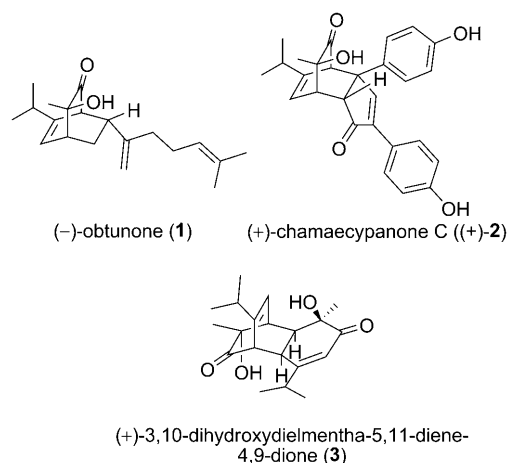


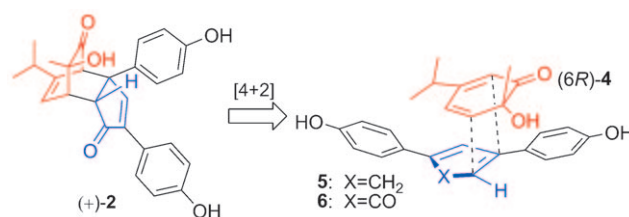
Enantioselective Synthesis of (+)-Chamaecyanone C: A Novel Microtubule Inhibitor**

Suwei Dong, Ernest Hamel, Ruoli Bai, David G. Covell, John A. Beutler, and John A. Porco, Jr.*

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 chamaecyanone 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; IC₅₀ = 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-bisaryl cyclopenta-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 chamaecyanone 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 (+)-chamaecyanone C involves mitotic arrest as a consequence of its binding in the colchicine site of tubulin.



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



Scheme 2. Plausible biosyntheses of (+)-chamaecyanone 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”

[*] S. Dong, Prof. Dr. J. A. Porco, Jr.

Department of Chemistry, Center for Chemical Methodology and Library Development (CMLD-BU), Boston University
590 Commonwealth Avenue, Boston, MA 02215 (USA)
Fax: (+1) 617-358-2847
E-mail: porco@bu.edu

Dr. J. A. Beutler
Molecular Targets Development Program
National Cancer Institute at Frederick, MD (USA)

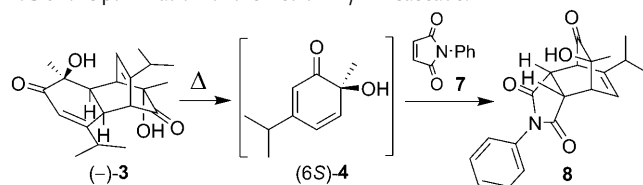
Dr. E. Hamel, Dr. R. Bai
Toxicology and Pharmacology Branch
National Cancer Institute at Frederick, MD (USA)

Dr. D. G. Covell
Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis
National Cancer Institute at Frederick, MD (USA)

[**] Financial support from the National Institutes of Health (GM-073855 and P50 GM067041), Wyeth Pharmaceuticals, Merck Research Laboratories, and the NCI intramural research program is gratefully acknowledged. We thank the Developmental Therapeutics Program at the NCI for the 60-cell assay, ThalesNano, Inc. (Budapest, Hungary) for assistance with the continuous-flow hydrogenation reactor, and Prof. Dr. Corey Stephenson (Boston University) for helpful discussions.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.200805486>.

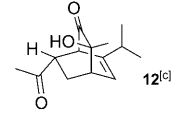
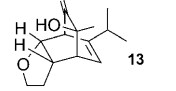
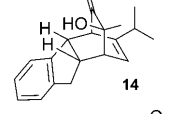
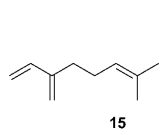
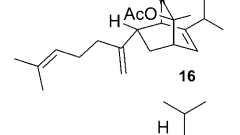
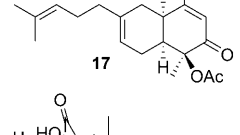
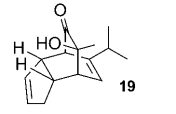
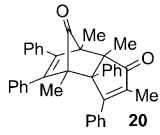
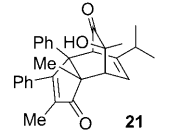
Table 1: Optimization of the Retro-DA/DA Cascade.



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 ¹H 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		5		3	92
2		20		12	84
3		10		3	99
4 ^[d]		10		4	42
				4	39
5		5		4	98
6		2.5		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 ¹H 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

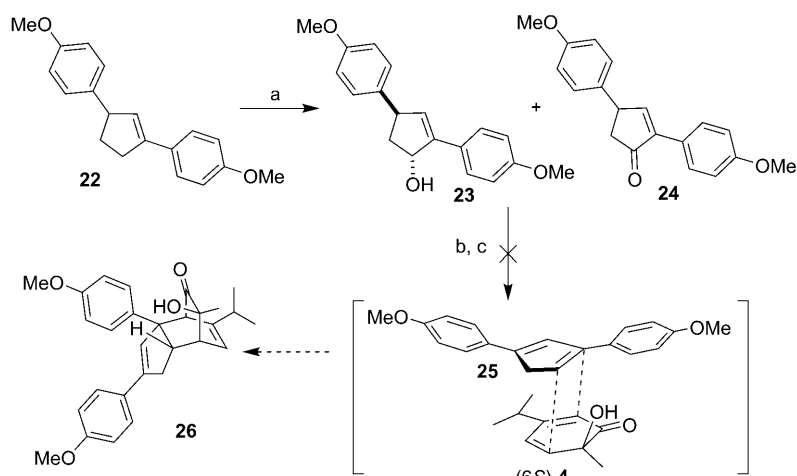
acetylated to afford **16** and **17**.^[9] Hydrolysis of **16** (aq. NaOH/MeOH) afforded optically pure *ent*-**1**.^[2,10] Furthermore, cyclopentadiene dimer **18** (Table 2, entry 5) was very reactive, and afforded the [4+2] adduct **19** as a single diastereomer in nearly quantitative yield.^[7a] 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**.

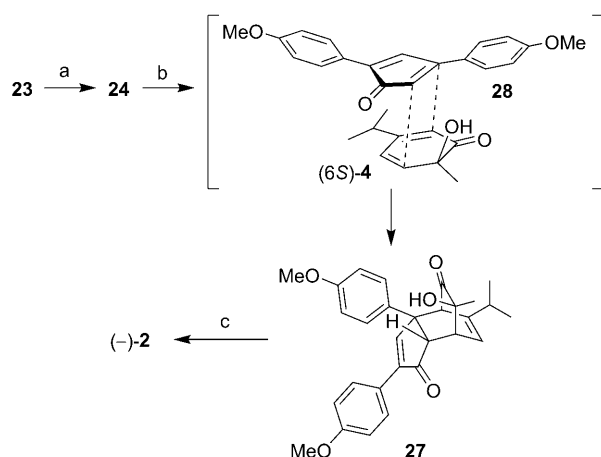
Alternatively, allylic alcohol **23** could be efficiently converted into cyclopentenone **24** using IBX as the oxidant^[15] (Scheme 4). After extensive experimentation, it was found that oxidation of **24** using DDQ^[16] in the presence of dimer **3** afforded the desired cycloadduct **27** in good 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 BBr₃ effected smooth demethylation to afford (–)-chamaecypaone 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 (+)-chamaecypaone 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 (+)-chamaecypaone C (53%, over 2 steps from enone **24**). Synthetic (+)-**2** was confirmed as being identical to natural chamaecypaone C by comparison of ¹H and ¹³C NMR spectra, the mass spectrum, IR, and [α]_D data, thus confirming its absolute configuration.^[10]

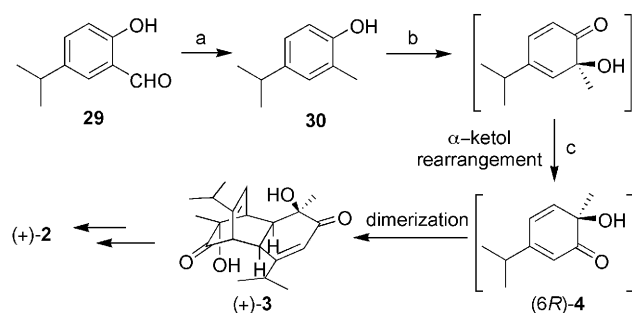
Both enantiomers of **2** were tested in the National Cancer Institute's 60-cell single dose assay at 10^{–5} M. Confirming earlier studies on the natural product,^[3] (+)-**2** inhibited tumor cell growth by an average of 71%, while (–)-**2** had no effect. (+)-**2** was then tested in a dose response 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 sulfuran, 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) $\text{LiOH}\cdot\text{H}_2\text{O}$ (1.0 equiv), EtOH/toluene, azeotrope; $\text{Cu}(\text{CH}_3\text{CN})_4\text{PF}_6$ (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 $0.21\text{ }\mu\text{M}$. 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 $2.0 \pm 0.1\text{ }\mu\text{M}$, while (–)-**2** had no effect at $40\text{ }\mu\text{M}$. (+)-**2** was also tested for inhibition of colchicine binding,^[23] where it was found to have moderate activity at $50\text{ }\mu\text{M}$ with $5\text{ }\mu\text{M}$ [^3H]colchicine and $1\text{ }\mu\text{M}$ 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 (–)-chamaecypa- none 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 (+)-chamaecypa- none C is a potent tumor cell growth inhibitor^[3] that acts primarily through inhibition of tubulin polymerization. Further studies

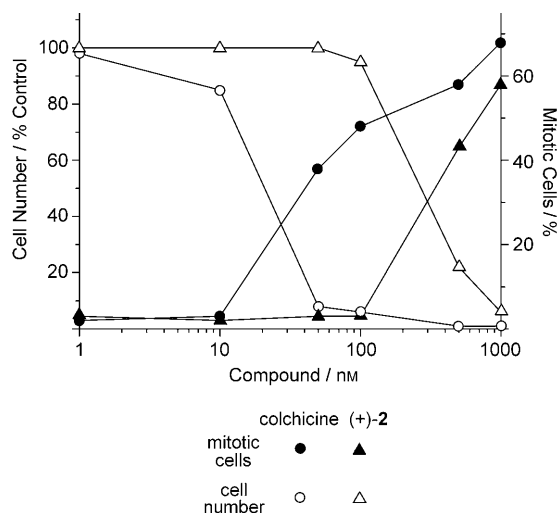


Figure 1. Human Burkitt lymphoma CA46 cells, obtained from the American Type Tissue Collection, were grown in a suspension culture for 24 hours at 37°C in a humidified, 5% CO_2 atmosphere. The medium was RPMI 1640 supplemented with 5% fetal bovine serum. Initially, the culture medium contained $20000\text{ cells mL}^{-1}$. 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.

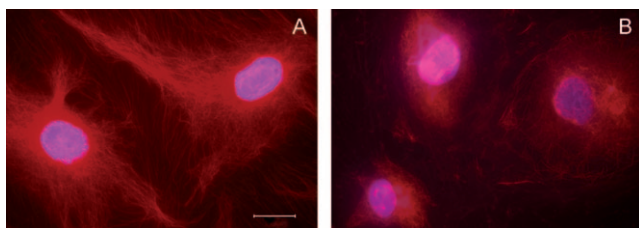


Figure 2. Disruption of the intracellular microtubule network by chamaecyanone C. *Potorus tridactylis* PtK2 kidney epithelial cells were obtained from the American Type Culture Collection and were cultured in minimal essential medium supplemented with 10% fetal bovine serum, 1 mM glutamine, and 1 mM 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 (+)-chamaecyanone 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 (+)-chamaecyanone 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.

Received: November 10, 2008

Published online: January 12, 2009

Keywords: cycloaddition · cyclopentadienone · microtubule inhibitor · natural product · total synthesis

- [1] For reviews on biosynthetic Diels–Alder reactions, see: a) E. M. Stocking, R. M. Williams, *Angew. Chem.* **2003**, *115*, 3186–3223; *Angew. Chem. Int. Ed.* **2003**, *42*, 3078–3115; b) H. Oikawa, T. Tokiwano, *Nat. Prod. Rep.* **2004**, *21*, 321–352.
- [2] Y. H. Kuo, C. H. Chen, S. L. Huang, *Chem. Pharm. Bull.* **1998**, *46*, 181–183.
- [3] S. C. Chien, J. Y. Chang, C. C. Kuo, C. C. Hsieh, N. S. Yang, Y. H. Kuo, *Tetrahedron Lett.* **2007**, *48*, 1567–1569.
- [4] R. M. Carman, L. K. Lambert, W. T. Robinson, J. M. A. M. Van Dongen, *Aust. J. Chem.* **1986**, *39*, 1843–1850.
- [5] S. Al-Busafi, J. R. Doncaster, M. G. B. Drew, A. C. Regan, R. C. Whitehead, *J. Chem. Soc. Perkin Trans. 1* **2002**, 476–484.
- [6] S. Dong, J. Zhu, J. A. Porco, Jr., *J. Am. Chem. Soc.* **2008**, *130*, 2738–2739.
- [7] For use of *ortho*-quinol dimers in a retro-DA/DA cascade, see: a) V. K. Singh, P. T. Deota, A. V. Bedekar, *J. Chem. Soc. Perkin Trans. 1* **1992**, 903–912; b) V. Singh, B. Samanta, *Tetrahedron Lett.* **1999**, *40*, 1807–1810; for use of MOB-derived dimers, see: c) C. C. Liao, R. K. Peddinti, *Acc. Chem. Res.* **2002**, *35*, 856–866;
- d) S. K. Chittimalla, H. Y. Shiao, C. C. Liao, *Org. Biomol. Chem.* **2006**, *4*, 2267–2277.
- [8] For reviews on *ortho*-quinol and MOB-derived [4+2] cyclo-dimers, see: a) D. Magdziak, S. J. Meek, T. R. R. Pettus, *Chem. Rev.* **2004**, *104*, 1383–1430; b) S. Quideau, L. Pouysegue, D. Deffieux, *Synlett* **2008**, 467–495.
- [9] B. B. Snider, Z. Chen, *Org. Prep. Proced. Int.* **1999**, *31*, 537–541.
- [10] See the Supporting Information for complete experimental details.
- [11] D. Hafner, K. Goliash, *Angew. Chem.* **1960**, *72*, 781.
- [12] M. Prashad, J. C. Tomesch, J. R. Wareing, H. C. Smith, S. H. Cheon, *Tetrahedron Lett.* **1989**, *30*, 2877–2880.
- [13] S. Datta, A. Odedra, R. S. Liu, *J. Am. Chem. Soc.* **2005**, *127*, 11606–11607.
- [14] J. Szymoniak, J. Besancon, A. Dormond, C. Moise, *J. Org. Chem.* **1990**, *55*, 1429–1432.
- [15] K. C. Nicolaou, Y. L. Zhong, P. S. Baran, *J. Am. Chem. Soc.* **2000**, *122*, 7596–7597.
- [16] a) D. R. Buckle in *Encyclopedia of Reagents for Organic Synthesis*, Vol. 3 (Ed.: L. A. Paquette), Wiley, Chichester, **1995**, pp. 1699; b) D. Walker, J. D. Hiebert, *Chem. Rev.* **1967**, *67*, 153–196.
- [17] For syntheses of cyclopentadienones from cyclopentenones, see: a) P. G. Baraldi, A. Barco, S. Benetti, G. P. Pollini, E. Polo, D. Simoni, *J. Chem. Soc. Chem. Commun.* **1984**, 1049–1050; b) M. Harmata, C. L. Barnes, J. Brackley, G. Bohnert, P. Kirchhoefer, L. Kuerti, P. Rashatasakhon, *J. Org. Chem.* **2001**, *66*, 5232–5236, and references therein.
- [18] For representative syntheses of cyclopentadienones, see: a) P. A. Wender, T. J. Paxton, T. J. Williams, *J. Am. Chem. Soc.* **2006**, *128*, 14814–14815, and references therein; for preparation and studies on 2,4-di-*tert*-butylcyclopentadienone, see: b) E. W. Garbisch, Jr., R. F. Sprecher, *J. Am. Chem. Soc.* **1969**, *91*, 6785–6800; c) R. C. DeSelms, W. R. Schleigh, *Synthesis* **1973**, 614–615; for a total synthesis of the 2,3-substituted cyclopentadienone-derived natural product manzamenone A, see Ref. [5].
- [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.
- [20] For deantiaromatization in electrocyclic reactions, see: a) M. Harmata, P. Zheng, P. R. Schreiner, A. Navarro-Vázquez, *Angew. Chem.* **2006**, *118*, 2000–2005; *Angew. Chem. Int. Ed.* **2006**, *45*, 1966–1971; for use of reactive cyclopentadienones in cycloaddition, see: b) M. Harmata, M. G. Gomes, *Eur. J. Org. Chem.* **2006**, 2273–2277.
- [21] K. D. Paull, C. M. Lin, L. Malspeis, E. Hamel, *Cancer Res.* **1992**, *52*, 3892–3900.
- [22] E. Hamel, *Cell Biochem. Biophys.* **2003**, *38*, 1–21.
- [23] P. Verdier-Pinard, J.-Y. Lai, H.-D. Yoo, J. Yu, B. Marquez, D. G. Nagle, M. Nambu, J. D. White, J. R. Falck, W. H. Gerwick, B. W. Day, E. Hamel, *Mol. Pharmacol.* **1998**, *53*, 62–72.
- [24] Chang and coworkers (National Health Research Institutes, Taipei, Taiwan) have also independently demonstrated that chamaecyanone C is a microtubule inhibitor, see: C. C. Hsieh, Y. H. Kuo, C. C. Kuo, C. Y. Chang, S. C. Chien, J. Y. Chang, *The 13th Joint Congress of Oncology Societies of Taiwan* (Taipei, Taiwan), May 3–4, **2008** (Abstract A-I-18).