Explorations of [4+2] and [5+2] Cycloadditions of Dienylcyclopropane Derived Enzymatically from Cyclopropylbenzene

Jason Reed Hudlicky, John Hopkins-Hill, Tomas Hudlicky*

Department of Chemistry and Centre for Biotechnology, Brock University, 500 Glenridge Avenue, St. Catharines, ON, L2S 3A1, Canada
E-mail: thudlicky@brocku.ca

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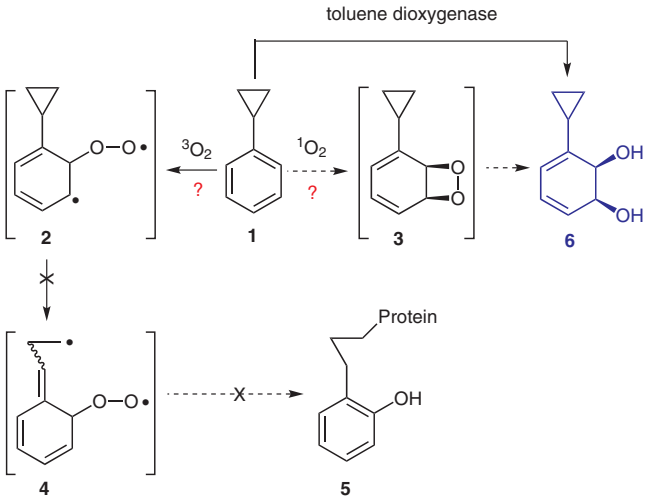
Abstract: Fermentation of cyclopropylbenzene with *E. coli* JM109(pDTG601a) furnished optically pure 1-cyclopropyl-2,3-dihydroxycyclohexa-4,6-diene whose reactivity in [4+2]- and [5+2]-cycloaddition chemistry was explored.

Key words: dienylcyclopropane, cyclopropylbenzene, [4+2] cycloadditions, [5+2] cycloadditions, enzymatic dihydroxylation of arenes

Some time ago we tested cyclopropylbenzene as a substrate for toluene dioxygenase in the whole-cell fermentation with *E. coli* JM109(pDTG601a).[1] The original reason for this investigation was based on an assumption that the enzymatic dihydroxylation may proceed via a radical intermediate such as **2**, rather than via a dioxetane intermediate of type **3**, originally proposed by Gibson.[2] The latter postulate would require a singlet oxygen species present in the active site of the enzyme. On the other hand, interaction of the substrate with a triplet species may generate a radical of type **2** that might undergo unraveling of the dienylcyclopropyl system to **4** and subsequent potential covalent trapping within the enzyme active site to a species such as **5**, as shown in Scheme 1.

Such mechanistic options have recently been summarized,[3] although the actual mechanism of the enzymatic dihydroxylation remains unknown. In spite of this fact the diols serve as convenient optically pure starting materials in creative syntheses of many natural products.[4]



Scheme 1 Speculations on possible intermediates in enzymatic dihydroxylations of arenes

We discovered that cyclopropylbenzene[5] was, in fact, an excellent substrate for toluene dioxygenase, initially leading to production of the 1-cyclopropylcyclohexadiene-*cis*-2,3-diol **6** in yields of ca. 2.5 g/L. With further optimization, we were able to prepare 60–80 grams of **6** on a 10-L fermenter scale. Such conversion was comparable to that obtained from toluene, styrene, or halobenzenes, which are among the best substrates for this enzyme, yielding >20 g/L of diols.

Calculations performed on the dienylcyclopropyl radical **2** indicated that the original anticipated unraveling would not take place: ΔG for the transformation was calculated to be 17.7 kcal/mol and the calculated rate constant k_r = 1.1 s^{-1}.[6] While the parent cyclopropylcarbinyl system rearranges with a rate constant of 10^8 s^{-1} the dienyl system enjoys the additional stabilization of the radical and thus the unraveling would be an energetically demanding process. It is well known that the presence of radical-stabilizing substituents near the carbinyl site effects lower the rates of ring opening.[7] Nevertheless, the availability of diol **6**, as well as other metabolites derived from functionalized cyclopropyl benzenes,[8] prompted us to investigate its reactive tendencies in various cycloadditions. In this paper we report the preliminary findings with regard to [4+2] and [5+2] cycloadditions.

The [4+2] cycloadditions of the diene unit in the protected diol **6** were investigated. The diol was protected as its acetonide **7**, a compound that proved to be relatively unstable. Small amounts of the 2-cyclopropylphenol generated during the protection led to further autocatalytic elimination. The acetonide was stable to degradation when kept as a solution in ethyl acetate over solid sodium carbonate. It was also prone to slow dimerization via Diels–Alder cycloaddition upon standing. Complete and stereoselective dimerization of **7** was achieved by heating **7** in xylene at reflux for 20 hours to yield **8** as a single stereoisomer in ca. 95% yield, in agreement with previously investigated dimerizations of this type. Past work from our laboratory has shown that acetonides derived from bromo- and chlorocyclohexadiene-*cis*-diols form the corresponding dimers stereo- and regioselectively and the structures were confirmed by X-ray crystallography.[9] These observations were previously made by Ley[10] for the acetonide of the diol derived from bromobenzene and by Roberts[11]

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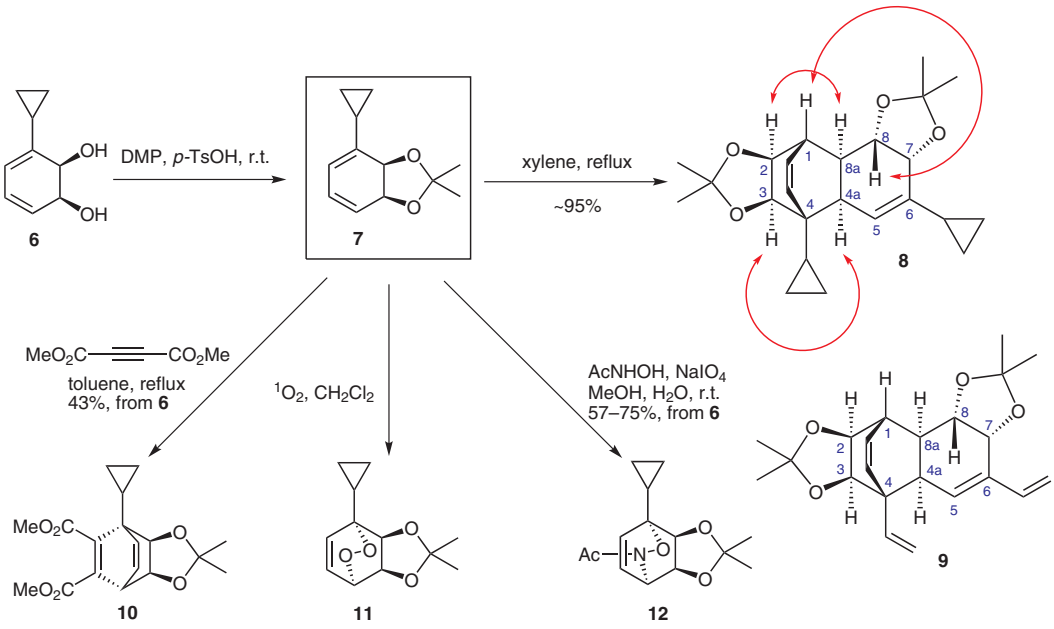


Scheme 2

for the acetonide of the diol obtained from trifluoromethylbenzene.

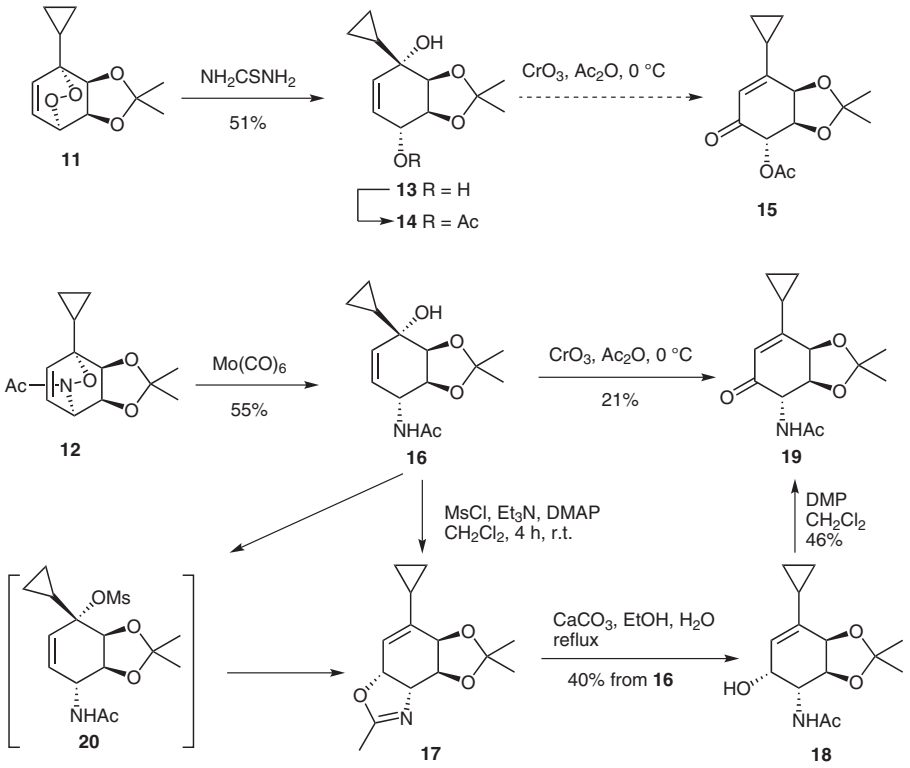
The stereochemistry of **8** was determined by ^{1}H NMR NOESY experiment, which indicated strong NOE between H-8a/H-2, H-4a/H-3, and H-1/H-8. The latter NOE confirmed the regiochemistry of the dimerization as drawn in Scheme 2. The observed NOEs as well as the value of the coupling constant between H-8a/H4a (J = 9.9 Hz) were identical to those observed in the NMR experiments (COSY and HETCOR) performed on the dimer **9**, which we have prepared from the acetonide-protected diol from styrene.[12] In those experiments irradiation of H-2 produced 8.3% enhancement at H-8a in **9**. Similarly, irradiation of H-3 resulted in 11.5% enhancement at H-4a. The structure of dimer **9** was also confirmed by X-ray analysis of the corresponding C-7/C-8 diol obtained by partial hydrolysis.

The bicyclic adduct **10** was obtained in 43% yield (over two steps) upon heating **7** in the presence of acetylene dicarboxylate in *m*-xylene for six hours. Singlet oxygen cycloaddition produced *endo*-peroxide **11** with complete conversion (this compound was not stable to isolation and was immediately reduced). Acetonide **7** underwent a rapid hetero-Diels–Alder cycloaddition with acyl nitroso dienophile generated in situ from the corresponding hydroxamic acid to yield oxazine **12** within a few minutes as a single isomer in 75% yield (small scale; 57% yield on 10-gram scale). The cycloadditions of nitroso dienophiles with the electronically polarized dienes in *cis*-diols derived from bromo- and chlorobenzene[13] or ethyl benzoate[14] produced single regioisomers of the corresponding oxazines. We did not expect complete regioselectivity in the cycloadditions of less polarized alkyl-substituted dienes such as that in **7**.

The *endo*-peroxide **11** was reduced with thiourea to furnish the conduritol derivative **13** and its acetate **14** in 51% yield over two steps. Reduction of the oxazine with $Mo(CO)_6$[15] gave the allylic alcohol **16** in 55% yield and this material was converted into enone **19** by modified Dauben–Michno oxidative transposition in 21% isolated yield, as shown in Scheme 3. The low yield in the conversion of **16** into **19** is the result of a rather arduous workup and does not reflect the otherwise complete conversion.

The Dauben–Michno oxidation was investigated in detail in our laboratories with regard to allylic alcohols adjacent to electron-withdrawing groups such as carboxylates[16] and nitriles.[17] Previously, this oxidation was reported only for allylic alcohols with electron-donating groups. It is assumed that it proceeds by a formation of a chromate ester that undergoes a [3,3]-sigmatropic rearrangement followed by the normal oxidation of the rearranged chromate. What is clear from the successful oxidation of **16** to **19** is that there very likely is no partial positive character at the site of the cyclopropylcarbinyl alcohol during the sigmatropic rearrangement otherwise substantial ring opening would be observed. This is an interesting, and somewhat surprising, result because the conditions of the Dauben–Michno oxidation are strongly acidic.

We have also prepared enone **19** in 45% yield by oxidation of the allylic alcohol **18**, which was obtained by a mild hydrolysis of oxazoline **17** (40% over two steps). Such oxazolines are produced by the rearrangement of mesylates, such as **20**, derived from 1,4-acetamido alcohols. We have investigated this rearrangement first in connection with a synthesis of oseltamivir[18] and later as a general method of synthesis for 1,2-amino alcohol derivatives from oxazines.[19] The yields of enone **19** by this method are slightly higher than those obtained by the Dauben–Michno transposition because of problems in

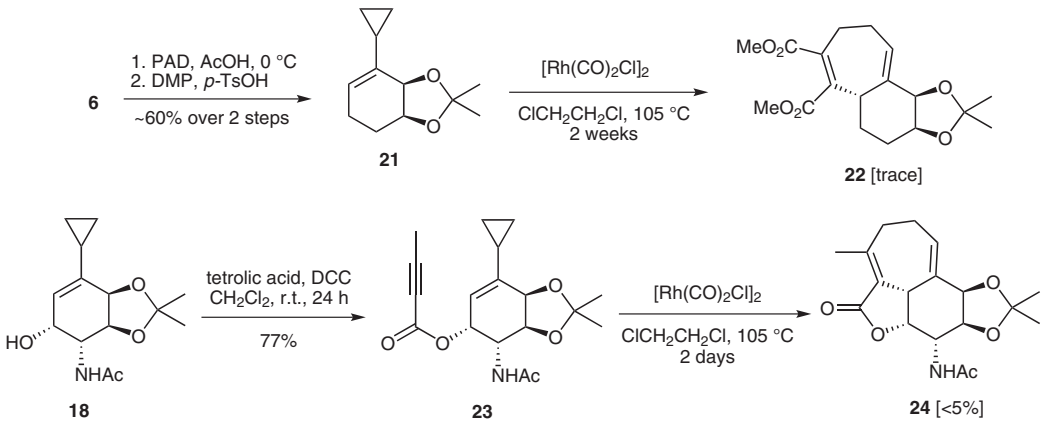


Scheme 3

workup. Although the conversion of **16** into **19** is complete, isolated yields tended to be quite low.

Enone **19** or its oxygen analogue **15** contain a vinylcyclopropane functionality amenable to either vinylcyclopropane–cyclopentene rearrangement or [5+2] cycloadditions. Both processes would lead to annulated conduritol and conduramine derivatives that have been shown to have antiglycosidic properties.[20] We therefore turned to investigations of the chemistry of the vinylcyclopropane moiety,[21] namely the rhodium-catalyzed [5+2] cycloadditions discovered and reported by Wender.[22] Diol **6** was reduced with potassium azodicarboxylate (PAD) as previously described and protected as its acetonide **21**[6] by treatment with 2,2-dimethoxypropane in the presence of *p*-TsOH, as shown in Scheme 4. Exposure of **21** to excess dimethyl acetylene dicarboxylate in dichloroethane and in the presence of $[Rh(CO)_2Cl]_2$ at 105 °C generated only traces of cycloheptadiene **22** after almost two weeks of heating in a sealed tube. Normally, the reactions of vinylcyclopropanes in [5+2] annulations reported by Wender are complete within several hours. However, the reported reactions did not involve vinylcyclopropanes where either component would be part of a ring, as is the case with **21**. We attribute this apparent lack of reactivity to the fact that the cyclopropyl ring does not appear to be 'in conjugation' with the olefin, and is likely in an essentially orthogonal arrangement with the alkene (vide infra NMR).

Assuming that the intramolecular process would be more feasible, we prepared ester **23** by acylation of **18** with methylpropiolic acid in the presence of DCC. When **23** was



Scheme 4

subjected to the [5+2]-annulation protocol under the same conditions employed with **21** the tetracyclic lactone **24** was detected to the tune of ca. 5% in a complex mixture containing at least three other products. We will return to the [5+2]-annulation issue with simpler model compounds to establish the reason for such lack of reactivity in vinylcyclopropanes in which one or the other moiety is confined in a ring.

In conclusion, preliminary investigations of cycloaddition chemistry of the enzymatically derived dienylcyclopropyl-*cis*-diol **6** were carried out.[23] New optically pure derivatives were obtained that can find utility in the synthesis of annulated conduritol and conduramine derivatives. The low reactivity of **21** and **23** in [5+2] cycloadditions will be revisited; our attempts constituted the first example of this process with substrates in which one of the moieties of vinylcyclopropane is constrained into a ring.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett. Experimental data for compounds **7**, **8**, **10**, **11**, **13**, **14**, **17**–**19**, and **23**, and ^{1}H NMR and ^{13}C NMR spectra for compounds **8**, **10**, **12**, **13**, **14**, **16**–**19**, and **23** are included.

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References and Notes

(1) Endoma, M. A.; Bui, V. P.; Hansen, J.; Hudlicky, T. *Org. Process Res. Dev.* **2002**, *6*, 525.
(2) (a) Gibson, D. T. *Zentralbl. Bakt.* **1976**, 157. (b) Jeffrey, A. M.; Yah, H. J. C.; Jerina, D. M.; Patel, T. R.; Davey, J. F.; Gibson, D. T. *Biochemistry* **1975**, *14*, 575.
(3) Hudlicky, T.; Reed, J. W. *Synlett* **2009**, 685.
(4) For reviews on applications of these compounds to synthesis, see: (a) Hudlicky, T.; Reed, J. W. *Chem. Soc. Rev.* **2009**, *38*, 3117. (b) Ref. 3 above. (c) Boyd, D. R.; Bugg, T. D. H. *Org. Biomol. Chem.* **2006**, *4*, 181. (d) Johnson, R. A. *Org. React. (N.Y.)* **2004**, *63*, 117. (e) Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1999**, *32*, 35. (f) Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. J. *Chem. Rev.* **1996**, *96*, 1195. (g) Hudlicky, T.; Reed, J. W. In *Advances in Asymmetric Synthesis*, Vol. 1; Hassner, A., Ed.; JAI Press: London, **1995**, 271. (h) Brown, S. M.; Hudlicky, T. In *Organic Synthesis: Theory and Applications*, Vol. 2; Hudlicky, T., Ed.; JAI Press: London, **1993**, 113.
(5) For large-scale preparation (ca. 100-g scale) of this compound from cinnamyl aldehyde, see: (a) Petersen, R. J.; Skell, P. S. *Org. Synth.* **1967**, *47*, 98. (b) Petersen, R. J.; Skell, P. S. *Org. Synth., Coll. Vol. V*; Wiley: New York, **1973**, 929.
(6) Bui, V. P.; Nguyen, M.; Hansen, J.; Baker, J.; Hudlicky, T. *Can. J. Chem.* **2002**, *80*, 708.
(7) Beckwith, A. L. J.; Bowry, V. W. *J. Am. Chem. Soc.* **1994**, *116*, 2710.
(8) Finn, K. J.; Rochon, L.; Hudlicky, T. *Tetrahedron: Asymmetry* **2005**, *16*, 3606.
(9) Hudlicky, T.; Boros, E. E.; Olivo, H. F.; Merola, J. S. *J. Org. Chem.* **1992**, *57*, 1026.
(10) Ley, S. V.; Redgrave, A. J.; Taylor, S. C.; Ahmed, S.; Ribbons, D. W. *Synlett* **1991**, 741.
(11) (a) Pittol, C. A.; Pryce, R. J.; Roberts, S. M.; Ryback, G.; Sik, V.; Williams, J. O. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1160. (b) Mahon, M. F.; Molloy, K.; Pittol, C. A.; Pryce, R. J.; Roberts, S. M.; Ryback, G.; Sik, V.; Williams, J. O.; Winders, J. A. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1255.
(12) Hudlicky, T.; Boros, C. H. *Tetrahedron Lett.* **1993**, *34*, 2557.
(13) (a) Hudlicky, T.; Olivo, H. F. *Tetrahedron Lett.* **1991**, *32*, 6077. (b) Hudlicky, T.; Olivo, H. F.; McKibben, B. *J. Am. Chem. Soc.* **1994**, *116*, 5108.
(14) Fabris, F.; Collins, J.; Sullivan, B.; Leisch, H.; Hudlicky, T. *Org. Biomol. Chem.* **2009**, *7*, 2619.
(15) (a) Marradi, M. *Synlett* **2005**, 1195. (b) Ritter, A. R.; Miller, M. J. *Org. Chem.* **1994**, *59*, 4602. (c) Zimmer, R.; Reissig, H.-U. *J. Org. Chem.* **1992**, *57*, 339.
(16) Werner, L.; Machara, A.; Hudlicky, T. *Adv. Synth. Catal.* **2010**, *352*, 195.
(17) Hudlicky, J. R.; Werner, L.; Semak, V.; Simionescu, R.; Hudlicky, T. *Can. J. Chem.* **2011**, *89*, 535.
(18) Sullivan, B.; Carrera, I.; Drouin, M.; Hudlicky, T. *Angew. Chem. Int. Ed.* **2009**, *48*, 4229.
(19) Werner, L.; Hudlicky, J. R.; Wernerova, M.; Hudlicky, T. *Tetrahedron* **2010**, 3761.
(20) See, for example: (a) Mehta, G.; Ramesh, S. S. *Can. J. Chem.* **2005**, *83*, 581. (b) Mehta, G.; Senaiar, R. S.; Bera, M. K. *Chem. Eur. J.* **2003**, *9*, 2264. (c) Desjardins, M.; Lallemand, M.-C.; Hudlicky, T.; Abboud, K. A. *Synlett* **1997**, 728. (d) For a definition of cyclitol, conduritol, and conduramine, see: Duchek, J.; Adams, D. R.; Hudlicky, T. *Chem. Rev.* **2011**, *111*, 4223.
(21) For a recent review of the chemistry of vinylcyclopropanes, see: Hudlicky, T.; Reed, J. W. *Angew. Chem. Int. Ed.* **2010**, *49*, 4864.
(22) For [5+2] annulation of vinylcyclopropanes with alkynes, see: (a) Wender, P. A.; Takahashi, H.; Witulski, B. *J. Am. Chem. Soc.* **1995**, *117*, 4720. (b) Wender, P. A.; Claudia, M.; Barzilay, C. M.; Dyckman, A. J. *J. Am. Chem. Soc.* **2001**, *123*, 179. (c) Liu, P.; Cheong, P. H.-Y.; Yu, Z.-X.; Wender, P. A.; Houk, K. N. *Angew. Chem. Int. Ed.* **2008**, *47*, 3939. (d) Wender, P. A.; Sirois, L. E.; Stemmler, R. T.; Williams, T. J. *Org. Lett.* **2010**, *12*, 1604.
(23) **Selected Experimental Procedures**
1-{4-Cyclopropyl-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-(epoxyimino)benzo[*d*][1,3]dioxol-8-yl}ethanone (12): To a solution of diol **6** (0.50 g, 3.28 mmol) in 2,2-dimethoxypropane was added a catalytic amount of *p*-toluenesulfonic acid. After 5 min, the reaction was quenched with the addition of solid $NaHCO_3$ (100 mg). The reaction mixture was then diluted with MeOH–H_2O (4:1; 30 mL) and sodium periodate (1.70 g, 8.13 mmol) was added in one portion. The resulting solution was then cooled to 0 °C and a solution of acetohydroxamic acid (0.61 g, 8.13 mmol) in MeOH (30 mL) was added dropwise over 10 min. The

solution was allowed to warm to r.t. and the stirring was continued for 17 h at r.t. After consumption of starting material, the reaction mixture was filtered and concentrated in vacuo. The oily residue was then diluted with EtOAc, and the organic solution was washed with sat. aq. $NaHCO_3$ followed by brine. The organic solution was dried over $MgSO_4$. The crude material was then purified by suction column chromatography to yield oxazine **12** (0.65 g, 75%) as an oil which slowly solidified.

Large-Scale Preparation of Oxazine 12:

Dimethoxypropane (15 mL) was cooled to 0 °C and a crystal of *p*-TsOH was added. After 2 min diol **6** (5.0 g, 0.033 mol) dissolved in acetone–EtOAc (15 mL) was added dropwise over 5 min. TLC analysis indicated full conversion to acetonide **7** accompanied by 5–10% of 2-cyclopropylphenol resulting from aromatization of diol **6**. The solution was diluted with EtOAc (20 mL), washed once with 1 N NaOH (3 mL), and added to a solution of $NaIO_4$ (17.0 g, 0.081 mol, 2.5 equiv) in MeOH–H_2O (4:1; 250 mL) cooled to 0 °C in 1-L Erlenmeyer flask. Acetohydroxamic acid (6.1 g, 0.081 mol, 2.5 equiv) dissolved in MeOH–H_2O (4:1; 100 mL) was added with vigorous stirring over 15 min. A thick white precipitate formed immediately. The reaction mixture was allowed to warm to r.t. over 1 h and the stirring was continued for 1 h at r.t. at which time the mixture was filtered, the precipitate was washed with EtOAc (200 mL) and the solution was washed with brine (2 × 100 mL), sat. $NaHCO_3$ (2 × 100 mL), dried over Na_2SO_4, and evaporated. The crude product, containing 5–10% of 2-cyclopropylphenol, was purified by chromatography (silica, gradient elution, hexane to hexane–EtOAc, 3:1) to furnish oxazine **12** (5.4 g, 64%) as an oil that slowly solidified. Repetition on 10-gram scale gave the oxazine (7.12 g, 57%). Recrystallization from EtOAc–pentane gave white needles.

12: R_f 0.27 (hexane–EtOAc, 4:1); mp 44–46 °C (EtOAc–pentane); $[\alpha]^{20}_D$ –20.5 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3691, 3011, 2419, 1731, 1655, 1618, 1375, 1270, 1088, 1064, 760 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 6.32 (dd, *J* = 8.0, 6.3 Hz, 1 H), 5.90 (d, *J* = 8.1 Hz, 1 H), 5.20–5.28 (m, 1 H), 4.32 (dd, *J* = 6.6, 4.5 Hz, 1 H), 4.13 (d, *J* = 6.9 Hz, 1 H), 1.79 (s, 3 H), 1.13 (s, 7 H), 0.37–0.59 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.2, 131.3, 131.0, 110.6, 79.9, 77.7, 73.3, 49.7, 25.7, 25.4, 21.5, 13.7, 1.1, 0.5. MS (EI+): *m*/*z* (%) = 265 (13), 250 (23), 207 (48), 178 (30), 135 (43), 123 (54), 118 (68), 107 (58), 91 (52), 85 (54). HRMS (EI+): *m*/*z* calcd for $C_{14}H_{19}NO_4$: 265.1314; found: 265.1317. Anal. Calcd for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22. Found: C, 62.92; H, 7.07.

***N*-{(3a*S*,4*R*,7*S*,7a*S*)-7-Cyclopropyl-7-hydroxy-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[*d*][1,3]dioxol-4-yl}acetamide (16)**: To a solution of oxazine **12** (248 mg, 0.94 mmol) in MeCN (7.5 mL) and distilled H_2O (0.5 mL) was added molybdenum hexacarbonyl (371 mg, 1.40 mmol) in one portion. The resulting suspension was immersed in an oil bath and heated to reflux. At reflux, the mixture changed from a clear suspension to a black solution, which was allowed to reflux for 2 h. Progress of the reaction was monitored by TLC (EtOAc). After consumption of the starting material was complete, the reaction mixture was removed from the oil bath and allowed to cool to r.t. The reaction mixture was then filtered through a plug of Celite using EtOAc as the eluent and concentrated in vacuo. The crude material was then purified via flash column chromatography (EtOAc) to yield amido alcohol **16** (138 mg, 55%) as a white powder; R_f 0.29 (EtOAc); mp 189–192 °C (EtOAc); $[\alpha]^{20}_D$ –93.8 (*c* = 1.0, $CHCl_3$). IR ($CHCl_3$): 3404, 3009, 2511, 1661, 1512, 1415, 1382, 1243, 1064, 759 cm^{-1}. ^1H NMR (600 MHz, MeOD): δ = 5.79 (d, *J* = 10.2 Hz, 1 H), 5.75 (dd, *J* = 10.2, 4.2 Hz, 1 H), 4.45 (td, *J* = 3.6, 1.2 Hz, 1 H), 4.27 (m, 2 H), 1.97 (s, 3 H), 1.43 (s, 3 H), 1.36 (s, 3 H), 1.19–1.26 (m, 1 H), 0.47–0.53 (m, 1 H), 0.35–0.47 (m, 3 H). ^{13}C NMR (150 MHz, MeOD): δ = 171.2, 133.9, 128.6, 108.3, 81.1, 76.9, 69.8, 48.8, 25.9, 23.8, 21.5, 16.3, –0.4, –0.9. MS (FAB+): *m*/*z* (%) = 268 (5), 250 (85), 192 (49), 150 (98), 105 (26), 69 (70), 43 (100). HRMS (FAB+): *m*/*z* calcd for $C_{14}H_{21}NO_4$: 267.1471; found: 267.1471. Anal. Calcd for $C_{14}H_{21}NO_4$: C, 62.90; H, 7.92. Found: C, 63.01; H, 7.93.