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

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A detailed account of the enantioselective total synthesis of (–)-zampanolide, a macrolide marine natural product with high anticancer activity, is described. For the synthesis of the 4-methylenetetrahydropyran unit of (–)-zampanolide, we initially 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 for the construction of a trisubstituted olefin, a ring-closing metathesis to form a highly functionalized macrolactone, and a chiral phosphoric acid promoted formation of an *N*-acyl aminal to furnish (–)-zampanolide stereoselectively and in good yield. The synthetic (–)-zampanolide had effects on cultured cells and on tubulin assembly consistent with the properties reported for the natural product.

Introduction

Zampanolide (**1**; Figure 1), an unsaturated 20-membered macrolide with an *N*-acyl hemiaminal side-chain, was initially isolated by Tanaka and Higa in 1996, from the marine sponge *Fasciospongia rimosa* taken from Okinawa, Japan.[1] More recently, (–)-zampanolide has been isolated from the Tongan marine sponge *Cacospongia mycofijiensis* by Northcote and co-workers.[2] Zampanolide exhibits potent cytotoxicity against a variety of cell lines. It has shown IC_{50} values of 1.1 and 2.9 nM against the SKM-1 and U937 cell lines, respectively. Moreover, it is very active against HL-60, 1A9, and, in particular, A2780AD cells, which show resistance to paclitaxel based on overexpression of the 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) has been isolated from the sponge *Dactylospongia* sp.[4] Interestingly, it displays only modest cytotoxicity, however, it contains opposite configurations of the macrolide core of (–)-zampanolide (**1**). The biological relevance of the macrolide core of (+)-dactylolide and (–)-zampanolide is not clear.

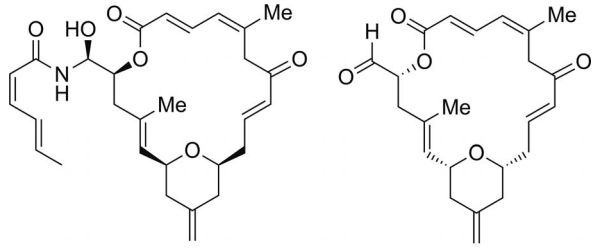

Figure 1. (–)-Zampanolide (**1**) and (+)-dactylolide (**2**).

Both zampanolide and dactylolide have attracted much interest in synthesis and in the biological studies of structural variants.[3] Smith et al. 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] have 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 by direct *N*-acyl aminal formation from unnatural (–)-dactylolide, albeit in only 12% yield. Other major products include the zampanolide epimer and bis-acylated products.[6] This direct transformation was achieved through a strategy promoted by an Al reagent[6a] or catalyzed by camphorsulfonic acid (CSA),[6b] although in both cases no stereoselectivity was observed. Herein, we

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report the details of our synthetic efforts that have led to a convergent total synthesis of (–)-zampanolide. The synthesis features a novel intramolecular oxidative cyclization reaction and the organocatalyic *N*-acyl aminal formation of dactylolide, which stereoselectively furnishes (–)-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 stereoselective amidation to provide (–)-zampanolide. Strategic disconnection of (–)-dactylolide at the C8–C9 and C1–C19 ester bonds results in the 4-methylenetetrahydropyran derivative **4** and the unsaturated carboxylic acid **3**. Our plan was to form an ester with the C19 alcohol and carboxylic acid **3** and then carry out ring-closing metathesis to construct the C20 macrolactone core of (–)-dactylolide. It was planned to functionalize 4-methylenetetrahydropyran **4** by an intramolecular oxidative cyclization of allyl ether **5**. This substrate can be synthesized from allylic alcohol **6** and optically active β-hydroxy ester **7**.

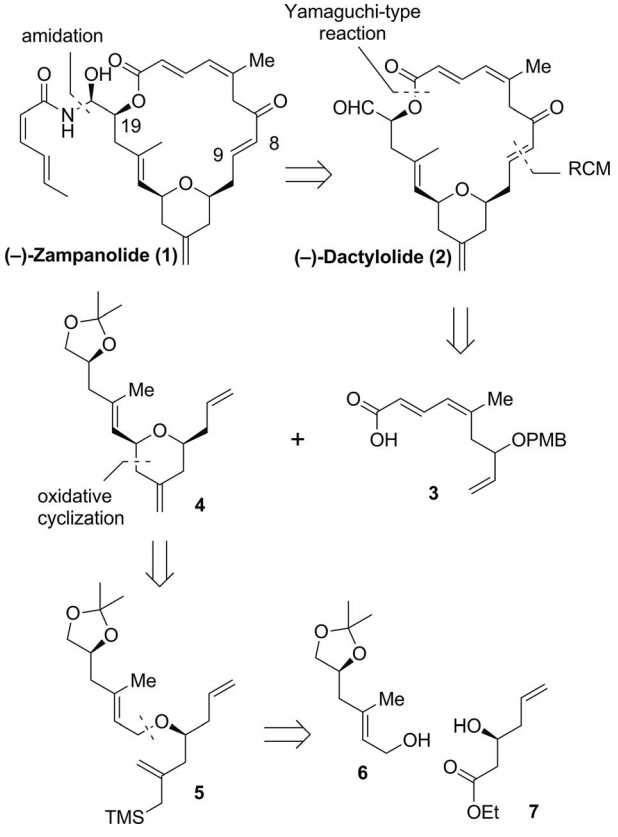
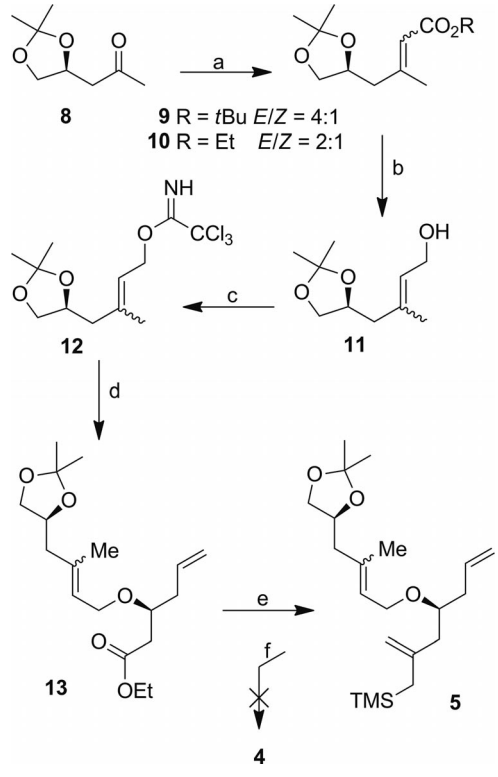


Figure 2. First-generation retrosynthetic analysis.

We first examined the feasibility of the 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 five steps from L-malic acid, as described previously.[9] Horner–Wadsworth–Emmons reaction of ketone **8** with NaH and *tert*-butyl phosphorylacetate at 23 °C afforded α,β-unsaturated ester **9** as a mixture of *E*/*Z* isomers (4:1). In contrast, the use of triethyl phosphonoacetate provided **10** with lower *E*/*Z* selectivity (2:1). The ester was reduced by exposure to excess DIBAL-H to give 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 Brønsted acids and Lewis acids such as TfOH, $BF_3{\cdot}Et_2O$, and TMSOTf. However, these conditions resulted in decomposition of the 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 into allylsilane derivative **5** by using the procedure developed by Narayanan and Bunnelle.[11] We attempted an oxidative cyclization reaction of this allylsilane substrate with DDQ under a variety of reaction condi-



Scheme 1. Attempted synthesis of dihydropyran **4**. Reagents and conditions: a) *tert*-butyl dimethoxyphosphorylacetate (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.), diethyl ether, 23 °C, 1 h, 98%; d) **7** (0.9 equiv.), TIPSOTf (0.34 equiv.), mol. sieves (4 Å), 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 to 23 °C, 4 h, decomposition.

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tions.[12] However, we did 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 macrolactone core **14**. Further disassembly of the macrolactone would give rise to tetrahydropyran derivative **15** and trisubstituted olefin **3**. It was planned to synthesize functionalized 4-methylenetetrahydropyran unit **15** from tetrahydropyran **16** by a cross-metathesis reaction. The tetrahydropyran **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 with 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 Reformatsky reaction of bromo olefin **19** and acrolein followed by a Wittig olefination.

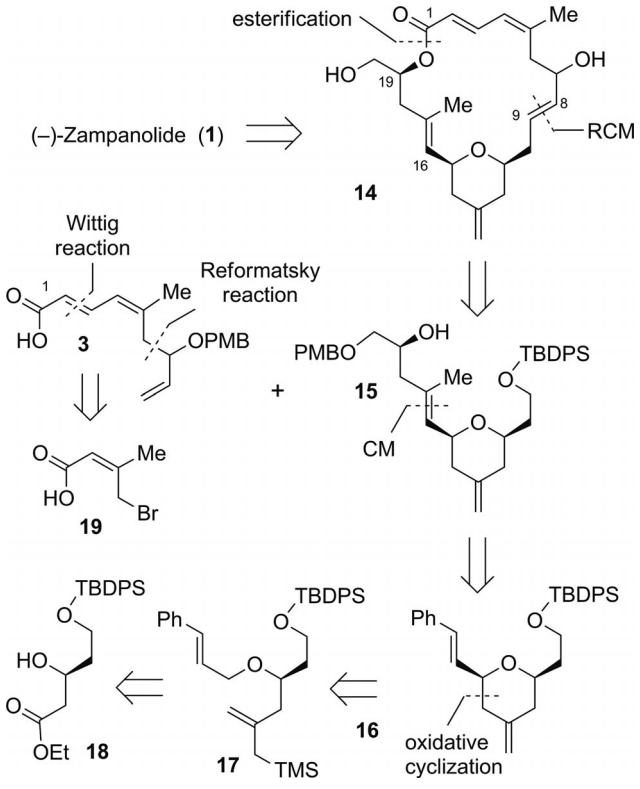
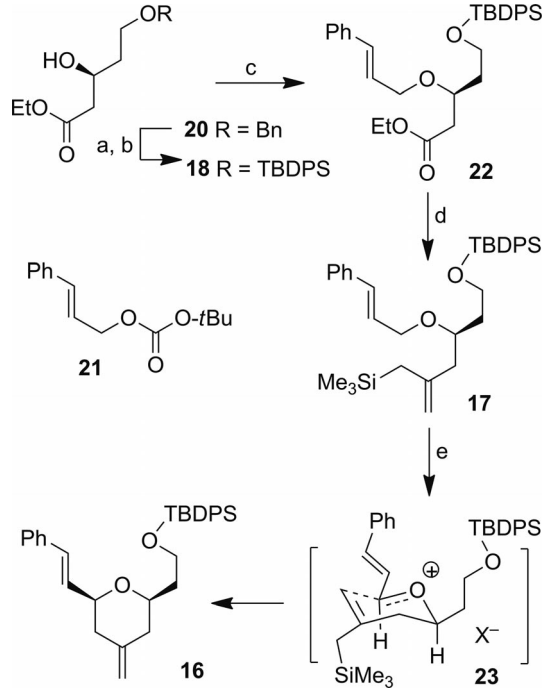


Figure 3. Alternative synthetic plan for (–)-zampanolide.

In accord with our revised plan, optically active β-hydroxy ester **20** was synthesized based upon a Noyori hydrogenation reaction.[13] This ester was converted into 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 hydroxy was then protected as a cinnamyl ether by Tsuji–Trost reaction with a catalytic amount of $[Pd(PPh_3)_4]$ and *tert*-butyl cinnamyl carbonate **21** to provide **22** in 71% yield.[14] The ester group in **22** was converted into allylsilane **17** by treatment with excess trimethylsilylmethylmagnesium chloride in the presence of $CeCl_3$, as described by Bunnelle and co-workers.[11] We then investigated a variety of reaction conditions for the effective oxidative cyclization of key substrate **17**. The results are shown in Table 1. As can be seen, our initial attempt using DDQ in the presence of $InCl_3$ as promoter resulted in the desired tetrahydropyran cyclization product **16** in 57% yield (entry 1) as a single diastereoisomer (by ^1H NMR analysis). The *cis* stereochemical identity of dihydropyran **16** was established by ^1H NMR NOESY experiments. The oxidative cyclization presumably proceeds through the Zimmerman–Traxler transition state shown as 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-g scale at –38 °C in CH_3CN to provide **16** in 81% yield.



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 to 23 °C overnight, 81%; e) see Table 1. TBDPS = *tert*-butyldiphenylsilyl, TMS = trimethylsilyl, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, PPTS = pyridinium *p*-toluenesulfonate.

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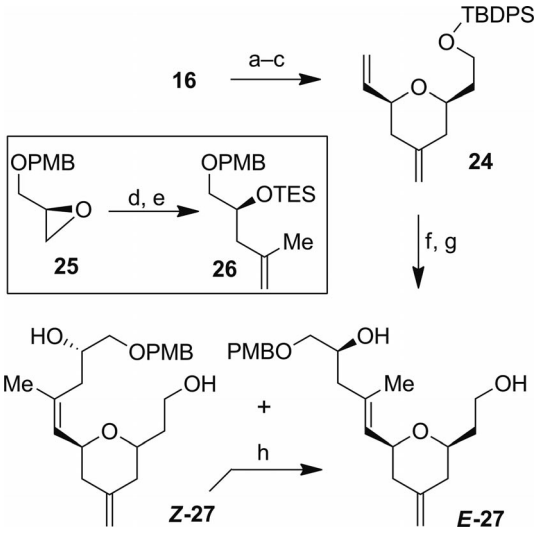
Table 1. Optimization of the oxidative cyclization of **17**.[a]

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]

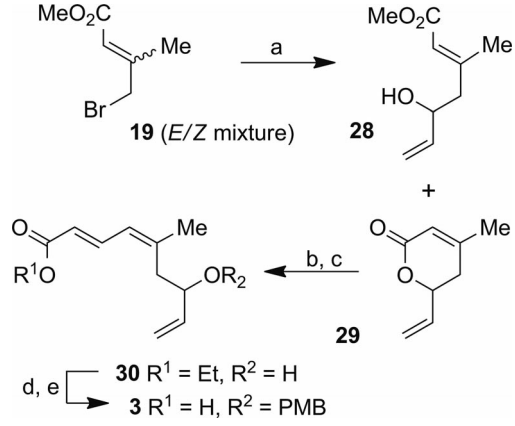
[a] The reaction was carried out on a 0.5 mmol scale. Reaction conditions: acid (1.5 equiv.), mol. sieves (4 Å), at –78 °C for CH_2Cl_2, at –38 °C for CH_3CN, 6–8 h. [b] Performed with 2,6-dichloropyridine (1.5 equiv.). [c] 20 mol-% DDQ and 2 equiv. CAN. [d] Performed with 4 g of **17**. CAN = ceric ammonium nitrate.

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 did not result in the 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 by 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 reaction of **24**, we prepared **26** by ring opening of 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 an *E*/*Z* mixture of isomers (ratio 1.7:1) in 57% combined yield. The mixture of olefins was treated with HF·Py to remove the two 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 [based on residual starting material (brsm), 68%].



Scheme 3. Synthesis of tetrahydropyran (*E*)-**27**. 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 catalyst (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.

The C1–C9 triene carboxylic acid **3** was synthesized by 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 Lei and Fallis.[17] Treatment of this *E*/*Z* mixture with Zn dust followed by reaction with acrolein afforded the *E*-alcohol **28** (47%) and α,β-unsaturated δ-lactone **29** (36%), which results 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 (ethoxycarbonylmethylene)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 over two steps.



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-%), mol. sieves (4 Å), CH_2Cl_2, 0 to 23 °C, 24 h, 80%; e) NaOH (3.0 equiv.), EtOH/H_2O, 0 to 23 °C, 12 h, 77%. DIBAL-H = diisobutylaluminium hydride, CSA = camphorsulfonic acid.

For elaboration of the macrolactone core of (–)-dactylolide and (–)-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 methylenetriphenylphosphorane af-

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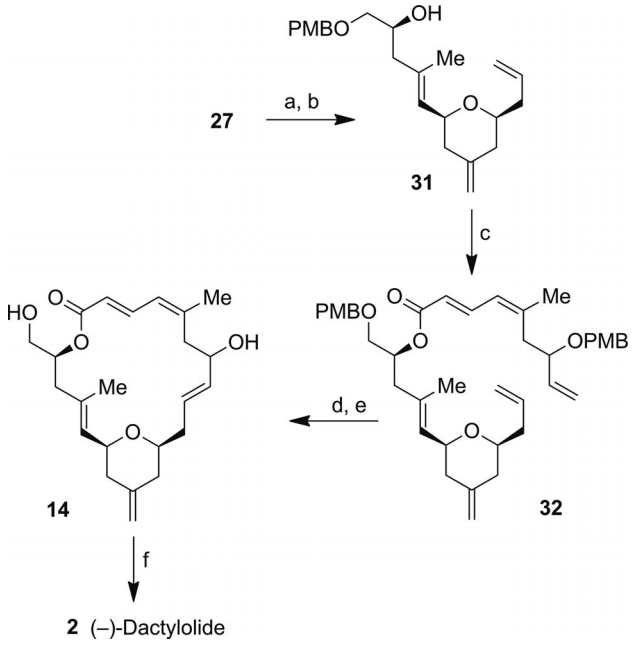
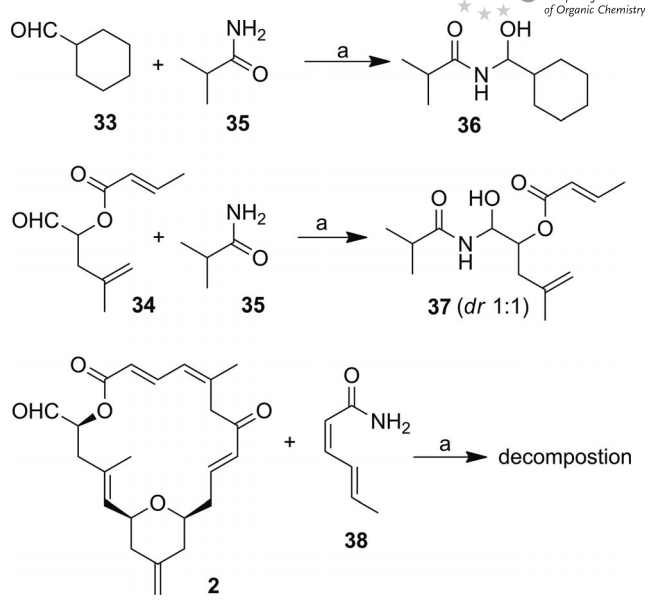
forded olefin **31** in 60% overall yield. The secondary alcohol in **31** was esterified under Yamaguchi conditions[19] with carboxylic acid **3** by using 2,4,6-trichlorobenzoyl chloride in the presence of DMAP to provide a mixture (1:1) of diastereomeric esters **32** in excellent yield. Subjection of this mixture of diastereomers to Grubbs II catalyst (12 mol-%) in benzene at 60 °C for 20 h furnished the corresponding 20-membered macrolactone. Exposure of the macrolactone mixture to DDQ in aqueous CH_2Cl_2 removed the PMB group, providing a diastereomeric mixture (1:1 at C7) of alcohols **14** in 65% yield over two steps. Oxidation of **14** with the Dess–Martin reagent[20] furnished (–)-dactylolide **2** in 80% yield. The spectroscopic data (^1H and ^{13}C NMR) of (–)-**2** is in full agreement with natural (+)-dactylolide except for the optical rotation of (–)-**2** {$[\alpha]_D^{24}$ = –148 (*c* = 0.09, MeOH)}.

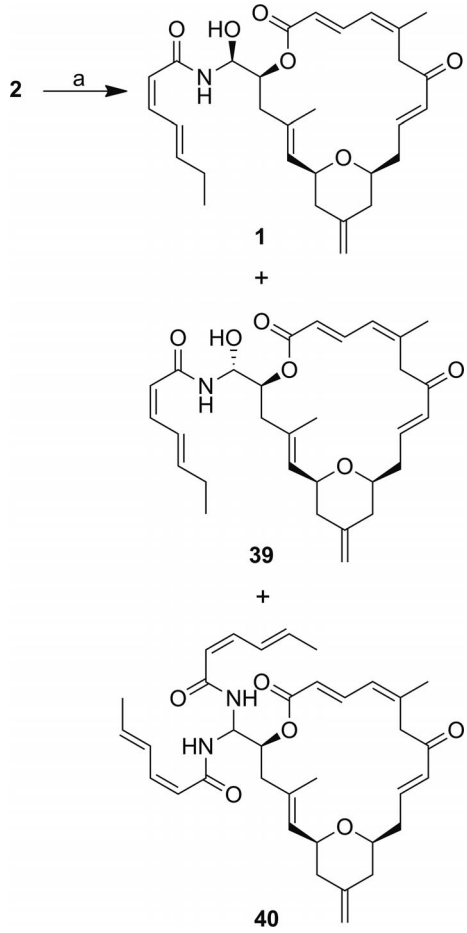


Scheme 5. The synthesis of (–)-dactylolide **2**. Reagents and conditions: a) $PhI(OAc)_2$ (2.3 equiv.), TEMPO (0.8 equiv.), CH_2Cl_2, 23 °C, 8.5 h; b) PPh_3CH_2 (3.5 equiv.), THF, 40 °C, 12 h, 60% in two steps; c) 2,4,6-trichlorobenzoyl chloride (1.5 equiv.), Et_3N (2.6 equiv.), DMAP (1 equiv.), toluene, 23 °C, 20 h, 91%; d) Grubbs II catalyst (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 = 4-(dimethylamino)pyridine. TEMPO = 2,2,6,6-tetramethylpiperidine 1-oxyl.

Previously, direct *N*-acyl aminal formation proceeded only with limited success, providing (–)-zampanolide (**1**) along with a mixture of C-20 diastereomers in poor yield (ca. 12%).[6] To improve this transformation, we initially selected cyclohexanecarbaldehyde (**33**) and aldehyde **34** as model substrates. We investigated *N*-acyl aminal formation by 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. How-



Scheme 6. Model *N*-acyl aminal reaction. Reagents and conditions: a) amide (10 equiv.), Cy_2BCl (9 equiv.), Et_3N (9 equiv.), Et_2O, 0 °C, 1 h.



Scheme 7. Brønsted acid catalyzed direct amidation of **2** and **38**. Reagents and conditions: a) amide **38**, diphenylphosphoric acid (10 mol-%), CH_2Cl_2, 23 °C, 14 h. The product distribution (**1**/**39**/**40** = 2.7:2.5:1) was measured by HPLC.

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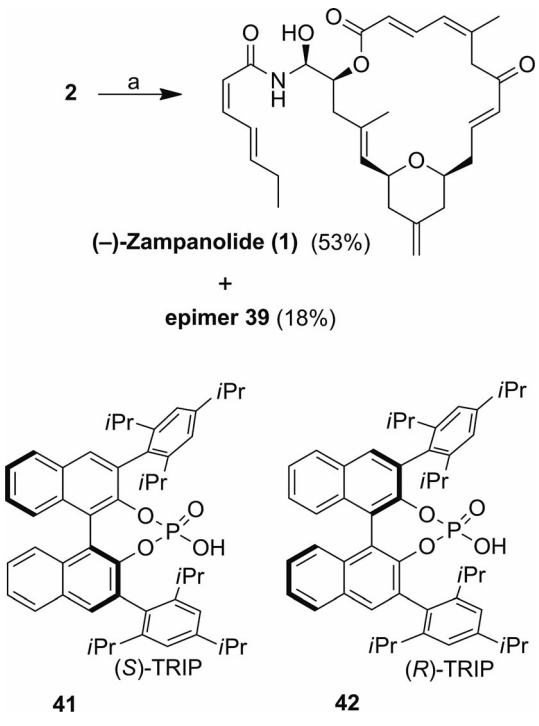
ever, our attempts to carry out this reaction with (–)-dactylolide (**2**) and (2*Z*,4*E*)-hexa-2,4-dienamide **38** were unsuccessful.

We subsequently planned to optimize the conditions for direct amidation reported by Uenishi et al.[6b] Reaction of (–)-dactylolide (**2**) and amide **38** with *p*TsOH was reported to provide (–)-zampanolide (**1**; 12%), the C-20 epimeric product (12%), and the corresponding bis-amide (23%).[6b] In this transformation, the lack of stereoselectivity and the formation of the bis-amide limited the yield of the desired (–)-zampanolide. In an effort to improve the reaction yield and minimize conversion into the bis-amide byproduct, we investigated the reaction of (–)-**2** and amide **38** in the presence of the Brønsted 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 conditions provided (–)-zampanolide (**1**), its epimer **39**, and bis-amide **40** in a 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] on the *N*-acyl aminal reaction of (–)-**2** and amide **38**. As shown in Scheme 8, the reaction of (–)-**2** in the presence of (*S*)-TRIP (**41**; 20 mol-%) at 23 °C for 12 h furnished *N*-acyl aminal derivatives diastereoselectively 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 the bis-amide byproduct **40** was not observed. The spectroscopic data (^1H and ^{13}C NMR) for synthetic (–)-zampanolide {**1**; $[\alpha]_D$ = –94, (*c* = 0.08, CH_2Cl_2)} are in full agreement with those of the natural zampanolide {ref.[1]: $[\alpha]_D$ = –101 (*c* = 0.12, CH_2Cl_2)}.



Scheme 8. Synthesis of (–)-zampanolide (**1**) by the (*S*)-TRIP-catalyzed amidation reaction. Reagents and conditions: a) amide **38** (3 equiv.), (*S*)-TRIP (20 mol-%), CH_2Cl_2, 23 °C, 14 h.

Biological Activity

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 by using several cell lines. Paclitaxel was used as a control in all experiments. With tubulin, **1** strongly induced assembly, as has been reported previously,[2] whereas compounds **39** and **40** showed 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 because such cell lines are highly resistant to paclitaxel and multidrug resistance may cause clinical loss of sensitivity to paclitaxel.

Table 2. Cytotoxic activity of compounds against three human cancer cell lines.[a]

	IC_{50} [nM]		
	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	>5000

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

Compound **1** shows similar 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 the cell lines examined. However, they were more active than paclitaxel in the NCI/ADR-RES cells, which indicates that they, too, are not good substrates for P-glycoprotein.

We also performed a limited study with **1**, **39**, and **40** in human CA46 Burkitt lymphoma cells because 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}. At 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, whereas with **39** and **40**, the concentration used was 5 μM (data not presented).

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Conclusions

A detailed account of the enantioselective total synthesis of (–)-zampanolide (**1**) has been described. Our initial synthetic 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 in which a cinnamyl ether was used in place of the allyl ether. This cyclization method provided the 4-methylenetetrahydropyran derivative in a highly stereoselective manner. The C1–C9 triene carboxylic acid unit **3** was synthesized by a Reformatsky reaction as the key step. Yamaguchi esterification and ring-closing olefin metathesis provided the zampanolide core. Organocatalytic *N*-acyl aminal formation with a chiral phosphoric acid proceeded stereoselectively and in good yield without the formation of the bisamide byproduct. The current synthesis is amenable to a variety of structural analogues of zampanolide. The synthetic zampanolide has 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 oven-dried flasks under argon. Anhydrous solvents were obtained as follows: THF, diethyl ether, and benzene were distilled from sodium and benzophenone, dichloromethane, pyridine, triethylamine, and diisopropylethylamine were distilled from CaH_2. All other solvents were of HPLC grade. ^1H and ^{13}C NMR spectra were recorded with Varian INOVA300-1 and Bruker Avance ARX-400 spectrometers. NMR spectroscopic data were resolved with Mestrec software. IR spectra were recorded with a Perkin–Elmer spectrometer. Optical rotations were recorded with 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 Merck silica gel 60 F_{254} plates. HPLC was performed with an Agilent 1100 instrument.

Compound 9: A solution of *tert*-butyl 2-(dimethoxyphosphoryl)-acetate (8.7 g, 38.8 mmol) in THF (10 mL) was added to a suspension of NaH (1.35 g, 60% in mineral oil, 33.7 mmol) in THF (40 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and at room temperature for 30 min. After cooling to 0 °C, ketone **8** (1.69 g, 10.7 mmol) in THF (10 mL) was added. 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 diethyl ether twice. The combined organic layers were 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 *tert*-butyl 2-(dimethoxyphosphoryl)-acetate. The mixture was subjected to silica gel column chromatography and eluted with hexanes/ethyl acetate (9:1) to yield the *Z* (0.4 g) and *E* isomers (1.7 g) in an overall yield of 74%. *Z* isomer: $[\alpha]_D^{23}$ = –43 (*c* = 2.3, ethyl acetate). R_f = 0.29 (hexanes/ethyl acetate = 90:10, $KMnO_4$ stain). ^1H NMR (400 MHz, $CDCl_3$): δ = 5.69 (s, 1 H), 4.36–4.27 (m, 1 H), 4.07 (dd, *J* = 6.0, 8.1 Hz, 1 H), 3.62 (dd, *J* = 7.1, 8.0 Hz, 1 H), 3.07 (dd, *J* = 4.7, 12.7 Hz, 1 H), 2.68 (dd, *J* = 7.8, 12.7 Hz, 1 H), 1.95 (d, *J* = 1.3 Hz, 3 H), 1.46 (s, 9 H), 1.42 (s, 3 H), 1.35 (s, 3 H) ppm. ^{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 ppm. IR (thin film): $\tilde{\nu}$ = 2981, 1706, 1646, 1500, 1368, 1254, 1221, 1072, 968, 860, 844 cm^{-1}. MS (ESI): *m*/*z* = 279 [M + Na]$^+$. *E* isomer: $[\alpha]_D^{23}$ = –11.7 (*c* = 2.2, ethyl acetate). R_f = 0.28 (hexanes/ethyl acetate = 90:10, $KMnO_4$ stain). ^1H NMR (400 MHz, $CDCl_3$): δ = 5.63 (d, *J* = 1.2 Hz, 1 H), 4.32–4.24 (m, 1 H), 4.06 (dd, *J* = 5.9, 8.1 Hz, 1 H), 3.57 (dd, *J* = 6.8, 8.1 Hz, 1 H), 2.46 (dd, *J* = 6.9, 13.4 Hz, 1 H), 2.27 (dd, *J* = 7.0, 14.1 Hz, 1 H), 2.17 (d, *J* = 1.3 Hz, 3 H), 1.48 (s, 9 H), 1.42 (s, 3 H), 1.36 (s, 3 H) ppm. ^{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 ppm. IR (thin film): $\tilde{\nu}$ = 2982, 1714, 1651, 1455, 1380, 1184, 1110, 1073, 862, 829 cm^{-1}. MS (ESI): *m*/*z* = 279 [M + Na]$^+$.

Compound 11: DIBAL-H (1.18 g, 1.47 mL, 8.3 mmol) was added to a solution of the *E* isomer of **9** (0.85 g, 3.3 mmol) in CH_2Cl_2 (20 mL) at –15 °C. 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 layers were dried (Na_2SO_4) and concentrated. Silica gel chromatography with hexanes/diethyl ether (2:3) as eluent yielded **11** as a colorless oil (0.56 g, 91%). $[\alpha]_D^{23}$ = +1.5 (*c* = 2.6, ethyl acetate). R_f = 0.27 (hexanes/diethyl ether = 2:3, $KMnO_4$ stain). ^1H NMR (400 MHz, $CDCl_3$): δ = 5.63 (t, *J* = 6.8 Hz, 1 H), 4.27–4.18 (m, 1 H), 4.12 (d, *J* = 6.6 Hz, 2 H), 4.01 (dd, *J* = 6.0, 8.0 Hz, 1 H), 3.53 (t, *J* = 7.5 Hz, 1 H), 2.34 (dd, *J* = 7.0, 13.9 Hz, 1 H), 2.17 (dd, *J* = 6.2, 14.0 Hz, 1 H), 1.98 (br. s, 1 H), 1.69 (s, 3 H), 1.39 (s, 3 H), 1.32 (s, 3 H) ppm. ^{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 ppm. IR (thin film): $\tilde{\nu}$ = 3413, 2985, 2935, 1668, 1455, 1371, 1216, 1157, 1062, 999, 831, 790 cm^{-1}. MS (ESI): *m*/*z* = 209 [M + Na]$^+$.

Compound 12: A solution of **11** (400 mg, 2.15 mmol) in diethyl ether (1 mL) was added dropwise 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. 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. The mixture was stirred at –40 °C for 1 h and then at room temperature for 1 h followed by the addition of MeOH (9 μL). The solution was concentrated, suspended in hexanes, and filtered through Celite. The filtrate was concentrated and analyzed by ^1H NMR spectroscopy. The resulting yellow oil **12** (697 mg, 98%) was sufficiently pure to be used in the next step without further purification. $[\alpha]_D^{23}$ = –1.9 (*c* = 2.0, ethyl acetate). R_f = 0.28 (hexanes/ethyl acetate = 90:10, $KMnO_4$ stain). ^1H NMR (400 MHz, $CDCl_3$): δ = 8.27 (s, 1 H), 5.54 (t, *J* = 6.8 Hz, 1 H), 4.82 (d, *J* = 6.8 Hz, 2 H), 4.30–4.21 (m, 1 H), 4.02 (dd, *J* = 5.9, 8.1 Hz, 1 H), 3.57 (t, *J* = 7.5 Hz, 1 H), 2.44 (dd, *J* = 6.7, 13.4 Hz, 1 H), 2.25 (dd, *J* = 6.9, 14.0 Hz, 1 H), 1.79 (s, 3 H), 1.42 (s, 3 H), 1.36 (s, 3 H) ppm. ^{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 ppm. IR (thin film): $\tilde{\nu}$ = 3470, 3344, 2986, 1738, 1662, 1455, 1370, 1291, 1229, 1157, 1068, 984, 827, 799, 650 cm^{-1}. MS (ESI): *m*/*z* = 352 [M + Na]$^+$.

Compound 13: TIPSOTf (25 μL, 93 μmol) was added to a suspension of **7** (40 mg, 0.25 mmol), **12** (90 mg, 0.27 mmol), and mol. sieves (4 Å, 40 mg) in CH_2Cl_2 (1 mL) at –78 °C and the mixture was stirred at room temperature overnight. The reddish suspension was quenched with Et_3N (0.1 mL) and concentrated. The residue was purified by silica gel chromatography with hexanes/diethyl ether (4:1) as eluent to give the target product **13** as a colorless oil (32 mg,

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E/*Z* = 2:1) containing residual **7** (20 mg). The yield was 79% (brsm). R_f = 0.40 (hexanes/ethyl acetate = 4:1, $KMnO_4$ stain). ^1H NMR (300 MHz, $CDCl_3$): δ = 5.87–5.73 (m, 2 H), 5.57–5.35 (m, 2 H), 5.12–5.06 (m, 4 H), 4.27–3.95 (m, 12 H), 3.88–3.79 (m, 2 H), 3.57–3.43 (m, 2 H), 2.55–2.15 (m, 12 H), 1.78 (s, 1.7 H), 1.69 (s, 4.3 H), 1.41 (s, 1.7 H), 1.40 (s, 4.3 H), 1.34 (s, 6 H), 1.25 (t, *J* = 7.2 Hz, 6 H) ppm.

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 THF (1 mL) 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 **13** (40 mg, 0.12 mmol) in THF (0.4 mL) was added. Then the mixture was stirred at –78 °C for 2 h and at room temperature overnight. The white suspension was cooled to 0 °C, diluted with anhydrous diethyl ether (5 mL) followed by the addition of HCl (1 mL, 2 M). Then the reaction mixture was diluted with water/diethyl ether. The aqueous layer was extracted with diethyl ether twice. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography with hexanes/ethyl acetate (93:7) as eluent to give the an *E*/*Z* mixture (ca. 2:1) of **5** (32 mg, 73%) as a colorless oil. R_f = 0.41 (hexanes/ethyl acetate = 4:1, PMA stain). MS (ESI): *m*/*z* = 389 [M + Na]$^+$. ^1H NMR (400 MHz, $CDCl_3$): δ = 5.90–5.80 (m, 2 H), 5.48 (t, *J* = 6.6 Hz, 0.66 H), 5.40 (t, *J* = 6.6 Hz, 1.38 H), 5.13–5.07 (m, 4 H), 5.10–5.04 (m, 4 H), 4.65 (s, 2 H), 4.59 (s, 2 H), 4.27–4.14 (m, 2 H), 4.05–3.98 (m, 6 H), 3.57–3.46 (m, 4 H), 2.46–2.37 (m, 2 H), 2.30–2.17 (m, 8 H), 2.08 (dd, *J* = 6.3, 14.1 Hz, 2 H), 1.78 (s, 2 H), 1.70 (s, 4 H), 1.58 (s, 1.4 H), 1.55 (s, 2.6 H), 1.42 (s, 2 H), 1.41 (4 H), 1.35 (s, 6 H), 0.02 (s, 18 H) ppm. ^{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 ppm. IR (thin film): $\tilde{\nu}$ = 3075, 2953, 1633, 1435, 1370, 1248, 1157, 1068, 857, 695 cm^{-1}.

Optimization of the Oxidative Cyclization Reaction: Solvent (4 mL), DDQ (34 mg, 0.15 mmol), and acid (1.5 equiv.) were added to a reaction vial charged with **17** (56 mg, 0.1 mmol) and mol. sieves (4 Å) (0.1 g) at –38 °C under argon. 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 Et_3N (0.2 mL) and filtered under pressure through a pad of silica gel. The concentrated residue was purified by silica gel chromatography with hexanes/ethyl acetate (95:5) as eluent to give **16** as a colorless oil.

Synthesis of Compound 16. Cyclization Reaction with a Stoichiometric Amount of DDQ (Table 1, Entry 10): Anhydrous acetonitrile (100 mL) was added to a flask charged with DDQ (1.56 g, 6.87 mmol), PPTS (1.78 g, 6.87 mmol), and mol. sieves (4 Å, 9.0 g) under argon at –38 °C. A solution of (*R*)-*tert*-butyl[3-cinnamyloxy-5-(trimethylsilylmethyl)hex-5-enyloxy]diphenylsilane (**17**; 2.63 g, 4.72 mmol, 97% *ee*) in acetonitrile (31 mL) was added dropwise over 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 (1.81 g, 81%). R_f = 0.36 (hexanes/ethyl acetate = 95:5, UV). The R_f is almost the same as that of the starting material, but development with hexanes/ethyl acetate = 99:1 three times gave a slightly higher R_f value than that of the starting material. $[\alpha]_D^{20}$ = +3.5 (*c* = 1.2, ethyl acetate). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.69–7.66 (m, 4 H), 7.45–7.21 (m, 11 H), 6.59 (d, *J* = 16.0 Hz, 1 H), 6.24 (dd, *J* = 16.0, 5.7 Hz, 1 H), 4.78 (s, 2 H), 3.99–3.86 (m, 2 H), 3.81–3.74 (m, 1 H), 3.70–3.62 (m, 1 H), 2.36 (d, *J* = 14.8 Hz, 1 H), 2.28 (d, *J* = 14.4 Hz, 1 H), 1.92–1.76 (m, 2 H), 1.05 (s, 9 H) ppm. ^{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 ppm. IR (thin film): $\tilde{\nu}$ = 3070, 2857, 1959, 1823, 1651, 1590, 1427, 1359, 1110, 966, 892, 741, 703, 614 cm^{-1}. MS (ESI): *m*/*z* 505 [M + Na$^+$]. HRMS (ESI): calcd. for $C_{32}H_{38}O_2SiNa$ 505.2539; found 505.2536.

Determination of the Enantiomeric Excess: TBAF (0.4 mL, 1 M in THF, 0.4 mmol) was added to a solution of **16** (30 mg, 60.4 μmol) in THF (1 mL). Then the solution was stirred at room temperature for 4 h and concentrated. The residue was purified by silica gel chromatography with hexanes/ethyl acetate (3:2, v/v) as eluent to give the free alcohol as a colorless oil (8.0 mg, 60%) with 97% *ee*. R_f = 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.^1H NMR (400 MHz, $CDCl_3$): δ = 7.39–7.23 (m, 5 H), 6.23 (dd, *J* = 6.1, 16.0 Hz, 1 H), 6.58 (d, *J* = 16.0 Hz, 1 H), 4.79 (s, 2 H), 4.03–3.99 (m, 1 H), 3.86–3.82 (m, 2 H), 3.68–3.62 (m, 1 H), 2.64 (s, 1 H), 2.36 (d, *J* = 13.3 Hz, 1 H), 2.23 (d, *J* = 13.3 Hz, 1 H), 2.20–2.08 (m, 2 H), 1.94–1.84 (m, 1 H), 1.83–1.76 (m, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 143.4, 130.6, 129.5, 128.4, 127.6, 126.4, 109.3, 79.0, 78.7, 61.2, 40.7, 40.4, 37.9 ppm. IR (thin film): $\tilde{\nu}$ = 3401, 2942, 2900, 1719, 1650, 1450, 1421, 1315, 1062, 966, 746, 693 cm^{-1}.

Catalyzed Synthesis of Compound 16 (Table 1, entry 9): A solution of **17** (1.80 g, 3.24 mmol) in CH_3CN (10 mL) was added 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 mol. sieves (4 Å, 3.6 g) in CH_3CN (120 mL). The suspension was stirred at –38 °C for 16 h and quenched with Et_3N (1 mL). The mixture was filtered through a column of silica gel with hexanes/ethyl acetate (95:5, v/v) as eluent. The concentrated product was purified by silica gel chromatography with hexanes/ethyl acetate (95:5, v/v) as eluent to give methylenetetrahydropyran **16** as a reddish oil (1.17 g, 71%). The analytical data comply with the data reported above.

Compound 36: Et_3N (56 μL, 0.4 mmol) and Cy_2BCl (0.24 mL, 1 M in hexanes, 0.24 mmol) were added in sequence to a reaction vial charged with isobutyramide (20.9 mg, 0.24 mmol) in diethyl ether (1.2 mL) to yield a white suspension. The mixture was stirred at 0 °C for 15 min and then cyclohexanecarbaldehyde (24 μL, 0.2 mmol) was added. After being stirred at 0 °C for 1 h, the reaction mixture was loaded onto a column of silica gel with CH_2Cl_2 (0.5 mL) 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%). R_f = 0.20 (hexanes/ethyl acetate = 3:2, PMA stain). ^1H NMR (400 MHz, $CDCl_3$): δ = 6.12 (d, *J* = 7.1 Hz, 1 H), 5.08–5.04 (m, 1 H), 3.95 (d, *J* = 3.7 Hz, 1 H), 2.39–2.32 (m, 1 H), 1.78–1.65 (m, 4 H), 1.54–1.44 (m, 1 H), 1.21–0.99 (m, 12 H) ppm. ^{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 ppm. IR (thin film): $\tilde{\nu}$ = 1693, 1639, 1512, 1366, 1249, 1171, 778 cm^{-1}. MS (ESI): *m*/*z* = 222 [M + Na]$^+$.

Compound 34: Li_2CuCl_4 (5.3 mL, 0.1 M in THF) and isoprenylmagnesium bromide (0.5 M in THF, 26 mL, 13 mmol) were added to a solution of TBS glycidol (2 g, 10.6 mmol) in THF (20 mL) at

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–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 layers were 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. R_f = 0.31 (hexanes/ethyl acetate = 97:3, PMA stain). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.98–6.91 (m, 1 H), 5.81 (dd, J = 1.6, 15.5 Hz, 1 H), 5.23–5.16 (m, 1 H), 4.77 (s, 1 H), 4.71 (s, 1 H), 3.66 (d, J = 5.2 Hz, 2 H), 2.36 (dd, J = 7.3, 13.9 Hz, 1 H), 2.24 (dd, J = 6.0, 13.8 Hz, 1 H), 1.86 (dd, J = 1.5, 6.9 Hz, 3 H), 1.75 (s, 3 H), 0.88 (s, 9 H), 0.02 (s, 6 H) ppm. ^{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 ppm. IR (thin film): $\tilde{\nu}$ = 2930, 1722, 1657, 1258, 1182, 1101, 836, 777 cm^{-1}. MS (ESI): m/z = 321 [M + Na]$^+$.

HCl (170 µL, 2 M) and water (170 µL) were added to a solution of TBS ether prepared as described above (50 mg, 0.168 mmol) in MeOH/CH_2Cl_2 (4:1, v/v, 5 mL). 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 layers were 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%). R_f = 0.28 (hexanes/ethyl acetate = 7:3, I_2 or $KMnO_4$ stain). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.05–6.93 (m, 1 H), 5.85 (dd, J = 1.7, 15.5 Hz, 1 H), 5.15–5.10 (m, 1 H), 4.81 (s, 1 H), 4.76 (s, 1 H), 3.78–3.72 (m, 1 H), 3.68–3.62 (m, 1 H), 2.37 (dd, J = 7.5, 14.1 Hz, 1 H), 2.30 (dd, J = 6.0, 14.2 Hz, 1 H), 2.06 (t, J = 6.0 Hz, 1 H), 1.88 (d, J = 5.9 Hz, 3 H), 1.77 (s, 3 H) ppm. ^{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 ppm. IR (thin film): $\tilde{\nu}$ = 3441, 2918, 1719, 1655, 1445, 1310, 1293, 1184, 1103, 1048, 969, 894, 838 cm^{-1}. MS (ESI): m/z = 207 [M + Na]$^+$.

Dess–Martin reagent (0.68 mL, 0.3 M in CH_2Cl_2, 0.204 mmol) was added 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). 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 layers were dried (Na_2SO_4) and concentrated. The residue was filtered through a plug of silica gel 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. R_f = 0.52 (hexanes/ethyl acetate = 7:3, I_2 stain or $KMnO_4$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.56 (s, 1 H), 7.13–7.01 (m, 1 H), 5.92 (dd, J = 1.7, 15.6 Hz, 1 H), 5.20 (dd, J = 5.7, 9.0 Hz, 1 H), 4.85 (s, 1 H), 4.78 (s, 1 H), 2.56 (dd, J = 4.6, 14.9 Hz, 1 H), 2.45 (dd, J = 8.9, 15.8 Hz, 1 H), 1.91 (dd, J = 1.6, 6.9 Hz, 3 H), 1.76 (s, 3 H) ppm. ^{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 ppm. IR (thin film): $\tilde{\nu}$ = 2943, 2851, 1722, 1656, 1444, 1377, 1294, 1263, 1178, 1105, 969, 898, 837, 688 cm^{-1}. MS (ESI): m/z = 205 [M + Na]$^+$.

Compound 37: Cy_2BCl solution (0.20 mL, 0.6 M in hexanes, 0.12 mmol) was added to a solution of amide **35** (10.4 mg, 0.12 mmol) and Et_3N (28 µL, 0.2 mmol) in diethyl ether (0.4 mL). The resulting white suspension was stirred at 0 °C for 15 min and then aldehyde **34** (28.4 mg, 0.1 mmol) in diethyl ether (0.2 mL) was added. After stirring at 0 °C for 1 h, the mixture was quenched with THF/phosphate buffer (pH = 7.4)/30% H_2O_2 (1:1:1, v/v/v, 2 mL). Then the aqueous layer was extracted with ethyl acetate three times. The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography with hexanes/ethyl acetate (1:1, v/v) as eluent to yield **37** as a mixture of diastereomers in the ratio of 1:1 (15 mg, 56%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 10.2 (s, 1 H), 9.9 (s, 1 H), 7.06–6.93 (m, 2 H), 6.70 (d, J = 7.6 Hz, 1 H), 6.60 (d, J = 8.3 Hz, 1 H), 5.86–5.84 (m, 1 H), 5.81–5.79 (m, 1 H), 5.16–5.07 (m, 2 H), 4.82–8.81 (m, 2 H), 4.75–4.74 (m, 2 H), 2.48–2.34 (m, 4 H), 2.15–2.02 (m, 2 H), 1.90–1.87 (m, 6 H), 1.75 (s, 3 H), 1.73 (s, 3 H), 1.20–1.16 (m, 12 H) ppm. IR (thin film): $\tilde{\nu}$ = 3339, 1711, 1659, 1531, 1444, 1293, 1183, 1103, 1045, 969, 893 cm^{-1}. MS (ESI): m/z = 292 [M + Na]$^+$. R_f = 0.35 (hexanes/ethyl acetate = 1:1, $KMnO_4$).

Synthesis of Compounds 1, 39, and 40 by Amidation Using Diphenyl Phosphate as Catalyst: A 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 column of silica gel with hexanes/ethyl acetate (1:1) as eluent to give a mixture of **1**, **38**, **39**, and **40**, which was further separated by HPLC (IC, hexanes/*i*PrOH = 1:1, 0.5 mL/min, 254 nm). The ratio of **1**/**39** was close to 1:1.

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

(–)-Zampanolide (1) and 39: (*S*)-TRIP (**41**; 2.7 mg, 20 mol-%) in CH_2Cl_2 (0.7 mL) was added 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). The resulting mixture was stirred at 23 °C for 12 h. After this period the reaction was loaded on a short column of silica gel and eluted with hexanes/ethyl acetate (3:2) to provide a crude mixture of (*S*)-TRIP (**41**), **1**, and **39**. The mixture was separated by HPLC to give (–)-zampanolide (**1**; 4.6 mg, 51%) and **39** (1.6 mg, 18%). HPLC: hexanes/*i*PrOH = 1:1, 0.5 mL/min, 254 nm, $t_{(S)\text{-TRIP}}$ = 7.4 min, t_{38} = 12.5 min, t_1 = 15.3 min, t_{39} = 23.3 min.

Zampanolide (1): R_f = 0.35 (hexanes/ethyl acetate = 1:1, UV, PMA). $[\alpha]_D^{20}$ = –94 (c = 0.08, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.65 (dd, J = 11.6, 14.9 Hz, 1 H), 7.45–7.40 (m, 1 H), 6.85–6.79 (m, 1 H), 6.46 (t, J = 11.3 Hz, 1 H), 6.31 (d, J = 7.8 Hz, 1 H), 6.11 (d, J = 11.9 Hz, 1 H), 6.08–6.02 (m, 1 H), 5.95 (d, J = 15.0 Hz, 1 H), 5.94 (d, J = 16.4 Hz, 1 H), 5.46–5.43 (m, 2 H), 5.31–5.27 (m, 1 H), 5.20 (d, J = 7.9 Hz, 1 H), 4.73 (s, 2 H), 4.13 (d, J = 13.0 Hz, 1 H), 3.98–3.94 (m, 1 H), 3.66 (br. s, 1 H), 3.29 (t, J = 10.5 Hz, 1 H), 3.04 (d, J = 13.6 Hz, 1 H), 2.41 (d, J = 13.6 Hz, 1 H), 2.38–2.29 (m, 1 H), 2.29–2.20 (m, 2 H), 2.14 (d, J = 13.4 Hz, 1 H), 2.09 (d, J = 13.4 Hz, 1 H), 1.97–1.91 (m, 2 H), 1.87 (d, J = 7.0 Hz, 3 H), 1.81 (s, 3 H), 1.72 (s, 3 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 197.9, 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 ppm. IR (thin film): $\tilde{\nu}$ = 3368, 2924, 2854, 2360, 1636, 1539, 1456, 1357, 1281, 1260, 1210, 1149, 1086, 979, 889, 803, 666 cm^{-1}. MS (ESI): m/z = 518 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{29}H_{37}NO_6Na$ 518.2519; found 518.2520.

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Zampanolide Diastereomer 39: R_f = 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, 1 H), 7.46–7.39 (m, 1 H), 6.86–6.80 (m, 1 H), 6.46 (t, J = 11.3 Hz, 1 H), 6.35 (d, J = 7.5 Hz, 1 H), 6.12 (d, J = 11.6 Hz, 1 H), 6.06 (dd, J = 6.9, 15.0 Hz, 1 H), 6.00 (d, J = 15.0 Hz, 1 H), 5.94 (d, J = 16.3 Hz, 1 H), 5.54–5.51 (m, 1 H), 5.45 (d, J = 11.2 Hz, 1 H), 5.37–5.31 (m, 1 H), 5.24 (d, J = 8.6 Hz, 1 H), 4.73 (br. s, 2 H), 4.11 (d, J = 13.7 Hz, 1 H), 3.98–3.94 (m, 1 H), 3.60 (br., 1 H), 3.34–3.27 (m, 1 H), 3.05 (d, J = 13.7 Hz, 1 H), 2.51 (dd, J = 11.0, 13.8 Hz, 1 H), 2.39–2.23 (m, 3 H), 2.15 (d, J = 13.7 Hz, 1 H), 2.10 (d, J = 13.1 Hz, 1 H), 2.00–1.93 (m, 2 H), 1.87 (d, J = 6.6 Hz, 3 H), 1.82 (s, 3 H), 1.71 (s, 3 H) ppm.

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] The cytotoxicities of the agents against the MCF-7, OVCAR-8, and NCI/ADR-RES human cancer cell lines were investigated by the sulforhodamine B method[25] in which the cellular protein is the parameter quantified.

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra for all new compounds and HPLC traces for compounds **1**, **16**, and **39**.

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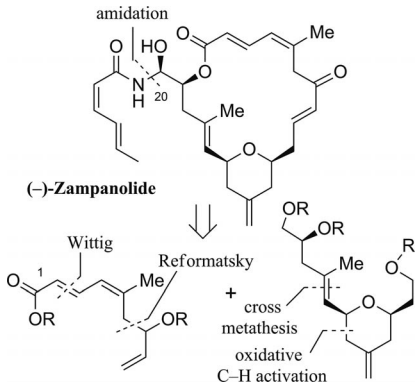
Natural Product Synthesis

An enantioselective synthesis of (–)-zampanolide has been achieved. This rare marine natural product shows microtubule-stabilizing properties. It also displays potent activity against taxol-resistant cells. This synthesis will provide a convenient access to analogues for important structure–activity relationship studies and less complex anticancer agents related to zampanolide.



Keywords: Natural products / Total synthesis / C–H activation / Metathesis / Stereoselective catalysis

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