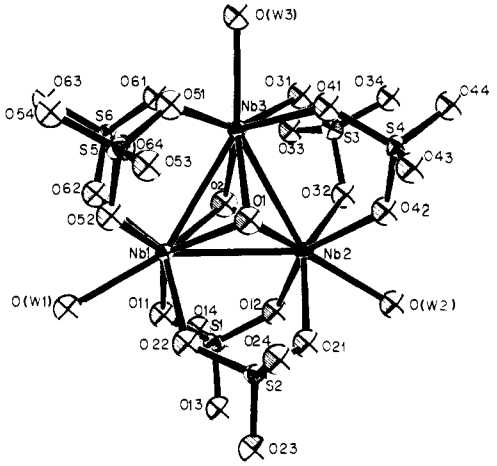

Figure 1. The structure of $[Nb_3O_2(SO_4)_6(H_2O)_3]^{5-}$.

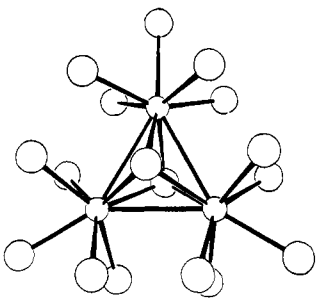

Figure 2. The skeletal structure of compounds with the general formula $[M_3X_2(O_2CR)_6L_3]^{n(\pm)}$.

Table I. Some Average Bond Distances (Angstroms) in $K_4(H_5O_2)[Nb_3O_2(SO_4)_6(H_2O)_3]\cdot 5H_2O$

Nb-Nb	2.886 (1)
Nb-(μ_3-O)	2.052 (9)
Nb-O(μ-SO_4)	2.136 (9)
Nb-O(H_2O)	2.241 (9)
O···H···O($H_5O_2^+$)	2.42 (1)

atoms and its seven surrounding oxygen atoms are similar to those found in the Mo and W complexes. Some pertinent bond distances are given in Table I.

The $H_5O_2^+$ ion occurs in this structure with an O···H···O distance of 2.42 (1) Å. This result is within the average range of 2.41–2.45 Å found in other compounds containing the diaquahydrogen ion.[16] The visible spectra of the niobium cluster in the solid state, in the mother liquid, and in a solution prepared by dissolving the solid in 60% H_2SO_4 were compared and found to be identical.[17] This, together with the fact that the red-brown solid is precipitated quantitatively from the red-brown solution by adding K_2SO_4, strongly supports the assumption that the species present in the solution is identical with the ion found in the crystal, namely, $[Nb_3O_2(SO_4)_6(H_2O)_3]^{5-}$. The 5- charge of the niobium cluster necessitates a nonintegral oxidation number of $3^2/_3$+ for the metal atoms. This result agrees with the results of Golibersuch and Young, who determined the oxidation state of the niobium atom in this complex by a permangantometric titration.[14e] These results suggest that there are four electrons involved in the Nb–Nb bonding. According to Cotton's MO calculations for metal clusters,[18] these four electrons occupy bonding orbitals and the resulting bond order in this complex is $^2/_3$. The same bond order was assigned to the structurally related triangular molybdenum cluster $[Mo_3(CCH_3)_2(O_2CCH_3)_6(H_2O)_3]^{2+}$;[1] the oxidation state of the molybdenum atoms is $4^2/_3$+ and four electrons are involved in the Mo–Mo bonding. The metal–metal bond length is 2.887 (1) Å, in good agreement with the Nb–Nb bond length reported here, 2.886 (1) Å, for the same bond order.

Supplementary Material Available: Positional and thermal parameters for the atoms of $K_4(H_5O_2)[Nb_3O_2(SO_4)_6(H_2O)_3]\cdot 5H_2O$ (2 pages). Ordering information is given on any current masthead page.

(16) Lundgren, J.-O.; Olovsson, I. "The Hydrogen Bond, Recent Developments in Theory and Experiment. Structure and Spectroscopy"; Shuster, P., Zundel, G., Sandorfy, C., Eds.; North-Holland Publishing Co.: Amsterdam, 1979; Vol. II, Chapter 10.

(17) Both solid (KBr pellet) and solution show absorption lines at 510 and 700 nm. The solution spectrum is in agreement with that reported earlier by Goroshchenko and Andreeva which was, however, wrongly assigned to a postulated $Nb_6O_3(SO_4)_8$.[14g]

(18) Cotton, F. A. *Inorg. Chem.* **1964**, *3*, 1217.

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Isolation and Structure of Spatol, a Potent Inhibitor of Cell Replication from the Brown Seaweed *Spatoglossum schmittii*

Sir:

Cytotoxicity is a frequently encountered pharmacological property associated with extracts of marine organisms,[1] and in several cases the cytotoxic agents have been purified and described.[2] The utilization of these cytotoxins in the selective inhibition of cancer cell replication represents a long-term goal of marine cytotoxicity investigations. To promote this application, we have adopted a convenient field-oriented bioassay involving the assessment of inhibition of the synchronous cell division of the fertilized sea urchin egg. Preliminary pharmacological studies have indicated that the urchin egg assay may be highly selective for compounds which inhibit mitotic spindle formation via inhibition of tubulin polymerization.[3] This mechanism of action

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(2) (a) F. J. Schmitz, K. H. Hollenbeak, and R. S. Prasad, *Tetrahedron Lett.*, 3387 (1979); (b) A. J. Weinheimer, J. A. Matson, D. van der Helm, and M. Poling, *ibid.*, 1295 (1977); (c) A. J. Weinheimer and J. A. Matson, *Lloydia*, **38**, 378 (1975); (d) A. J. Weinheimer, J. A. Matson, M. B. Hossain, and D. van der Helm, *Tetrahedron Lett.*, 2923 (1977); (e) M. T. Cheng and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **100**, 7409 (1978); (f) G. R. Pettit, C. L. Herald, M. S. Allen, R. B. Von Dreele, L. D. Vanelle, J. P. Y. Kao, and W. Blake., *ibid.*, **99**, 262 (1977); (g) F. J. Schmitz, K. H. Hollenbeak, D. C. Carter, M. B. Hossain, and D. van der Helm, *J. Org. Chem.*, **44**, 2445 (1979); (h) J. S. Mynderse, R. E. Moore, M. Kashiwagi, and T. R. Norton, *Science*, **196**, 538 (1977); (i) B. M. Howard, K. Clarkson, and R. Bernstein, *Tetrahedron Lett.*, 4449 (1979); (j) Y. Gopichand and F. J. Schmitz, *ibid.*, 3921 (1979); (k) G. T. Carter and K. L. Rinehart, Jr., *J. Am. Chem. Soc.*, **100**, 7441 (1978).

(3) The urchin egg assay is conducted by measuring inhibition of the first cleavage of the fertilized egg. Data reported in this paper were obtained by using eggs from the Pacific urchin *Lytechinus pictus* (Verrill). Aspects of the pharmacology of this assay have been recently reported: R. S. Jacobs, S. White, and L. Wilson, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, in press.

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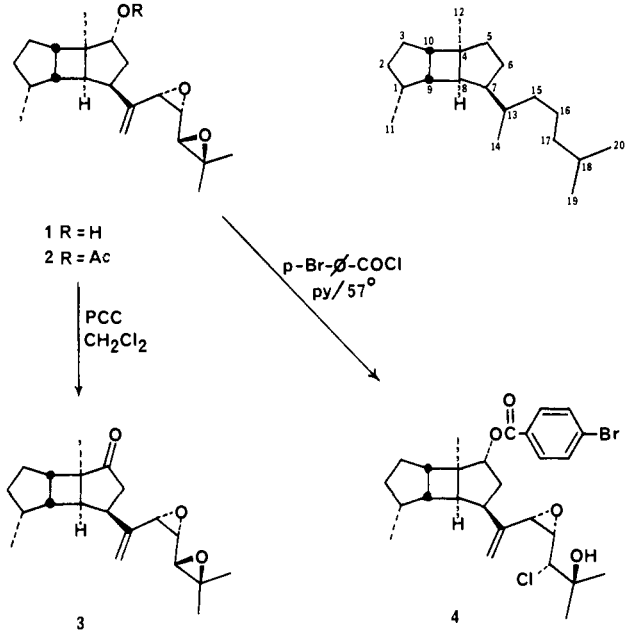
is widely recognized for several potent antitumor agents, including colchicine and the *Vinca*-derived alkaloids vincristine and vinblastine,[4] which have proven clinically useful.

In this communication, we wish to report the isolation and structure elucidation of a potent new cytotoxic diepoxide, spatol (**1**). Spatol was isolated from the brown seaweed *Spatoglossum schmittii* Taylor (Dictyotaceae) from the Galapagos Islands and shows an ED_{50} = 1.2 μg/mL in the urchin egg assay. Further, at the preliminary cell culture testing concentration of 16 μg/mL, spatol completely inhibits cell division in human T242 Melanoma and 224C Astrocytoma neoplastic cell lines.[5] The tricyclic and regular diterpenoid structure of spatol is unprecedented in the terpenes; however, the tricycle nucleus is uniquely similar (epimeric at C-7) to the skeleton of the bourbonene class of sesquitepernoids.[6] We wish to suggest the name "spatane" for this new class of diterpenoid molecules, and the numbering scheme shown is in analogy to the numbering accepted for bourbonene.[6]



Spatoglossum schmittii was collected along the western coast of Isla Isabella, Archipelago de Colon (Galapagos Islands), in Feb 1978 and immediately stored in isopropyl alcohol. An extract (10.7 g, 3% dry wt) was subsequently prepared by repeated $CHCl_3$/MeOH extraction, followed by removal of the solvents from the combined extracts. Isolation of the cytotoxic diepoxide was accomplished via repeated silica gel chromatography, first by using open-column techniques (10% EtOAc/CH_2Cl_2) and secondly, liquid chromatography (μ-Porasil, 50% EtOAc/isooctane). Spatol crystallized from the concentrated LC eluant to form fine white needles, mp 100–102 °C. The cytotoxin showed $[\alpha]_D$ +45.6° (*c* 1.56, $CHCl_3$) and analyzed for $C_{20}H_{30}O_3$ by mass spectrometry. The nature of the six degrees of unsaturation inherent in this molecular formula could be well defined by ^1H and ^{13}C NMR analysis.[7] One terminal or exocyclic double bond was present,

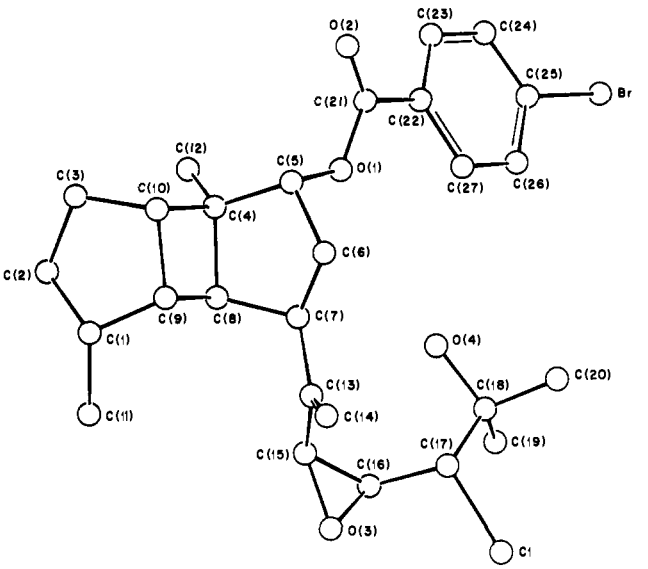


Figure 1. Computer-generated perspective drawing of spatol derivative **4**. The absolute configuration shown is that predicted from anomalous X-ray scattering. Hydrogens are omitted for clarity.

along with a secondary alcohol and two epoxide groups. The remaining three degrees of unsaturation were then assigned to a tricyclic carbon skeleton.

The 220-MHz ^1H NMR spectrum of spatol, in conjunction with spin-decoupling experiments, gave considerable insight into the structure of this metabolite. A one-proton band at δ 3.64 (d, *J* = 4.0 Hz) was readily assigned to the alcohol methine hydrogen at C-5, and this assignment was confirmed by acetylation of **1** (Ac_2O/pyridine/room temperature) to yield the monoacetate **2**. In the acetate the C-5 methine proton was shifted to δ 4.85. By decoupling both **1** and **2**, protons at C-5 through C-7 were interrelated, and the presence of the side chain was established at C-7. On the basis of further decoupling data, the terminal olefin and the two epoxide functionalities were placed in adjacent proximity along the side chain.

Oxidation of spatol with pyridinium chlorochromate in CH_2Cl_2 smoothly converted **1** to the corresponding ketone, **3**. The ketone showed an infrared carbonyl absorption at 1740 cm^{-1}, indicating the production of a cyclopentanone. This information, together with the ^1H NMR spin-decoupling results for the natural product, allowed the basic formulation of spatol as a tricyclic diterpenoid related, in part, to the sesquiterpene bourbonene.

Unfortunately, crystals of spatol were not suitable for X-ray analysis due to their small size. Hence, a heavy-atom derivative of **1** was envisioned via esterification of the C-5 alcohol. Treatment of spatol with *p*-bromobenzoyl chloride in benzene, in the presence of equimolar quantities of pyridine, led to the production of the crystalline derivative **4**, which had undergone both esterification and epoxide ring opening.[8] Orthorhombic crystals of **4**, suitable for X-ray determination, were obtained from isooctane. Accurate lattice parameters, determined by a least-squares fit of 15 diffractometer-measured 2θ values between 35 and 45° were *a* = 9.624 (1), *b* = 11.846 (2), and *c* = 23.303 (2) Å. Systematic extinctions and the presence of chirality were uniquely accommodated by space group $P2_12_12_1$, and density considerations indicated one molecule of composition $C_{27}H_{34}BrClO_4$ per asymmetric unit.

The final results of the X-ray diffraction experiment are illustrated in Figure 1, and the following stereochemical descriptors define the absolute stereochemistry of **4**: C-1 (*R*), C-4 (*R*), C-5 (*R*), C-7 (*R*), C-8 (*R*), C-9 (*R*), C-10 (*S*), C-15 (*S*), C-16 (*S*), and C-17 (*R*). When an inversion at C-17 in the conversion of

(4) R. L. Margolis and L. Wilson, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 3466 (1977).

(5) We thank Dr. Robert Jacobs, University of California, Santa Barbara, for providing cytotoxicity measurements. Human cancer cell lines, T242 Melanoma and 224C Astrocytoma, were kindly provided by Drs. Gordon Sato and Hideo Masui, University of California, San Diego. Complete cytotoxicity testing results will be presented in a forthcoming paper.

(6) J. Křepinský, Z. Samek, F. Šorm, D. Lamparsky, P. Ochsner, and Y. R. Naves, *Tetrahedron*, **8**, 53 (1967).

(7) For spatol (**1**): ^1H NMR (220 MHz, $CDCl_3$) δ 5.07 (1 H, *d*, *J* = 0.8 Hz), 4.98 (1 H, s), 3.64 (1 H, d, *J* = 4.0 Hz), 3.31 (1 H, dd, *J* = 4.0, 0.8 Hz), 2.98 (1 H, m), 2.71 (1 H, dd, *J* = 4.0, 8.5 Hz), 2.34 (1 H, d, *J* = 8.5 Hz), 2.24 (1 H, ddd, *J* = 13.3, 5.5, 4.0 Hz), 1.6–2.2 (10 H, m), 1.45 (3 H, s), 1.30 (3 H, s), 0.97 (3 H, s), 0.94 (3 H, d, *J* = 7.0 Hz); ^{13}C NMR (20 MHz, benzene-d_6): 148.6 (s), 110.8 (t), 79.7 (d), 58.4 (s), 58.4 (d), 57.1 (d), 54.9 (d), 47.5 (s), 44.0 (d), 43.6 (d), 43.5 (t), 38.0 (d), 37.2 (t), 36.8 (d), 35.4 (d), 28.1 (t), 24.1 (q), 19.2 (q), 14.6 (q), 13.4 (q).

(8) A similar unconventional reaction seems to occur in the acid-catalyzed epoxide ring opening of a structurally related diepoxide, crotepoxide: S. M. Kupchan, R. J. Hemmingway, P. Coggon, A. T. McPhail, and G. A. Sim, *J. Am. Chem. Soc.*, **90**, 2982 (1968).

spatol to **4** is assumed, the C-17 stereochemistry in **1** must be *S*. The cyclobutane ring has both cyclopentane rings joined in a cis fashion, and each ring is oriented anti in a manner identical with that found in bourbonene.

Epoxides, and diepoxides in particular,[9] have long been known to possess cytotoxic activity resulting from protein binding via displacement reactions with sulfhydryl groups.[10] In the case of spatol, the unsaturation in proximity to the epoxide groups may enhance the reactivity of this metabolite to nucleophilic addition.

Acknowledgment. Research at the Scripps Institution of Oceanography was financially supported by the Sea Grant Program, Department of Commerce (Grant No. 04-8-M01-189). Research at Cornell University was supported, in part, by NIH Grant CA-24487. We thank Dr. Jim Norris for the opportunity to participate in field research in the Galapagos Islands and also for taxonomic advice and guidance with marine algae.

Supplementary Material Available: Details of the X-ray crystallographic experiment, tables of fractional coordinates, thermal parameters, bond distances, bond angles, and observed and calculated structure factors for compound **4** (16 pages). Ordering information is given on any current masthead page.

(9) (a) J. L. Everett and G. A. R. Kon, *J. Chem. Soc.*, 3131 (1950); (b) S. M. Kupchan, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **33**, 2288 (1974).
(10) E. Fujita and Y. Nagao, *Bioorg. Chem.* **6**, 287 (1977).

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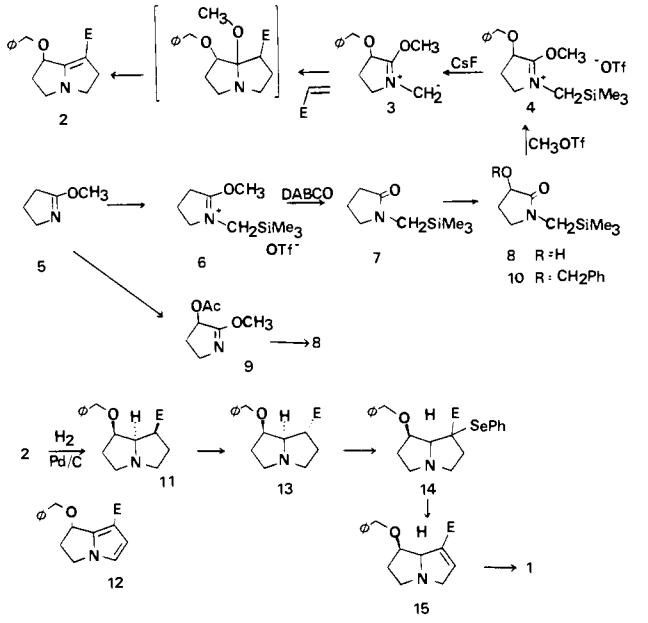
Stereospecific Synthesis of Retronecine by Imidate Methylide Cycloaddition

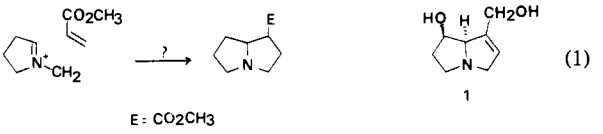
Sir:

We have recently described the first synthetically viable route to nonstabilized iminium ylides, as well as their 1,3-dipolar cycloadditions to give pyrrolines.[1] This study is now extended to construction of the pyrrolizidine nucleus, culminating in an efficient, stereospecific synthesis of retronecine (**1**).[2,3] Derivatives of retronecine are of some interest as antitumor agents.[4]

Although the simplest conceivable route to pyrrolizidines appeared to involve cycloadditions such as eq 1,[5] stereochemical considerations in the context of the retronecine problem suggested than an alternative oxidation state of the cycloadduct would be more desirable. Specifically, our plans were based on the assumption that an ester-conjugated enamine **2** could be reduced from the least hindered side to control stereochemistry.

Scheme I





For preparation of **2**, we envisioned a cycloaddition between methyl acrylate and the imidate methylide 3, a member of a hitherto unknown class of nonstabilized nitrogen ylides.[5-7] As in our previous study,[1] the crucial ylide intermediate can be generated by CsF desilylation from the (trimethylsilyl)methyl salt **4**.

The retronecine sequence depends on the hydroxy lactam **8** as the first nontrivial intermediate. Two routes to **8** have been developed. The shortest route starts with alkylation of imidate **5** by $CF_3SO_3CH_2SiMe_3$[1] (20 °C, 0.5 h, CH_2Cl_2) to give a salt **6** (not isolated) which can be demethylated with Dabco (18 h, 20 °C, CH_2Cl_2) to give the lactam **7** (mp 30–32 °C). Enolate hydroxylation of **7** using the LDA,MoO_5·Py·HMPA method[8] (−78 °C, 1.5 h; allow to warm to 20 °C) affords **8**, mp 71–72 °C (60%). An alternative route which has some advantages on a large scale is based on the known conversion of **5** into **9** via bromination (NBS) and nucleophilic displacement with $(C_2H_5)_4N^+OAc^-$.[9] Treatment of **9** with $Me_3SiCH_2OSO_2CF_3$ and Dabco as before, followed by acetate hydrolysis ($NaOCH_3$ in 2% aqueous ethanol, room temperature, 18 h), gives hydroxy lactam **8** in 70% yield overall from **9**.

After a brief survey of other hydroxyl protecting groups,[10] the benzyl ether **10** ($PhCH_2Br$; NaH; DME, 20 °C; 90% after Kugelrohr distillation, 115–120 °C at 0.1 mm) was selected for conversion to retronecine. The cycloaddition sequence begins with O-alkylation of **10** by treatment with $CH_3OSO_2CF_3$ (CH_2Cl_2, 20 °C, 18 h). The crude salt **4** is then dissolved in DME and is stirred

(1) Vedejs, E.; Martinez, G. R. *J. Am. Chem. Soc.* **1979**, *101*, 6452.
(2) Review: Bull, L. B.; Culvenor, C. C.; Dick, A. T. "The Pyrrolizidine Alkaloids"; North-Holland Publishing Co.: Amsterdam, 1968.
(3) Recent work on total synthesis of retronecine: (a) Tufariello, J. J.; Lee, G. E. *J. Am. Chem. Soc.* **1980**, *102*, 373. (b) Keck, G. E.; Nickell, D. G. *Ibid.* **1980**, *102*, 3634. (c) First total synthesis: Geissman, T. A.; Weiss, A. C., Jr. *J. Org. Chem.* **1962**, *27*, 139.
(4) Kugelman, M.; Lin, W. C.; Axelrod, M.; McBride, T. J.; Rao, K. V. *Lloydia* **1976**, *39*, 125. Powis, G.; Ames, M. M.; Kovach, J. S. *Cancer Res.* **1979**, *39*, 3564. Kovach, J. S.; Ames, M. M.; Powis, G.; Moertel, C. G.; Hahn, R. G.; Creagan, E. T. *Ibid.* **1979**, *39*, 4540.
(5) The conceptually similar cycloaddition of ylides generated in situ from *N*-acylamino acids + acetic anhydride can be used to prepare aromatized pyrrolizidines,[6] which can then be hydrogenated to saturated analogues. This approach has not yet been demonstrated for substrates having an oxygen function as required for retronecine.
(6) (a) Pizzorno, M. T.; Albonico, S. M. *J. Org. Chem.* **1974**, *39*, 731. *Ibid.* **1977**, *42*, 909. (b) Robins, D. J. *J. Chem. Soc., Perkin Trans. 1*, **1979**, 1734. (c) *J. Chem. Soc., Chem. Commun.* **1979**, 1181.
(7) Related, stabilized azomethine ylides are well-known. Reviews: (a) Lown, J. W. *Rec. Chem. Prog.* **1971**, *32*, 51. (b) Kellogg, R. M. *Tetrahedron* **1976**, *32*, 2165. (c) Huisgen, R. *J. Org. Chem.* **1976**, *41*, 403. See also: Hermann, H.; Huisgen, R.; Mader, H. *J. Am. Chem. Soc.* **1971**, *93*, 1779 and references therein.
(8) Vedejs, E.; Engler, D. A.; Telschow, J. E. *J. Org. Chem.* **1978**, *43*, 188.
(9) Yamada, Y.; Okada, H. *Agric. Biol. Chem.* **1976**, *40*, 1437.
(10) The pivalate was unsatisfactory due to apparent elimination during ylide generation; other ethers such as CH_2OCH_3 gave lower yields in the cycloaddition step.

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