purdue University.

References

1. Tanaka J, Higa T. Tetrahedron Lett. 1996; 37:5535–5538.
2. Field JJ, Singh AJ, Kanakkanthara A, Halafihi T, Northcote PT, Miller JH. J Med Chem. 2009; 52:7328–7332. [PubMed: 19877653]
3. Qi YK, Ma ST. ChemMedChem. 2011; 6:399–409. [PubMed: 21302362]
4. Cutignano A, Bruno I, Bifulco G, Casapullo A, Debitus C, Gomez-Paloma L, Riccio R. Eur J Org Chem. 2001:775–778.
5. a) Smith AB III, Safonov IG, Corbett RM. J Am Chem Soc. 2001; 123:12426–12427. [PubMed: 11734051] b) Smith AB III, Safonov IG, Corbett RM. J Am Chem Soc. 2002; 124:11102–11113. [PubMed: 12224958]
6. a) Hoye TR, Hu M. J Am Chem Soc. 2003; 125:9576–9577. [PubMed: 12904009] b) Uenishi J, Iwamoto T, Tanaka J. Org Lett. 2009; 11:3262–3265. [PubMed: 19586001]
7. Ghosh AK, Cheng X. Org Lett. 2011; 13:4108–4111. [PubMed: 21749032]
8. a) Smith AB III, Safonov IG. Org Lett. 2002; 4:635–637. [PubMed: 11843610] b) Ding F, Jennings MP. Org Lett. 2005; 7:2321–2324. [PubMed: 15932188] c) Aubele DL, Wan S, Floreancig PE. Angew Chem. 2005; 117:3551–3554.Angew Chem Int Ed. 2005; 44:3485–3488.d) Sanchez CC, Keck GE. Org Lett. 2005; 7:3053–3056. [PubMed: 15987203] e) Louis I, HungeRford NL, Humphries EJ, McLeod MD. Org Lett. 2006; 8:1117–1120. [PubMed: 16524282] f) Zurwerra D, Gertsch J, Altmann KH. Org Lett. 2010; 12:2302–2305. [PubMed: 20415434] g) Yun SY, Hansen

EC, Volchkov I, Cho EJ, Lo WY, Lee D. Angew Chem. 2010; 122:4357–4359.Angew Chem Int Ed. 2010; 49:4261–4263.

9. Bode ML, Gates PJ, Gebretnsae SY, Vleggaar R. Tetrahedron. 2010; 66:2026–2036.
10. Doroh B, Sulikowski GA. Org Lett. 2006; 8:903–906. [PubMed: 16494470]
11. Narayanan BA, Bunnelle WH. Tetrahedron Lett. 1987; 28:6261–6264.
12. For related DDQ cyclizations, see references: Hayashi Y, Mukaiyama T. Chem Lett. 1987; 9:1811–1814.Ying BP, Trogden BG, Kohlman DT, Liang SX, Xu YC. Org Lett. 2004; 6:1523–1526. [PubMed: 15128226] Zhang Y, Li CJ. Angew Chem. 2006; 118:1983–1986.Angew Chem Int Ed. 2006; 45:1949–1952.Tu W, Liu L, Floreancig PE. Angew Chem. 2008; 120:4252–4255.Angew Chem Int Ed. 2008; 47:4184–4187.Tu W, Floreancig PE. Angew Chem. 2009; 121:4637–4641.Angew Chem Int Ed. 2009; 48:4567–4571.Yu B, Jiang T, Li J, Su Y, Pan X, She X. Org Lett. 2009; 11:3442–3445. [PubMed: 19603799]
13. Claffey MM, Hayes CJ, Heathcock CH. J Org Chem. 1999; 64:8267–8274. [PubMed: 11674747]
14. Chrobok A, Gossinger E, Grunberger K, Kahlig H, White MJ, Wuggenig F. Tetrahedron. 2007; 63:8336–8350.
15. Zhang W, Carter RG. Org Lett. 2005; 7:4209–4212. [PubMed: 16146389]
16. Chatterjee AK, Choi TL, Sanders DP, Grubbs RH. J Am Chem Soc. 2003; 125:11360–11370. [PubMed: 16220959]
17. Lei B, Fallis AG. Can J Chem. 1991; 69:1450–1456.
18. Jauch J. Angew Chem Int Ed. 2000; 39:2764–2765.
19. Inanaga J, Hirata K, Saeki H, Katsuki T, Yamaguchi M. Bull Chem Soc Jpn. 1979; 52:1989–1993.
20. Dess DB, Martin JC. J Org Chem. 1983; 4:4155.
21. Kiren S, Shangguan N, Williams LJ. Tetrahedron Lett. 2007; 48:7456–7459.
22. For a review of TRIP in the organocatalysis, see: Akiyama T. Chem Rev. 2007; 107:5744–5758. [PubMed: 17983247] for application examples, see: Hoffmann S, Seayad AM, List B. Angew Chem. 2005; 117:7590–7593.Angew Chem Int Ed. 2005; 44:7424–7427.
23. Hamel E, Lin CM. Biochemistry. 1984; 23:4173–4184. [PubMed: 6487596]
24. Pryor DE, O'Brate A, Bilcer G, Díaz JF, Wang Y, Wang Y, Kabaki M, Jung MK, Andreu JM, Ghosh AK, Giannakakou P, Hamel E. Biochemistry. 2002; 41:9109–9115. [PubMed: 12119025]
25. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, Langley J, Cronise P, Vaigro-Wolff A, Gray-Goodrich M, Campbell H, Mayo J, Boyd M. J Natl Cancer Inst. 1991; 83:757–766. [PubMed: 2041050]

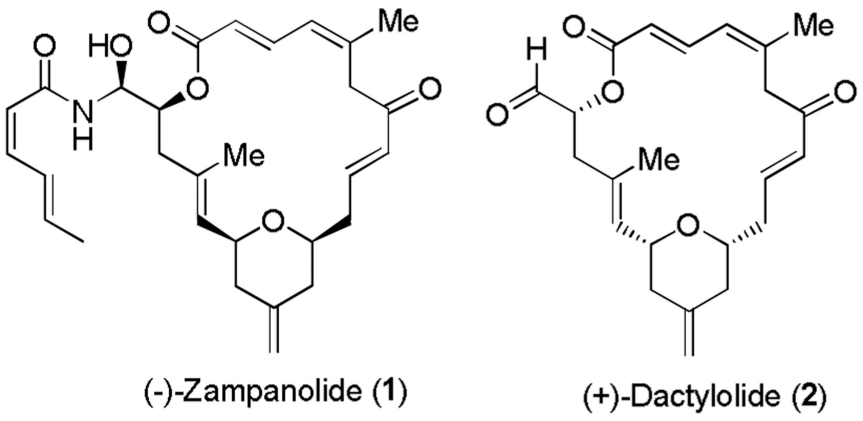


Figure 1. (-)-Zampanolide 1 and (+)-dactylolide 2

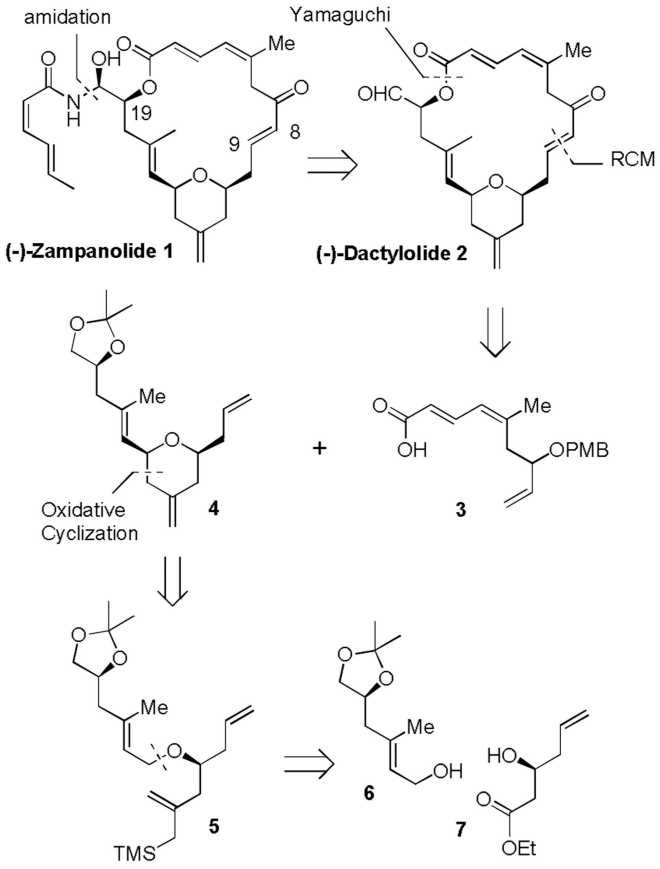


Figure 2.
First generation retrosynthetic analysis.

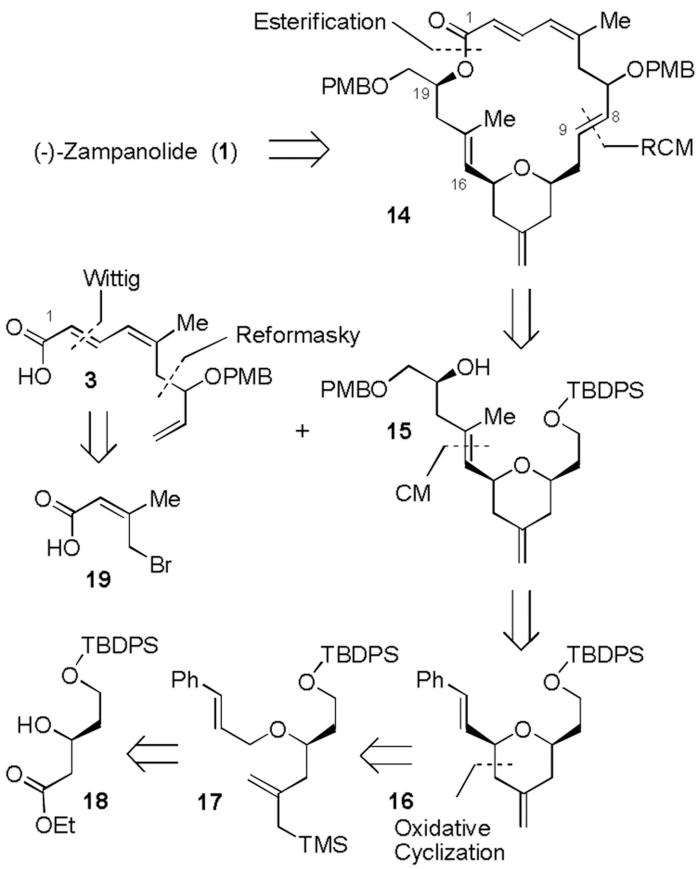
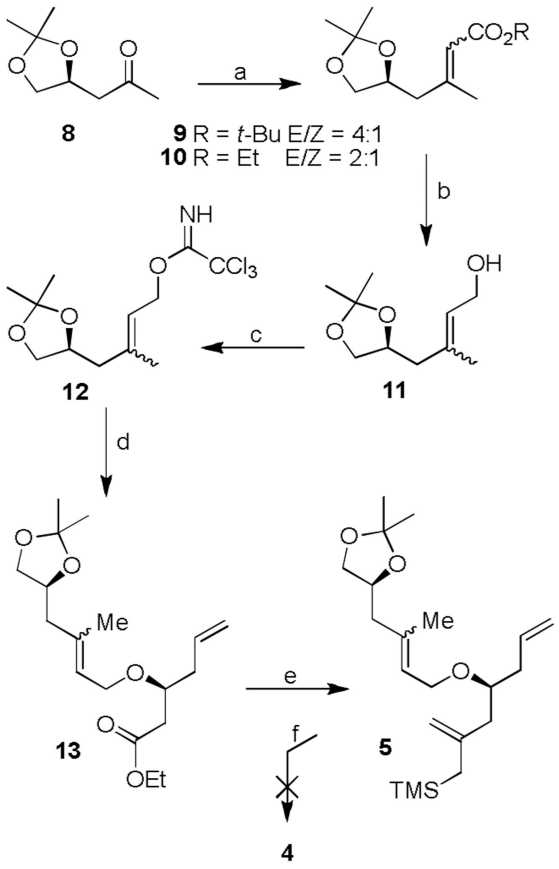
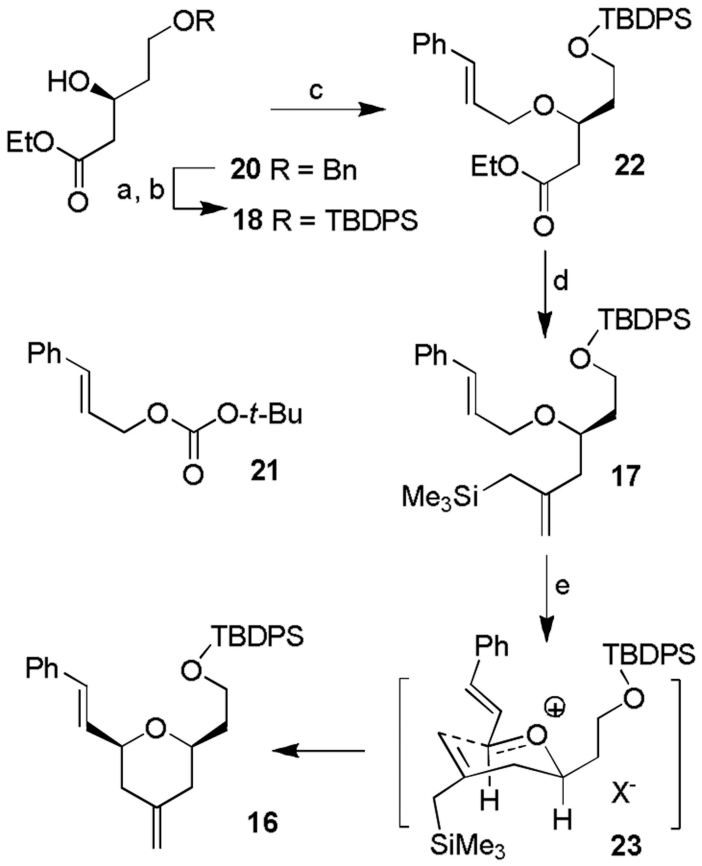


Figure 3.
Our alternative synthetic plan.



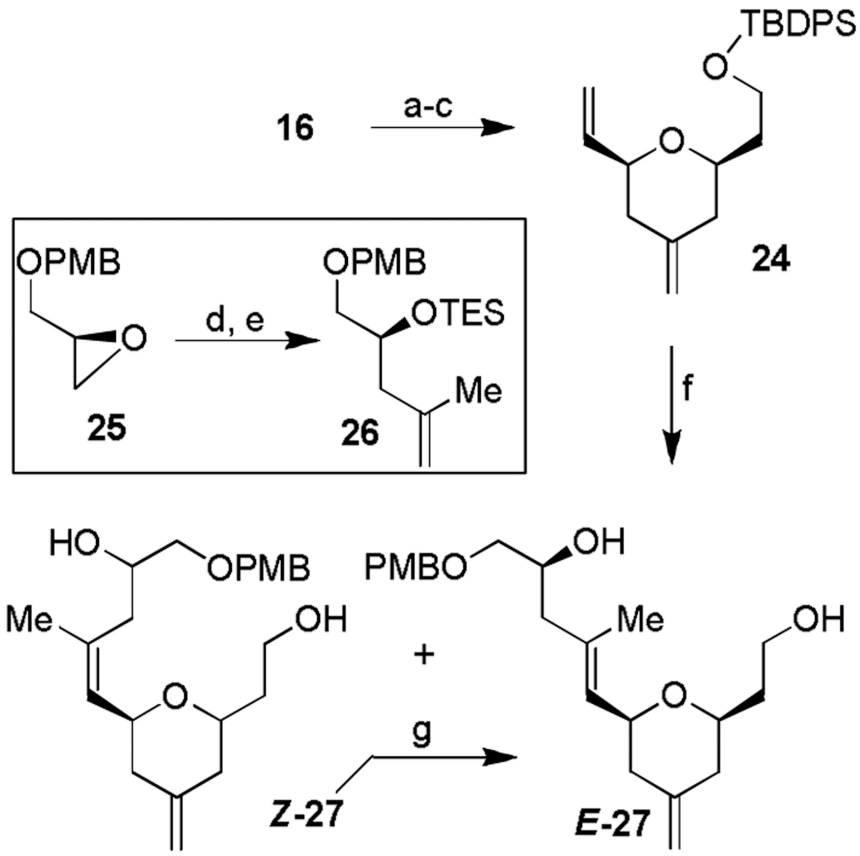
Scheme 1.

Attempted synthesis of dihydropyran **4**. Reagents and Conditions: a) dimethyl-*t*-butyl phosphonoacetate (4.4 equiv.), NaH (3.8 equiv.), THF, 23 °C, 17 h, 74%; b) DIBAL-H (8 equiv.), CH_2Cl_2, −15 °C, 5 min, 91%; c) NaH (10 mol-%), CCl_3CN (1.0 equiv.), ether, 23 °C, 1 h, 98%; d) TIPSOTf (1.1 equiv.), CH_2Cl_2, 23 °C, 12 h, 79% (brsm); e) $CeCl_3$ (5 equiv.), $TMSCH_2MgCl$ (5 equiv.), THF, 23 °C, 12 h, 73%; f) DDQ (2 equiv.), Lewis acid, CH_2Cl_2, −78 °C to 23 °C, 4 h, decomposition.



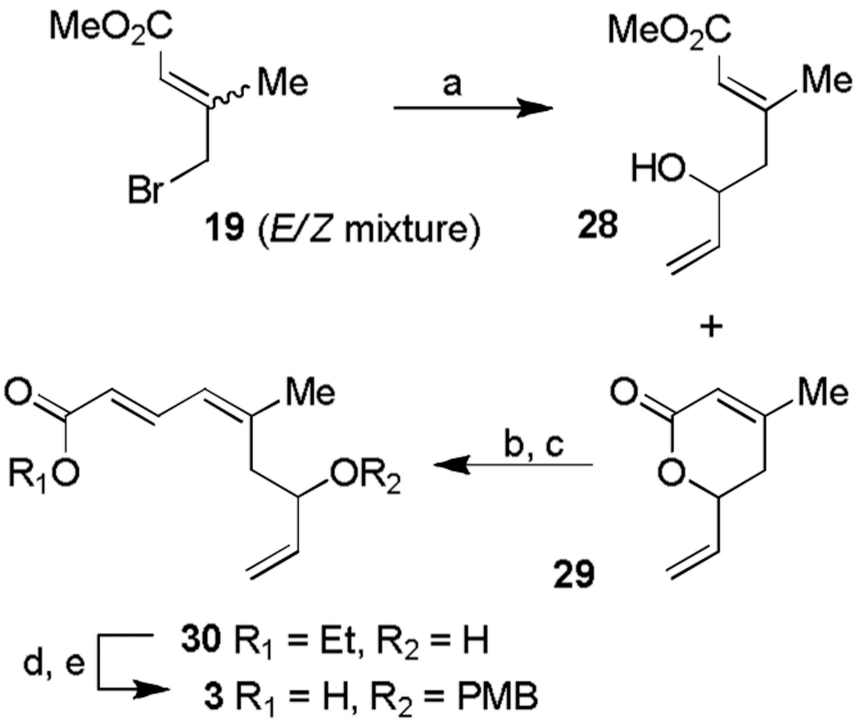
Scheme 2.

Synthesis of tetrahydropyran **16**. Reagents and conditions: a) Pd/C (3 mol-%), H_2, EtOH, 23 °C, 4 h; b) TBDPSCl (1.5 equiv.), imidazole (1.5 equiv.), THF, 0 °C, 1 h, 71% in two steps; c) **21** (2 equiv.), $Pd(PPh_3)_4$ (5.5 mol-%), THF, reflux, 36 h, 71%; d) $CeCl_3$ (5 equiv.), $TMSCH_2MgCl$ (5 equiv.), THF, −78 °C to 23 °C overnight, 81%; e) see Table 1. TBDPS = *tert*-butyldiphenylsilyl, TMS = trimethylsilyl, DDQ = 2,3-dicloro-5,6-dicyano-1,4-benzoquinone, PPTS = pyridinium *p*-toluenesulfonate.



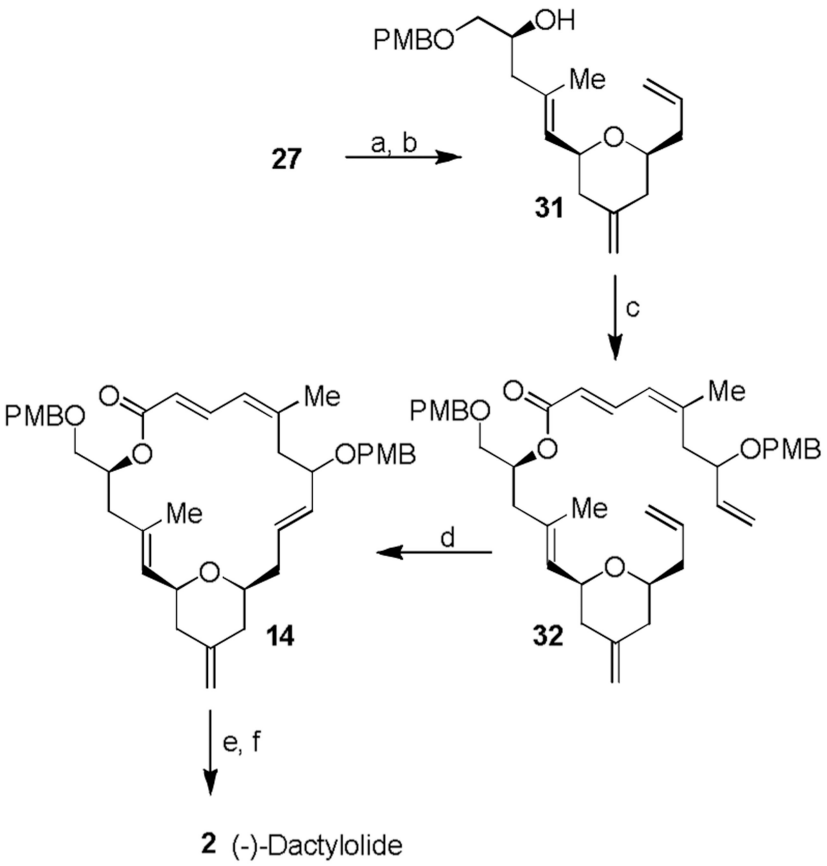
Scheme 3.

Synthesis of tetrahydropyran ***E*-15**. Reagents and conditions: a) AD-mix-α (5 mol-% based on osmium), $MeSO_2NH_2$ (1.5 equiv.), *t*-BuOH/H_2O (1:1), 23 °C, 1 h; b) $NaIO_4$ (1.2 equiv.), MeOH/H_2O, 23 °C, 2 h; c) $Ph_3P{=}CH_2$ (4.5 equiv.), THF, 40 °C, 16 h. 78% overall yield three steps; d) isopropenylMgBr, (2 equiv.), Li_2CuCl_4 (5 mol-%), THF, 0 °C, 2 h, 91%; (e) TESOTf (1 equiv.), 2,6-lutidine (1.5 equiv.), CH_2Cl_2, −30 °C, 1 h, 94%; f) Grubbs II (10 mol-%), **26** (10 equiv.), CH_2Cl_2, reflux, 9 h, 57%; g) HF/Py, THF, 23 °C, 14 h, *E*/*Z* = 1.7:1, overall 81%; h) *hv*, benzene, 23 °C, 4 h, 51% after two runs.



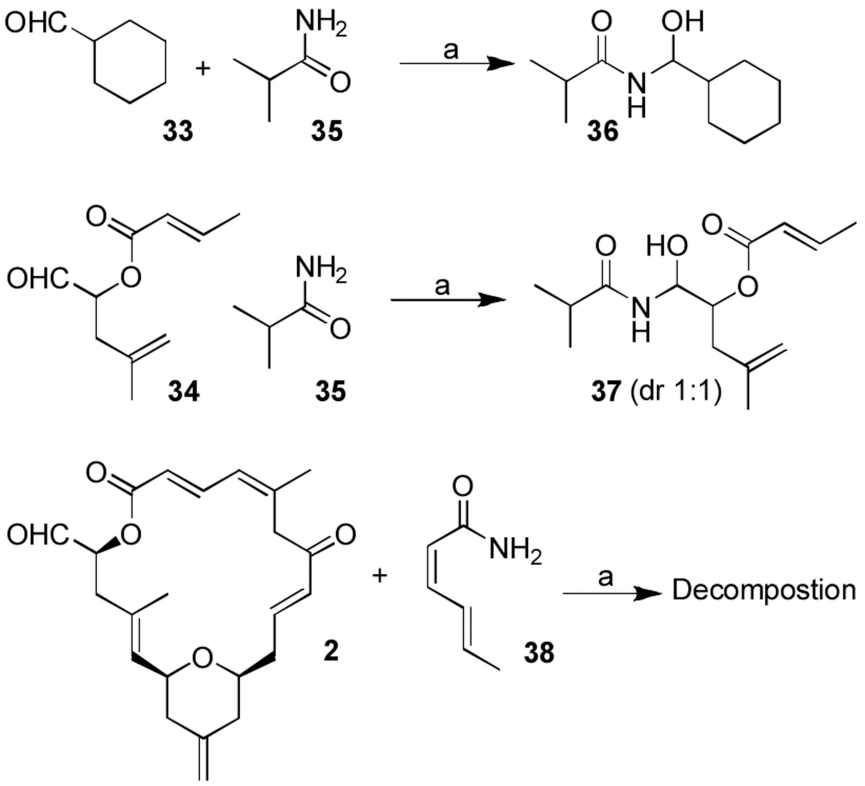
Scheme 4.

Synthesis of polyene fragment **3**. Reagents and conditions: a) Zn (10 equiv.), acrolein (1.0 equiv.), THF, reflux, 2 h, 84% based on effective starting material; b) DIBAL-H (1.0 equiv.), CH_2Cl_2, −78 °C, 2 h; c) Ph_3P=CHCOOEt (1.3 equiv.), toluene, 100 °C, 16 h, 33% two steps; d) 4-methoxybenzyl 2,2,2-trichloroacetimidate (1.3 equiv.), (+)-CSA (10 mol %), 4 Å MS, CH_2Cl_2, 0 °C to 23 °C, 24 h, 80%; e) NaOH (3.0 equiv.), EtOH/H_2O, 0 °C to 23 °C, 12 h, 77%. DIBAL-H = diisobutyl aluminium hydride, CSA = camphor sulfonic acid.



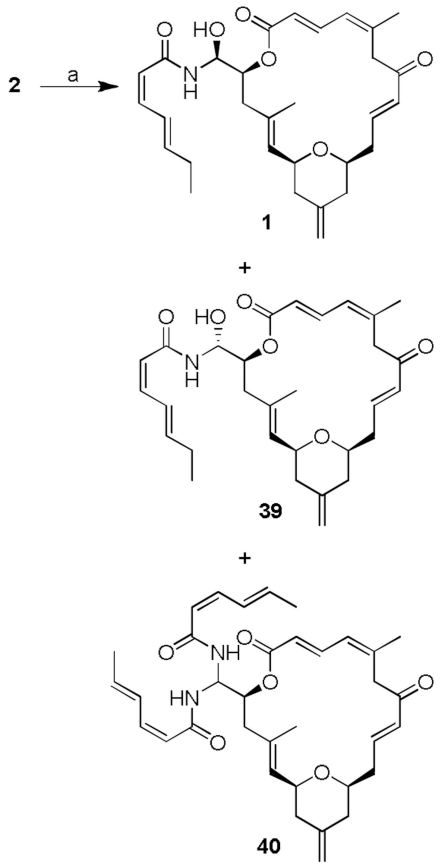
Scheme 5.

The synthesis of (-)-dactylolide **2**. Reagents and conditions: a) $PhI(OAc)_2$ (2.3 equiv.), TEMPO (0.8 equiv.), CH_2Cl_2, 23 °C, 8.5 h; b) PPh_3CH_2 (3.5 equiv.), THF, 40 °C, 12 h, 60% in two steps; c) 2,4,6-trichlorobenzoyl chloride (1.5 equiv.), Et3N (2.6 equiv.), DMAP (1 equiv.), toluene, 23 °C, 20 h, 91%; d) Grubbs II (12 mol-%), benzene, 60 °C, 20 h; e) DDQ (2.2 equiv.), CH_2Cl_2/water, 23 °C, 30 min, 65% in two steps; f) Dess-Martin reagent (6.0 equiv.), $NaHCO_3$ (13.8 equiv.), CH_2Cl_2, 23 °C, 3.5 h, 80%. DMAP = N, N-dimethyl-4-aminopyridine. TEMPO = 2,2,6,6-Tetramethylpiperidine 1-oxyl.



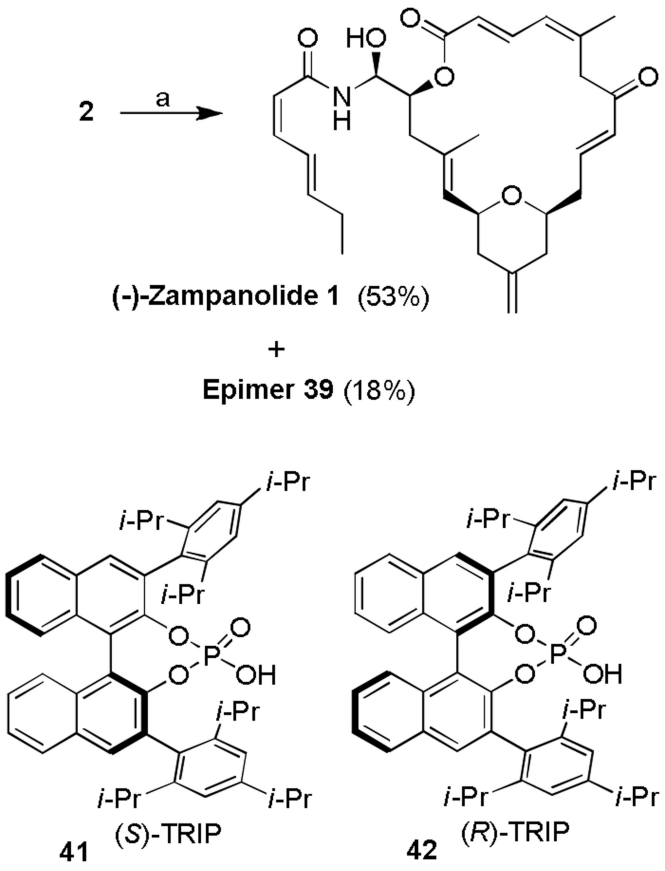
Scheme 6.

Model *N*-acylaminal reaction. Reagents and conditions: a) amide (10 equiv.), Cy_2BCl (9 equiv.), Et_3N (9 equiv.), Et_2O, 0 °C, 1 h.



Scheme 7.

Brφnsted acid catalyzed direct amidation of **2** and **38**. a) diphenylphosphoric acid (10 mol-%), CH_2Cl_2, 23 °C, 14 h. Product distribution (**1 : 39 : 40** = 2.7 : 2.5 : 1) was measured by HPLC.



Scheme 8.

Synthesis of (-)-zampanolide using (*S*)-TRIP catalyzed amidation: a) amide **38** (3 equiv.), (*S*)-TRIP (20 mol-%), CH_2Cl_2, 23 °C, 14 h.

Table 1

Reaction optimization for oxidative cyclization of 17[a]

Entry	Acid	Solvent	Yield
1	$InCl_3$	CH_2Cl_2	57%
2	$InCl_3$[b]	CH_2Cl_2	55%
3	$AlCl_3$	CH_2Cl_2	N.D.
4	$LiClO_4$	CH_2Cl_2	41%
5	$LiClO_4$[b]	CH_2Cl_2	46%
6	$TiCl_4$	CH_2Cl_2	N.D.
7	CSA	CH_2Cl_2	65%
8	CSA	CH_3CN	69%
9	PPTS	CH_3CN	71% [c]
10	PPTS	CH_3CN	81% [d]

[a]Reaction conditions: the reaction was carried out on a 0.5 mmol scale, acid (1.5 equiv.), 4 Å MS, for CH_2Cl_2 at −78 °C, for CH_3CN at −38 °C, 6-8 h;

[b]with 2,6-dichloropyridine (1.5 equiv.);

[c]20 mol-% DDQ and 2 equiv CAN

[d]4 grams scale **17**. CAN = ceric ammonimum nitrate.

Table 2

The cytotoxic activity of compounds against three human cancer cell lines.[a]

	IC_{50} (nM) ± Standard deviation		
Compounds	**MCF-7**	**OVCAR 8**	**NCI/ADR-RES**
1	4.0 ± 0.5	20 ± 0	25 ± 7
39	200 ± 0	250 ± 70	750 ± 200
40	430 ± 200	300 ± 0	750 ± 400
Paclitaxel	2.0 ± 0	7.5 ± 2	>5,000

[a]A standard deviation of 0 indicates the same value was obtained in all experiments (usually two independent determinations)