Amphidinol, a Polyhydroxypolyene Antifungal Agent with an Unprecedented Structure, from a Marine Dinoflagellate, Amphidinium klebsii

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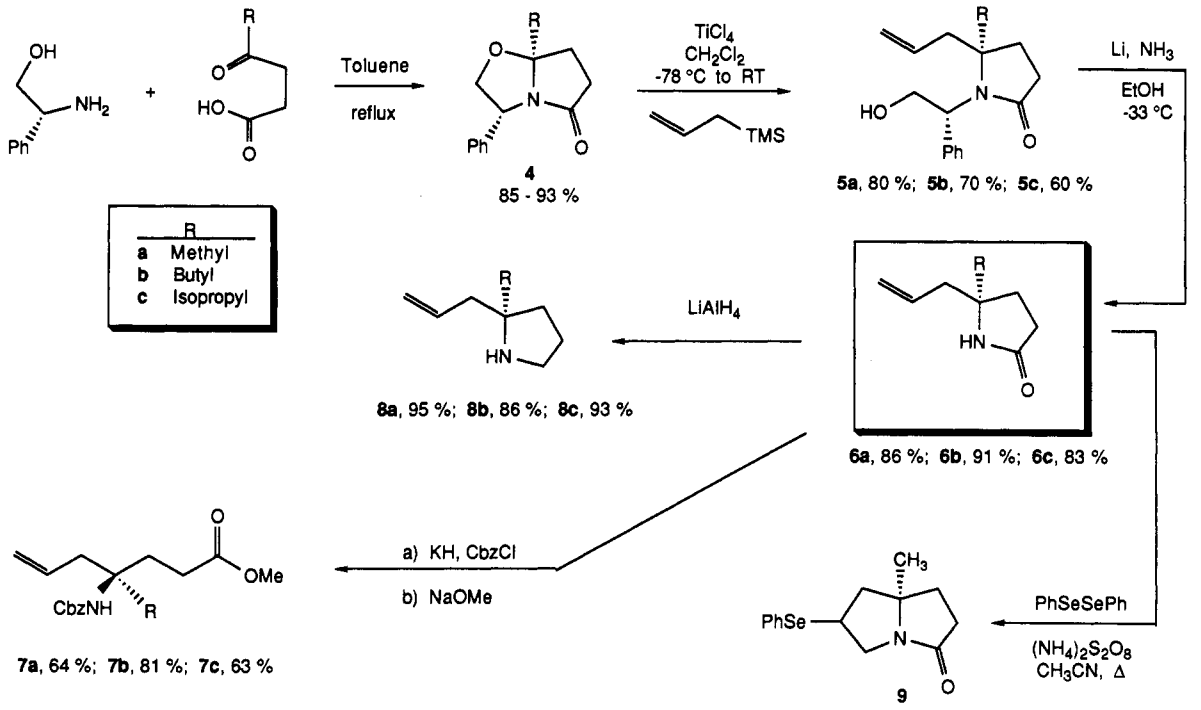
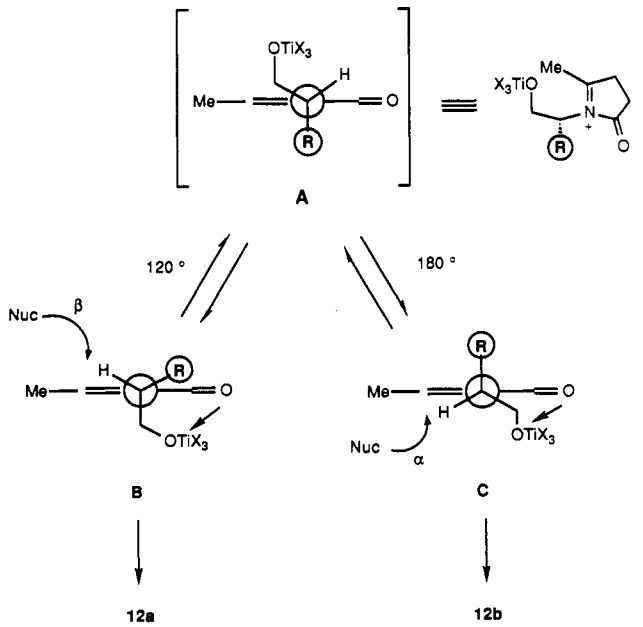
Scheme I



Scheme II



and isopropyl), entry by silane occurs from the face opposite this large group (C) to generate the product (α-entry) of inversion (**12b**). Alternatively, when R is the smaller group (methyl or phenyl), the alkoxytitanium assumes the role of the large group and occupies the antiperiplanar position (B), thus directing the entry from the β-face to provide **12a**. The acyliminium ion derived from the phenylglycinol moiety may also involve several stereoelectronic factors.[13] Finally, and perhaps of equal significance, there exists the possibility of a seven-membered-ring chelate in B and C derived from the alkoxytitanium and the carbonyl oxygen.

The present study provides a remarkable example of how the stereochemistry of Lewis acid–allylsilane alkylations may be altered (inversion or retention at the electrophilic carbon) by simply changing the nature of the auxiliary group from small (methyl) to large (*tert*-butyl), rather than altering the stereocenter. Additionally, the stereoselectivity leading to **12a** or **12b** is found to increase significantly (5:1 versus 8:1 in phenyl to methyl and 1:2 versus 1:11 in isopropyl to *tert*-butyl), suggesting a protocol to reach maximum selectivity. Further studies are in progress to fully evaluate the potential of this system.

Acknowledgment. Support for this research by grants from the National Institutes of Health is gratefully acknowledged.

Supplementary Material Available: Complete experimental details for the preparation of compounds **4a–c** through **8a–c** and **9**, including all physical data (14 pages). Ordering information is given on any current masthead page.

(12) Ahn, N. T. *Top. Curr. Chem.* **1980**, *88*, 145.
(13) Polniaszek, R. P.; Belmont, S. E.; Alvarez, R. *J. Org. Chem.* **1990**, *55*, 215.

Amphidinol, a Polyhydroxypolyene Antifungal Agent with an Unprecedented Structure, from a Marine Dinoflagellate, *Amphidinium klebsii*

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Marine dinoflagellates are attracting much attention as a rich source of bioactive compounds, e.g. brevetoxins, ciguatoxins, maitotoxin, and okadaic acid.[1] While screening dinoflagellate cultures for bioactive compounds, we discovered a potent antifungal agent, amphidinol (**1**) in cultures of the dinoflagellate *Amphidinium klebsii*. In this communication we report the structural elucidation of amphidinol, which is the first member

(1) (a) Lin, Y.-Y.; Risk, M.; Ray, S. M.; Engen, D. V.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773–6775. (b) Murata, M.; Legrand, A. M.; Ishibashi, Y.; Fukui, M.; Yasumoto, T. *J. Am. Chem. Soc.* **1990**, *112*, 4381–4386. (c) Murakami, Y.; Oshima, Y.; Yasumoto, T. *Bull. Jpn. Soc. Sci. Fish.* **1982**, *48*, 69–72.

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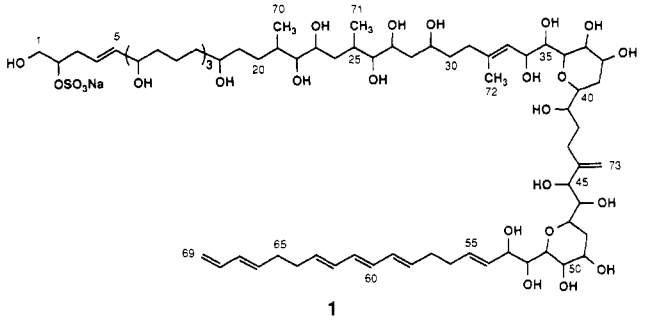
of a new class of polyhydroxypolyene compounds.

A. klebsii collected at Ishigaki Island, Japan, was cultured for 21 days at 25 °C in an ES-1 medium.[2] To part of the culture (40 L) was added $NaH^{13}CO_3$ (50 mg/L). The cells were extracted with MeOH. Solvent partition and column chromatography[3] of the extract yielded ca. 3 mg of ^{13}C-enriched **1**. From the ^{13}C-unenriched culture, 2.3 mg of **1**[4] was obtained as a pale yellow amorphous solid: $[\alpha]^{23}_D$ −25° (*c* 0.18, MeOH); UV_{max} (MeOH) 259 (ε 37500), 270 (ε 41900), and 282 nm (ε 37500); IR (KBr) 3400, 1620, 1240, and 1220 cm^{-1}; HR-FABMS $[M - Na]^-$ *m/z* 1465.8120 for a molecular formula of $C_{73}H_{125}O_{27}SNa$ (calculated for M − Na, 1465.8129). The IR bands at 1240 and 1220 cm^{-1} are typical for sulfate esters. The sulfate group was confirmed by HPLC analysis of the hydrolyzates.[5] Judging from FABMS, ^{13}C abundance was enhanced to ca. 3.5%, thus greatly reducing the time for measuring hetero 2D spectra, e.g. phase-sensitive ^{13}C–^1H COSY, long-range ^{13}C–^1H COSY, and ^{13}C–^1H HOHAHA.

Large segments of the structure (C-1–C-6 and C-18–C-55) were elucidated by detailed analyses of homo 2D experiments,[6] e.g. conventional COSY, double quantum filtered (DQF) COSY, and HOHAHA. The number of hydroxyl groups was estimated by measuring the ^{13}C NMR deuterium shift of the hydroxy-bearing carbons using CD_3OH/C_5D_5N (2:1) and CD_3OD/C_5D_5N (2:1) as solvents. Of 26 signals in the region of oxygenated carbons (65.3–81.4 ppm), 21 signals were shifted by 0.1 ppm or more after deuterium replacement, suggesting that **1** had 21 hydroxyl groups. Five signals (C-2, C-36, C-40, C-47, and C-51) were virtually unchanged. Four of these (C-36, C-40 and C-47, C-51) were located such that two tetrahydropyrans were formed readily. This was confirmed by NOE experiments[7] and $^3J_{H,H}$ measurements[8] around the rings. The remaining C2 resonance was assigned to the carbon bearing the sulfate ester.



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Of five unassigned carbon–carbon double bonds, three were accounted for by a conjugated triene residing in the middle of the molecule and giving rise to UV maxima at 259, 270, and 282 nm.[9]

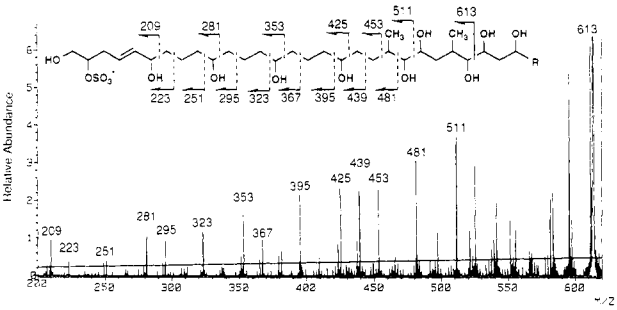

Figure 1. Negative FAB MS/MS for ions at *m/z* 613 of amphidinol **1** and fragmentation pattern of the polyhydroxyl moiety.

^1H–^1H COSY indicated the presence of the conjugated double bonds at the terminus of the molecule. ^1H NMR data pointed to two sets of vicinal methylenes (C-56,57 and C-64,65) placed between the terminal diene and the triene and between the triene and C-54=C-55; their ^1H NMR chemical shifts (δ 2.08–2.09) are typical for methylenes adjacent to a single olefin (methylenes sandwiched between two olefins are observed at around 2.8 ppm). Geometry of all seven double bonds (C-4, C-32, C-54, C-58, C-60, C-62, and C-66) was determined to be *E* by $^3J_{H,H}$ and NOE values,[10] and by comparison of ^1H NMR chemical shifts with those of known compounds.[10] The rest of the molecule (C-7–C-17) gave severely overlapping signals in both ^1H and ^{13}C NMR spectra. In the ^{13}C NMR spectrum, eleven carbons remained unassigned: two oxymethines at δ 72.95, six methylenes around δ 39, and three methylenes around δ 24. 2D ^{13}C–^1H HOHAHA (mixing time 23 ms) indicated the oxymethines were adjacent to the methylenes at δ 39, which were further connected to the methylenes at δ 24. Hence repetitive *n*-butanol units (–$CH(OH)CH_2CH_2CH_2$–) were suggested.

Further verification of the structure in the C-6–C-20 polyhydroxylated and the C-54–C-69 conjugated ene regions was carried out by tandem mass spectrometry.[11] The negative collisionally activated dissociation (CAD) produced ions derived from cleavages at positions α and β to OH groups, while the positive CAD mode gave peaks diagnostic of the conjugated ene system.

In the negative ion FABMS, fragment ions at *m/z* 1221, 1045, 785, 613, and 511 due to 1,2-diol cleavages were observed. The *m/z* 613 ion was selected to determine the location of hydroxyl groups by negative FAB MS/MS (Figure 1).[12] A series of intense peaks resulting from cleavages α and β to hydroxyl groups showed the presence of three repeating subunits between C-6 and C-17. Locations of diene and triene systems were clarified by the positive FAB MS/MS[12] with an $[M + Li]^+$ peak at *m/z* 1479. Fragment ions at *m/z* 1375 due to cleavage of the sulfate group and ions

(2) Provasoli, L. In *Proceedings of the U.S.-Japan Conference Held at Hakone*; Watanabe, A., Hattori, A., Eds.; Tokyo, 1966; pp 63–75.

(3) The aqueous suspension of the extract was extracted with Et_2O and 1-butanol, and the butanol extract was purified over columns of HW-40 (Toyopearl, Tosoh) with $MeOH/H_2O$ (1:1) and then Sephadex LH-20 (Pharmacia) with MeOH. Either antifungal tests against *Aspergillus niger* or hemolysis tests using mouse blood cells were used to detect **1** in the eluates.

(4) Growth inhibiting activity (6 μg/disk) of **1** against *A. niger* was 3 times that of amphotericin B; the hemolytic activity was 120 times that of standard saponin (Merck).

(5) Hydrolysis of sulfate ester was carried out in 2 N HCl at room temperature for 2 h; the sulfate was determined by HPLC (Murata, M.; Kumagai, M.; Lee, J. S.; Yasumoto, T. *Tetrahedron Lett.* **1987**, *28*, 5869–5872).

(6) All NMR spectra were recorded on a JEOL GSX-400 (400 MHz) spectrometer. ^1H–^1H COSYs were measured in four different solvents: CD_3OD, C_5D_5N, CD_3OD/C_5D_5N (2:1), and $CD_3OD/C_6D_6/D_2O$ (10:10:1). DQF-COSY and 2D-HOHAHA were determined in CD_3OD/C_5D_5N (2:1). Data sizes of 2D matrices were usually 1K × 256 points except for conventional COSYs measured with 2K × 512 points.

(7) Significant NOEs were observed for H-35–H-38, H-35–H-40, and H-38–H-40 upon measurement of phase-sensitive NOESY (400 MHz, C_5D_5N, −20 °C). The NOEs indicated that C-35, H-38, and H-40 had axial orientation on a six-membered ring.

(8) H-48 is part of a four-spin system (quartet, *J* = 12–13 Hz). Two vicinal *J*s are typical for diaxial coupling in a six-membered ring.

(9) (a) Spangler, C. W.; Jondahl, T. P.; Spangler, B. *J. Org. Chem.* **1973**, *38*, 2478–2484. (b) Damberg, M.; Russ, P.; Zeeck, A. *Tetrahedron Lett.* **1982**, *23*, 59–62.

(10) The geometry of double bonds, C-4=C-5, C-54=C-55, C-58=C-59, C-62=C-63 and C-66=C-67, was assigned to be *E* on the basis of their $^3J_{H,H}$ (ca. 16 Hz) determined by a 2D *J* spectrum. Olefin C-32 was *E*, because a significant NOE was observed between Me-72 and H-34 in an NOE difference spectrum with irradiation at Me-72 (δ 1.72). Olefin C-60 was *E* based on ^1H NMR chemical shifts of the adjacent protons; in the case of conjugated *E,Z,E*-olefins, the chemical shifts of the inner protons on outer double bonds (corresponding to H-59 at δ 6.03 and H-62 at δ 6.02) should be observed at δ 6.6–6.8 (Hokanson, G. C.; French, J. C. *J. Org. Chem.* **1985**, *50*, 462–466).

(11) Biemann, K.; Hubert, H. A. *Science* **1987**, *237*, 992–998.

(12) Tandem MS were measured by a JEOL JMS-HX110/HX110 (MS_1/MS_2) mass spectrometer equipped with EBEB geometry high performance TMS, 10 kV accelerating voltage, JMS DA-5000 data system, and Xenon FAB gun (6 kV). Both parts were run at ca. 1500 resolution. The negative HRFABMS was obtained by the double target method with use of PEG-1500 succinate as standard (Hemling, M. E. Presented at the 36th ASMS Conference on Mass Spectrometry and Applied Topics, Tucson, AZ, June 1990; paper 174), and detection was done at the first multiplier with resolution of 5000. Experiments were performed on the modified *B/E* linked scan mode of MS_2 via the collision cell in the third field-free region (MS/MS interface), floated at 3 kV. He collision gas was used with the pressure to reduce the selected precursor ion intensity by 70%. The sample (ca. 10 μg) in CH_3CN was mixed with the glycerol matrix for the negative ion mode, and 2,2-dithiodiethanol for the positive mode; in the latter case, a small amount of NaCl or LiCl was added to enhance ion intensity. MS/MS of the Li adduct exhibited more intense ions than the Na adduct, presumably due to its stronger binding energy.

at m/z 1411 and 1305 accounting for allylic cleavages at C-64/C-65 and C-56/C-57 were prominent in the spectra. These MS results coupled with NMR data lead to the whole structure of amphidinol. The stereochemistry of **1** remains unknown because its 27 chiral centers are remote and most of them reside on acyclic parts.

Amphidinol (**1**) is the first representative of a new class of polyketide metabolites and exhibits potent antifungal and hemolytic activities.

Acknowledgment. We are grateful to JEOL LTD. for technical assistance and to Prof. K. Nakanishi, Drs. Y. Naya and R. Orlando, the Suntory Institute, and Prof. P. J. Scheuer (University of Hawaii) for discussions. This work was supported by the Ministry of Education, Science and Culture, Japan.

Registry No. 1, 132930-70-2.

Supplementary Material Available: All ^1H and ^{13}C NMR assignments of **1** and ^1H NMR spectrum, ^1H–^1H and ^{13}C–^1H COSY, ^1H–^1H and ^{13}C–^1H HOHAHA, ^{13}C NMR spectra for deuterium shifts, 2D J (resolution) spectrum, and 1D/2D NOE spectra of amphidinol (13 pages). Ordering information is given on any current masthead page.

Alkoxyvinyl Thionium Ions in Intramolecular 4 + 3 Cycloaddition Reactions

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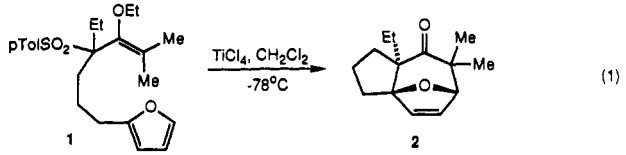
We recently reported new methodology for the intramolecular 4 + 3 cycloaddition reaction based on the paradigm defining the "chameleon" nature of the sulfone functional group.[1-3] Thus, treatment of a solution of sulfone **1** in CH_2Cl_2 with $TiCl_4$ gave cycloadduct **2** as a single isomer in good yield (eq 1).[1] Unfortunately, the generality of this process is limited by the reluctance of alkoxyallylic sulfones with less alkyl substitution to undergo the reaction.[4] It appeared that a means to circumvent this problem could be found using alkoxyallylic sulfones which possessed substituents capable of assisting the ionization process

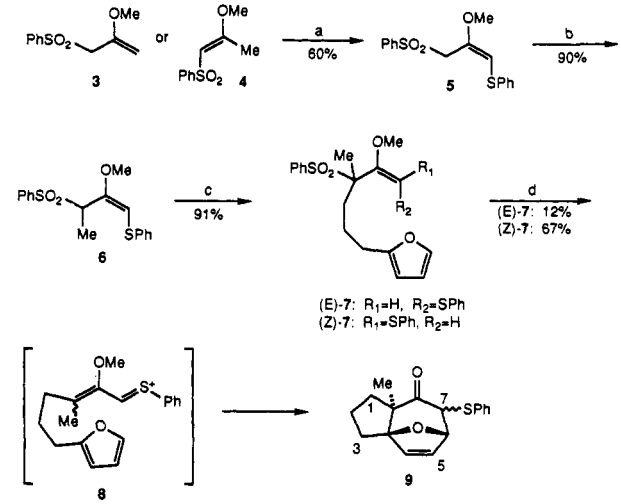


1 2 (1)

through resonance delocalization of the incipient positive charge.[5] Obvious choices included sulfur-, oxygen-, or nitrogen-containing functional groups as substituents. We chose to examine sulfur initially.

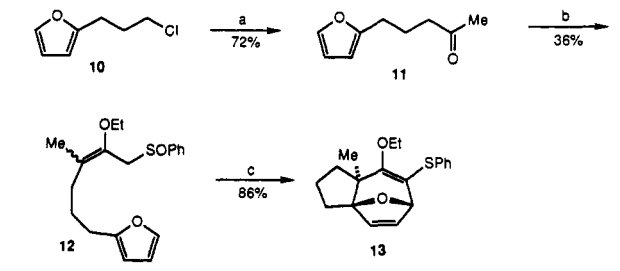
To that end, treatment of either 2-methoxy-3-(phenylsulfonyl)-1-propene (**3**) or its double-bond isomer **4** with phenylsulfenyl chloride followed by DBU gave **5** in 60% isolated yield after trituration with *tert*-butyl methyl ether.[6-8] Deprotonation with *n*-BuLi and alkylation with methyl iodide gave **6** in 90% yield with complete regiocontrol.[9] Subsequent deprotonation and alkylation with 2-(3-iodopropyl)furan gave (*E*)- and (*Z*)-**7** in 91% combined yield, along with recovered starting material[10] (Scheme I).

Scheme I[a]



[a] (a) (1) PhSCl, −78 °C (10 min) to 25 °C (1 h), CH_2Cl_2. (2) DBU, −78 °C (5 min), then 25 °C (30 min). (b) (1) *n*-BuLi, THF, −78 °C. (2) MeI. (c) (1) *n*-BuLi, THF, −78 °C. (2) 2-(3-Iodopropyl)furan, −78 to 25 °C, slowly. (d) $TiCl_4$, CH_2Cl_2, −78 °C, inverse addition.

Scheme II[a]



[a] (a) (1) Mg, ether. (2) Add to excess Ac_2O, ether, −78 °C. (b) (1) (Ethoxyvinyl)lithium THF, −78 °C. (2) *n*-BuLi, then PhSCl, THF, −78 °C. (c) Tf_2O (1 equiv), 2,6-lutidine (2 equiv), CH_2Cl_2, 25 °C.

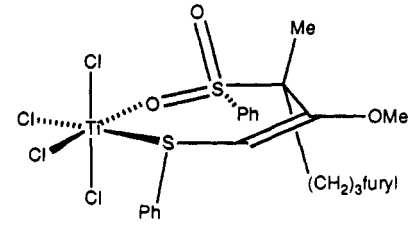


Figure 1. Putative complex between **7a** and $TiCl_4$.

Interestingly, treatment of (*E*)-**7** with $TiCl_4$ (0.02 M, CH_2Cl_2, −78 °C, inverse addition) resulted in only a 12% yield (based on recovered starting material) of cycloadduct **9** as a mixture of two

(1) (a) Harmata, M.; Gamlath, C. B. *J. Org. Chem.* **1988**, *53*, 6154. (b) Harmata, M.; Gamlath, C. B.; Barnes, C. L. *Tetrahedron Lett.* **1990**, 5981.
(2) Hoffmann, H. M. R. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 1. (b) Mann, J. *Tetrahedron* **1986**, *42*, 4611. (c) Noyori, R.; Hayakawa, Y. *Org. React. (N.Y.)* **1983**, *29*, 163.
(3) Trost, B. M. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 107.
(4) Harmata, M.; Gamlath, C. B., unpublished results from these laboratories.
(5) Heteroatom-stabilized oxyallyl cations and related species have received some attention in the context of intermolecular 4 + 3 cycloadditions. See: (a) Föhlisch, B.; Krimmer, D.; Gehrlach, E.; Kashammer, D. *Chem. Ber.* **1988**, *121*, 1585. (b) Murray, D. H.; Albizati, K. F. *Tetrahedron Lett.* **1990**, 4109. (c) Hanke, K.; Gotthardt, L. *J. Chem. Soc., Chem. Commun.* **1984**, 1682. (d) Sasaki, T.; Ishibashi, Y.; Ohno, M. *Tetrahedron Lett.* **1982**, 1693.
(6) Stirling, C. J. M. *J. Chem. Soc.* **1964**, 5856.
(7) Barrett, A. G. M.; Dhanak, D.; Graboski, G. G.; Taylor, S. J. *Org. Synth.* **1989**, *68*, 8.
(8) The nature of the rearrangement in the conversion of **4** to **5** has not been investigated.
(9) The stereochemistry of the double bond of **6** was established by X-ray crystallography: Enraf-Nonius CAD4 diffractometer, Mo Kα radiation, $C_{17}H_{18}O_3S_2$, space group $P\bar{1}$, a = 10.007 (4) Å, b = 10.922 (6) Å, c = 8.353 (5) Å, V = 830.2 (7) Å^3, Z = 2, d_{calcd} = 1.338. The structure was solved (2348 reflections, $I > 2.5\sigma(I)$) by direct methods and refined to R = 0.037 (R_w = 0.056). See supplementary material for more details.
(10) Recovered starting material was not stereochemically homogeneous.

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