Panepophenanthrin Total Synthesis

Total Synthesis of the Ubiquitin-Activating Enzyme Inhibitor (+)-Panepophenanthrin**

*Xiaoguang Lei, Richard P. Johnson, and John A. Porco, Jr.**

The ubiquitin-proteasome pathway (UPP) regulates a variety of cellular processes by degradation of targeted proteins.[1] Ubiquitin (Ub) is first activated by ubiquitin-activating enzyme (E1), and the activated ubiquitin is transferred by transacylation to an active cysteine residue of ubiquitin-conjugating enzymes (E2s). The E2 enzymes then cooperate with ubiquitin ligases (E3s) to attach Ub to the amino group of lysine residues on protein targets, leading ultimately to protein degradation by the proteasome. It has been shown that abnormal ubiquitination-mediated protein degradation may be associated with human cancers, inflammation, and neurodegenerative disease.[2] Small-molecule inhibitors of ubiquitination may thus find clinical applications, provided that selective compounds may be uncovered.[3] Recently, the first naturally occurring inhibitor of ubiquitin-activating enzyme, panepophenanthrin (**1**, Figure 1), was isolated from the mushroom strain *Panus rudis* Fr. IFO8994.[4] This molecule falls into a general class of epoxyquinoid natural products produced by Diels–Alder-type dimerization,[5] including torreyanic acid (**2**)[6] and epoxyquinols A (**3**) and B (**4**)[7] (Figure 1). Herein, we report the first total synthesis of (+)-panepophenanthrin, which utilizes a highly stereoselective Diels–Alder dimerization of an epoxyquinol dienol monomer, as well as initial experimental and computational studies to probe the dimerization mechanism.

Our retrosynthetic route for panepophenanthrin is depicted in Figure 2a. Target molecule **1**[28] may be derived from hemiacetal formation of the hydroxy ketone **5.** The propensity for epoxyquinol derivatives to form both hydrates and hemiacetals by reaction of water and alcohols with the electrophilic carbonyl has been documented.[8] The X-ray

[*] Prof. Dr. J. A. Porco, Jr., X. Lei
Department of Chemistry and
Center for Chemical Methodology and Library Development
Boston University, 590 Commonwealth Avenue
Boston, MA 02215 (USA)
Fax: (+1) 617-353-6466
E-mail: porco@chem.bu.edu
Prof. Dr. R. P. Johnson
Department of Chemistry, University of New Hampshire
Durham, NH 03824 (USA)

[**] We thank Chaomin Li for helpful discussions, Dr. Ryuichi Sekizawa (Showa Pharmaceutical University) for providing authentic panepophenanthrin, and Dr. Emil Lobkovsky (Cornell University) for X-ray crystal structure analysis. Financial support from the American Cancer Society (RSG-01-135-01, J.A.P, Jr.) and the NIH (P50 GM067041) is gratefully acknowledged. R.P.J. acknowledges the Norman and Anna S. Waite Professorship for support.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

 DOI: 10.1002/ange.200351862

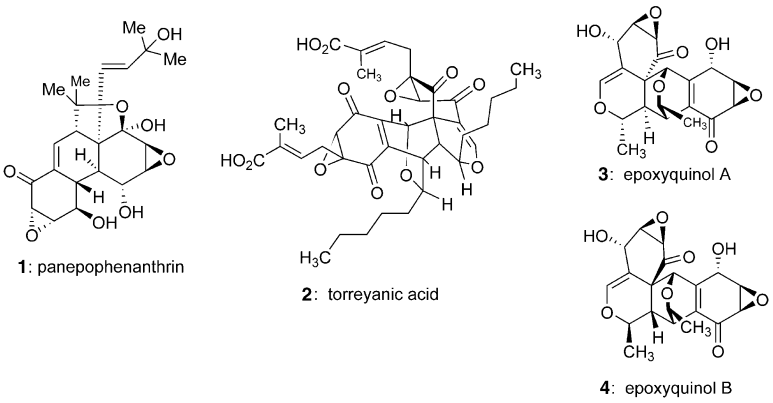


Figure 1. Dimeric epoxyquinoid natural products **1**–**4**.

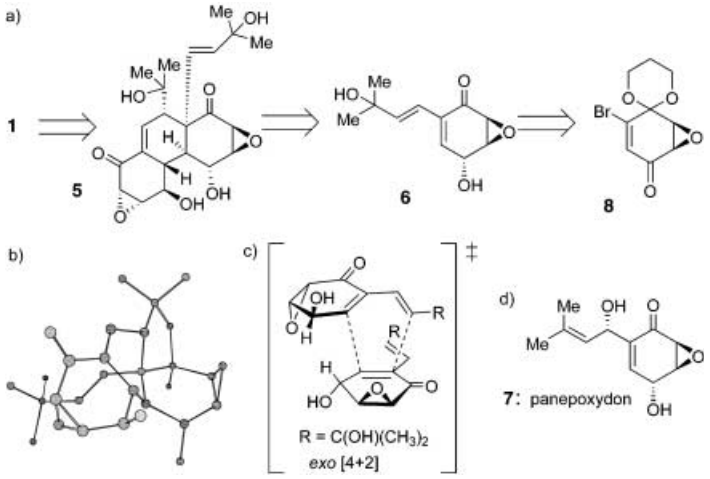
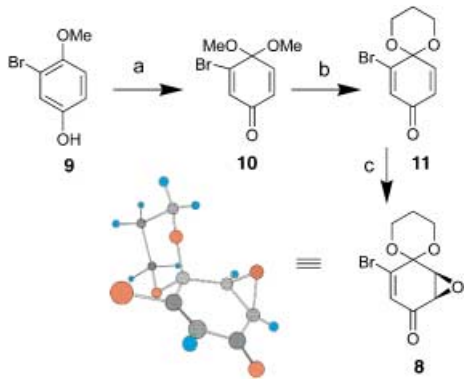


Figure 2. a) Retrosynthetic analysis for panepophenanthrin (**1**), b) X-ray crystal structure of **1**, c) transition state structure for the dimerization of **6**, d) panepoxydon (**7**).

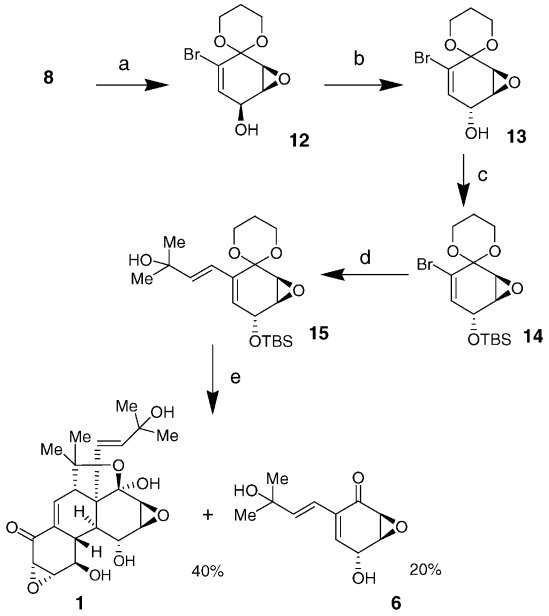
crystal structure of **1** (Figure 2b) also shows the close proximity of the tertiary hydroxy group and ketone, which should substantially favor formation of a hemiacetal bridge.[9] In principle, hemiacetal formation may precede intramolecular [4+2] cycloaddition.[10] The open-form precursor **5** may be derived from *exo*-Diels–Alder dimerization[11] of epoxyquinol monomer **6** (Figure 2c), the conjugated diene isomer of the natural product panepoxydon (**7**, Figure 2d).[12] Recent reports by Shotwell et al. have documented the facile rearrangement of **7** to give conjugated isomers such as **6** under mildly acidic conditions.[12c] Epoxyquinol diene monomer **6** may be derived from transformations of chiral, nonracemic epoxy ketone **8**, including a Heck-type coupling to install the dienol. Compound **8** may be prepared as either antipode by using tartrate-mediated asymmetric nucleophilic epoxidation[13] of a quinone monoketal precursor.

The synthesis of epoxyquinol diene monomer **6** was initiated by oxidation of the readily available monomethoxy hydroquinone **9**[14] with $PhI(OAc)_2$[15] to afford dimethoxyketal **10** (Scheme 1). Transketalization of **10** with 1,3-propanediol under Pirrung's conditions[16] afforded 1,3-dioxane **11**, which was found to be a suitable substrate for nucleophilic epoxidation. Tartrate-mediated nucleophilic epoxidation of **11** using NaHMDS as base cleanly produced epoxy ketone **8** (80% yield, 95% *ee*). The absolute stereochemistry of **8** was confirmed by X-ray crystal structure analysis[27] (Scheme 1) and is consistent with our proposed transition-state model for tartrate-mediated nucleophilic epoxidation.[13a]



Scheme 1. a) $PhI(OAc)_2$, MeOH, RT, 1 h, 96%; b) 1,3-propanediol, $BF_3{\cdot}Et_2O$, DME, RT, 2 h, 75%; c) Ph_3COOH, NaHMDS (1 M in THF), L-DIPT, 4-Å molecular sieves, toluene, −55 °C, 48 h, 80%, 95% *ee*. DIPT = diisopropyl tartrate, DME = dimethoxyethane, NaHMDS = sodium bis(trimethylsilyl)amide, RT = room temperature.

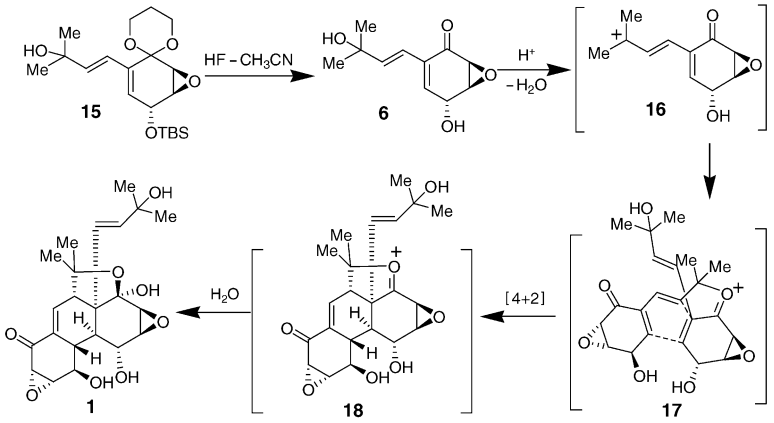
Advancement of **8** to the epoxyquinol monomer **6** is shown in Scheme 2. Attempted chelation-controlled reduction (with e.g. DIBAL-H[17] or $Zn(BH_4)_2$[18]) led to poor diastereoselectivity (1:1). In contrast, reduction of **8** with Super-Hydride cleanly afforded *syn*-epoxy alcohol **12**, which



Scheme 2. a) $LiEt_3BH$, THF, −78 °C, 1 h, 98%; b) PPh_3, DIAD, 4-nitrobenzoic acid, THF, −50 °C→RT, 1 h; NaOMe 1 M, MeOH, RT, 30 m, 80%; c) TBSCl, imidazole, DMF, RT, 10 h, 90%; d) $Pd(OAc)_2$, Ag_2CO_3, 2-methyl-3-buten-2-ol, DMF, 90 °C, 16 h, 80%; e) 10% HF, CH_3CN/CH_2Cl_2, RT, 1 h. DIAD = diisopropyl diazodicarboxylate, DMF = dimethylformamide, TBS = *tert*-butyldimethylsilyl.

was subsequently converted into the *anti* diastereomer **13** by a Mitsunobu protocol[19] (80%, two steps). Silylation of the secondary hydroxy group afforded **14**, which was subjected to Heck-type coupling with 2-methyl-3-buten-2-ol[12c] to afford dienol **15** (80%). Double deprotection of **15** with aqueous HF afforded the desired epoxyquinol monomer **6** (20%) and panepophenanthrin (**1,** 40%). Synthetic **1** was confirmed to be identical to natural panepophenanthrin by ^{1}H and ^{13}C NMR data, mass spectrometry, and optical rotation, and by TLC comparison in three solvent systems.

Our initial supposition was that the tandem deprotection/Diels–Alder dimerization (**15**→**1**)may proceed through an ionic process (Scheme 3).[20] Acid-catalyzed ionization of the tertiary allylic alcohol of epoxyquinol **6** could in principle lead

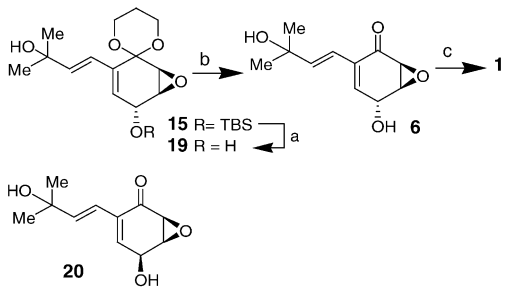


Scheme 3. Deprotection of **15** and subsequent Diels–Alder dimerization to give **1**.

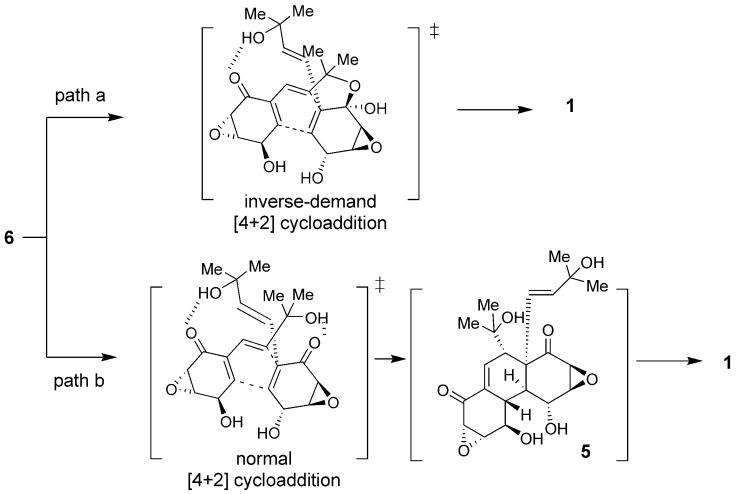
to allylic cation **16,** which may react with the carbonyl group of another monomer to produce the tethered intermediate **17**.[10,21] Intramolecular ionic Diels–Alder reaction of **17** to give **18** followed by addition of water *anti* to the epoxide would afford **1** directly. To further judge the validity of an ionic Diels–Alder process, we revised our synthetic approach in order to prepare **6** for alternative dimerization experiments (Scheme 4). Desilylation of **15** with TBAF cleanly produced epoxy alcohol **19** (90%). Gratifyingly, treatment of **19** with 0.2 M HCl in CH_3CN/CH_2Cl_2 (1:1) cleanly effected ketal hydrolysis to afford epoxyquinol monomer **6** (95%). Interestingly, Diels–Alder dimerization of **6** to give panepophenanthrin (**1**) also proceeded when the monomer was allowed to stand at 25 °C without solvent (24 h, 80%).[7d] The reaction was conducted effectively in both standard laboratory glassware and Teflon vials, which confirms that an ionic Diels–Alder process is not strictly required for the [4+2] dimerization. Subsequent experiments also revealed that *syn*-epoxyquinol monomer **20** (Scheme 4) did not undergo Diels–Alder dimerization under conditions reported for *anti* isomer **6** (neat, room temperature).

We next considered alternative mechanisms for the Diels–Alder dimerization of monomer **6** (Scheme 5). In one case (path a), the tertiary hydroxy group of one monomer adds to the carbonyl group of another to generate a hemiacetal intermediate. An intramolecular, inverse-demand Diels–Alder reaction then affords the dimeric natural product.[22] An alternative mechanism (path b) may involve transition-state hydrogen bonding[23] of the epoxyquinol monomers in a "pseudo-transannular,"[24] normal Diels–Alder cycloaddition. Subsequent ring closure to form a five-membered ring hemiacetal leads to **1**.

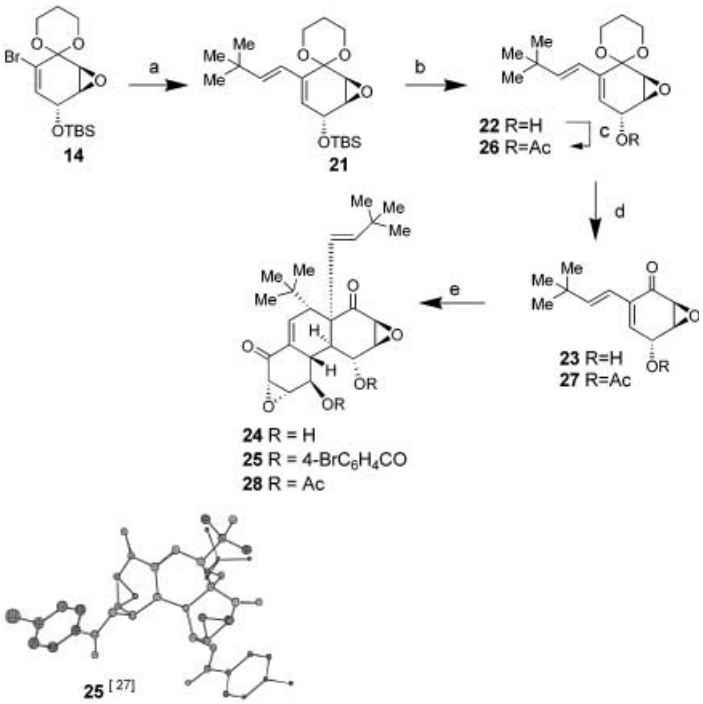
To further probe the role of the tertiary hydroxy group in the Diels–Alder dimerization of **6**, we prepared an epoxyquinol monomer lacking this functionality (Scheme 6). Heck-type coupling of **14** with 3,3-dimethylbutene afforded **21** (80%). Reaction of **21** with TBAF produced **22**, which was treated with 0.2 M HCl to effect ketal hydrolysis forming monomer **23**. Epoxyquinol **23** was cleanly dimerized to **24** (neat, 24 h). The regio- and stereochemistry of **24** was confirmed by X-ray structure analysis of bis-*para*-bromobenzoate **25**. Interestingly, **25** crystallized as a centrosymmetric racemate ($P\bar{1}$ space group).[25] Production of dimer **24** confirms that that tertiary hydroxy group of monomer **6** and hydrogen-bond organization is not essential for successful Diels–Alder dimerization. Acetylation of monomer **22** led to **26**, which was hydrolyzed to give monomer **27**. This com-



Scheme 4. a) TBAF 1 M in THF, THF, RT, 3 h, 90%; b) 0.2 M HCl, CH_3CN/CH_2Cl_2 (1:1), RT, 2 h, 95%; c) no solvent, RT, 24 h, 80%. TBAF = tetrabutylammonium fluoride.



Scheme 5. Possible mechanisms for the Diels–Alder dimerization.



Scheme 6. Top: a) $Pd(OAc)_2$, Ag_2CO_3, 3,3-dimethylbutene, DMF, 90 °C, 24 h, sealed tube, 80%; b) TBAF 1 M in THF, THF, RT, 3 h, 90%; c) AcOH, DIC, DMAP, CH_2Cl_2, RT, 30 m, 91%; d) 0.2 M HCl, CH_3CN/CH_2Cl_2 (1:1), RT, 2 h, 95%; e) neat, RT, 24 h, 90%. DIC = 1,3-diisopropylcarbodiimide, DMAP = 4-dimethylaminopyridine. Bottom: X-ray structure of **25**.[27]

pound also dimerized to afford **28**, which was identical to material obtained by acetylation of **24**. These studies support the normal [4+2] pathway (Scheme 5, path b), in which Diels–Alder dimerization is followed by hemiacetal formation.

To further understand the timing of the [4+2] dimerization and hemiacetal formation, we performed computational studies by disconnecting panepophenanthrin in a retro-[4+2] fashion and optimizing each stage at the B3LYP/6-31G* level of theory.[26] Our results are summarized in Figure 3. Initial formation of the hemiacetal link provides the advantage of an intramolecular [4+2] reaction, but renders the dienophile much less reactive because it is no longer an enone. The predicted overall free-energy barrier is 39.2 kcal mol^{-1}. In

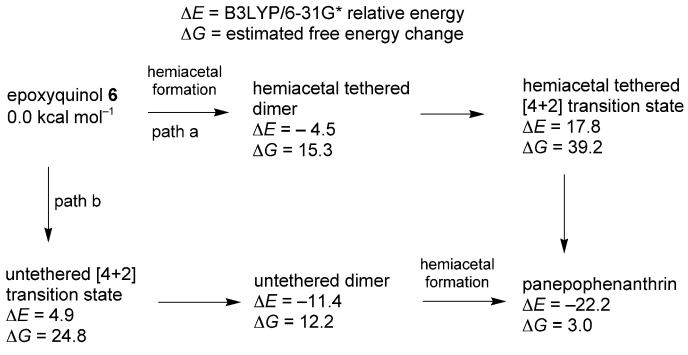


Figure 3. Relative energies (in kcal mol^{-1}, B3LYP/6-31G*) for the intermediates in the two paths for Diels–Alder dimerization.

contrast, direct intermolecular cycloaddition of two epoxyketones proceeds through a reactive dienophile and thus a much lower intrinsic barrier of 24.8 kcal mol^{-1}. Figure 4 shows the predicted transition-state structure. The initial stage of

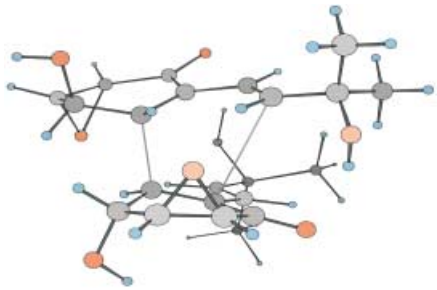
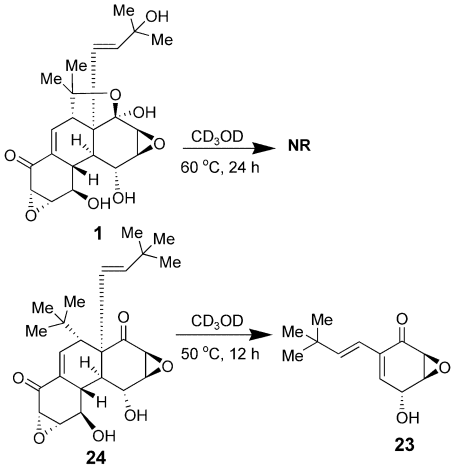


Figure 4. B3LYP/6-31G*-optimized transition-state structure for the formation of panepophenanthrin (**1**).

intermolecular cycloaddition is slightly endothermic, and presumably reversible, but hemiacetal formation "locks" the structure and thus renders panepophenanthrin isolable. Thermolysis experiments on panepophenanthrin are consistent with these general energetic considerations (Scheme 7). When dimer **1** was heated (CD_3OD, 60 °C, 24 h), starting material was recovered; however, thermolysis of untethered dimer **24** (CD_3OD, 50 °C, 12 h) led to quantitative production (^{1}H NMR) of monomer **23** in solution.



Scheme 7. Thermolysis of hemiacetal-bridged (**1**) and nonbridged (**24**) dimers.

In summary, the first enantioselective total synthesis of the ubiquitin-activating enzyme inhibitor (+)-panepophenanthrin (**1**) has been achieved employing tartrate-mediated asymmetric nucleophilic epoxidation and stereoselective Diels–Alder dimerization of an epoxyquinol dienol monomer. Modification of the epoxyquinol monomer leading to panepophenanthrin by replacing a tertiary hydroxy group with a methyl group gave mechanistic insight into the critical [4+2] dimerization. Additional experimental and computational studies related to panepophenanthrin and applications

of the methodology to produce highly functionalized molecules are in progress and will be reported in due course.

Received: May 12, 2003 [Z51862]

Keywords: cycloaddition · epoxyquinoids · natural products · total synthesis

[1] For recent reviews on the ubiquitin-proteasome pathway, see: a) A. Hershko, A. Ciechanover, *Annu. Rev. Biochem.* **1998**, *67*, 425; b) J. Myung, K. B. Kim, C. M. Crews, *Med. Res. Rev.* **2001**, *21*, 245.

[2] For information regarding ubiqutin ligases and relevance to human disease, see: http://www.ligase.com/ligase/si_ligased-iseases.php

[3] D. C. Swinney, *Drug Discovery Today* **2001**, *6*, 244.

[4] R. Sekizawa, S. Ikeno, H. Nakamura, H. Naganawa, S. Matsui, H. Iinuma, T. Takeuchi, *J. Nat. Prod.* **2002**, *65*, 1491.

[5] For a recent review of Diels–Alder-type natural products, see: A. Ichihara, H. Oikawa, *Curr. Org. Chem.* **1998**, *2*, 365.

[6] a) C. Li, E. Lobkovsky, J. A. Porco, Jr., *J. Am. Chem. Soc.* **2000**, *122*, 10484; b) C. Li, R. P. Johnson, J. A. Porco, Jr., *J. Am. Chem. Soc.* **2003**, *125*, 5095.

[7] Isolation: a) H. Kakeya, R. Onose, H. Koshino, A. Yoshida, K. Kobayashi, S.-I. Kageyama, H. Osada, *J. Am. Chem. Soc.* **2002**, *124*, 3496; epoxyquinol B: b) H. Kakeya, R. Onose, A. Yoshida, H. Koshino, H. Osada, *J. Antibiot.* **2002**, *55*, 829; synthesis: c) C. Li, S. Bardhan, E. Pace, M. Liang, T. Gilmore, J. A. Porco, Jr., *Org. Lett.* **2002**, *4*, 3267; d) M. Shoji, J. Yamaguchi, H. Kakeya, H. Osada, Y. Hayashi, *Angew. Chem.* **2002**, *114*, 3324; *Angew. Chem. Int. Ed.* **2002**, *41*, 3192; e) M. Shoji, S. Kishida, M. Takeda, H. Kakeya, H. Osada, Y. Hayashi, *Tetrahedron Lett.* **2002**, *43*, 9155; f) G. Mehta, K. Islam, *Tetrahedron Lett.* **2003**, *44*, 3569.

[8] Cf.: P. Wipf, P. Jeger, Y. Kim, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 351.

[9] For examples of molecules with a hemiacetal bridge formed from a tertiary hydroxy group, see: a) M. Matsuo, S. Matsumoto, Y. Iitaka, A. Hanaki, T. Ozawa, *Chem. Commun.* **1979**, 105; b) D. Martins, E. Osshiro, N. F. Roque, V. Marks, H. E. Gottlies, *Phytochemistry* **1998**, *48*, 677.

[10] For an ionic Diels–Alder reaction involving a hemiacetal tether, see: R. K. Haynes, K.-P. Lam, K.-Y. Wu, I. D. Williams, L.-L. Yeung, *Tetrahedron* **1999**, *55*, 89.

[11] The *endo* [4+2] dimerization appears to be precluded by steric interactions between the epoxide on the dienophile and the diene. For Diels–Alder reactions of 2-vinylcyclohexenones and related dienes, see: a) C. Spino, M. Pesant, Y. Dory, *Angew. Chem.* **1998**, *37*, 3454; *Angew. Chem. Int. Ed.* **1998**, *37*, 3262; b) J. T. Kuethe, C. A. Brooks, D. L. Comins, *Org. Lett.* **2003**, *5*, 321.

[12] a) Z. Kis, A. Closse, H. P. Sigg, L. Hruban, G. Snatzke, *Helv. Chim. Acta* **1970**, *53*, 1577; b) G. Erkel, T. Anke, O. Sterner, *Biochem. Biophys. Res. Commun.* **1996**, *226*, 214; c) J. B. Shotwell, S. Hu, E. Medina, M. Abe, R. Cole, C. M. Crews, J. L. Wood, *Tetrahedron Lett.* **2000**, *41*, 9639.

[13] a) C. Li, E. Pace, M. Liang, E. Lobkovsky, T. D. Gilmore, J. A. Porco, Jr., *J. Am. Chem. Soc.* **2001**, *123*, 11308; For tartrate-mediated epoxidation of acyclic enones, see: b) C. L. Elston, R. F. W. Jackson, S. J. F. MacDonald, P. J. Murray, *Angew. Chem.* **1997**, *36*, 379; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 410; c) O. Jacques, S. J. Richards, R. F. W. Jackson, *Chem. Commun.* **2001**, 2712.

[14] A. B. Hughes, M. V. Sargent, *J. Chem. Soc. Perkin Trans. 1* **1989**, *10*, 1787.

[15] a) A. Pelter, S. Elgendy, *Tetrahedron Lett.* **1988**, *29*, 677; b) A. E. Fleck, J. A. Hobart, G. W. Morrow, *Synth. Commun.* **1992**, *22*, 179.

[16] M. C. Pirrung, D. S. Nunn, *Tetrahedron Lett.* **1992**, *33*, 6591.

[17] J. P. Ragot, C. Steeneck, M.-L. Alcaraz, R. J. K. Taylor, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1073.

[18] T. Nakata, T. Tanaka, T. Oishi, *Tetrahedron Lett.* **1981**, *22*, 4723.

[19] S. F. Martin, J. A. Dodge, *Tetrahedron Lett.* **1991**, *32*, 3017.

[20] For a review on ionic Diels–Alder reactions, see: R. Sanghi, P. S. Vankar, Y. D. Vankar, *J. Indian Chem. Soc.* **1998**, *75*, 709.

[21] For ionic Diels–Alder reactions involving hydroxy dienes and cyclic enones, see: a) P. G. Gassman, D. A. Singleton, *J. Am. Chem. Soc.* **1984**, *106*, 6085; b) R. K. Haynes, G. R. King, S. C. Vonwiller, *J. Org. Chem.* **1994**, *59*, 4743.

[22] For a review, see: D. L. Boger, M. Patel, *Prog. Heterocycl. Chem.* **1989**, *1*, 30.

[23] a) R. Tripathy, P. J. Carroll, E. R. Thornton, *J. Am. Chem. Soc.* **1990**, *112*, 6743; b) Y. Huang, V. H. Rawal, *J. Am. Chem. Soc.* **2002**, *124*, 9662.

[24] Recent review: E. Marsault, A. Toro, P. Nowak, P. Deslongchamps, *Tetrahedron* **2001**, *57*, 4243.

[25] Analysis by chiral HPLC verified that the crystal was racemic, while the mother liquor was optically pure (see Supporting Information). For an example of a "racemic crystal" formed by crystallization of an optically enriched compound, see: A. Mishnev, I. Iovel, J. Popelis, I. Vosekalna, E. Lukevics, *J. Organomet. Chem.* **2000**, *608*, 1.

[26] All calculations were performed with Spartan'02, Wavefunction Inc., Irvine, CA, 2002; see Supporting Information.

[27] CCDC-206033 (**8**) and -206034 (**25**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

[28] Note added in proof: A racemic synthesis of **1** was recently reported: J. E. Moses, L. Commeiras, J. E. Baldwin, R. M. Adlington, *Org. Lett.* **2003**, *5*, 2987.

