Natural Product Synthesis

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Enantioselective Synthesis of (+)-Chamaecypanone C: A Novel Microtubule Inhibitor**

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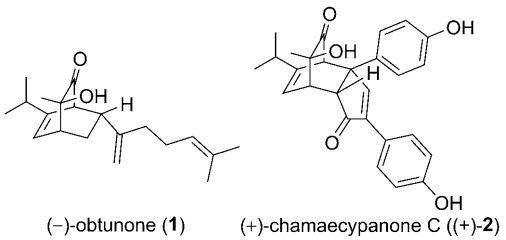
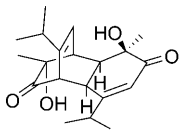
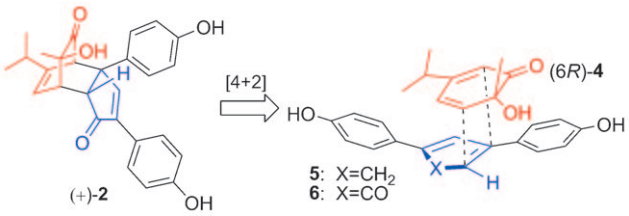
A number of bicyclo[2.2.2]octenone-containing natural products have been isolated from the heartwood of *Chamaecyparis obtusa* var. *formosana* (Scheme 1), including the Diels–Alder (DA) adducts[1] obtunone (**1**) [2] and chamaecypanone C (**2**),[3] as well as the [4+2] dimer (+)-**3**.[2,4] (+)-**2** has been shown to exhibit potent cytotoxicity against several human cancer cells, including human oral epidermoid carcinoma (KB; $IC_{50} = 190$ nM).[3] The biosynthesis of **2** is proposed[3] to occur through *endo* [4+2] cycloaddition between cyclohexa-2,4-dienone **4** (Scheme 2) and 1,3-bisarylcyclopenta-1,3-diene **5**, and subsequent oxidation to an enone—in accordance with literature reports of cyclopentadienes as biosynthetic precursors to natural products.[1b] An alternative biosynthetic possibility involves the corresponding cyclopentadienone **6** as the dienophile, and which may also be considered in light of known biosyntheses that involve reactive cyclopentadienones.[5] Herein, we report a concise synthesis of both enantiomers of chamaecypanone C (**2**). The synthesis involves a retro-Diels–Alder/Diels–Alder cascade of dimer **3**, which is obtained by utilizing copper-mediated asymmetric oxidative dearomatization.[6] Also presented are biological studies which document that the cytotoxic action of (+)-chamaecypanone C involves mitotic arrest as a consequence of its binding in the colchicine site of tubulin.





(+)-3,10-dihydroxydielmentha-5,11-diene-4,9-dione (**3**)

Scheme 1. Representative bicyclo[2.2.2]octenone-containing natural products.



Scheme 2. Plausible biosyntheses of (+)-chamaecypanone C ((+)-**2**).

Inspired by literature reports on tandem retro-DA/DA reactions of dimers derived from *ortho*-quinols and masked *ortho*-benzoquinones (MOBs),[7,8] we first evaluated reactions between the readily accessible dimer (−)-**3**[6] and *N*-phenylmaleimide (**7**) under thermolytic reaction conditions in different solvents (Table 1). Although reactions in toluene (Table 1, entry 1) and chlorobenzene (Table 1, entry 2) generated the desired cycloadduct **8** in moderate to good conversion (12 h), reactions in mesitylene at 150 °C gave both excellent conversion and yield of isolated **8** in 1.5 hours (Table 1, entries 3 and 4).

By using these optimized reaction conditions, a number of representative dienophiles were thermolyzed in the presence of dimer (−)-**3** in mesitylene (Table 2). Thermolysis reactions with methyl vinyl ketone (**9**; Table 2, entry 1), 2,3-dihydrofuran (**10**; Table 2, entry 2), and indene (**11**; Table 2, entry 3) successfully generated bicyclo[2.2.2]octenones **12**–**14** in good to excellent yields. These results underscore the reactivity of *ortho*-quinols as both "normal" and "inverse-demand"

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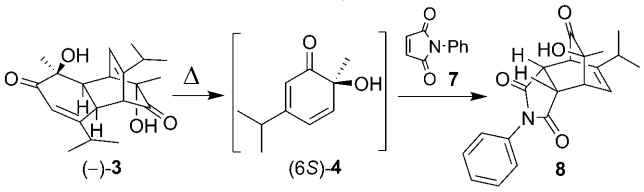
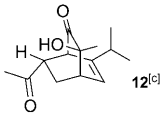
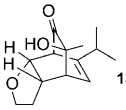
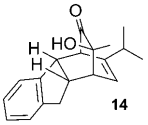
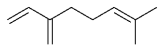
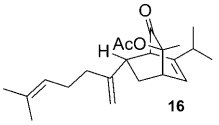
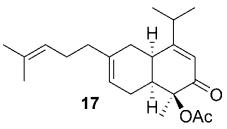
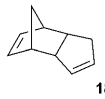
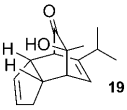
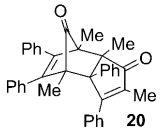
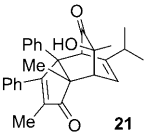
Table 1: Optimization of the Retro-DA/DA Cascade.



Entry	Solvent	*T* [°C]	Dienophile **7** [equiv]	*t* [h]	Conv. [%][a]
1	toluene	110	5	12	69
2	chlorobenzene	130	5	12	92
3	mesitylene	150	5	1.5	>99 (98)[b]
4	mesitylene	150	3	1.5	>99 (97)[b]

[a] Conversion is based on ^{1}H NMR analysis of **8** and starting material (−)-**3**. [b] Yield of isolated **8**.

Table 2: Tandem retro-DA/DA reactions using bicyclooctenone (−)-**3**.[a]

Entry	Dienophile	Equiv	Cycloadduct	*t* [h]	Yield [%][b]
1	 **9**	5	 **12**[c]	3	92
2	 **10**	20	 **13**	12	84
3	 **11**	10	 **14**	3	99
4[d]	 **15**	10	 **16**	4	42
			 **17**	4	39
5	 **18**	5	 **19**	4	98
6	 **20**	2.5	 **21**	5	57

[a] Reaction conditions: dimer (−)-**3**, dienophile, mesitylene, 150°C. [b] Yield of isolated product after column chromatography. [c] Approximately 6% of an inseparable minor product was detected by ^{1}H NMR spectroscopy. [d] Acetylation was required for product separation.

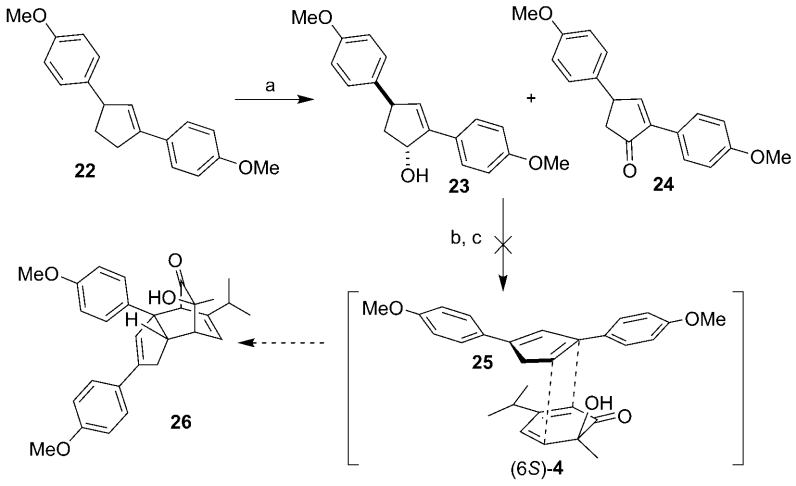
dienes. The observed regioselectivity for products **12**–**14** is in agreement with those reported for related cyclohexadienones (MOBs).[7c,d] Treatment of (−)-**3** with β-myrcene (**15**; Table 2, entry 4) smoothly generated an inseparable 1:1 mixture of *ent*-obtunone (**1**) and a decalin product, both of which were acetylated to afford **16** and **17**.[9] Hydrolysis of **16** (aq. NaOH/MeOH) afforded optically pure *ent*-**1**.[2,10] Furthermore, cyclopentadiene dimer **18** (Table 2, entry 5) was very reactive, and afforded the [4+2] adduct **19** as a single diastereomer in nearly quantitative yield.[7a] In contrast, use of cyclopentadienone dimer **20** (Table 2, entry 6) produced **21** only in moderate yield, probably as a result of side reactions (including decarbonylation) of **20** at high temperature.[11]

Based on our ability to trap (6*S*)-**4** with a number of dienophiles, we proceeded to evaluate both cyclopentadienes and cyclopentadienones for the synthesis of **2**. Accordingly, we targeted a single starting material for the preparation of both precursors. Starting from the known bisarylcyclopentene derivative **22**,[12] allylic oxidation using selenium dioxide afforded alcohol **23** as the major product (50% yield) along with a small amount of enone **24** (Scheme 3). Although diaryl cyclopentadienes[13] were detected by GC-MS analysis under acid-catalyzed dehydration conditions (cat. MP-TsOH, toluene, 110°C, 1 h),[14] all attempts to isolate pure product **25**, or trap it with reactive dienophiles (e.g. maleic anhydride, tetracyanoethylene) failed. Moreover, thermolysis of the crude mixture from either dehydration of **23** or base-promoted elimination of the derived mesylate derivative with dimer **3** also did not afford the desired cycloadduct **26**.

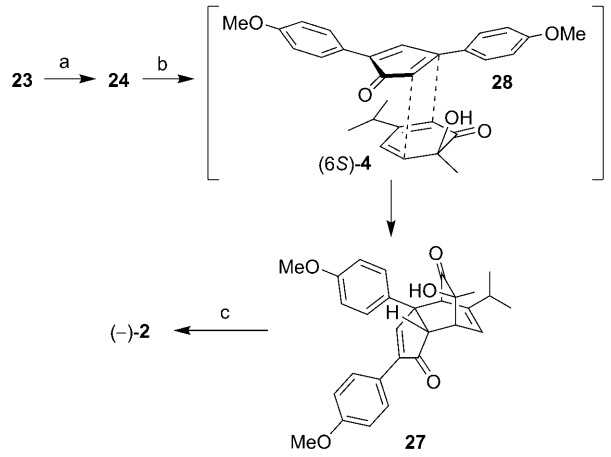
Alternatively, allylic alcohol **23** could be efficiently converted into cyclopentenone **24** using IBX as the oxidant[15] (Scheme 4). After extensive experimentation, it was found that oxidation of **24** using DDQ[16] in the presence of dimer **3** afforded the desired cycloadduct **27** in good yield. The *endo* configuration of **27** was unambiguously assigned by NOE experiments.[10] The transformation presumably proceeds through the initial formation of the reactive cyclopentadienone **28** from cyclopentenone **24**.[17] Unfortunately, all efforts to isolate either the cyclopentadienone monomer or derived dimers have thus far failed in control experiments. Finally, treatment of **27** with BBr_3 effected smooth demethylation to afford (−)-chamaecypanone C ((−)-**2**; 86%). To the best of our knowledge, this is the first example of the generation of a 2,4-diarylcyclopentadienone and its usage in natural product synthesis.[18] The instability and high reactivity of the diarylcyclopentadienone intermediate[19] is likely due to the relief of antiaromaticity upon cycloaddition as suggested by Harmata et al.[20]

In a similar manner, we prepared (+)-chamaecypanone C ((+)-**2**; Scheme 5). Hydrogenation of **29** quantitatively generated 2,4-disubstituted phenol **30**. An asymmetric hydroxylation/α-ketol rearrangement/dimerization sequence[6] afforded (+)-dimer **3** in moderate yield over two steps (>99% *ee*), and which was further elaborated into (+)-chamaecypanone C (53%, over 2 steps from enone **24**). Synthetic (+)-**2** was confirmed as being identical to natural chamaecypanone C by comparison of ^{1}H and ^{13}C NMR spectra, the mass spectrum, IR, and $[\alpha]_D$ data, thus confirming its absolute configuration.[10]

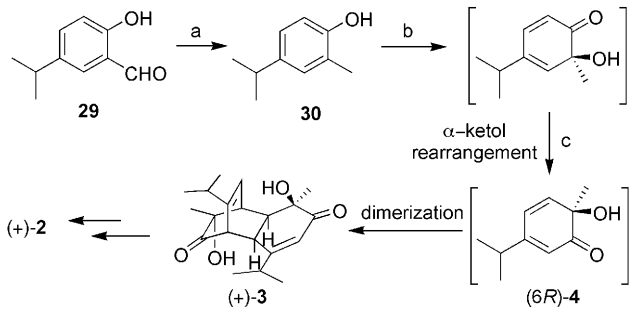
Both enantiomers of **2** were tested in the National Cancer Institute's 60-cell single dose assay at 10^{-5} M. Confirming earlier studies on the natural product,[3] (+)-**2** inhibited tumor cell growth by an average of 71%, while (−)-**2** had no effect. (+)-**2** was then tested in a dose response format, where it



Scheme 3. Reagents and conditions: a) SeO_2 (0.5 equiv), TBHP (2 equiv), DCE, 60 °C, 2 h, 50% (**23**), and 5% (**24**); b) cat. MP-TsOH, toluene, 110 °C, 1 h; or Martin sulfurane, CH_2Cl_2, RT, 0.5 h; c) (−)-**3** (0.2 equiv), mesitylene, 150 °C. DCE = 1,2-dichloroethane, MP = macroporous polymer, TBHP = *tert*-butyl hydroperoxide, Ts = 4-toluenesulfonyl.



Scheme 4. Reagents and conditions: a) IBX (2.0 equiv), toluene/DMSO (1 M, 2:1), 50 °C, 30 min, 90%; b) (−)-**3** (1.5 equiv), DDQ (2.0 equiv), *o*-dichlorobenzene, 150 °C, 1 h, 61%; c) BBr_3 (8.0 equiv) CH_2Cl_2, −78 °C to RT, 4 h, 86%. DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone, DMSO = dimethyl sulfoxide, IBX = *o*-iodoxybenzoic acid.



Scheme 5. Reagents and conditions: a) H-Cube (Pd/C), H_2 (40 bar), MeOH (0.03 M), 50 °C, 0.5 mL min^{-1}, quantitative; b) $LiOH \cdot H_2O$ (1.0 equiv), EtOH/toluene, azeotrope; $Cu(CH_3CN)_4PF_6$ (2.2 equiv), (−)-sparteine (2.3 equiv), 3 Å molecular sieves, O_2, THF, −78 °C, 16 h; c) benzene, reflux, 12 h, 47% (over 2 steps). H-Cube = continuous-flow hydrogenation reactor, THF = tetrahydrofuran.

displayed robust selectivity with a mean GI_{50} value of 0.21 μM. COMPARE analysis of the data[21] at the total growth inhibition level suggested that (+)-**2** might act through interference with tubulin function, as high correlations were seen to the data for seven established tubulin inhibitors.[10] Examination of this hypothesis by using an in vitro tubulin polymerization assay[22] found this to be the case, with an IC_{50} value of 2.0 ± 0.1 μM, while (−)-**2** had no effect at 40 μM. (+)-**2** was also tested for inhibition of colchicine binding,[23] where it was found to have moderate activity at 50 μM with 5 μM [^{3}H]colchicine and 1 μM tubulin. Finally, we confirmed that (+)-**2** had an effect on cells consistent with its inhibitory effects on the assembly of tubulin. A cytotoxic concentration of (+)-**2** arrested cells in mitosis concordant with inhibition of cell growth (Figure 1) and caused the disassembly of the intracellular microtubule network (Figure 2).[24]

In conclusion, we have accomplished the total syntheses of (+)- and (−)-chamaecypanone C. The key transformation involved a Diels–Alder cycloaddition between a diarylcyclopentadienone, which was generated in situ, and a chiral *ortho*-quinol derived from a retro-Diels–Alder reaction of its dimeric form. Initial biological studies indicate that (+)-chamaecypanone C is a potent tumor cell growth inhibitor[3] that acts primarily through inhibition of tubulin polymerization. Further studies

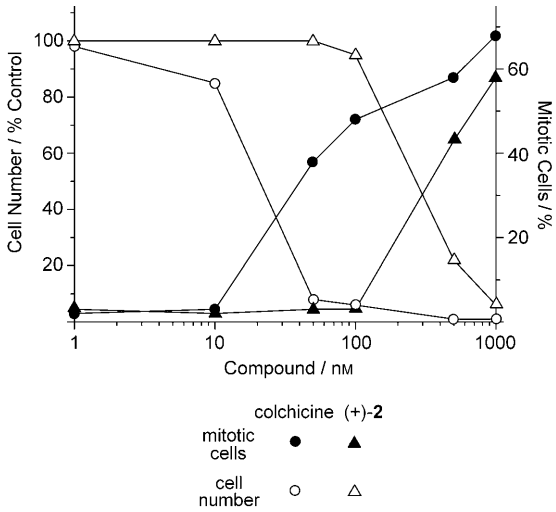


Figure 1. Human Burkitt lymphoma CA46 cells, obtained from the American Type Tissue Collection, were grown in a suspension culture for 24 hours at 37 °C in a humidified, 5% CO_2 atmosphere. The medium was RPMI 1640 supplemented with 5% fetal bovine serum. Initially, the culture medium contained 20000 cells mL^{-1}. For determination of the cell growth, the increase in cell number was determined with the cells counted in a model Z1 particle counter (Beckman Coulter). For determination of mitotic cells, cells were harvested by centrifugation, briefly swollen in a hypotonic solution, fixed on a glass slide, and stained with Giemsa. The percentage of cells with condensed chromosomes was then determined.

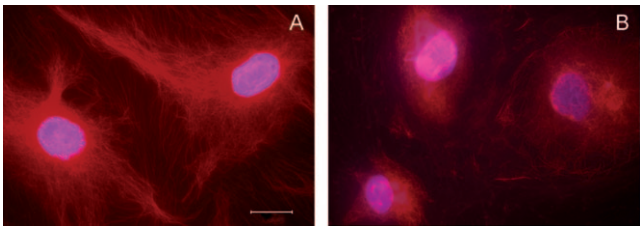


Figure 2. Disruption of the intracellular microtubule network by chamaecypanone C. *Potorus tridactylis* PtK2 kidney epithelial cells were obtained from the American Type Culture Collection and were cultured in minimal essential medium supplemented with 10% fetal bovine serum, 1 mM glutamine, and 1 mM sodium pyruvate. The cells were grown to confluence, disrupted by trypsinization, and seeded at about 35 000 cells into each compartment of a chambered coverglass system (Nunc) with A) no compound or B) 0.5 μM of (+)-chamaecypanone C added to the culture medium. After growth for 16 h at 37 °C in a humidified, 5% CO_2 atmosphere, the cells on the coverglass were fixed with acetone at −20 °C, washed with phosphate-buffered saline, and stained with a DNA stain and with a monoclonal antibody to β-tubulin conjugated to the fluorescent dye Cy3 (Sigma product C4585: instructions provided by the manufacturer were followed). The coverglass was mounted on a slide with antifade mounting solution and examined in a Nikon Eclipse E800 microscope with a 100-times oil objective and by using appropriate epifluorescence accessories. Images were captured with a Spot digital camera. The scale bar shown in A) represents 20 μm.

on the preparation of (+)-chamaecypanone C analogues by using a retro-DA/DA cascade process, as well as biological evaluation of these compounds, are currently in progress and will be reported in due course.

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[19] The presumed cyclopentadienone **28** was also successfully trapped with dimethyl acetylenedicarboxylate to afford a tetrasubstituted aromatic product.[10] For examples of cyclopentadienones as 4π partners in cycloadditions, see: a) T. Ban, Y. Wakita, K. Kanematsu, *J. Am. Chem. Soc.* **1980**, *102*, 5415–5416; b) T. Jikyo, M. Eto, K. Harano, *J. Chem. Soc. Perkin Trans. 1* **1998**, 3463–3470.
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