Neocarzinostatin Chromophore:
Structural, Mechanistic, and Synthetic Studies

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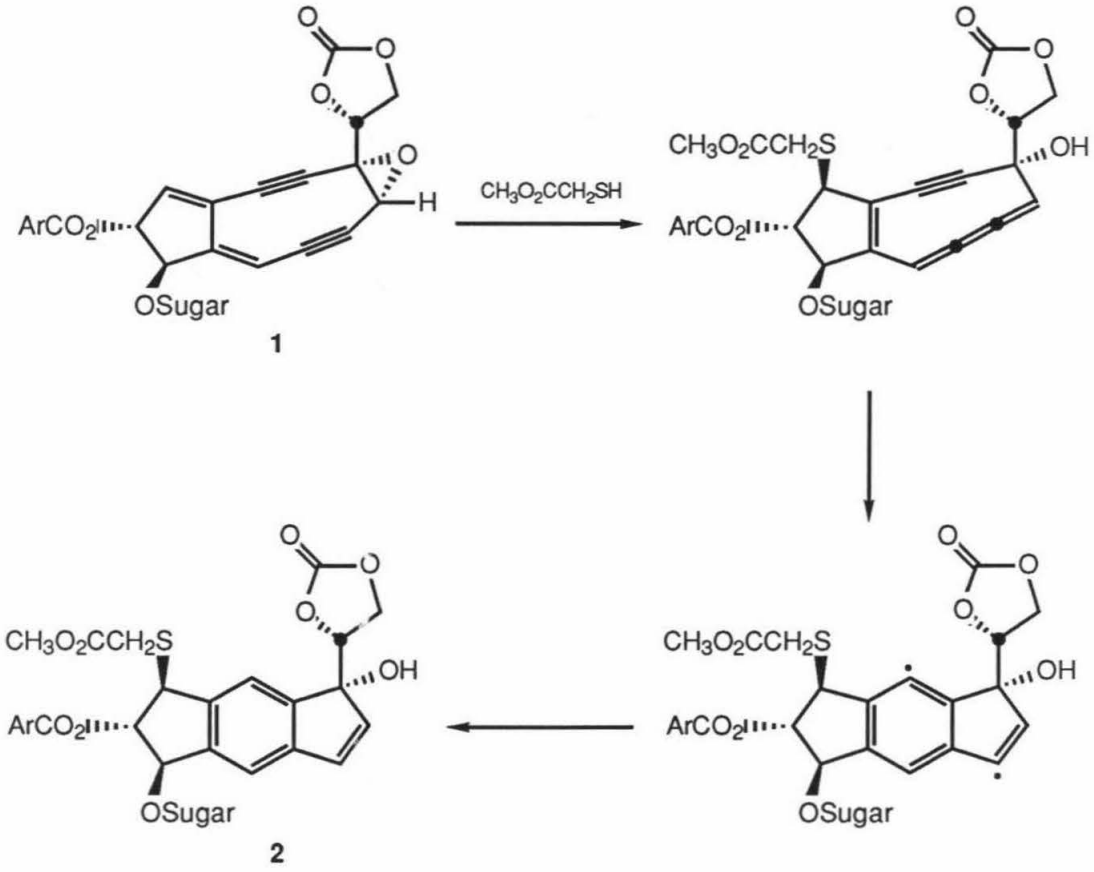
Abstract

Neocarzinostatin chromophore **1** is the active component of the antitumor antibiotic neocarzinostatin (NCS). The chromophore reacts with thiols to form a highly strained cumulene-enyne species which rapidly rearranges to a biradical intermediate which can abstract hydrogen atoms from DNA, leading to strand cleavage. DNA damage is the proposed source of biological activity for NCS. The structure of the methyl thioglycolate monoadduct **2** of NCS chromophore, including the absolute stereochemistry, was determined by NMR studies. The presence of the cumulene-enyne intermediate and the rearrangement to a biradical were supported by data from low temperature NMR investigations. Also included are synthetic approaches to NCS chromophore model compounds based on intramolecular addition of an acetylide to an aldehyde.



1

2

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Introduction

Neocarzinostatin (NCS) is an antitumor antibiotic which is isolated from *Streptomyces carzinostaticus* var. F-41.[1] It consists of a protein component and a nonprotein chromophore.[2] Neocarzinostatin is able to cleave DNA and it is proposed that DNA damage is the source of its biological activity.[3] NCS chromophore, the nonprotein component, has been found to possess the full activity of the whole antibiotic.[4] Since the chromophore alone is fully active, it is this portion of the antibiotic complex that we have studied.

We first confirmed the proposed gross structure of a neocarzinostatin chromophore-methyl thioglycolate adduct[5] and then extended this effort by determining the absolute stereochemistry of the adduct. Since there is no stereochemical loss in formation of the adduct, we also know the absolute stereochemistry of NCS chromophore itself. The proposed mechanistic pathway[5] to the thiol adduct is intriguing because it involves a novel rearrangement. We studied this mechanism by investigating the low-temperature formation of a highly reactive intermediate. Evidence is presented which supports the presence of a biradical precursor to the adduct. The details of these two studies is summarized in the two publications which follow. Since both articles are communications, experimental details have been omitted. In this report, however, an experimental section for both projects is included.

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[1]Ishida, N.; Miyazaki, K.; Kumagai, K.; Rikimaru,M. *J. Antibiot.* **1965**, *18*, 68.

[2]Napier, M.A.; Holmquist, B.; Strydom, D.J.; Goldberg, I.H. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 635.

[3]Goldberg, I.H. In "Mechanisms of DNA Damage and Repair"; Simic, M.G.; Grossman, L.; Upton, A.D.; Eds.; Plenum Press, New York, 1986; pp. 231-244.

[4]Kappen, L.S.; Napier, M.A.; Goldberg, I.H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 1970.

[5]Myers, A.G. *Tetrahedron Lett.* **1987**, *28*, 4493.

Synthetic analogs of NCS chromophore would be valuable for mechanistic as well as medicinal reasons. Research on synthetic approaches to analogs based on intramolecular acetylide addition to an aldehyde are reported. In addition, a model study is presented which is aimed at avoiding some of the problems inherent with the intramolecular reaction described above.

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Stereochemical Assignment of Neocarzinostatin Chromophore. Structures of Neocarzinostatin Chromophore–Methyl Thioglycolate Adducts†

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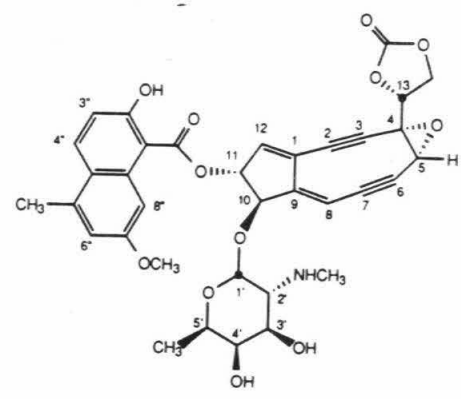
The nonprotein component of the antitumor antibiotic neocarzinostatin[1] (neocarzinostatin chromophore, **1**)[2,3] undergoes rapid and irreversible reaction with thiols to produce a species which is capable of cleaving DNA upon aerobic incubation.[4] Goldberg and co-workers first demonstrated that reaction of **1** with methyl thioglycolate produces a 1:1 adduct with the added incorporation of two hydrogen atoms.[5] Subsequently, we proposed structure **2** (planar form) for this adduct and presented the mechanism outlined in Scheme I to account for its formation.[6] We describe herein the isolation and complete characterization of **2** and a new product, the bisthiol adduct **3**. The full stereochemical assignment of **2** and, by induction, of **1** is also reported.

Dissociation of **1** from its protein complex was achieved with >85% efficiency by a modification of the procedure of Goldberg et al.[5,7] A freshly prepared solution of **1** ($6.6 \cdot 10^{-4}$ M) in 0.5 M methanolic acetic acid was deoxygenated at −78 °C and treated with excess distilled, deoxygenated methyl thioglycolate (**4**, 300 equiv) with reaction at −78 °C for 2 h followed by slow warming to 0 °C (10 °C/h). Volatiles were removed at 0 °C, 0.05 mm to provide a mixture containing **2** and **3** (ca. 1:1) as the major products. Preparative thin-layer chromatography (48:48:4 ethanol:benzene:acetic acid, R_f values 0.31 and 0.43 for **2** and **3**, respectively) with subsequent and final purification over Sephadex LH-20 (dichloromethane) provided **2** and **3** as amorphous films.[8]



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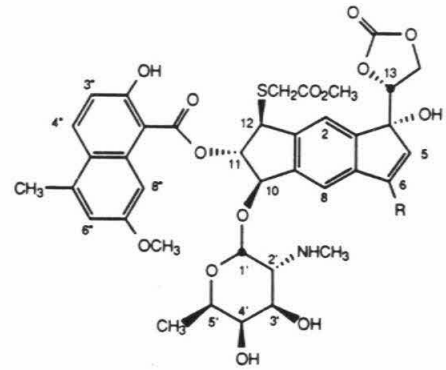


2 R = H

3 R = $SCH_2CO_2CH_3$

High resolution FABMS (calcd for $[M + H]^+$, $C_{38}H_{42}NO_{14}S$: 768.2326; found: 768.2429) of **2** confirmed its formulation as **1** + $HSCH_2CO_2CH_3$ + H_2. The FTIR spectrum (neat film) showed that the carbonate and naphthoate groups had been preserved (1807 and 1644 cm^{-1}, respectively) and indicated incorporation of **4** (1738 cm^{-1}). All carbon-bound proton resonances were well-resolved in the 400 MHz ^1H NMR spectrum ($CDCl_3$). In addition to readily discernible signals for the *N*-methylfucosamine, naphthoate, methyl thioglycolate, and carbonate appendages, seven resonances attributable to the rearranged core were visible: two aromatic and three nonaromatic singlets (δ 7.77, 7.23, 5.78, 5.24, 4.63) and two coupled olefinic doublets (δ 6.94, 6.30, J = 5.6 Hz). 2D-COSY revealed a coupling pathway linking the five singlets (C2–C12–C11–C10–C8–C2).[9] Whereas vanishingly small positive NOEs were observed in $CDCl_3$ ($\omega\tau_c \approx 1$), large negative NOEs could be obtained in dimethyl-d_6 sulfoxide (DMSO-d_6):D_2O (2:1, 23 °C, Figure 1).[10] Qualitatively similar information was obtained in a two-dimensional version of the CAMELSPIN experiment ($CDCl_3$).[11,12] The NOE studies corroborate the linkage established in the 2D-COSY experiment and further define the substitution pattern along the entire periphery of the nucleus, confirming the two-dimensional structure of **2** previously set forth.[6] The data also allow complete determination of the stereochemistry of the left-hand portion of the molecule. The trans,trans-arrangement of the substituents at C10, C11, and C12 was apparent from proton–proton coupling constants ($J_{10,11}$ and $J_{11,12} \leq 1$ Hz)[13] and multiple confirming NOEs (Figure

† Dedicated to Professor E. J. Corey on the occasion of his 60th birthday.

(1) Isolation of neocarzinostatin: Ishida, N.; Miyazaki, K.; Kumagai, K.; Rikimaru, M. *J. Antibiot.* **1965**, *18*, 68.

(2) Isolation of **1**: (a) Napier, M. A.; Holmquist, B.; Strydom, D. J.; Goldberg, I. H. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 635. (b) Koide, Y.; Ishii, F.; Hasuda, K.; Koyama, Y.; Edo, K.; Katamine, S.; Kitame, F.; Ishida, N. *J. Antibiot.* **1980**, *33*, 342.

(3) (a) In determination of structure **1**, the C10 and C11 substituents were shown to be trans. The stereochemistry at C4, C5, C10, C11, and C13 was not assigned: Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331. (b) The *N*-methylfucosamine appendage has been shown unambiguously to be a D-sugar: Edo, K.; Akiyama, Y.; Saito, K.; Mizugaki, M.; Koide, Y.; Ishida, N. *J. Antibiot.* **1986**, *39*, 1615. (c) The stereochemistry at C10 and C11 was recently suggested to be *R,R* based on a model of the binding of **1** to DNA: Schreiber, S. L.; Kiessling, L. L. *J. Am. Chem. Soc.* **1988**, *110*, 631.

(4) (a) Beerman, T. A.; Goldberg, I. H. *Biochem. Biophys. Res. Commun.* **1974**, *59*, 1254. (b) Beerman, T. A.; Poon, R.; Goldberg, I. H. *Biochim. Biophys. Acta* **1977**, *475*, 294. (c) Kappen, L. S.; Goldberg, I. H. *Nucleic Acids Res.* **1978**, *5*, 2959.

(5) Hensens, O. D.; Dewey, R. S.; Liesch, J. M.; Napier, M. A.; Reamer, R. A.; Smith, J. L.; Albers-Schönberg, G.; Goldberg, I. H. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 538.

(6) Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493.

(7) Neocarzinostatin powder (0.500 g) was suspended in ice-cold 0.5 M methanolic acetic acid solution (20 mL), and the mixture was gently agitated for 2 h. The suspension was centrifuged and filtered, and the clear filtrate was stored on ice in the dark. The extraction was repeated by resuspending the protein pellet in fresh cold solvent. The filtrates were combined, and the content of **1** was determined by UV absorbance at 340 nm.[2a] We are indebted to Dr. Matthew Suffness, the National Cancer Institute, and Kayaku Co., Ltd. for generous supplies of neocarzinostatin powder.

(8) We obtained 1.5–2.5 mg (5–10%) each of pure **2** and **3**. These yields are for scrupulously purified samples and, as such, represent lower limits on the actual values.

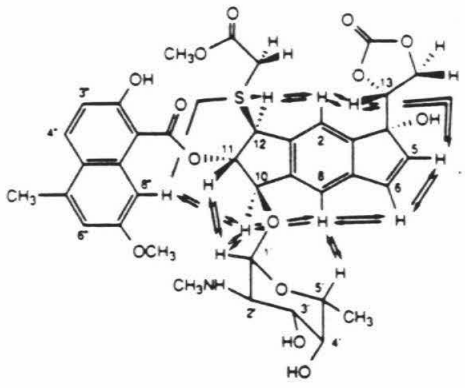
(9) For clarity, we have numbered **2** and **3** to correspond with **1**.

(10) Fesik, S. W.; Olejniczak, E. T. *Magn. Reson. Chem.* **1987**, *25*, 1046.

(11) (a) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811. (b) Rance, M. *J. Magn. Reson.* **1987**, *74*, 557.

(12) We would like to express our sincere gratitude to Dr. Mark Rance for his assistance in obtaining 2-D CAMELSPIN spectra.

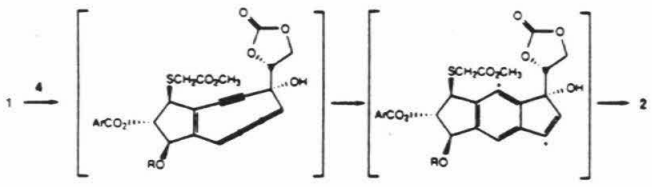
(13) Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; Pergamon Press: New York, 1969; pp 286–288, and references therein.



hν	NOE	%
2	12	7
2	13	4
5	6	19
5	13	10
6	5	12
6	8	8
8	6	14
8	10	6
8	5'	5
10	8	7
10	1'	14
10	8"	3
11	1'	14
12	2	9
13	2	4
13	5	5
1'	10	18
1'	11	10
5'	8	10
8"	10	2
8"	12	3

Figure 1. Summary of DNOE experiments in DMSO-d_6:D_2O (2:1, 23 °C).

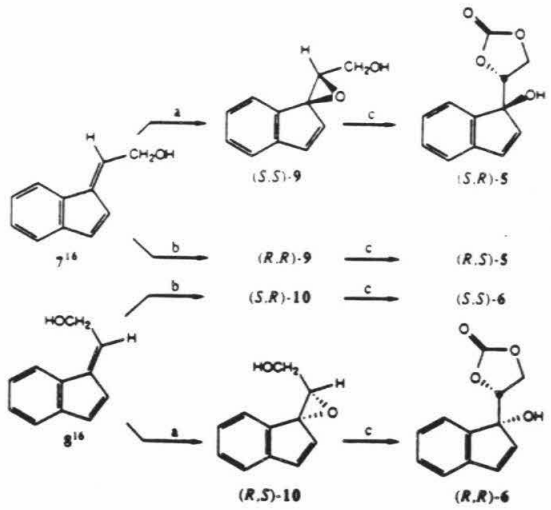
Scheme I. Proposed Mechanism for the Formation of **2**.



1). The absolute stereochemistry at these centers was revealed by three reciprocal NOEs between proton pairs H5–H8, H1′–H10, and H1′–H11, allowing correlation of the stereochemistry at C10 and C11 with the D-sugar appendage.[3b] Analysis of coupling constants ($J_{1',2'}$ and $J_{3',4'}$ = 3.2 Hz, $J_{2',3'}$ = 10.5 Hz, $J_{4',5'}$ < 1 Hz) shows that the fucosamine ring occupies a chair conformation with an axially oriented C1′ alkoxy substituent, as anticipated from the anomeric effect.[14] Imposing the exo-anomeric effect,[14] that is, a gauche orientation of O–C1′–O–C10 with dihedral angle +60°, then reveals simultaneous proximity of the three interacting proton pairs in the (10*R*, 11*S*, 12*S*)-diastereomer but not the (10*S*, 11*R*, 12*R*)-diastereomer (proximal proton pairs: H1′–H8, H1′–H10, and H5′–H11).[15] The latter diastereomer is further excluded upon examination of molecular models; we find no reasonable conformer which places either of the H5′–H8 or H1′–H11 pairs in proximity.

Absolute and relative stereochemistry at C4 and C13 of **2** was determined as follows. Each of the four diastereomers of model compounds **5** and **6** was synthesized in nonracemic form as outlined in Scheme II and separately converted into its (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenyl acetic acid ester (MTPA derivative, Mosher ester)[18] with excess MTPA chloride and 4-(dimethylamino)pyridine (0.6 M each) in methylene chloride at 23 °C for 1.5 h. Subjection of **2** to identical reaction conditions with (*R*)- and (*S*)-MTPA chloride produced, in each case, a triester derivative in which the C3′ equatorial alcohol, phenol, and C4 tertiary hydroxyl groups had been acylated (FABMS: 1416). The 400 MHz ^{1}H NMR spectrum of each Mosher ester derivative of **2**, **5**, and **6** was recorded in both $CDCl_3$ and C_6D_6 under conditions of high dilution ($2\cdot10^{-3}$ M).[21] Chemical shifts of the

Scheme II. Asymmetric Synthesis of Diastereomeric Model Compounds **5** and **6**[a]



[a] (a) stoichiometric (+)-diethyl tartrate, Ti(O*i*Pr)$_4$, *t*-BuOOH, CH_2Cl_2, −20 °C, 11 h;[17] (b) as in (a) with (−)-diethyl tartrate (ee **9** = 84%, ee **10** = 88%);[18,19] (c) CO_2, Cs_2CO_3, 3-Å molecular sieves, DMF, 23–40 °C.[20]

three carbonate protons and two cis-coupled olefinic resonances in each derivative of the four diastereomeric model compounds were then tabulated, providing 20 points for comparison with the corresponding derivatives of **2** (five proton resonances, two Mosher ester derivatives, two solvents). Quantitative assessment of the data was achieved by computing the χ_{20}^2 function[22] taking the reasonable value of 0.10 ppm for σ. We find χ_{20}^2 = 15.9, 69.0, 71.2, and 81.6 for the (*R*,*R*)-, (*S*,*S*)-, (*S*,*R*)- and (*R*,*S*)-diastereomers, respectively. The latter three diastereomers can therefore be rejected with >99.5% confidence (χ_{20}^2 (0.995) = 40.0), and the complete stereochemical assignment of **2** is then 4*R*, 10*R*, 11*S*, 12*S*, 13*R*. It follows that the corresponding (previously unassigned) stereocenters of **1** are 4*S*, 5*R*, 10*R*, 11*R*, 13*R*, and the stereochemistry of thiol attack on **1** is defined as anti to both the C11 naphthoate appendage and the epoxide oxygen.

Product **3** was shown to be a 2:1 adduct of **4** and **1** by HRFABMS (calcd for $[M + H]^+$, $C_{41}H_{46}NO_{16}S_2$ 872.2258; found: 872.2366). The FTIR spectrum (neat film) revealed a close correspondence with **2**: 1815 and 1792 cm^{-1} (carbonate), 1647 cm^{-1} (hydroxynaphthoate), and 1734 cm^{-1} (methyl ester). The 400 MHz ^{1}H NMR spectrum ($CDCl_3$) was nearly superimposable with that of **2** with the following distinguishing features: (1) resonances for a second methyl thioglycolate substituent were apparent; and (2) the doublet pair corresponding to H5 and H6 was absent, while a new, sharp singlet appeared at δ 5.90. Irradiation of this singlet (DMSO-d_6:D_2O 2:1, 23 °C) led to NOE of the carbonate methine and the more downfield $SCH_2CO_2CH_3$ protons, while irradiation of the latter led to NOE of the δ 5.90 singlet and H8. These experiments establish the point of attachment of the second methyl thioglycolate as C6; the assignment of structure **3** is otherwise identical with **2**.[23]

(14) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: New York, 1983; Chapter 2.

(15) It is perhaps noteworthy that application of this same conformational analysis to **1** leads to a structure in which the amino nitrogen lies in close proximity to H11, in near alignment with the C11–H11 bond axis. This suggests an intriguing possibility for the extreme base-sensitivity of **1** ($t_{1/2}$ = 0.6 min at 0 °C, pH 8), i.e., an internal eliminative pathway for cumulene formation, rather than the substitutive pathway which operates in the formation of **2** and **3** [base-sensitivity of **1**: Povirk, L. F.; Goldberg, I. H. *Biochemistry* **1980**, *19*, 4773].

(16) Prepared from indene and 1-chloro-1,2-ethanediol diacetate [Nagasawa, J.-i.; Araki, Y.; Ishido, Y. *J. Org. Chem.* **1981**, *46*, 1734] after the following: Neuenschwander, M.; Vögeli, R.; Fahrni, H.-P.; Lehmann, H.; Ruder, J.-P. *Helv. Chim. Acta* **1977**, *60*, 1073.

(17) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765 and references therein.

(18) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

(19) Stereochemical assignments are based on the Sharpless model for asymmetric epoxidation: Katsuki, T.; Sharpless, K. B. *J. Am. Chem. Soc.* **1980**, *102*, 5974.

(20) Myers, A. G.; Widdowson, K. L., submitted for publication in *Tetrahedron Lett.*

(21) Appropriate control experiments established that chemical shift values were essentially invariant over at least a tenfold range in concentration.

(22)

$$\chi^2_{20} = \sum_{i=1}^{20} (\delta_{i\text{Adduct}} - \delta_{i\text{Model}})^2 / \sigma^2$$

(23) We presently consider two mechanisms as likely for the formation of **3**: (1) a hydrogen atom abstraction (by C-2)–radical recombination (at C-6) reaction of the biradical intermediate of Scheme I with **4** and (2) addition of thiol radical to the cumulene intermediate of Scheme I (at C-6), transannular ring closure, and hydrogen atom abstraction (by C-2). Experiments designed to distinghish between these mechanisms are in progress.

We find that incubation of **1** with 0.2 M $DSCH_2CO_2CH_3$ in both 9:1 CD_3OD:CD_3CO_2D and 9:1 CH_3OD:CH_3CO_2D, as described above, leads to >80% incorporation of deuterium at C-2 and C-6 in isolated **2**.[24] These observations provide strong support for the biradical intermediate of Scheme I. Its formation from the cumulene-enyne of Scheme I, as compared with the Bergman reaction[25] postulated to occur in the calichemicin[26] and esperamicin[27] antibiotics, represents a new molecular rearrangement and a distinct strategy for the spontaneous generation of carbon-centered free radicals at or below ambient temperature.

Acknowledgment. Generous financial assistance from the National Institutes of Health (CA-47148-01) and Merck & Co., Inc., a Dreyfus New Faculty Award (to A.G.M.), and a National Science Foundation predoctoral fellowship (to P.J.P.) are gratefully acknowledged. We thank David Wheeler, Scott Ross, Dr. James Yesinowski, and Dr. Eric Anslyn for their assistance in obtaining NMR spectra and Richard Barrans for assistance with statistical analyses. We are indebted to our colleagues Professor Peter Dervan, Professor Dennis Dougherty, Professor Robert Grubbs, and Professor John Roberts for many fruitful discussions.

Supplementary Material Available: A tabulation of complete and assigned ^1H NMR spectral data for **2** and **3** and the 2-D COSY spectrum of **2** and chemical shift comparisons for (*R*)- and (*S*)-Mosher ester derivatives of **2**, **5**, and **6** (3 pages). Ordering information is given on any current masthead page.

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(24) Goldberg et al. report that incubation of **1** with $DSCH_2CO_2CH_3$ (concentration not specified) in 0.1 M CD_3CO_2D in CH_3OD leads to production of a monoadduct displaying signals at δ 7.83 (s) and δ 7.01 (d) "at reduced intensity" with "incomplete deuterium incorporation from deuteriothioglycolate".[5]

(25) (a) Bergman, R. G.; Jones, R. R. *J. Am. Chem. Soc.* **1972**, *94*, 660. (b) Lockhart, T. P.; Comita, P. B.; Bergman, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 4082. (c) Lockhart, T. P.; Bergman, R. G. *J. Am. Chem. Soc.* **1981**, *103*, 4091.

(26) (a) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464. (b) Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466.

(27) (a) Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.-i.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3461. (b) Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.-i.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462.

Supplementary Material

Proton NMR spectra recorded on a JEOL JNM-GX400 instrument.

Reference: $CHCl_3$ at 7.26 ppm. Digital resolution: 0.24 Hz.

Compound **2**: 8.01 (d, 1H, *J*=9.3 Hz, H4"), 7.77 (s, 1H, H2), 7.50 (d, 1H, *J*=2.2 Hz, H8"), 7.23 (s, 1H, H8), 7.02 (d, 1H, *J*=9.3 Hz, H3"), 6.94 (d, 1H, *J*=5.6 Hz, H6), 6.77 (d, 1H, *J*=2.2 Hz, H6"), 6.30 (d, 1H, *J*=5.6 Hz, H5), 5.78 (s, 1H, H11), 5.57 (d, 1H, *J*=3.2 Hz, H1'), 5.24 (s, 1H, H10), 4.85 (dd, 1H, *J*=6.4, 8.5 Hz, H13), 4.63 (s, 1H, H12), 4.44 (t, 1H, *J*=8.5 Hz, *anti*-H14), 4.22 (dd, 1H, *J*=6.4, 8.5 Hz, *syn*-H14), 4.00 (q, 1H, *J*=6.6 Hz, H5'), 3.80 (d, 1H, *J*=3.2 Hz, H4'), 3.74 (s, 3H, CO_2CH_3), 3.62 (d, 1H, *J*=15.1 Hz, $SC\underline{H}_aH_b$), 3.54 (dd, 1H, *J*=3.2, 10.5 Hz, H3'), 3.46 (d, 1H, *J*=15.1 Hz, $SCH_a\underline{H}_b$), 3.28 (s, 3H, Ar-OCH_3), 2.86 (dd, 1H, *J*=3.2, 10.5 Hz, H2'), 2.58 (s, 3H, NCH_3), 2.56 (s, 3H, Ar-CH_3), 1.37 (d, 3H, *J*=6.6 Hz, CH_3 at C5').

Compound **3**: 8.02 (d, 1H, *J*=9.3 Hz, H4"), 7.73 (s, 1H, H2), 7.51 (bs, 1H, H8"), 7.23 (s, 1H, H8), 7.02 (d, 1H, *J*=9.3 Hz, H3"), 6.78 (bs, 1H, H6"), 5.90 (s, 1H, H5), 5.80, (s, 1H, H11), 5.54, (d, 1H, *J*=3.2 Hz, H1') 5.26 (s, 1H, H10), 4.90 (dd, 1H, *J*=6.4, 8.5 Hz, H13), 4.63 (s, 1H, H12), 4.41 (t, 1H, *J*=8.5, *anti*-H14), 4.10 (dd, 1H, *J*=6.4, 8.5 Hz, *syn*-H14), 3.99 (q, 1H, *J*=6.6 Hz, H5'), 3.82 (d, *J*=3.2 Hz, H4'), 3.80 (s, 3H, C6-$SCH_2CO_2C\underline{H}_3$), 3.74 (s, 3H, C12-$SCH_2CO_2C\underline{H}_3$), 3.65 (d, 1H, *J*=15.1 Hz, C12-$SC\underline{H}_aH_b$), 3.55 (dd, 1H, *J*=3.2, 10.5 Hz, H3'), 3.48 (d, 1H, *J*=15.1 Hz, C12-$SCH_a\underline{H}_b$), 3.32 (s, 3H, Ar-OCH_3), 2.87 (dd, 1H, *J*=3.2, 10.5 Hz, H2'), 2.57 (s, 3H, NCH_3), 2.56 (s, 3H, Ar-CH_3), 1.40 (d, 3H, *J*=6.6 Hz, CH_3 at C5'). C6-$SC\underline{H}_2$ not resolved in $CDCl_3$.

Chemical Shifts of Mosher Ester Derivatives of 2, 5 and 6*

Compound	Derivative	Solvent	Proton 3	2	8	*anti*-9	*syn*-9
2††	(*R*)-MTPA Ester	$CDCl_3$	7.146	6.385	5.022	4.328	3.754
		C_6D_6	6.464	5.889	3.833	3.091	2.969
	(*S*)-MTPA Ester	$CDCl_3$	7.112	6.177	4.820	4.367	3.913
		C_6D_6	6.445	5.625	3.829	3.149	3.269
(*R*,*S*)-5	(*R*)-MTPA Ester	$CDCl_3$	7.036	6.510	4.859	4.376	4.067
		C_6D_6	6.346	6.096	3.987	3.201	3.442
	(*S*)-MTPA Ester	$CDCl_3$	7.060	6.344	5.102	4.371	3.843
		C_6D_6	6.342	5.793	4.241	3.199	3.132
(*S*,*R*)-5	(*R*)-MTPA Ester	$CDCl_3$	7.058	6.342	5.099	4.367	3.840
		C_6D_6	6.358	5.806	4.258	3.228	3.149
	(*S*)-MTPA Ester	$CDCl_3$	7.038	6.510	4.860	4.376	4.067
		C_6D_6	6.351	6.099	3.994	3.210	3.446
(*R*,*R*)-6	(*R*)-MTPA Ester	$CDCl_3$	7.141	6.274	4.910	4.191	3.738
		C_6D_6	6.395	5.744	3.904	2.958	3.020
	(*S*)-MTPA Ester	$CDCl_3$	7.110	6.078	4.688	4.286	3.909
		C_6D_6	6.404	5.508	3.735	3.074	3.277
(*S*,*S*)-6	(*R*)-MTPA Ester	$CDCl_3$	7.109	6.079	4.689	4.284	3.907
		C_6D_6	6.408	5.495	3.717	3.064	3.273
	(*S*)-MTPA Ester	$CDCl_3$	7.142	6.275	4.910	4.191	3.737
		C_6D_6	6.399	5.751	3.919	2.972	3.033

$(\delta_{Adduct} - \delta_{Model})$*

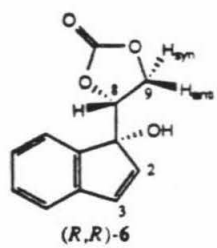
Compound	Derivative	Solvent	Proton 3†	2†	8†	*anti*-9†	*syn*-9†
(*R*,*S*)-5	(*R*)-MTPA Ester	$CDCl_3$	+0.110	-0.125	+0.163	-0.048	-0.313
		C_6D_6	+0.118	-0.207	-0.154	-0.110	-0.473
	(*S*)-MTPA Ester	$CDCl_3$	+0.052	-0.167	-0.282	-0.004	+0.070
		C_6D_6	+0.103	-0.168	-0.412	-0.050	+0.137
(*S*,*R*)-5	(*R*)-MTPA Ester	$CDCl_3$	+0.088	+0.043	-0.077	-0.039	-0.086
		C_6D_6	+0.106	+0.083	-0.425	-0.137	-0.180
	(*S*)-MTPA Ester	$CDCl_3$	+0.074	-0.333	-0.040	-0.009	-0.154
		C_6D_6	+0.094	-0.474	-0.165	-0.061	-0.177
(*R*,*R*)-6	(*R*)-MTPA Ester	$CDCl_3$	+0.005	+0.111	+0.112	+0.137	+0.016
		C_6D_6	+0.069	+0.145	-0.071	+0.133	-0.051
	(*S*)-MTPA Ester	$CDCl_3$	+0.002	+0.099	+0.132	+0.081	+0.004
		C_6D_6	+0.041	+0.117	+0.094	+0.075	-0.008
(*S*,*S*)-6	(*R*)-MTPA Ester	$CDCl_3$	+0.037	+0.306	+0.333	+0.044	-0.153
		C_6D_6	+0.056	+0.394	+0.116	+0.027	-0.304
	(*S*)-MTPA Ester	$CDCl_3$	-0.030	-0.098	-0.090	+0.176	+0.176
		C_6D_6	+0.046	-0.126	-0.090	+0.177	+0.236

*Chemical shifts are in ppm. All spectra recorded at 400 MHz; digital resolution = 0.0006 ppm (0.24 Hz).

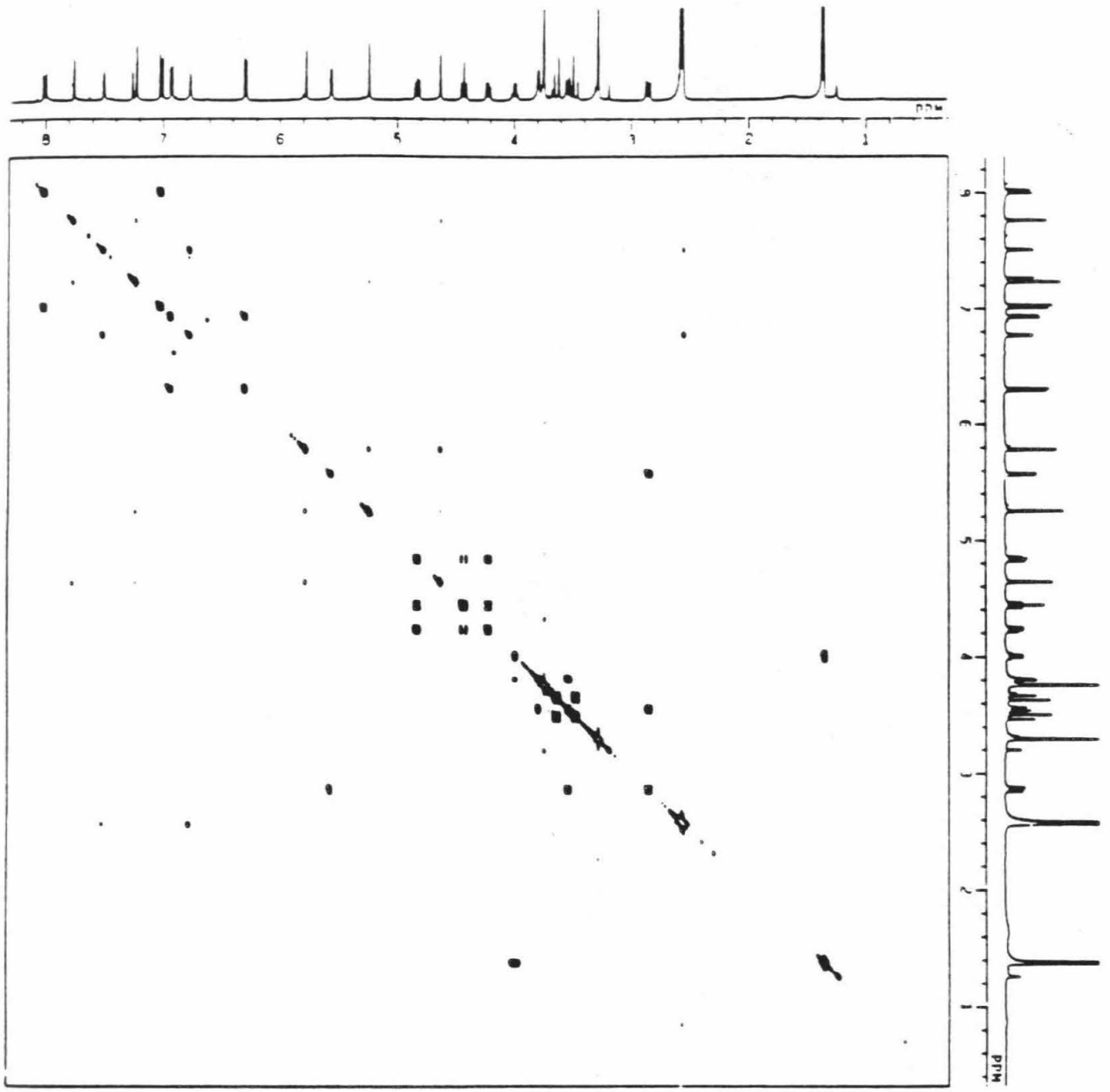
†Coupling constants for the tabulated protons in all four model compounds, 2 and all Mosher Ester derivatives are equal within digital resolution: H3 (d, *J*=5.6 Hz), H2 (d, *J*=5.6 Hz), H8 (dd, *J*=6.4, 8.5 Hz), *anti*-H9 (t, *J*=8.5 Hz), *syn*-H9 (dd, *J*=6.4, 8.5 Hz).

††The chemical shifts of these derivatives are listed to correspond with the numbering system of the model compounds. The actual designations are: H6, H5, H13, *anti*-H14, *syn*-H14.

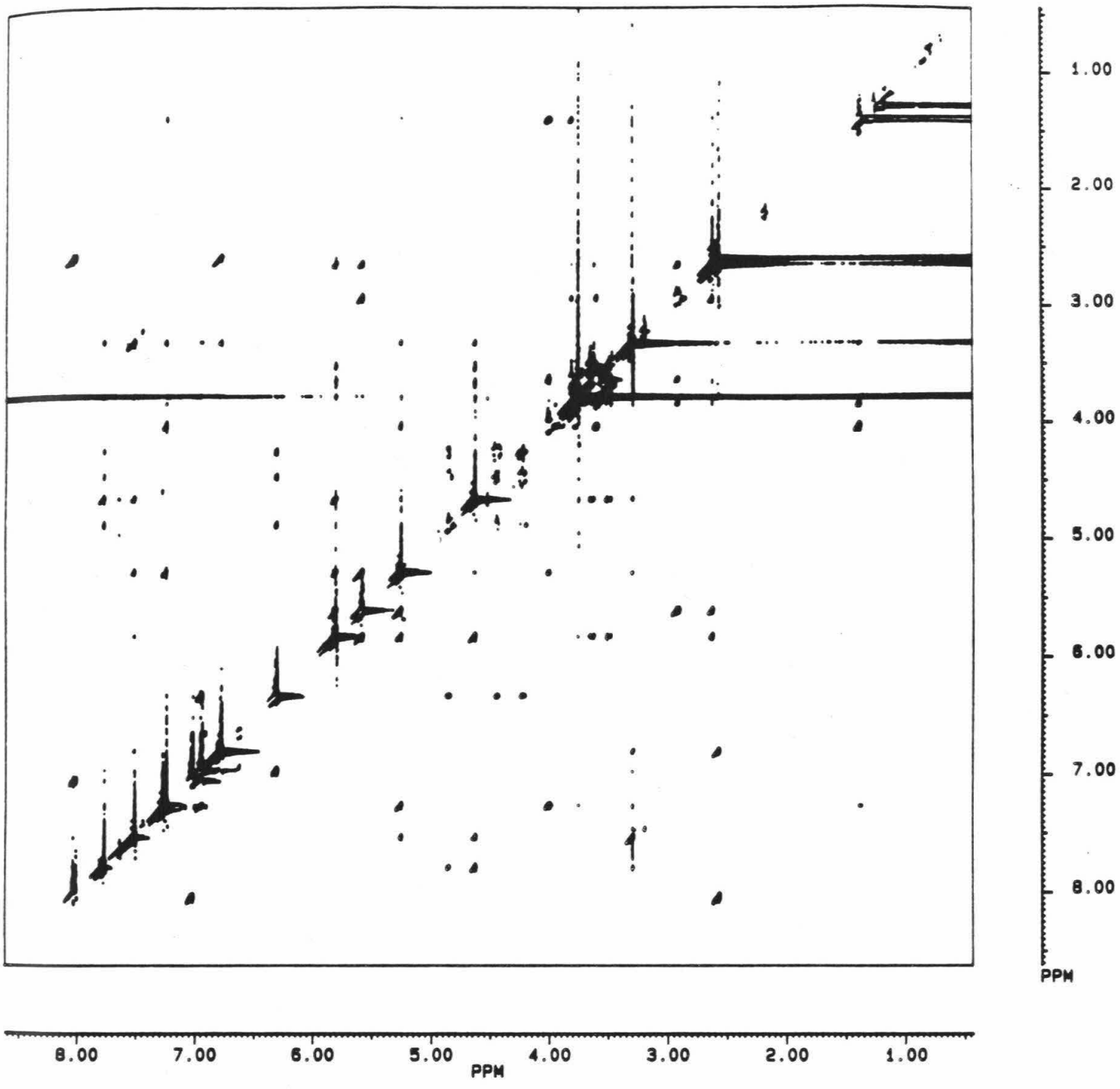
Numbering:



(*R*,*R*)-6



2D-COSY Spectrum of **2** ($CDCl_3$, 400 MHz)



2D-CAMELSPIN Spectrum of **2** ($CDCl_3$, 500MHz)

Reprinted from the Journal of the American Chemical Society, 1989, *111*, 1146.

Evidence for Spontaneous, Low-Temperature Biradical Formation from a Highly Reactive Neocarzinostatin Chromophore–Thiol Conjugate

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Neocarzinostatin chromophore (**1**) and methyl thioglycolate (**2**) combine at −70 °C to form the observable intermediate **3** which, upon warming to −38 °C, decays in a first-order process ($t_{1/2}$ = 2 h). Evidence is presented to support the biradical **4** as the direct product of this unimolecular decomposition.

Reaction of the antitumor antibiotic neocarzinostatin chromophore (**1**) with thiol **2** (0.2 M) in 0.5 M methanolic acetic acid (−78 → 0 °C) has been shown to produce the stable mono- and bisthiol adducts **5** and **6**, respectively, in 1:1 ratio.[1] The sequence **1** → **3** → **4** → **5** (Scheme I) was suggested to account for the formation of **5**.[2] In this pathway, biradical **4** is of particular significance since free-radical intermediates have been implicated in the cleavage of DNA by thiol-activated **1**.[3] In order to gain further insight into the mechanistic details of the transformation **1** + **2** → **5** + **6**, we have studied this reaction by low-temperature ^{1}H NMR spectroscopy.

A solution of **1** (0.01 M) and **2** (0.2 M) in 9:1 tetrahydrofuran-d_8:CD_3CO_2H at −78 °C showed distinct signals for each component in the 400-MHz ^{1}H NMR spectrum. Upon warming to −70 °C, a pseudo-first-order transformation of resonances for **1** to those of a new compound was observed [$t_{1/2}$(−70 °C, 0.2 M **2**) = 1.5 h, $k_1 = (1.2 \pm 0.1) \times 10^{-4}$ s^{-1}, Figure 1]. The chemical shift changes that signalled this conversion were entirely consistent with the proposal **1** → **3**. Thus, signals for H12 (δ 6.80), H11 (δ 6.12), H8 (δ 5.66), and H5 (δ 4.11) of **1** diminished while four new peaks at δ 4.20, 5.72, 6.24, and 5.81 (assigned as H12, H11, H8, and H5 of **3**, respectively) increased. The latter two resonances were observed as a pair of coupled doublets (verified by low-temperature irradiation), J = 5.1 Hz, confirming their assignment as H8 and H5 and providing support for the presence of the cumulene functional group.[4] While stable for days under argon at −70 °C, intermediate **3** rapidly decayed at −38 °C and above to produce as major products (ca. 50% yield) a 1:1 mixture of the mono- and bisthiol adducts **5** and **6**. Careful separation of **5** and **6** and subsequent ^{1}H NMR analysis of each pure compound showed that deuterium had been incorporated at C2 and C6 of **5** to the extent of 35 ± 5% and at C2 of **6** to the same degree, within experimental error. When the above experiment was conducted at 7-fold lower concentration of **2** (0.03 M, ca. 3 equiv), the ratio of **5** to **6** increased to 4:1 and the incorporation of solvent (carbon-bound) deuterium was increased to 80 ± 5% at each of these three positions. These data clearly support the existence of free-radical precursors to **5** and **6** with odd electron density at the labeled carbon atoms. The data also suggest that the bulk of bisthiol adducts **6** does not arise by a cage abstraction–recombination reaction of **4** with **2**, since this pathway would predict incorporation of protium at C2.[5] The data do support the formation of **6** by a process involving initial thiol radical addition into C6 of the cumulene **3** with transannular ring closure (Scheme I). Also consistent with this hypothesis is the fact that the kinetics of decay of **3** under conditions that produced equivalent amounts of **5** and **6** (0.2 M **2**) were complex, approximately second-order in **3**.

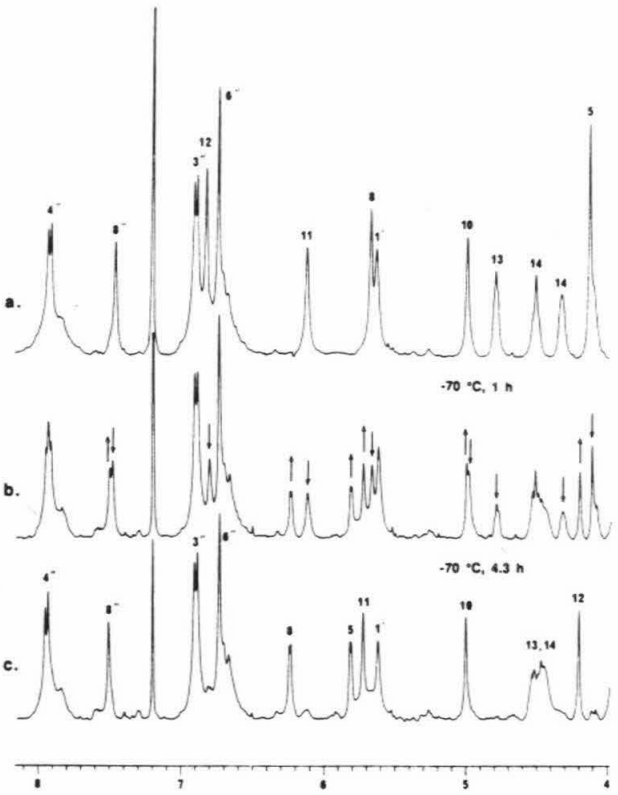

Figure 1. Reaction of **1** (0.01 M) and **2** (0.20 M) at −70 °C (9:1 tetrahydrofuran-d_8:CD_3CO_2H) to produce **3**, as monitored by 400-MHz ^{1}H NMR (δ 4.0–8.1): (a) **1**, −78 °C, prior to addition of **2**; (b) **1** + **2**, −70 °C, 1 h; (c) **1** + **2**, −70 °C, 4.3 h.

By careful optimization of parameters we were able to effectively suppress formation of **6** and thereby obtain first-order kinetics for the decomposition of **3**. Incubation of **1** (0.01 M) with **2** (0.03 M) in 9:1 tetrahydrofuran-d_8:CD_3CO_2H containing 1,4-cyclohexadiene (0.2 M) led to complete conversion of **1** to the cumulene **3** after 74 h at −70 °C. Warming to −38 °C in the probe of a high-field ^{1}H NMR spectrometer (400 MHz, *trans*-1,2-dichloroethylene as internal standard) then led to smooth first-order decay of **3** [$k_{obsd} = (1.0 \pm 0.2) \times 10^{-4}$ s^{-1}] with concomitant production of **5**. The yield of **5** was approximately 68%, the ratio of 5:6 was >10:1, and purified **5** had 40 ± 5% incorporated deuterium at C2 and C6. Following the precedent of Bergman, ΔH for the transformation of **3** to **4** can be estimated to be ≃ +6 kcal/mol, less the strain energy of the nine-membered ring of **3**.[6] Any reasonable estimate of the latter will undoubtedly place **4** below **3** in energy. The reaction **4** → **3** can therefore be excluded

(1) Myers, A. G.; Proteau, P. J.; Handel, T. M. *J. Am. Chem. Soc.* **1988**, *110*, 7212.

(2) Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493.

(3) Goldberg, I. H. *Free Radical Biology & Medicine* **1987**, *3*, 41 and references therein.

(4) The theoretical (Karplus, M. *J. Am. Chem. Soc.* **1960**, *82*, 4431) and experimental (Montijn, P. P.; Brandsma, L.; Arens, J. F. *Recl. Trav. Chim. Pays-Bas.* **1967**, *86*, 129) value of $J_{1,4}$ in [3]-cumulenes is about 7.8 Hz. It is anticipated that cyclic cumulenes will exhibit somewhat smaller values: Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; Pergamon Press: New York, 1969; p 303 and references therein.

(5) For the same reason, mechanisms involving polar addition of thiol to **3** can be ruled out. We are grateful to Professor Jack Baldwin for stimulating discussions on this topic.

(6) Jones, R. R.; Bergman, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 660. For this calculation, we used the model transformation of 1,2,3,5-cyclononatetraen-7-yne to 3,7-dehydroindene. ΔH_f for the former was determined to be 149 kcal/mol using group activities (Benson, S. W. *Thermochemical Kinetics*, 2nd ed.; Wiley: New York, 1976) and as 155 kcal/mol for the latter by subtraction of the bond dissociation energy of molecular hydrogen (104.2 kcal/mol, Herzberg, G. *J. Mol. Spectrosc.* **1970**, *33*, 147) from the sum of ΔH_f indene (39.08 ± 0.43 kcal/mol; Pedley, J. B.; Naylor, R. D.; Kirby, S. P. *Thermochemical Data of Organic Compounds*, 2nd ed.; Chapman and Hall: New York, 1986) and two benzene CH bond dissociation energies (2 × 110.2 ± 2.0 kcal/mol; Chamberlain, G. A.; Whittle, E. *Trans. Faraday Soc.* **1971**, *67*, 2077).

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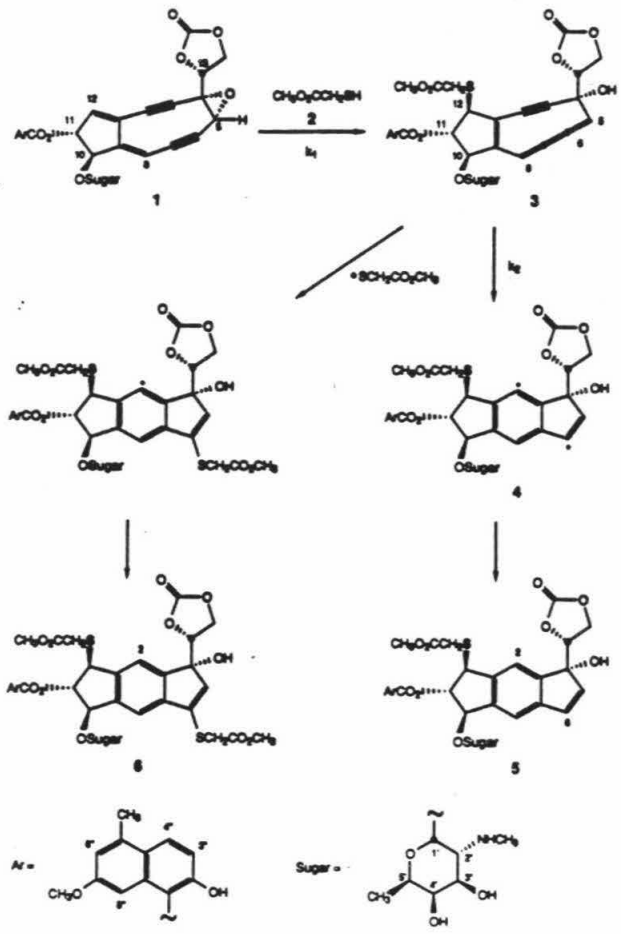
Scheme I



from consideration in light of more rapid trapping of **4** by solvent and we thus conclude that $k_{obsd} = k_2$ (Scheme I) and that $\Delta G^{\ddagger}$ for the process **3** → **4** is 18.0 ± 0.1 kcal/mol.[7]

While the conditions of the experiments outlined above are far from physiological, our results bear on questions regarding the relevance of pathways such as that outlined in Scheme I to the mechanism of action of neocarzinostatin in vivo. In the initial activation event, it is clear that **1** possesses a remarkable affinity for thiols, combining readily with methyl thioglycolate (30 mM, $pK_a = 7.9$)[8] at −70 °C. Glutathione ($pK_a = 8.7$), present in mammalian cells at concentrations of 0.5–10 mM,[9] has been strongly implicated as the activating nucleophile in studies of neocarzinostatin toxicity in intact cells.[10] With regard to the second event in activation, aromatization of a thiol–chromophore adduct such as **3** to the corresponding tetrahydroindacenediyl, we calculate a half-life of ~0.5 s for the transformation of **3** to **4** at 37 °C.[11]

Acknowledgment. Generous financial assistance from the National Institutes of Health (CA-47148-01) and Merck & Co., Inc., a Dreyfus New Faculty Award (to A.G.M.), and a National Science Foundation predoctoral fellowship (to P.J.P.) are gratefully acknowledged. We are indebted to our colleague Professor Dennis Dougherty for many helpful discussions.

(7) The rate of trapping of **4** can be estimated by the rate of reaction of phenyl radical with diphenylmethane (7.7×10^6 M^{-1} s^{-1} at 60 °C): Lockhart, T. P.; Mallon, C. B.; Bergman, R. G. *J. Am. Chem. Soc.* **1980**, *102*, 5976. Ingold, K. U. In *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. I, Chapter 2.

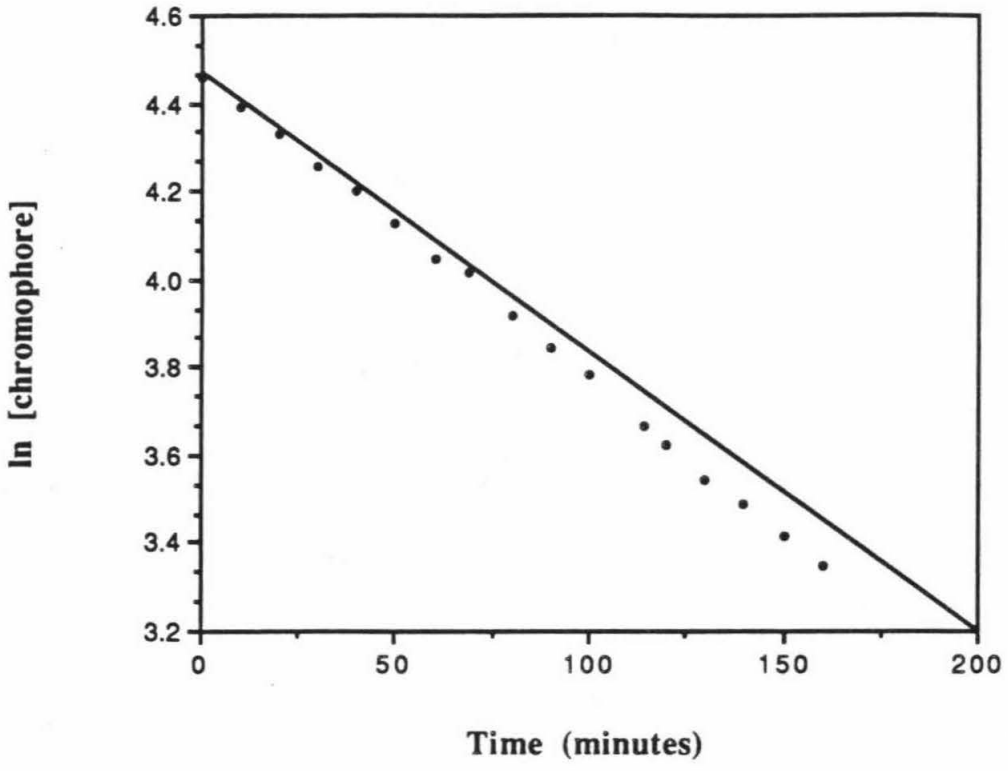
(8) Jencks, W. P.; Salvesen, K. *J. Am. Chem. Soc.* **1971**, *93*, 4433.

(9) Meister, A.; Anderson, M. E. *Annu. Rev. Biochem.* **1983**, *52*, 711.

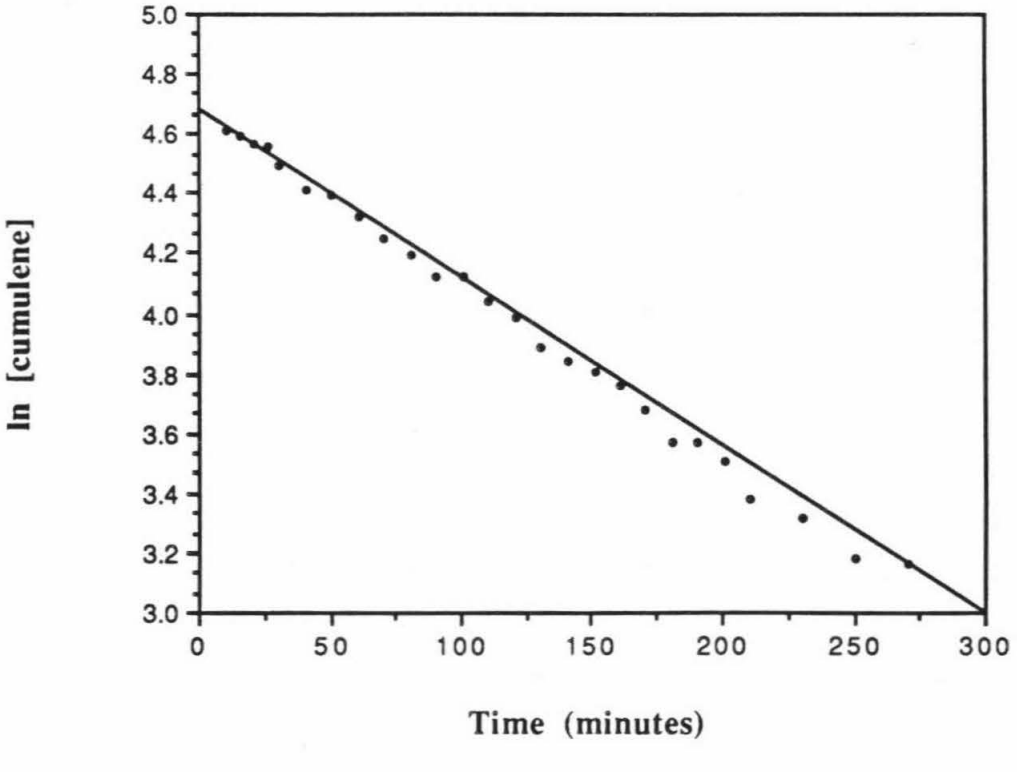
(10) DeGraff, W. G.; Russo, A.; Mitchell, J. B. *J. Biol. Chem.* **1985**, *260*, 8312.

(11) This assumes a (likely) weak temperature dependence of $\Delta G^{\ddagger}$.

Pseudo First-order, -70° C



First-order, -38° C



Experimental Section

Lyophilized neocarzinostatin powder (Lot #160) was obtained from Kayaku Co., Ltd. and was stored at -78° C in a brown container. Acetic acid-d_3 was purchased from Stohler/Kor Stable Isotopes, a division of ICN Biomedicals, Inc.

Neocarzinostatin Chromophore-Methyl Thioglycolate Adducts: Monoadduct **2** and Bisthiol Adduct **3**

Lyophilized neocarzinostatin powder (0.504 g) was suspended in ice-cold 0.5 M acetic acid in methanol (20 ml) and stirred in the dark at 0° C for 2 h. Centrifugation and filtration provided a pale yellow filtrate which was chilled in an ice bath in the dark. The extraction procedure was repeated employing only 1 h of stirring. The filtrates were combined and the content of chromophore **1** was determined by UV absorbance at 340 nm ($\varepsilon_{340\ nm} \approx 8000$).‡ The resulting solution of **1** (6.6×10^{-4} M) was deoxygenated, then cooled to -78° C before addition of methyl thioglycolate (1.0 ml, 9.4 mmol, 300 equiv). The temperature was maintained at -78° C for 2 h followed by slow warming to 0° C (10° C/h). Concentration *in vacuo* (0° C, 0.05 mm Hg) afforded a crude brownish-yellow residue. Initial purification was done by preparative thin-layer chromatography (48/48/4 ethanol/benzene/acetic acid). The desired compounds were eluted from the silica gel using an ethanol/benzene/acetic acid (80/19/1) solution. Concentration *in vacuo* gave the compounds in silica gel matrices. Each matrix was dissolved in 15% brine (15 ml) and methylene chloride/benzene/isopropanol (50/40/10, 10 ml) and then the phases were separated. The aqueous layer was extracted with five portions of methylene chloride/benzene/isopropanol (50/40/10) and then one portion of methylene chloride. The combined organic layers were dried with sodium sulfate and then concentrated *in vacuo* to yield **2** (3.2 mg) and **3** (3.4 mg). Final purification was achieved by Sephadex LH-20 chromatography (6 g, 1 cm diameter column, pre-swelled for 2-3 h) using methylene chloride as the eluting solvent. For compound **2**, ~2 ml fractions were collected and analyzed by TLC (366 nm UV detection using silica gel 60 F-254 plates). Smaller

fractions (~1 ml) were collected for compound **3**. Both **2** (1.9 mg, 6.7%) and **3** (2.5 mg, 7.7%) were obtained as amorphous white films.

Note: It may be possible to avoid the extraction step to remove silica by directly loading the silica matrix mixture onto the Sephadex column.

Monoadduct **2**

NMR (400 MHz, $CDCl_3$) :	See supplementary material.
FTIR (neat film, cm^{-1}) :	3435 (m), 3310 (shoulder), 1807 (s), 1738 (s), 1733 (s), 1644 (s), 1616 (s), 1204 (s), 1174 (s), 1158 (s), 1086 (s), 1030 (s).
HRFABMS (glycerol) :	calcd for $[M+H]^+$: 768.2326 found: 768.2429
TLC ($EtOH/C_6H_6/AcOH$) : 48 / 48 / 4	Monoadduct $R_f = 0.31$

Bisthiol Adduct **3**

^1H NMR (400 MHz, $CDCl_3$) :	See supplementary material.
FTIR (neat film, cm^{-1}) :	3392 (w), 1815 (m), 1792 (m), 1739 (s), 1734 (s) 1647 (m), 1616 (s), 1267 (s), 1204 (s), 1173 (s), 1158 (s), 1085 (s), 1029 (s).
HRFABMS (PEG) :	calcd for $[M+H]^+$: 872.2258 found: 872.2366
TLC ($EtOH/C_6H_6/AcOH$) : 48 / 48 / 4	Bisadduct $R_f = 0.43$

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‡Napier, M.A.; Holmquist, B.; Strydom, D.J.; Goldberg, I.H. *Biochem. Biophys. Res. Commun.* **1979**, *89*, 635. The extinction coefficient of the chromophore used is approximate. A coefficient of 9500 at 340 nm was employed in another Goldberg article: Napier, M.A.; Goldberg, I.H. *Molecular Pharm.* **1983**, *23*, 500. The paper referenced in this article, however, does not contain the value $\varepsilon_{340} \approx 9500$: Povirk, L.F.; Dattagupta, N.; Warf, B.C.; Goldberg, I.H. *Biochemistry* **1981**, *20*, 4007. The extinction coefficient reported in the the latter reference is for the protein-bound chromophore ($\varepsilon_{340} = 10{,}900$). A re-determination of the extinction coefficient for unbound NCS chrompore is necessary.

NCS-Methyl thioglycolate Adduct, Mosher Ester Derivative

(*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenyl acetic acid (MTPA, Mosher acid; 66.7 mg, 285 μmol, 1 equiv) in benzene (1 ml) was treated with oxalyl chloride (0.20 ml, 2.3 mmol, 8 equiv), followed by a catalytic amount of dimethylformamide. Concentration *in vacuo* commenced after 45 minutes, providing a colorless oil. Dissolution of this oil in methylene chloride (1 ml) produced a Mosher acid chloride (MTPA-Cl) solution.

NCS adduct **2** (ca. 0.5 mg, 0.7 μmol, 1 equiv) was concentrated from methylene chloride/methanol (300 μl) in a dry Schlenk flask, yielding a white solid film. 4-(Dimethylamino)pyridine (10 mg, 82 μmol, 117 equiv) in dry toluene (1 ml) was added and the solution was concentrated *in vacuo*. The resulting solid was dissolved in methylene chloride (1 ml) and MTPA-Cl solution (0.25 ml, 71 μmol, 100 equiv) was introduced at 23° C. After 1.75 h, the reaction mixture was cooled to -20° C and stored for 24 h. Water (20 μl) was added and initial purification was done by Sephadex LH-20 chomatography (7 g, 1 cm diameter column, CH_2Cl_2). The crude product was purified by preparative thin-layer chromatography (60% ethyl acetate/hexanes) to yield a clear film (0.4 mg).
Note: This procedure also applies to formation of the (*S*)-(-)-MTPA derivative of **2**.

Hydroxymethylfulvenes **7** and **8**

A solution of 1-chloro-1,2-ethanediol diacetate (1.75 g, 9.69 mmol, 1 equiv) in tetrahydrofuran (THF, 40 ml) was degassed at -78° C. Indene (1.25 ml, 10.7 mmol, 1.11 equiv) in THF (10 ml) was degassed at 0° C and treated with potassium *tert*-butoxide (7 ml of a 1.5 M solution in THF, 10.5 mmol, 1.08 equiv) over a two minute period, providing a clear green solution. After 5 minutes of stirring, a portion of this indenyl anion solution was transferred to the chloride solution at -78° C. The remainder of the anion solution was added over a three minute interval forty minutes after the initial injection; warm to 0° C and stir for 10 minutes. Upon complete addition of the anion, the solution became viscous,

making stirring difficult. The mixture was poured into saturated, aqueous, ammonium chloride/water (1:1, 200 ml) and then extracted with 50% ethyl acetate/hexanes. The organic layer was dried with sodium sulfate and concentrated. The crude oil was dissolved in ethanol (40 ml), cooled to 0° C, degassed, and treated with aqueous sodium hydroxide (50% w/w, 1 ml). Saponification/elimination was complete in 0.5 h. The reaction mixture was partitioned between saturated, aqueous, ammonium chloride/water (1:1, 260 ml) and 50% ethyl acetate/hexanes (E/H). The organic phase was dried (sodium sulfate) and concentrated. Flash chromatography (20-30% E/H; isomers **7** and **8** co-eluted) provided a yellow orange solid (653 mg, 43%), which was a 4:1 ratio of **7**:**8**. Separation of the isomers was achieved by medium pressure liquid chromatography (20% ethyl acetate/ cyclohexane). These hydroxymethylfulvene compounds were unstable, readily polymerizing when concentrated. Storage of the compounds at -20° C frozen in benzene with 2,6-di-*tert*-butyl-4-methylphenol (BHT) added inhibited decomposition.

Compound **7**

^{1}H NMR (400 MHz, $CDCl_3$) :	7.58 (d, 1H, *J*=8 Hz, aromatic H), 7.31-7.18 (m, 3H, aromatic H's), 6.92 (br d, 1H, *J*=6 Hz, H3), 6.76 (d, 1H, *J*=6 Hz, H2), 6.68 (t, 1H, *J*=7 Hz, H8), 4.65 (d, 2H, *J*=7 Hz, CH_2), 1.86 (br s, 1H, OH).
HRMS :	calcd: 158.0732 found: 158.0743
MS (EI, 20 eV) : m/e (relative intensity)	158 (38), 141 (9), 129 (100), 116 (82).
TLC (7% EtOAc/Toluene) :	S.M. $R_f = 0.38$ **7**,**8** $R_f = 0.2$

Compound **8**

^{1}H NMR (400 MHz, $CDCl_3$) :	7.49 (d, 1H, *J*=8 Hz, aromatic H), 7.31-7.17 (m, 3H, aromatic H's), 6.81 (d, 1H, *J*=6 Hz, H3), 6.44 (d, 1H, *J*=6 Hz, H2), 6.42 (t, 1H, *J*=6 Hz, H8),

4.92 (d, 2H, J=6 Hz, CH_2), 1.80 (br s, 1H, OH).

HRMS : calcd: 158.0732
found: 158.0751 (12.2 ppm error)

MS (EI, 20 eV) : m/e (relative intensity) 158, 141, 129 (100), 116

Epoxy Alcohol (*R, R*)-**9**

Methylene chloride (5 ml) and crushed 3-Å molecular sieves (180 mg) were combined in a Schlenk flask and cooled to -22° C. (-)-Diethyl tartrate (0.24 ml, 1.4 mmol, 7.3 equiv), titanium(IV) isopropoxide (0.33 ml, 1.2 mmol, 6.2 equiv), and *tert*-butyl hydroperoxide (0.5 ml of a 4.12 M solution in CH_2Cl_2, 2.1 mmol, 10.7 equiv) were added, sequentially spaced by 5-7 minutes, and the mixture was stirred for 30 minutes at -22° C. Hydroxymethylfulvene **7** (30.3 mg, 0.192 mmol, 1 equiv) was then introduced. After 15.5 h the temperature was raised to 0° C and the reaction mixture was poured into an ice-cold solution of iron(II) sulfate heptahydrate (7.7 g, 27.7 mmol) and tartaric acid (2.3 g, 15.3 mmol) in water (55 ml). The phases were separated after 10 minutes and the aqueous layer was extracted with diethyl ether (2 x 25 ml). The combined organic layers were cooled to 0° C and treated with 50% sodium hydroxide/brine (1:2, 15 ml) for 1 h. This solution was diluted with water (80 ml) and the aqueous phase was extracted with diethyl ether (2 x 30 ml). The combined organics were dried with sodium sulfate and concentrated. Flash chromatography (40% ethyl acetate/hexanes) employing a silica gel plug afforded a yellow oil (13.2 mg, 40%).

^{1}H NMR (400 MHz, $CDCl_3$) : 7.32-7.15 (m, 4H, aromatic H's), 6.99 (d, 1H, J=5.9 Hz, H3), 6.25 (d, 1H, J=5.9 Hz, H2), 4.07 (m, 1H, CH_aH_b), 3.95 (m, 2H, H8 and CH_aH_b), 1.87 (br s, 1H, OH).

FTIR (neat film, cm^{-1}) : 3403 (m, brd), 1457 (m), 1037 (s), 888 (s), 757 (s).

HRMS : calcd: 174.0681
found: 174.0671

MS (EI, 20 eV) : m/e (relative intensity)	174 (6), 156 (32), 144 (37), 128 (48), 116 (100), 115 (86), 102 (11).
TLC (40% EtOAc/Hexanes) :	Epoxy alcohol **9** $R_f = 0.27$

Epoxy Alcohol (*S, R*)-**10**

A Schlenk flask containing crushed 3-Å molecular sieves (55 mg) was flame dried. Methylene chloride (10 ml) was added, an argon-filled balloon was attached, and the flask was cooled to -22° C. (-)-Diethyl tartrate (0.24 ml, 1.4 mmol, 5.6 equiv) and titanium(IV) isopropoxide (0.33 ml, 1.2 mmol, 4.8 equiv) were added to the stirring solution, then *tert*-butyl hydroperoxide (0.5 ml of a 4.12 M solution in CH_2Cl_2, 2.1 mmol, 8.4 equiv) was introduced. After the reaction mixture stirred for 35 minutes at -22° C, fulvene **8** (ca. 40 mg, 0.25 mmol, 1 equiv) in methylene chloride (3 ml) was transferred via cannula to the flask. An argon-filled balloon was attached and the flask was placed in a -18° C cooling bath. The reaction mixture was stirred for 12.5 h, then poured into an ice-cold solution of iron(II) sulfate heptahydrate (16.5 g, 59 mmol) and tartaric acid (5 g, 33 mmol) in water (50 ml). After 10 minutes of stirring, the phases were separated and the aqueous layer was extracted with diethyl ether (2 x 30 ml). The combined organic layers were cooled to 0° C and treated with 50% sodium hydroxide/brine (1:2, 7.5 ml) for 1 h. The mixture was diluted with water (50 ml) and then transferred to a separatory funnel. The aqueous phase was extracted with diethyl ether (2 x 30 ml). The organics were dried (sodium sulfate), filtered, and concentrated *in vacuo* to afford a dark lime green film. The crude product was purified by preparative thin-layer chromatography (40% ethyl acetate:hexanes) to provide **10** (12.0 mg, 25%) as a faint lime green film.

^{1}H NMR (400 MHz, $CDCl_3$) :	7.33-7.16 (m,4H, aromatic H's), 6.90 (d, 1H, *J*=5.6 Hz, H3), 6.09 (d, 1H, *J*=5.6 Hz, H2), 4.16 (br dd, 1H, *J*=6.8, 12.2 Hz, CH_aH_b), 4.04 (br d, 1H, *J*=12.2 Hz, CH_aH_b), 3.88 (dd, 1H, *J*=4.2, 6.8 Hz, H8), 1.93 (br s, OH).

FTIR (neat film, cm^{-1}) :	3397 (m, broad), 1458 (m), 1020 (s), 888 (s), 756 (s).
HRMS :	calcd: 174.0681 found: 174.0676
MS (EI, 20 eV) : m/e (relative intensity)	174 (10), 158 (6), 144 (45), 131 (77), 116 (80), 115 (100), 102 (22), 89 (7).

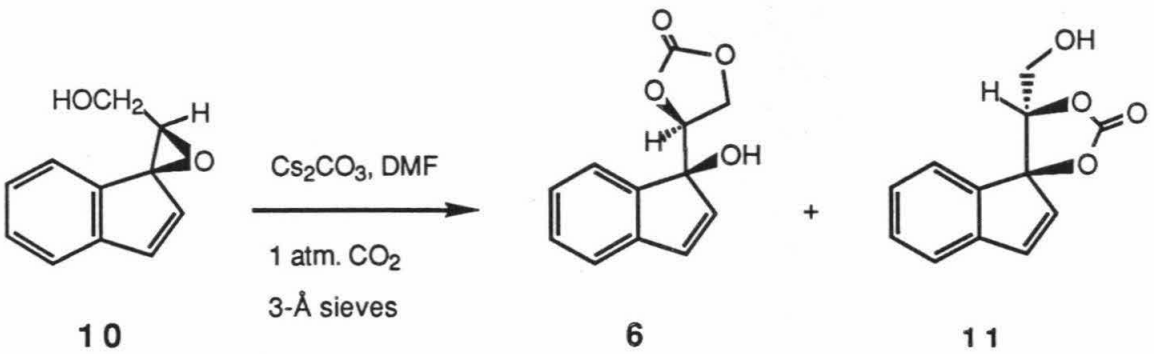
Hydroxy Carbonate **5**

Crushed 3-Å molecular sieves (50 mg) and Cs_2CO_3 (74.7 mg, 0.23 mmol, 0.45 equiv) were combined in a flame-dried 10 ml Schlenk flask. The flask and contents were then flame-dried and cooled under argon. Epoxy alcohol **9** (89.3 mg, 0.51 mmol, 1 equiv) was dried by azeotroping from toluene (3 x 1 ml), then dissolved in dry dimethylformamide (DMF, 1 ml). This solution was transferred to the Schlenk flask via cannula, followed by two DMF rinse solutions (each 0.5 ml) from the epoxy alcohol flask. The reaction vessel was charged with CO_2 (1 atm) and the mixture was stirred at 40° C for 40 h. The heating bath was removed and saturated, aqueous, ammonium chloride solution (5 ml) was added. After 5 minutes of stirring, the mixture was diluted with 10% brine solution (100 ml) and extracted with 50% ethyl acetate/hexanes (E/H, 3 x 50 ml). A crude dark greenish brown oil was obtained after drying (sodium sulfate) and concentration *in vacuo*. Purification by flash chromatography (30-40% E/H) afforded a viscous orange oil (95.6 mg, 85%).

^1H NMR (400 MHz, $CDCl_3$) :	7.43-7.23 (m, 4H, aromatic H's), 6.86 (d, 1H, *J*=5.6 Hz, H3), 6.31 (d, 1H, *J*=5.6 Hz, H2), 4.81 (dd, 1H, *J*=6.6, 8.5 Hz, H8), 4.47 (t, 1H, *J*=8.5 Hz, *anti*-H9), 4.32 (dd, 1H, *J*=6.6, 8.5, *syn*-H9), 2.35 (s, 1H, OH).
FTIR (neat film, cm^{-1}) :	3421 (m, broad), 1805 (s), 1783 (s), 1175 (m), 1081 (m), 761 (m).
HRMS :	calcd: 218.0577 found: 218.0579
MS (EI, 20 eV) :	218 (6), 156 (13), 144 (11), 131 (100), 115 (17),

m/e (relative intensity) 103 (14), 77 (7).

TLC (40% EtOAc/Hexanes) : S.M. $R_f = 0.28$
Product $R_f = 0.20$



10 **6** **11**

Hydroxy Carbonate **6**

Crushed 3-Å molecular sieves (12.1 mg) and Cs_2CO_3 (28.5 mg, 87.5 μmol, 2.8 equiv) were flame dried together in a dry Schlenk flask, cooled under argon, then cooled to 0° C. Epoxy alcohol **10** (5.4 mg, 31.0 mmol, 1 equiv) in dimethylformamide (1 ml) and subsequent flask rinses (dimethylformamide, 2 x 0.3 ml) were transferred to the Schlenk flask and then the flask was purged with CO_2. After 19 h (cooling bath removed after 2.5 h), dried crushed 4-Å sieves (38 mg) and Cs_2CO_3 (36 mg, 110.5 μmol, 3.6 equiv) were added. Saturated, aqueous, ammonium chloride solution (5 ml) was poured into the flask after a total reaction time of 53 h and the mixture was stirred for 5 minutes. Dilution with 10% brine solution (50 ml), extraction with 50% ethyl acetate/hexanes (E/H, 3 x 25 ml), drying (sodium sulfate), and concentration *in vacuo* provided a crude oil. Purification by preparative thin-layer chromatography (50% ethyl acetate/hexanes) afforded two products: desired carbonate **6** (4.9 mg, 72%) and rearranged carbonate **11** (0.6 mg, 9%).

Hydroxy Carbonate **6**

^1H NMR (400 MHz, $CDCl_3$) : 7.54 (d, 1H, *J*=7.3 Hz, aromatic H), 7.37-7.24 (m, 3H, aromatic H's), 6.92 (d, 1H, *J*=5.6 Hz, H3), 6.22 (d, 1H, *J*=5.6 Hz, H2), 4.81 (dd, 1H, *J*=6.4, 8.5 Hz, H8), 4.32 (t, 1H, *J*=8.5, *anti*-H9), 4.06 (dd, 1H, *J*=6.4, 8.5 Hz, *syn*-H9), 2.38 (s, 1H, OH).

FTIR (neat film, cm^{-1}) :	3421 (m, broad), 1801 (s), 1781 (s), 1174 (m), 1085 (s), 758 (m).
HRMS :	calcd: 218.0579 found: 218.0576
MS (EI, 20 eV) : m/e (relative intensity) :	218 (5), 156 (11), 131 (100), 115 (8), 103 (13), 77 (7).

TLC (40% EtOAc/Hexanes) :

S.M.	$R_f = 0.26$		
Product **6**	$R_f = 0.18$	(50% E/H)	$R_f = 0.31$
Product **11**	$R_f = 0.17$	"	$R_f = 0.28$

Rearranged Hydroxy Carbonate **11**

^1H NMR (400 MHz, $CDCl_3$) :	7.45 (d, 1H, *J*=7.3 Hz, aromatic H), 7.38-7.24 (m, 3H, aromatic H's), 6.87 (d, 1H, *J*=5.6 Hz, H3), 6.46 (d, 1H, *J*=5.6 Hz, H2), 4.86 (dd, 1H, *J*=3.4, 4.4 Hz, H8), 3.83(m, 2H, $C\underline{H_a}\underline{H_b}$), 1.99 (t, 1H, *J*=6.4 Hz, OH).
FTIR (neat film, cm^{-1}) :	3454 (broad w), 1801 (s), 1784 (s), 1230 (m), 1194 (m), 1061 (m), 763 (m).
HRMS :	calcd: 218.0579 found: 218.0584
MS (EI, 20 eV) : m/e (relative intensity)	218 (11), 156 (12), 144 (14), 131 (100), 115 (31), 103 (14), 77 (8).

Hydroxy Carbonate (*S,R*)-**5**, (*R*)-(+)-Mosher Ester Derivative

A stock solution of 4-(dimethylamino)pyridine (DMAP) was prepared by dissolving DMAP (397 mg) in methylene chloride (10 ml). The DMAP had been dried by azeotroping from dry toluene (3 x 5 ml).

(*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenyl acetic acid (MTPA, Mosher acid; 120 mg, 0.51 mmol, 1 equiv) in benzene (2 ml) was treated with oxalyl chloride (0.30 ml, 3.4 mmol, 6.7 equiv), followed by a catalytic amount of dimethylformamide. Concentration *in vacuo* commenced after 45 minutes, providing a colorless oil. Dissolution

of this oil in methylene chloride (1 ml) produced a working Mosher acid chloride (MTPA-Cl) solution.

Hydroxy carbonate **5** (4 mg, 18.3 μmol, 1 equiv) was concentrated from tetrahydrofuran/toluene (1:4, 200 μl) in a flame-dried flask. Methylene chloride (0.5 ml) and DMAP solution (0.5 ml, 20 mg, 164 μmol, 9 equiv) were introduced to the flask, which was then equipped with an argon-filled balloon. MTPA-Cl solution (0.2 ml, 95 μmol, 5.2 equiv) was added and the mixture was stirred for 2 h. The reaction was quenched with water, the layers were separated and the organic phase was treated with saturated aqueous citric acid solution:50% ethyl acetate/hexanes (1:1). The combined organic layers were dried with sodium sulfate and then solvents were removed *in vacuo*. Purification was done by preparative thin-layer chromatography (40% ethyl acetate/ hexanes) to afford the pure Mosher ester derivative of **5** (7.0 mg, 88%).

Hydroxy Carbonate **5**, Mosher Ester Derivative (*S,R,R*) or (*R,S,S*)

^{1}H NMR (400 MHz, $CDCl_3$) :	7.51-7.27 (m, 9H, aromatic H's), 7.06 (d, 1H, *J*=5.6 Hz, H3), 6.34 (d, 1H, *J*=5.6 Hz, H2), 5.10 (dd, 1H, *J*=6.4, 8.5 Hz, H8), 4.37 (t, 1H, *J*=8.5 Hz, *anti*-H9), 3.84 (dd, 1H, *J*=6.4, 8.5 Hz, *syn*-H9), 3.53 (s, 3H, OCH_3).
(400 MHz, C_6D_6) :	7.70 (d, 2H, *J*=7.6 Hz, aromatic H's), 7.36 (d, 1H, aromatic H), 7.17-6.95 (m, 4H, aromatic H's), 6.87 (d, 2H, *J*=7.6, aromatic H's), 6.36 (d, 1H, *J*=5.6 Hz, H3), 5.81 (d, 1H, *J*=5.6 Hz, H2), 4.26 (dd, 1H, *J*=6.4, 8.5 Hz, H8), 3.23 (t, 1H, *J*=8.5 Hz, *anti*-H9),3.15 (dd, 1H, *J*=6.4, 8.5 Hz, *syn*-H9), 3.39 (s, 3H, OCH_3).
FTIR (neat film, cm^{-1}) :	1816 (s), 1791 (m), 1761 (s), 1170 (s), 1082 (s).
HRMS :	calcd: 434.0977 found: 434.0964
MS (EI, 20 eV) : m/e (relative intensity)	434 (<2), 189 (100), 129 (9), 115 (12), 105 (6).

Hydroxy Carbonate **5**, Mosher Ester Derivative (*R,S,R*) or (*S,R,S*)

^{1}H NMR (400 MHz, $CDCl_3$) :	7.49-7.37 (m, 7H, aromatic H's), 7.25-7.13 (m, 2H, aromatic H's), 7.04 (d, 1H, *J*=5.6 Hz, H3), 6.51 (d, 1H, *J*=5.6 Hz, H2), 4.86 (dd, 1H, *J*=6.4, 8.5 Hz, H8), 4.38 (t, 1H, *J*=8.5 Hz, *anti*-H9), 4.07 (dd, 1H, *J*= 6.4, 8.5 Hz,*syn*-H9), 3.56 (s, 3H, OCH_3).
(400 MHz, C_6D_6) :	7.66 (d, 2H, *J*=7.8 Hz, aromatic H's), 7.14-6.83 (m, 7H, aromatic H's), 6.35 (d, 1H, *J*=5.6 Hz, H3), 6.10 (d, 1H, *J*=5.6 Hz, H2), 3.99 (dd, 1H, *J*=6.4, 8.5 Hz, H8), 3.49 (s, 3H, OCH_3), 3.44 (dd, 1H, *J*=6.4, 8.5 Hz, *syn*-H9), 3.20 (t, 1H, *J*=8.5 Hz, *anti*-H9).
FTIR (neat film, cm^{-1}) :	1816 (s), 1790 (m), 1764 (s), 1170 (s), 1082 (s).
HRMS :	calcd: 434.0977 found: 434.0950
MS (EI, 20 eV) : m/e (relative intensity)	434 (3), 189 (100), 170 (22), 156 (19), 140 (8), 128 (35), 115 (22), 105 (22), 91 (15), 77 (22), 44 (15).

Hydroxy Carbonate (*S,S*)-**6**, (*R*)-(+)-Mosher Ester Derivative

For preparation of DMAP and MTPA-Cl solutions, see the experimental section for the Mosher ester derivative of **5**.

Hydroxy carbonate **6** (ca. 1 mg, 4.6 μmol, 1 equiv) was concentrated from methylene chloride/toluene (1:1, 150 μl) in a flame-dried flask. Methylene chloride (0.5 ml) and DMAP solution (0.5 ml, 20 mg, 164 μmol, 36 equiv) were introduced to the flask, which was subsequently equipped with an argon-filled balloon. MTPA-Cl solution (0.2 ml, 95 μmol, 21 equiv) was added and the mixture was stirred for 1.25 h. The reaction was quenched with water, the layers were separated, and the organic phase was treated with saturated, aqueous, citric acid solution:50% ethyl acetate/hexanes (1:1, 20 ml). After vigorous shaking, the organic layer was removed, dried (sodium sulfate), and concentrated *in vacuo*. The crude product was purified by flash chromatography (30% ethyl acetate/ hexanes) through a short silica gel plug, affording a clear oil (1.2 mg).

Note: These reaction conditions are applicable to the formation of all (*R*)-(+)- and (*S*)-(-)-Mosher ester derivatives of both enantiomers of **6.**

Hydroxy Carbonate **6**, Mosher Ester Derivative (*S,S,R*) or (*R,R,S*)

^{1}H NMR (400 MHz, $CDCl_3$) :	7.52-7.29 (m, 9H, aromatic H's), 7.11 (d, 1H, *J*=5.6 Hz, H3), 6.08 (d, 1H,*J*=5.6 Hz, H2), 4.69 (dd, 1H, *J*=6.4, 8.5 Hz, H8), 4.28 (t, 1H, *J*=8.5 Hz, *anti*-H9), 3.91 (dd, 1H, *J*= 6.4, 8.5 Hz,*syn*-H9), 3.56 (s, 3H, OCH_3).
(400 MHz, C_6D_6) :	7.68 (d, 2H, *J*=7.8 Hz, aromatic H's), 7.43 (d, 1H, aromatic H), 7.16-6.91 (m, 6H,aromatic H's), 6.41 (d, 1H, *J*=5.6 Hz, H3), 5.50 (d, 1H, *J*=5.6 Hz, H2), 3.72 (dd, 1H, *J*=6.4, 8.5 Hz, H8), 3.46 (s, 3H, OCH_3), 3.27 (dd, 1H, *J*=6.4, 8.5 Hz, *syn*-H9), 3.06 (t, 1H, *J*=8.5 Hz, *anti*-H9).
FTIR (neat film, cm^{-1}) :	1816 (s), 1790 (m), 1764 (s), 1168 (s), 1086 (s).
HRMS :	calcd: 434.0977 found: 434.0961
MS (EI, 20 eV) : m/e (relative intensity)	434 (2), 189 (100), 170 (35), 156 (34), 141 (14), 128 (50), 115 (23), 105 (32), 91 (19), 77 (31), 44 (31).
TLC (40% EtOAc/Hexanes) :	S.M. **6** R_f = 0.22 Product **17** R_f = 0.33

Hydroxy Carbonate **6**, Mosher Ester Derivative (*S,S,S*) or (*R,R,R*)

^{1}H NMR (400 MHz, $CDCl_3$) :	7.47-7.38 (m, 7H, aromatic H's), 7.25-7.18 (m, 2H, aromatic H's), 7.14 (d, 1H, *J*=5.6 Hz, H3), 6.27 (d, 1H,*J*=5.6 Hz, H2), 4.91 (dd, 1H, *J*=6.4,8.5 Hz, H8), 4.19 (t, 1H, *J*=8.5 Hz, *anti*-H9), 3.74 (dd, 1H, *J*=6.4, 8.5 Hz, *syn*-H9), 3.56 (s, 3H, OCH_3).
(400 MHz, C_6D_6) :	7.65 (d, 2H, *J*=7.6 Hz, aromatic H's), 7.15-6.86 (m, 7H, aromatic H's), 6.39 (d, 1H, *J*=5.6 Hz, H3), 5.74 (d, 1H, *J*=5.6 Hz, H2), 3.90 (dd, 1H, *J*=6.4, 8.5 Hz, H8), 3.45 (s, 3H, OCH_3), 3.02 (dd, 1H, *J*=6.4, 8.5 Hz, *syn*-H9), 2.96 (t, 1H, *J*=8.5 Hz, *anti*-H9).

FTIR (neat film, cm^{-1}) :	1816 (s), 1791 (m), 1762 (s), 1168 (s), 1086 (s).
HRMS :	calcd: 434.0977 found: 434.0985
MS (EI, 20 eV) : m/e (relative intensity)	434 (10), 189 (100), 129 (6), 115 (12), 105 (4).

Purification of NCS Chromophore for VT NMR Experiments

Attempts were made at all steps of this process to avoid exposure to light. Neocarzinostatin chromophore was extracted as described in the procedure for preparation of monoadduct **2**. The methanolic solution of the chromophore was concentrated *in vacuo* to yield an orange-brown residue. This crude mixture was purified by low temperature (4° C) Sephadex chromatography. The Sephadex LH-20 (6 g) was pre-swelled in the elution solvent, methylene chloride/methanol/acetic acid (93:5:2). The eluant was analyzed for chromophore content by ultraviolet spectroscopy. The collected eluant was divided into four portions and the solvents were removed *in vacuo* to yield tan, powdery residues (ca. 6 mg each). The individual flasks were wrapped in foil and stored at -100° C.
Note: Less Sephadex can probably be used because it seems to act only as a filtration aid to remove highly polar components (protein) from the crude mixture.

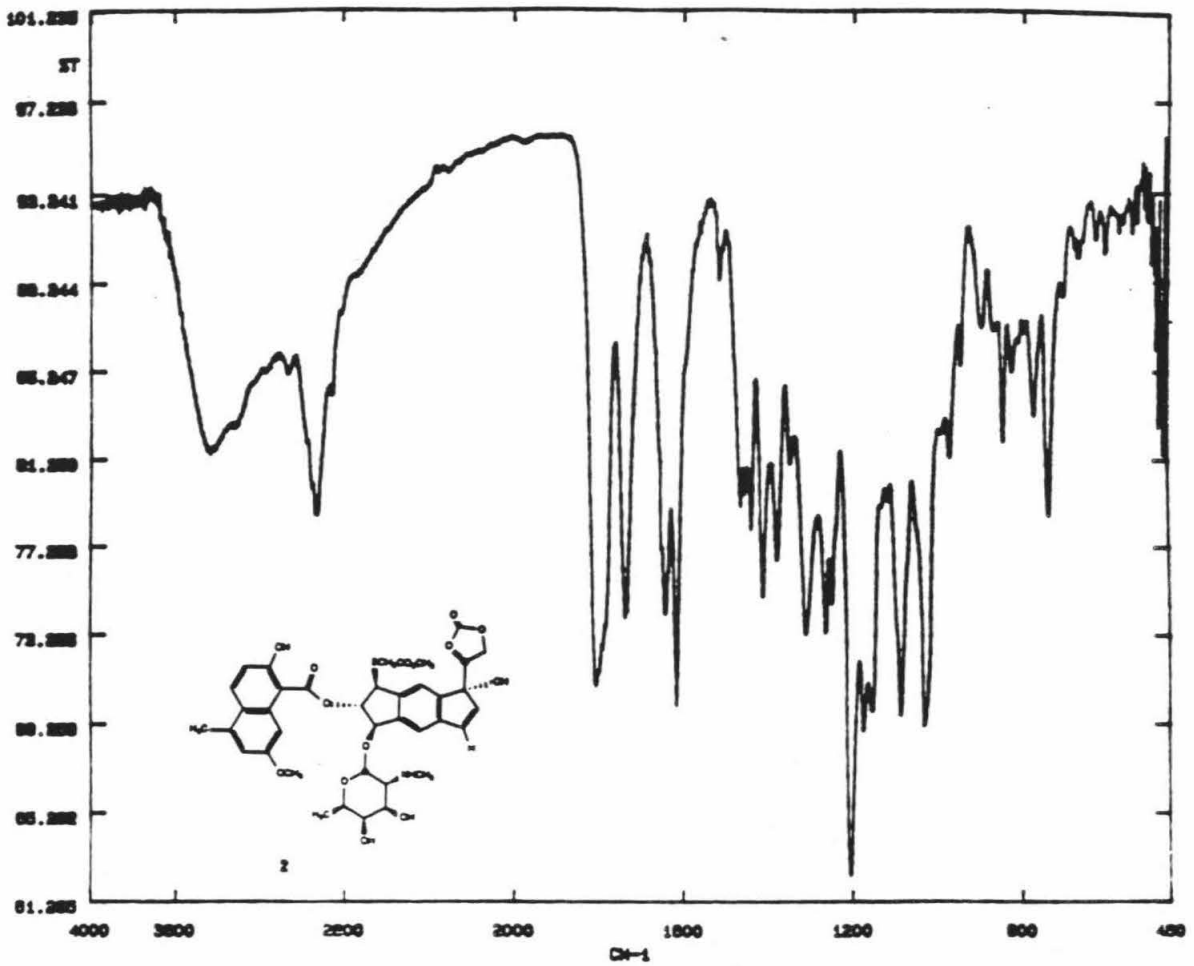
VT NMR Study. High Thiol Concentration (0.2 M)

Powdered NCS chromophore (ca. 6 mg) was dissolved in tetrahydrofuran-d_8/CD_3COOH (9:1, 400 µl) and then transferred to an NMR tube. Benzene-d_6 (14 µl, 99.5% D) was added as a reference and a concentration standard. The sample was cooled to -78° C in the NMR probe and a spectrum was recorded. The sample tube was removed from the probe, cooled in a dry ice-acetone bath, and degassed. Methyl thioglycolate (7 µl, 0.2 M) was then introduced. The tube was quickly placed back into the cooled NMR probe. Spectra were recorded every ten minutes for 4.3 h with the probe at -70° C. The

probe was then warmed to -40° C and spectra were again recorded every ten minutes for 4 h. The sample was then removed from the probe and stored at -20° C overnight. Mono- and bisthiol adducts were isolated as described previously. Deuterium incorporation was determined by NMR integration.

VT NMR Study, Low Thiol Concentration (0.03 M)

Powdered NCS chromophore (ca. 6 mg) was dissolved in tetrahydrofuran-d_8:CD_3COOH (9:1, 400 µl) containing a trace amount of *trans*-1,2-dichloroethylene as an internal standard. After transferring the brown solution to an NMR tube, freshly distilled 1,4-cyclohexadiene (7.5 µl, 0.2 M) was added. The sample was cooled to -78° C and then degassed. An NMR spectrum was recorded at -78° C. While the tube was cooling in a dry ice-acetone bath, methyl thioglycolate (1 µl, 11.2 µmol, 0.03 M) was introduced. The sample was then transferred to a -70° C bath. After 74 h the tube was placed in a -70° C NMR probe and a spectrum was recorded. The sample was warmed to -40° C and spectra were recorded every ten minutes for 3.3 h. The isolation of monoadduct was achieved as above. The amount of bisthiol adduct was too small to isolate.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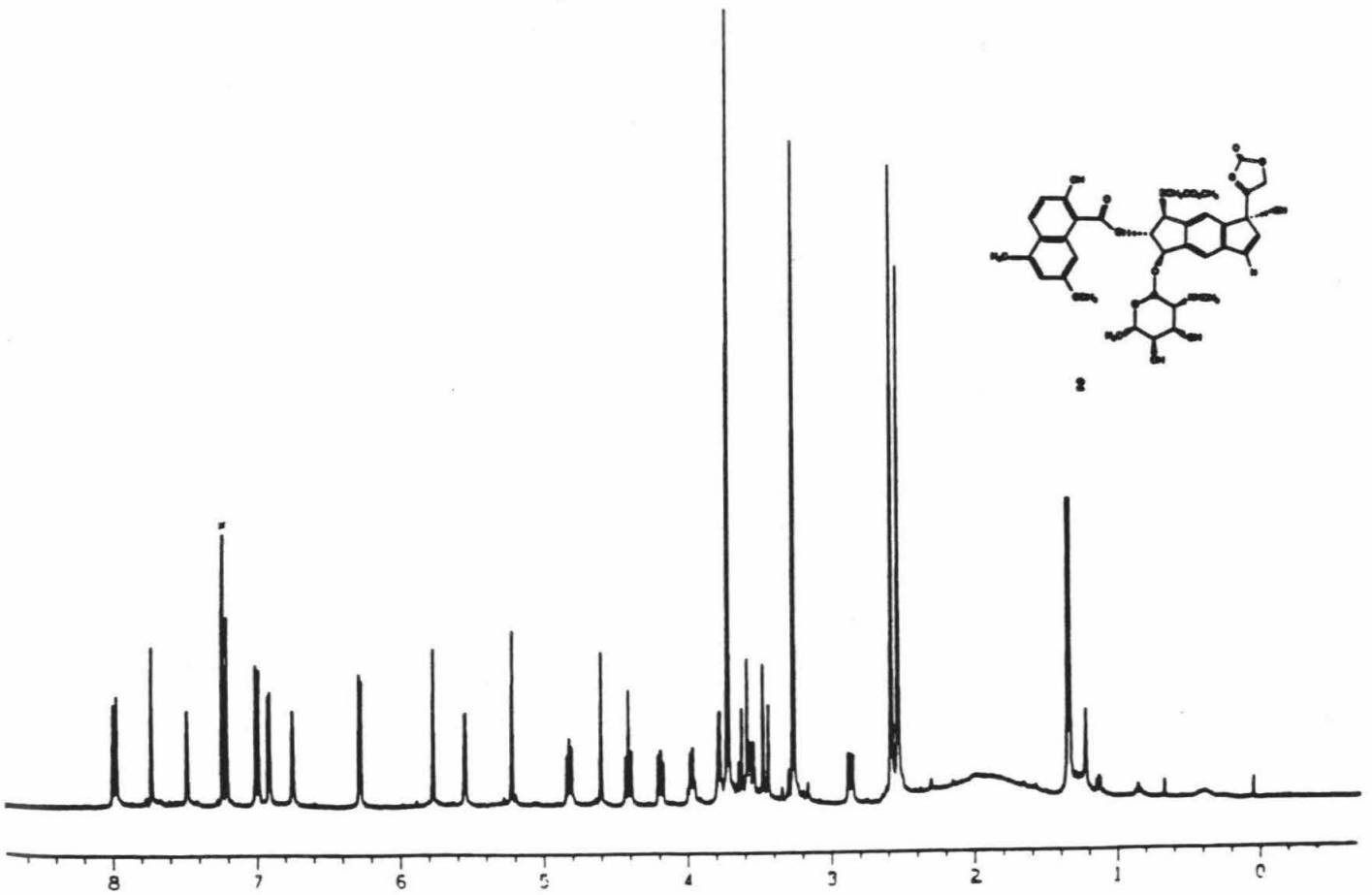
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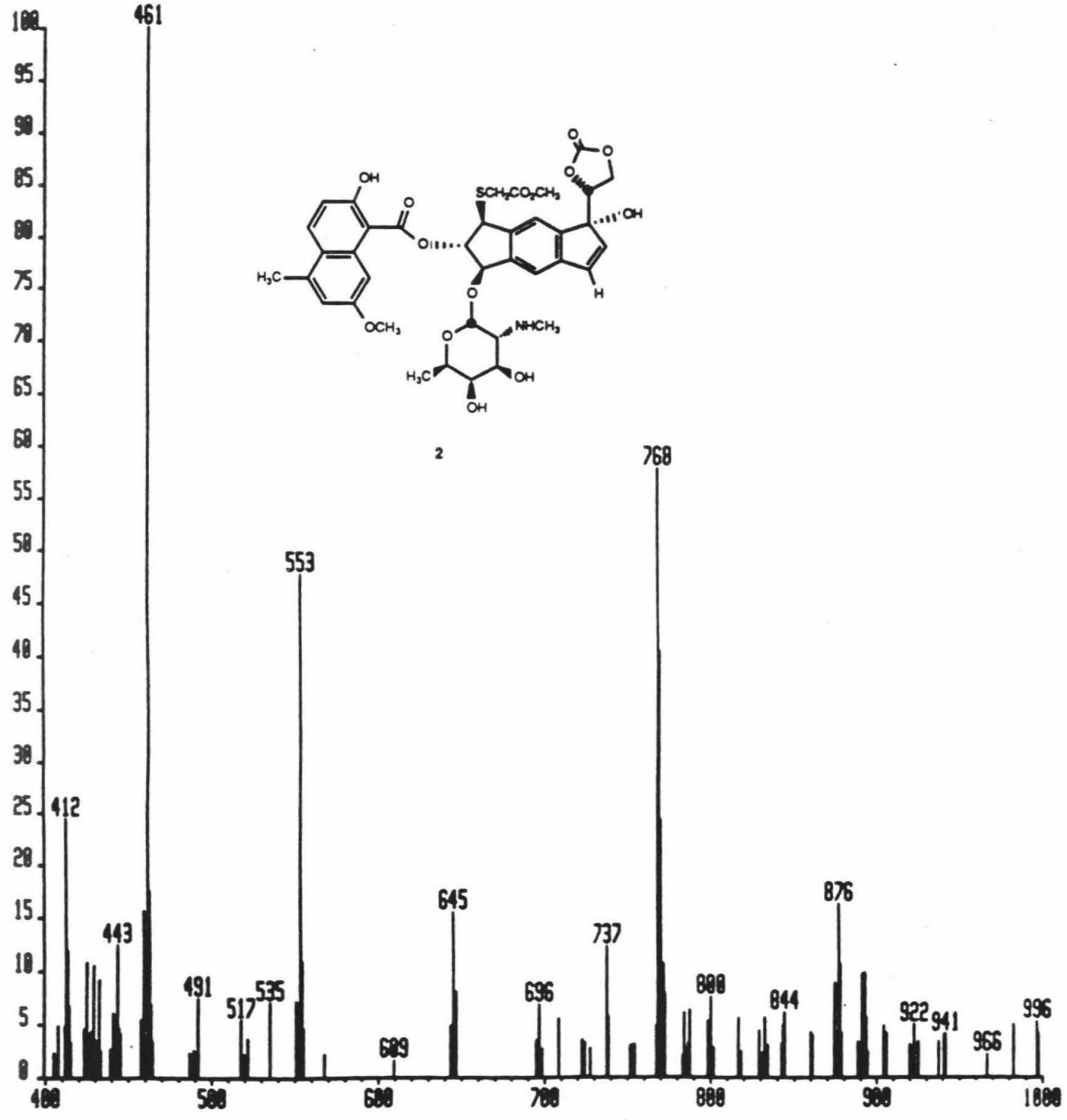
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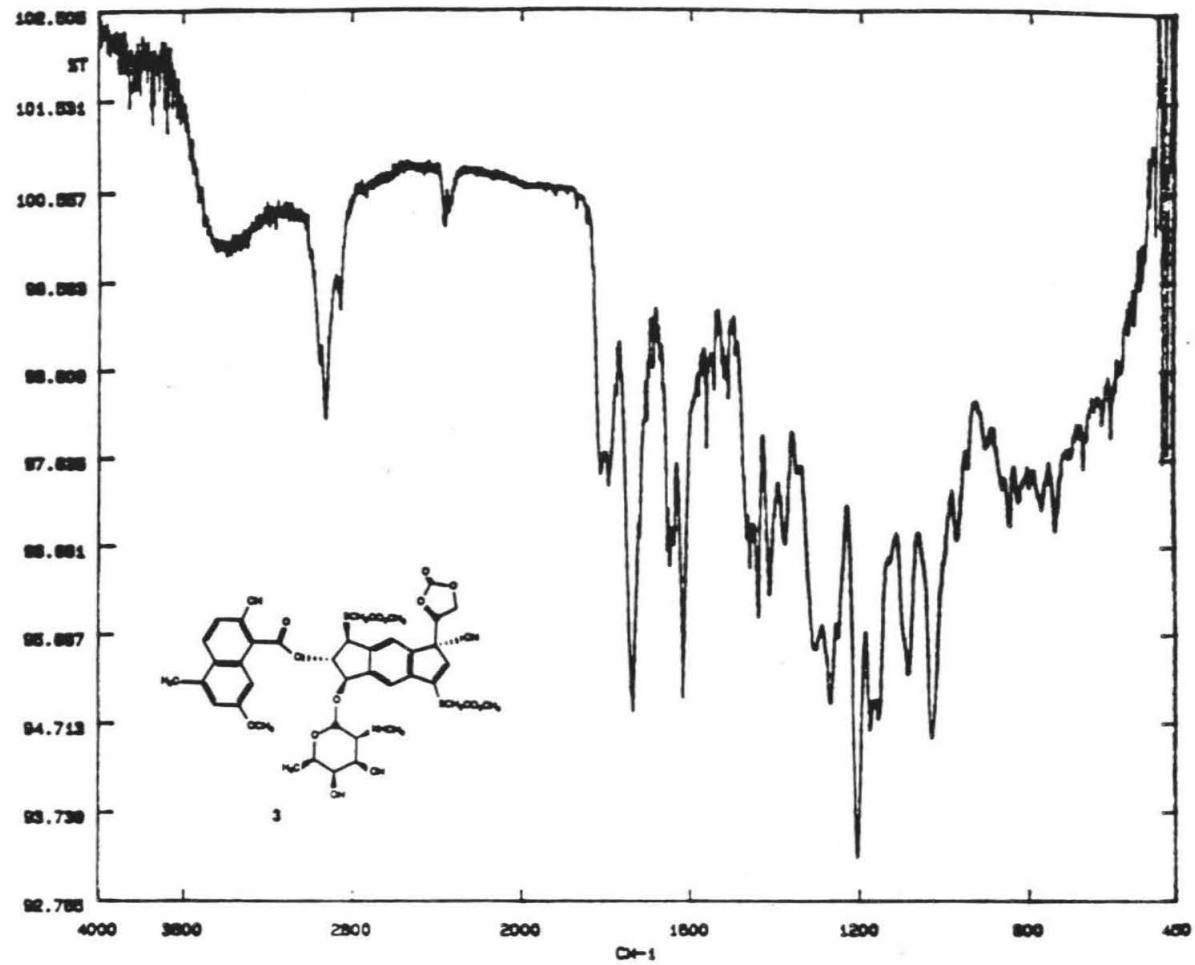
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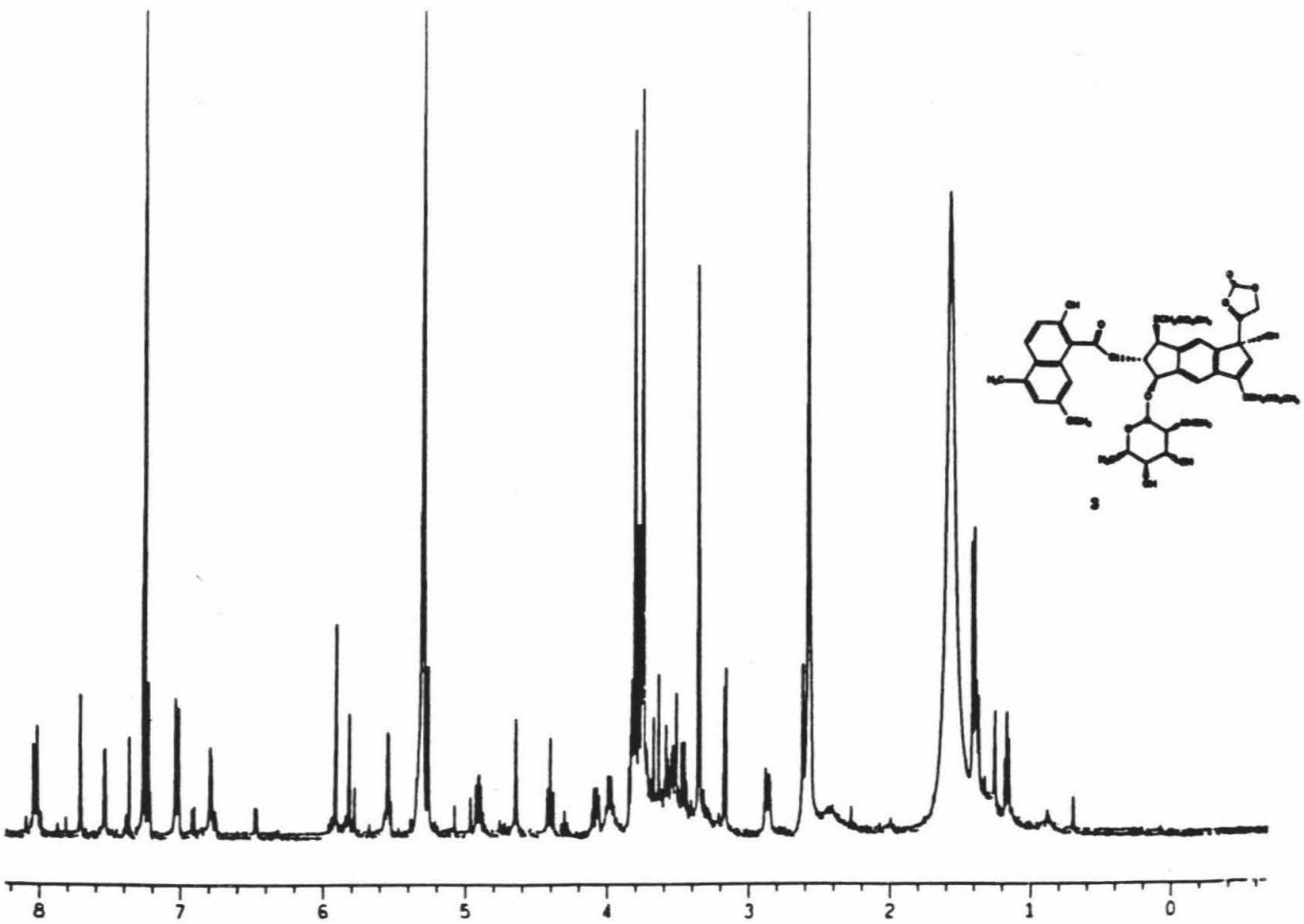

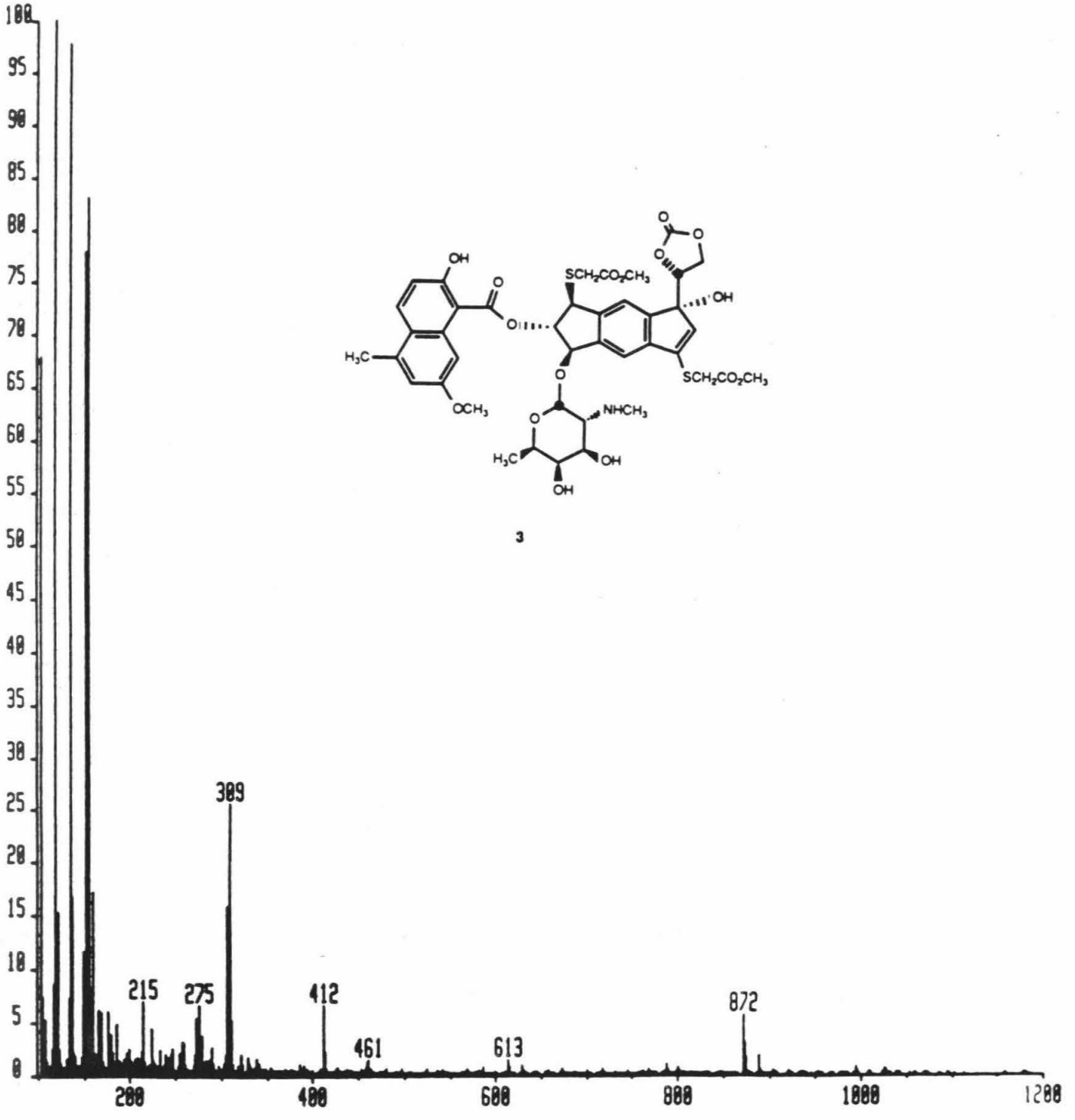
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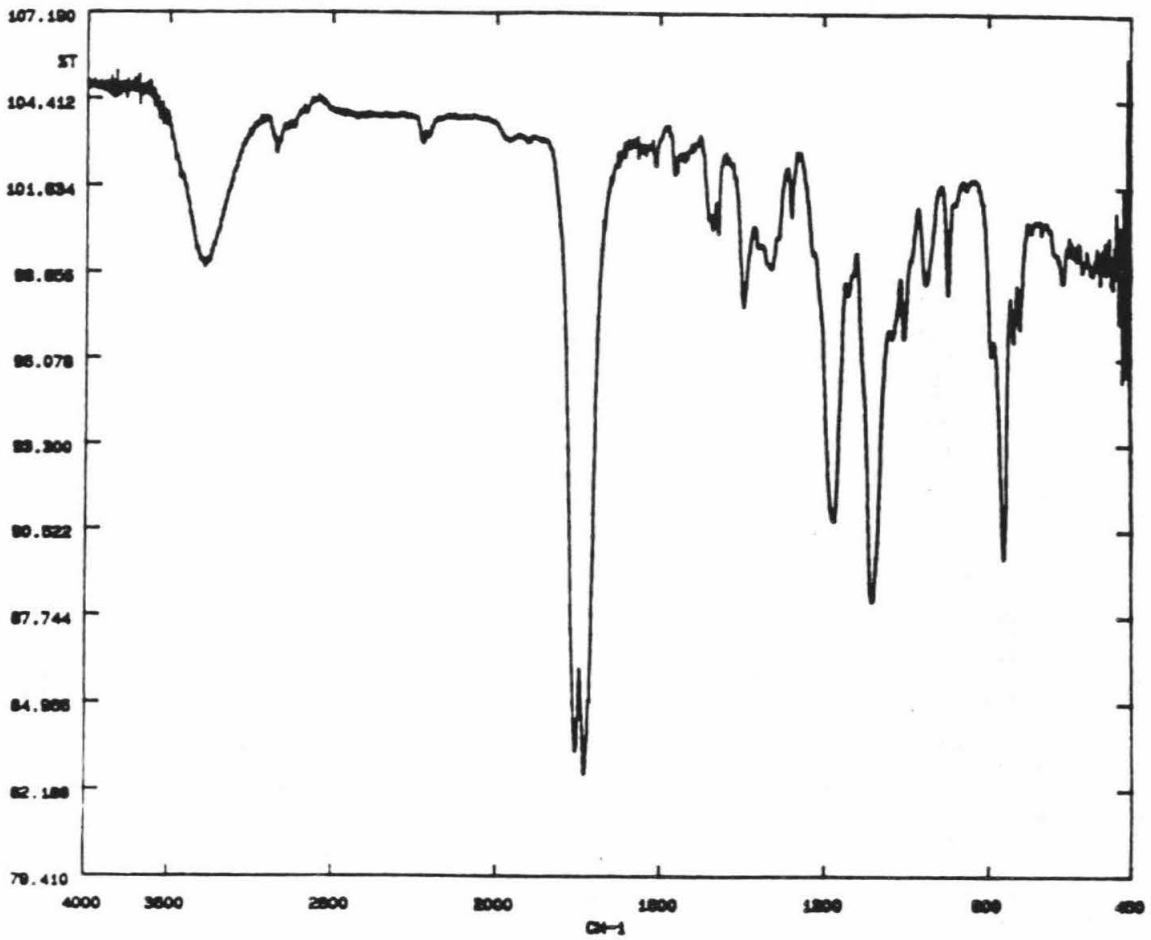
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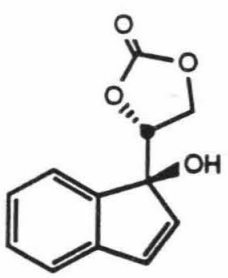
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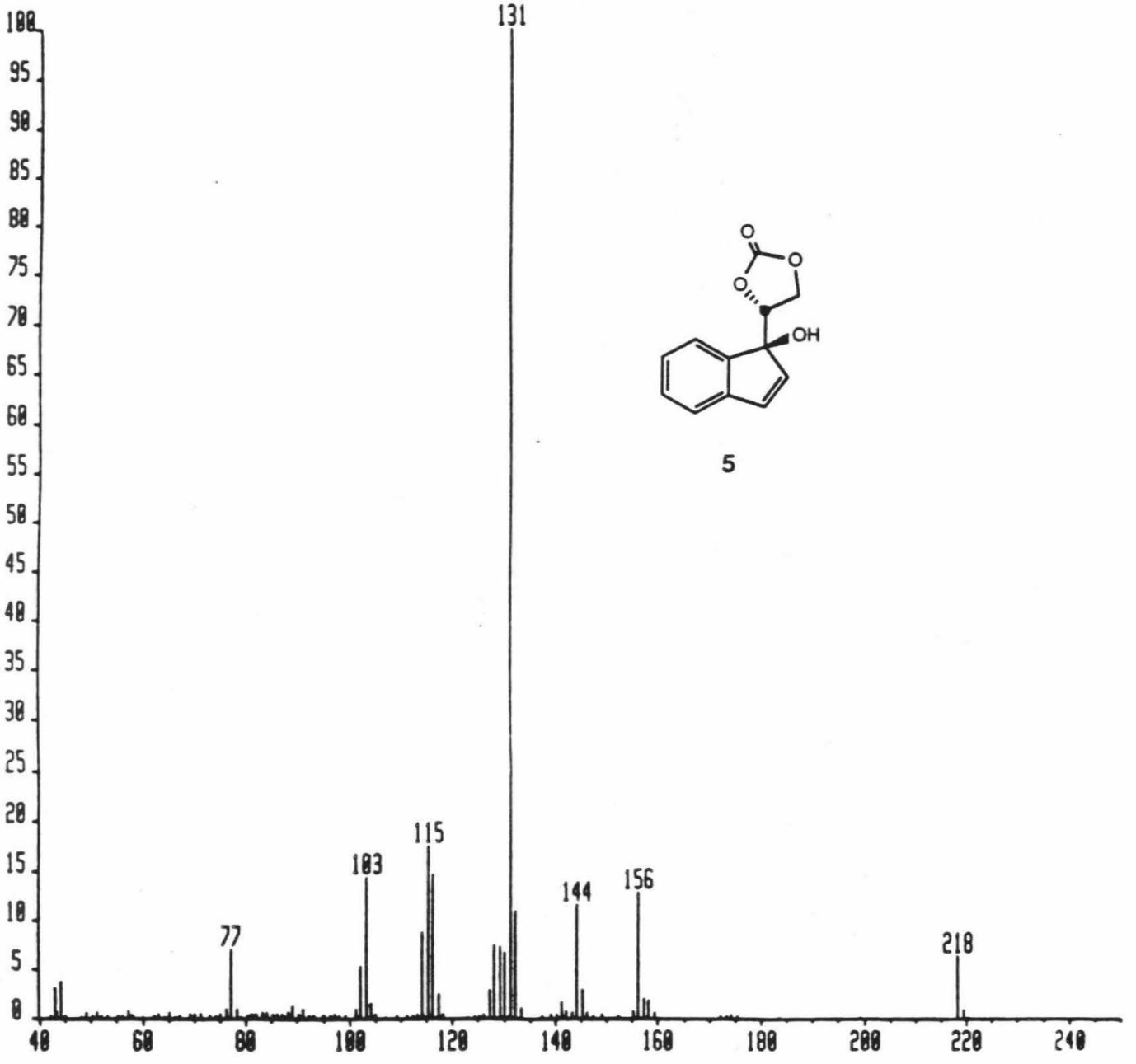
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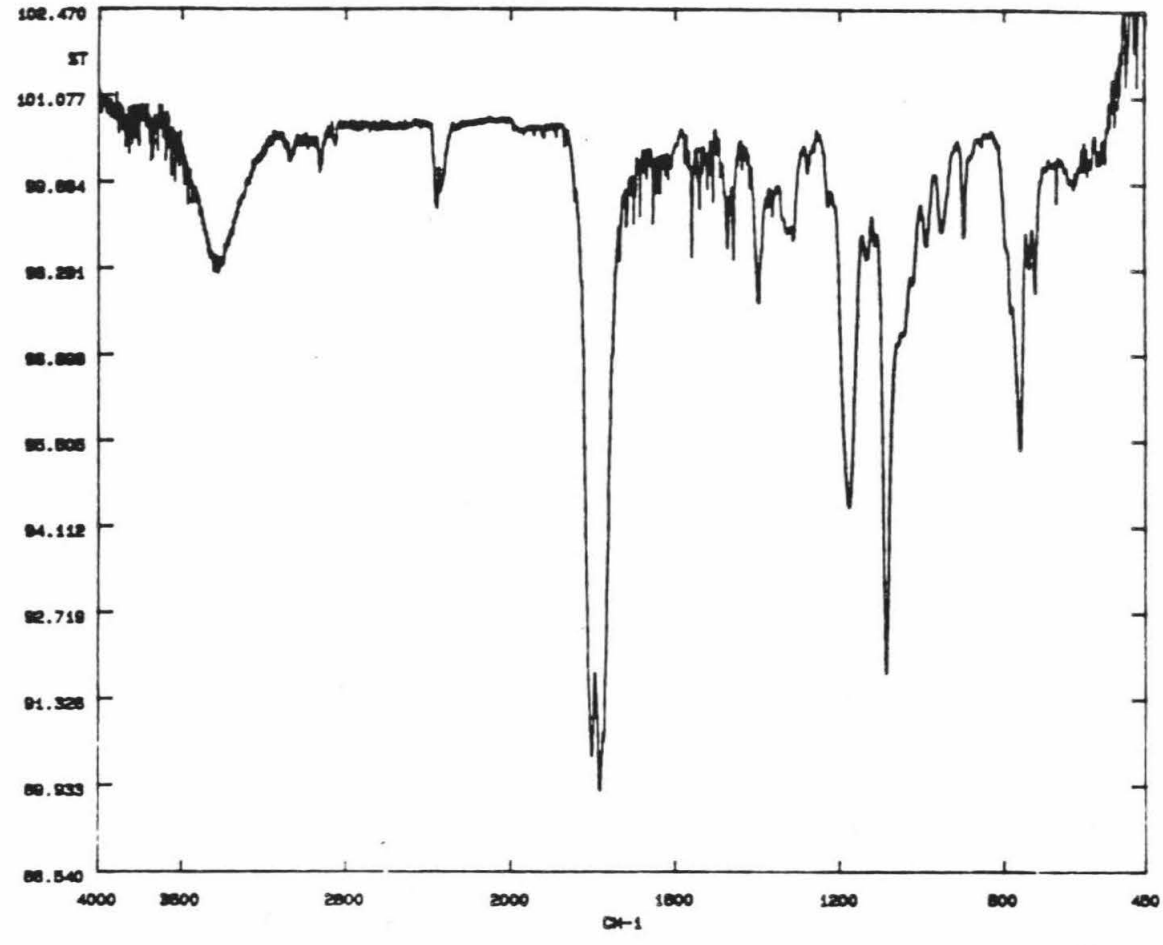
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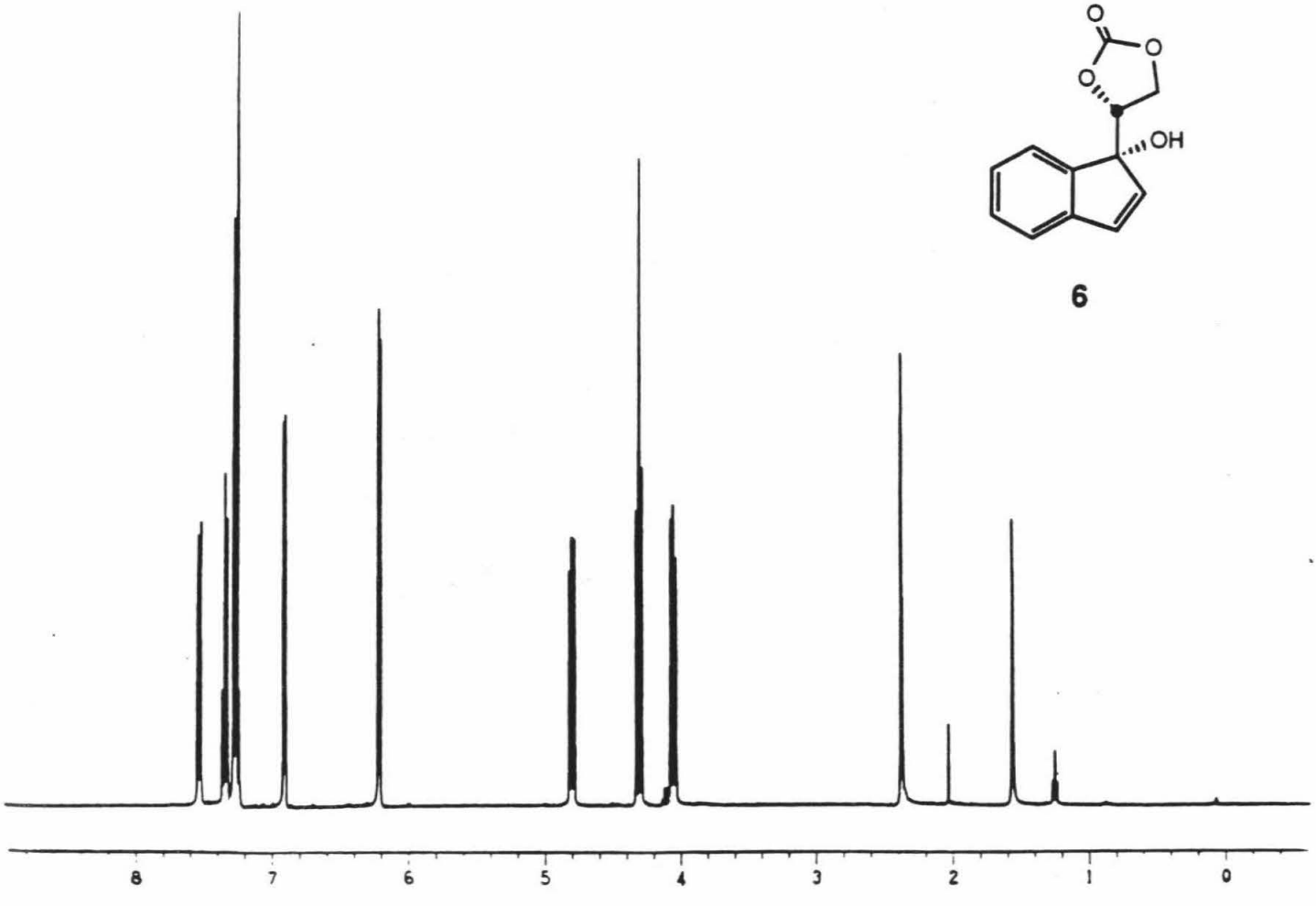
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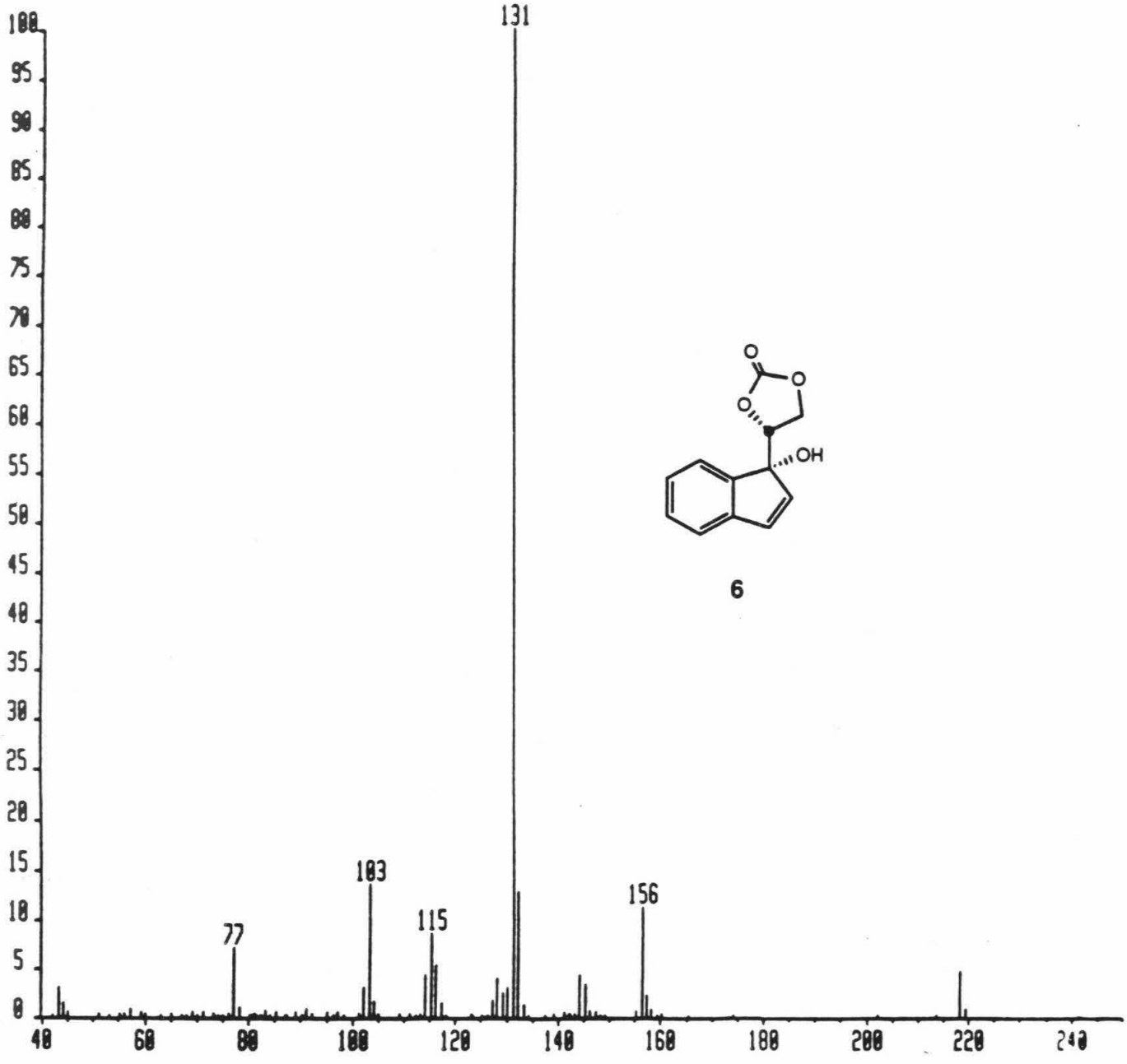
6



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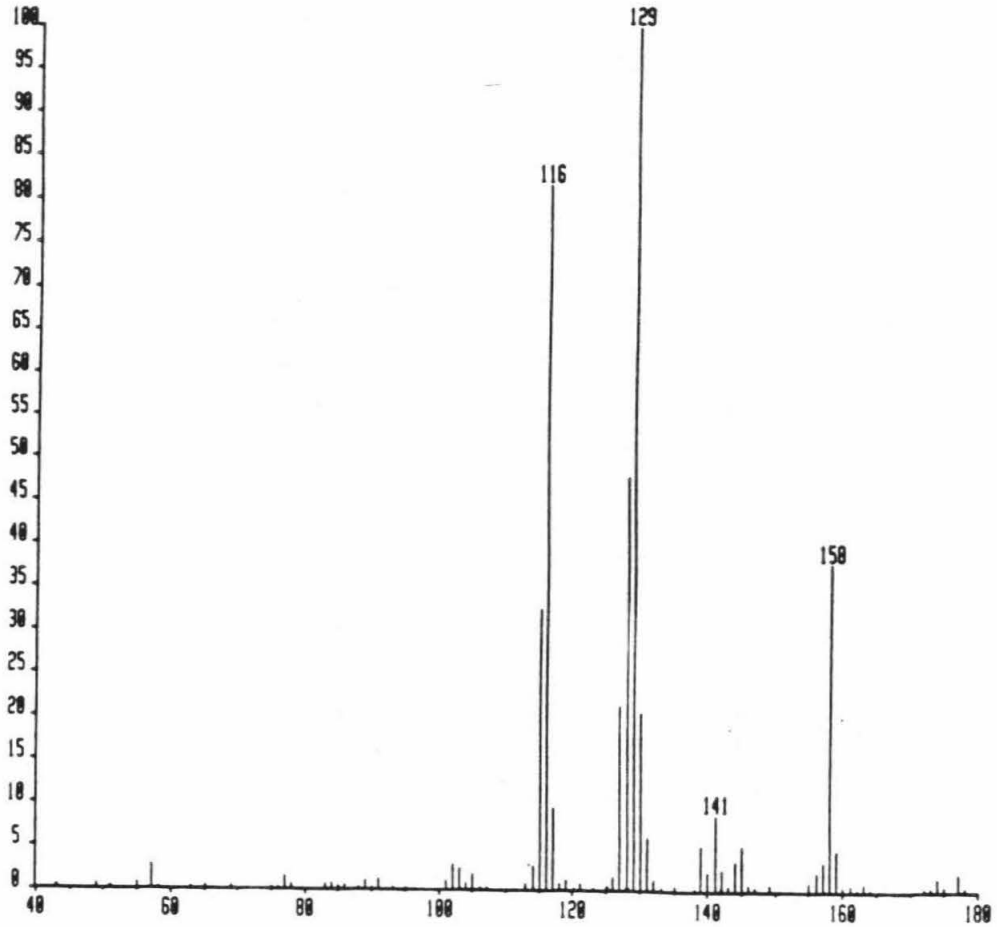

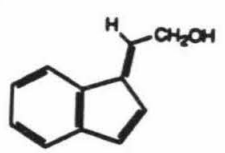

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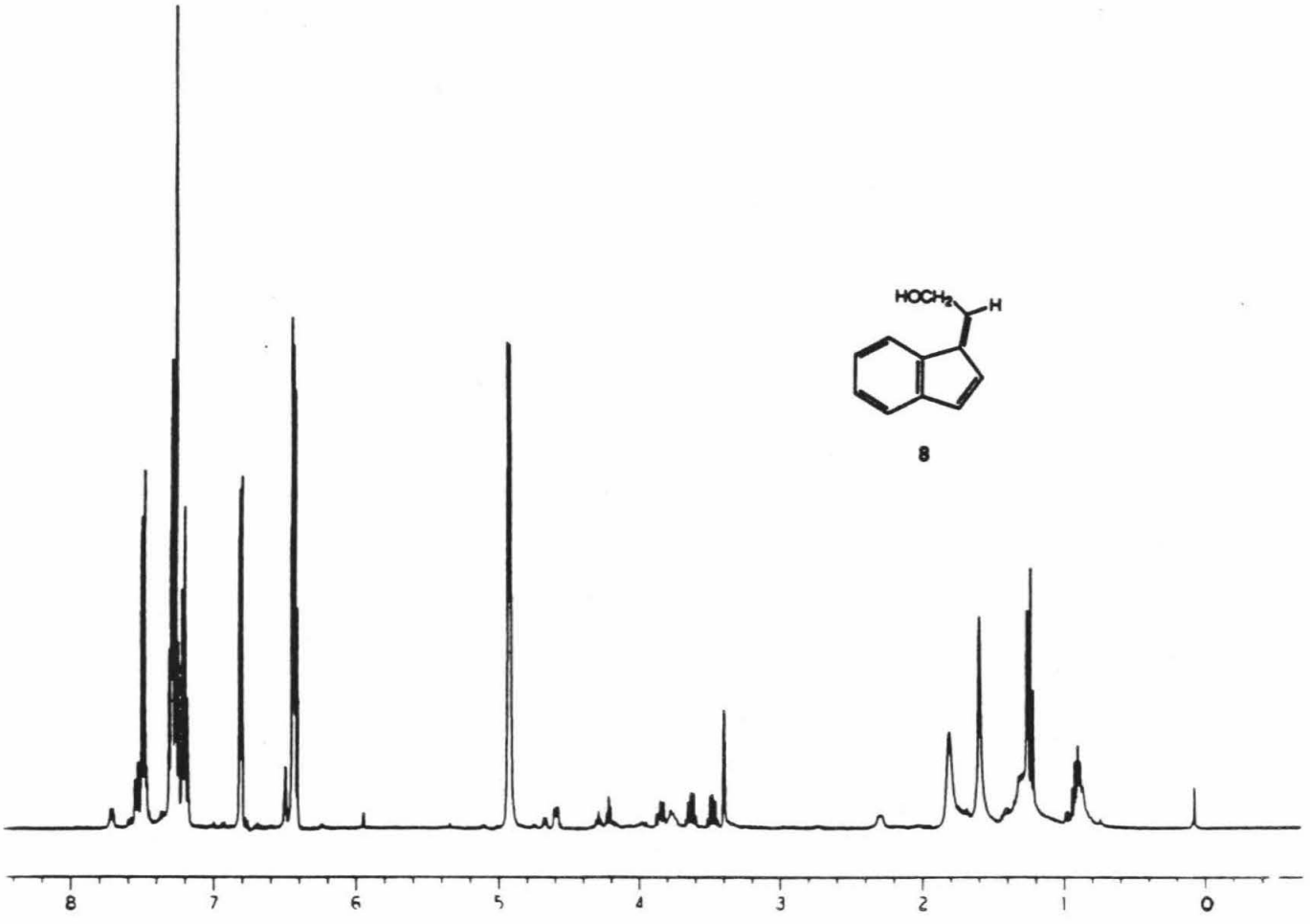
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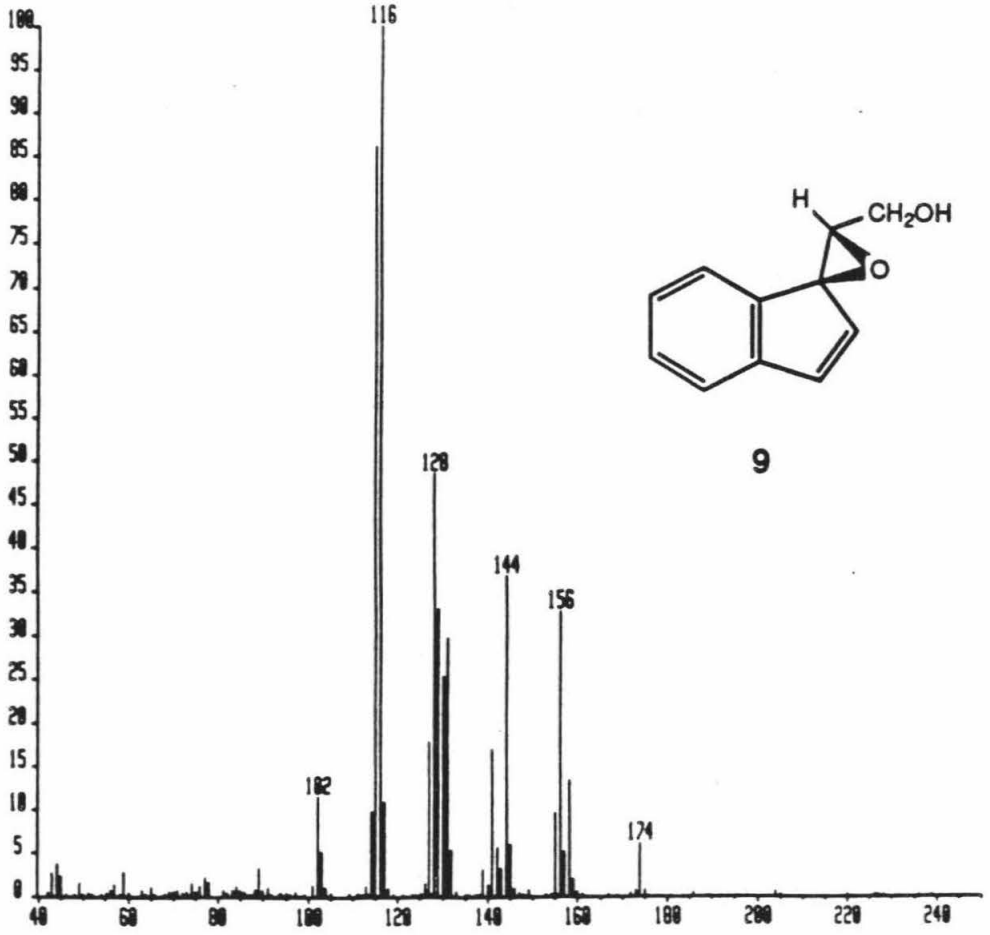
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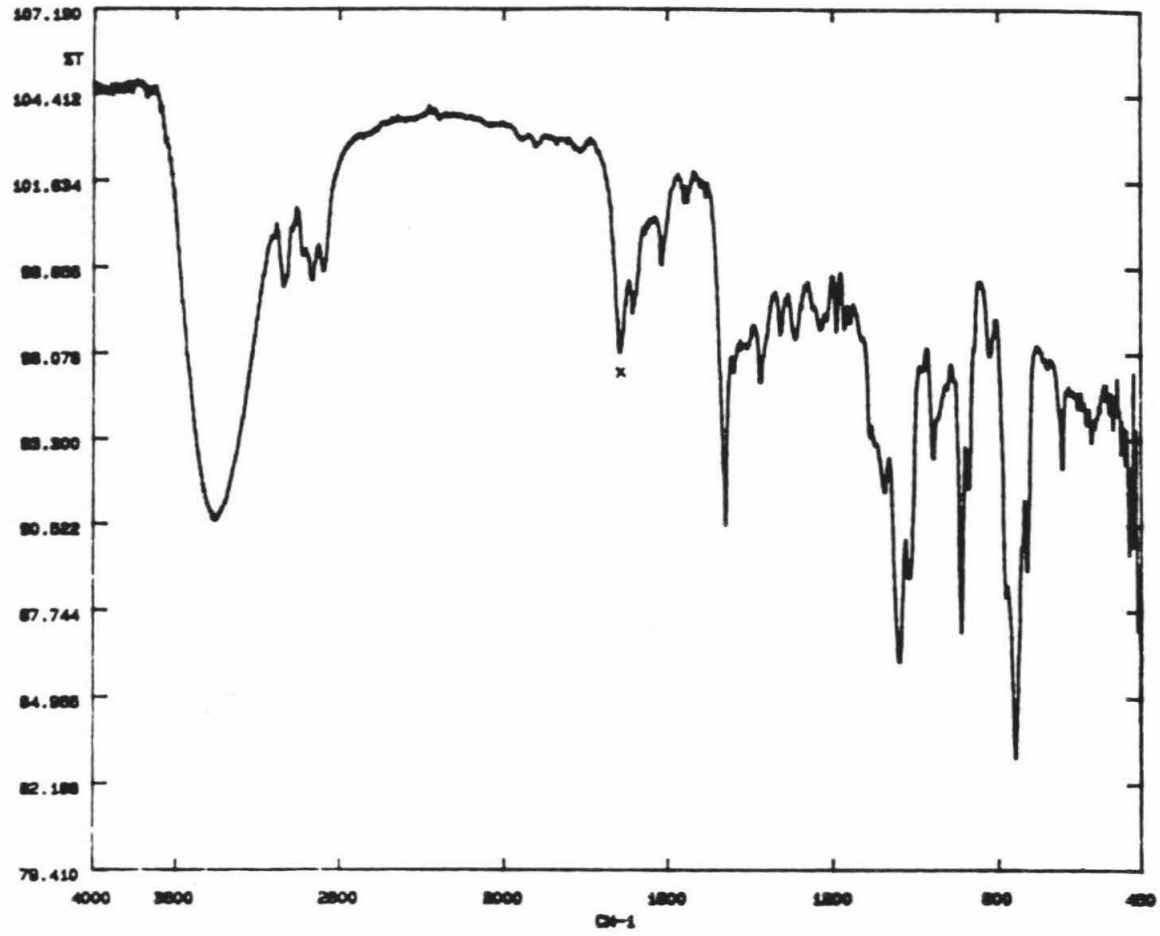
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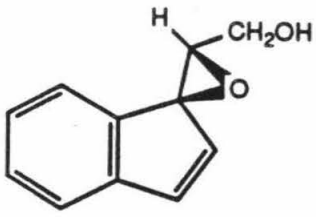
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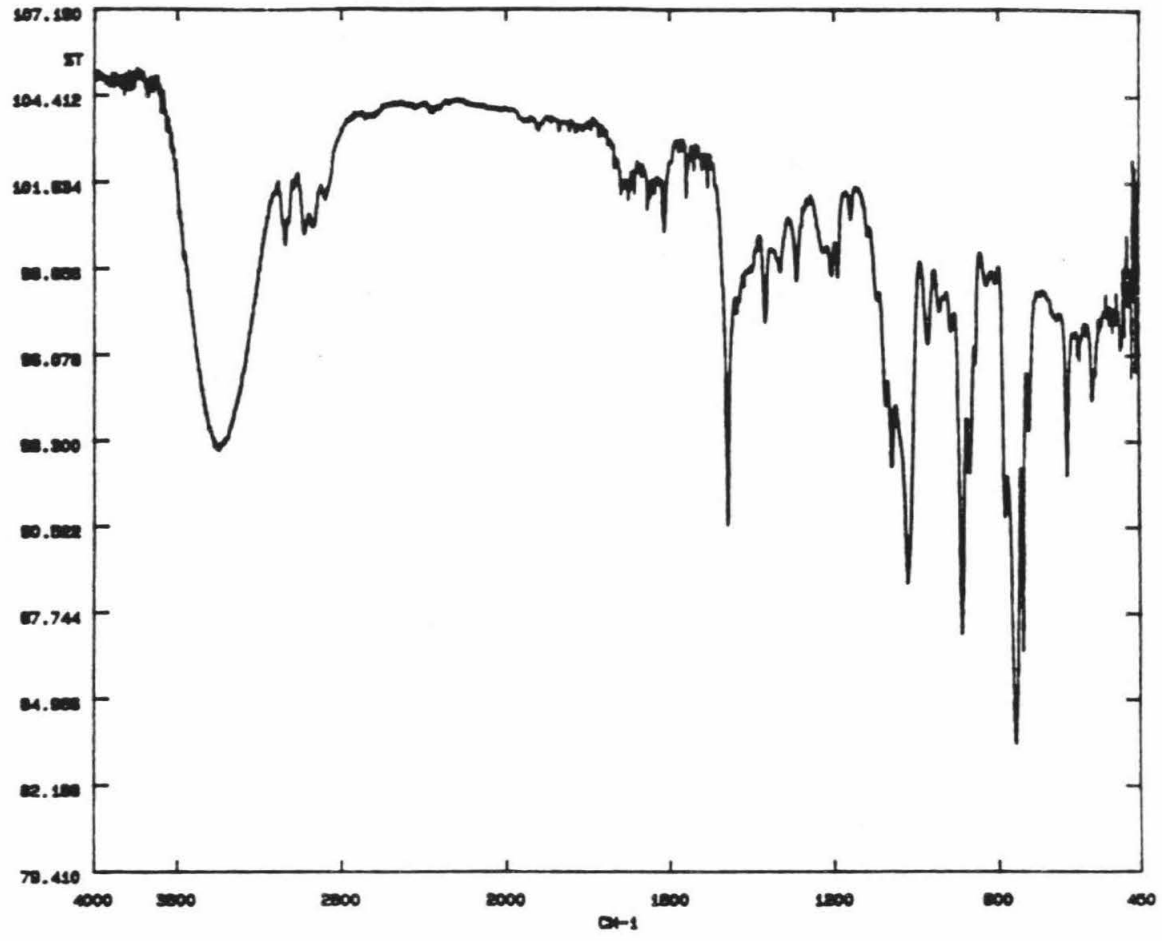
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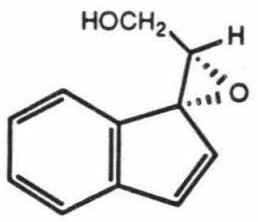
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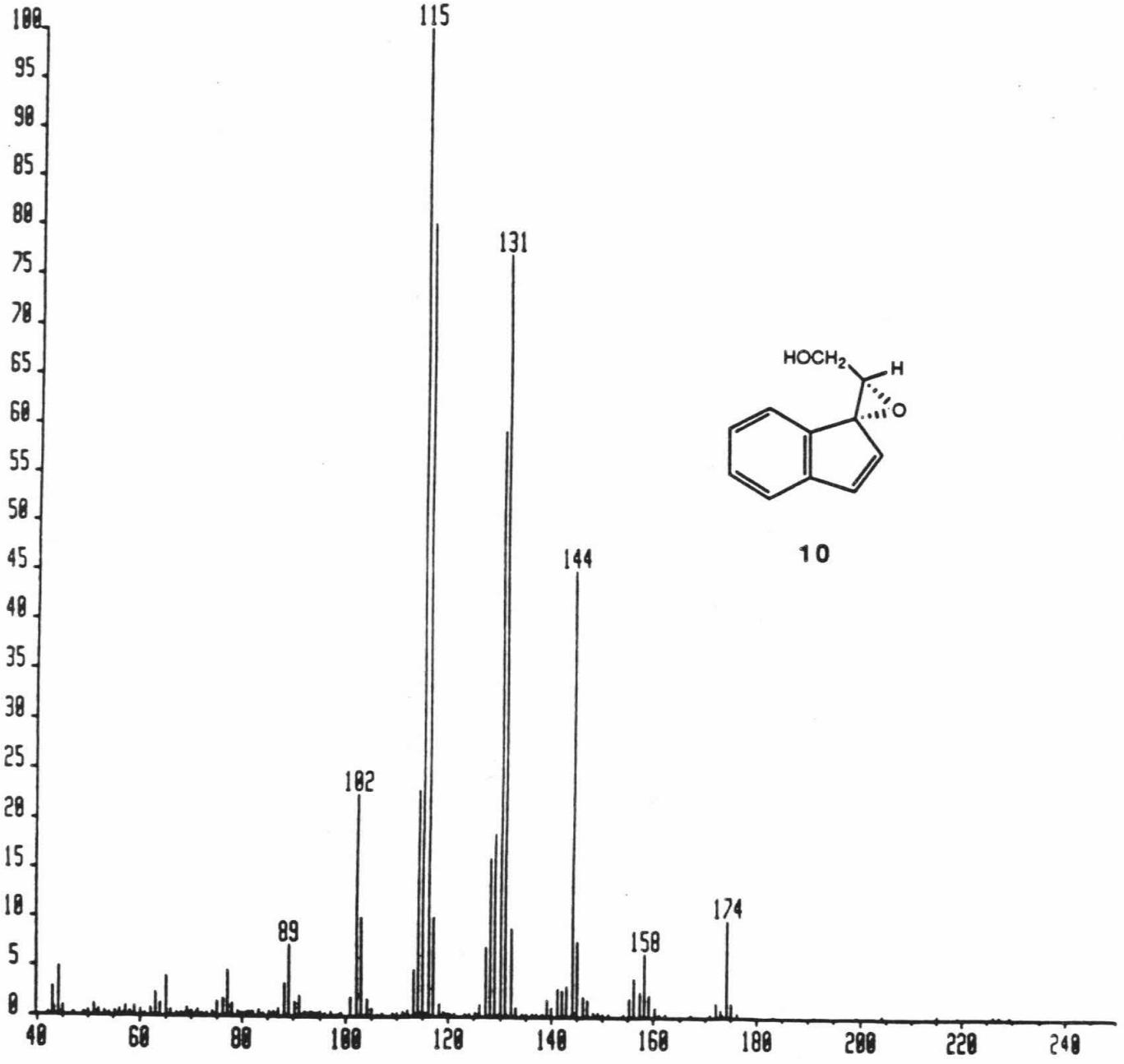
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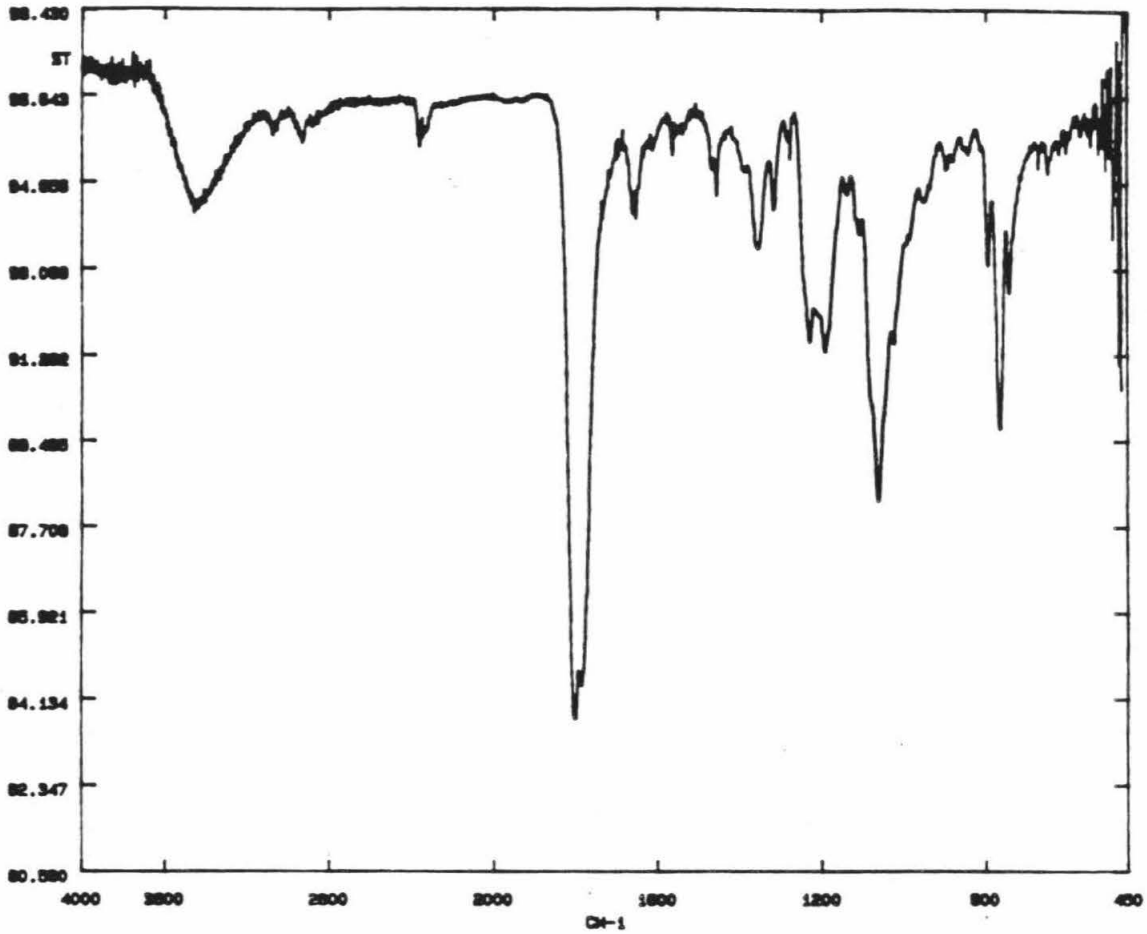
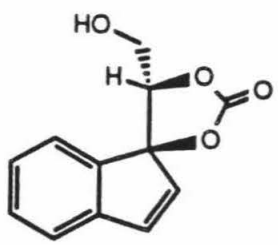
O

10





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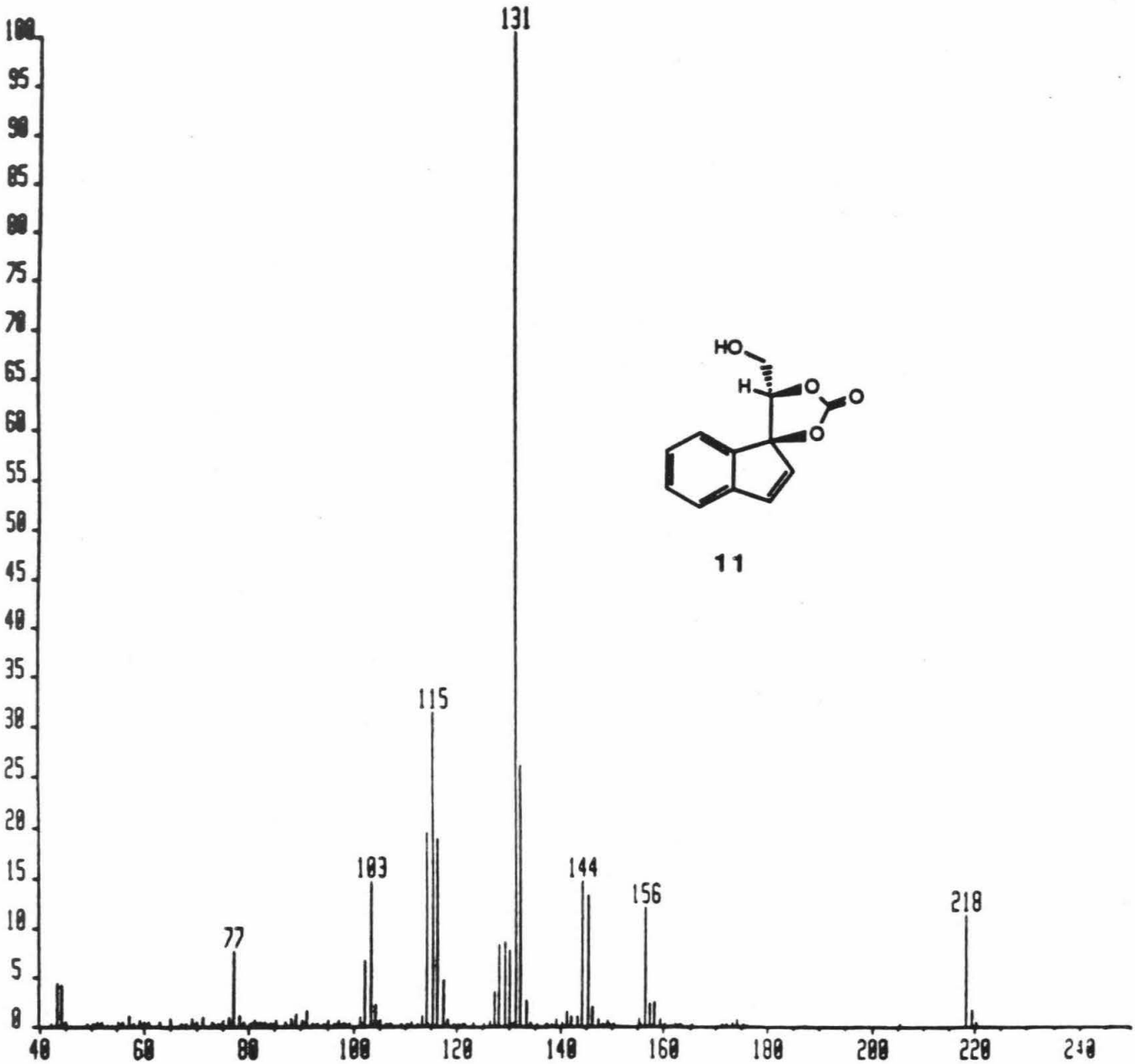
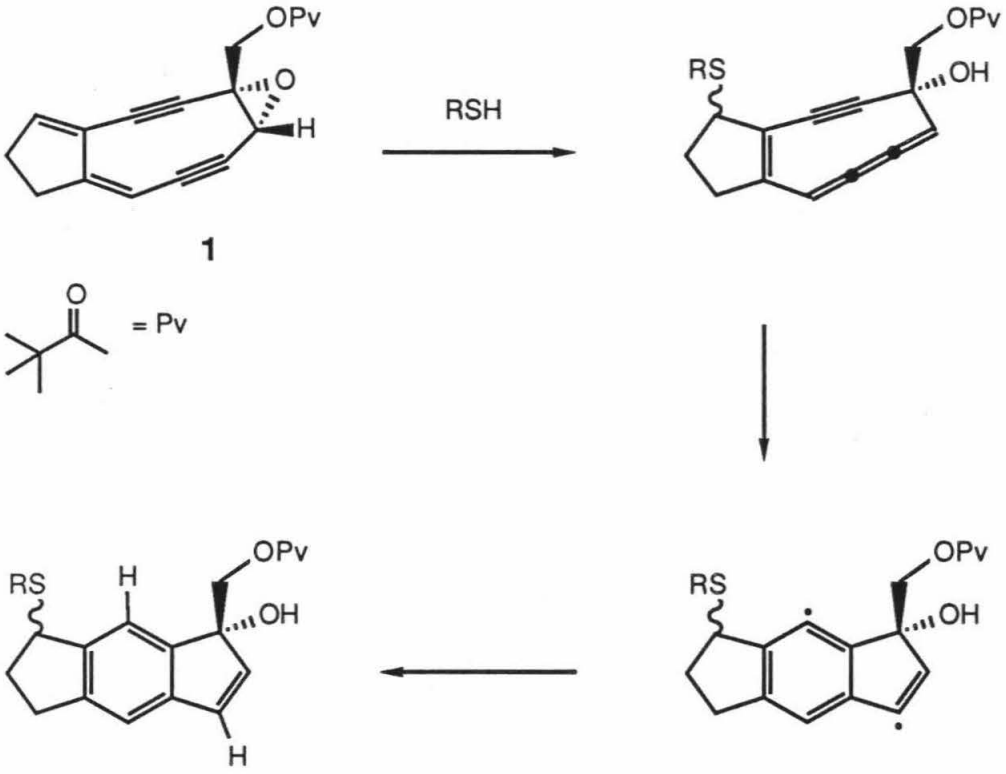


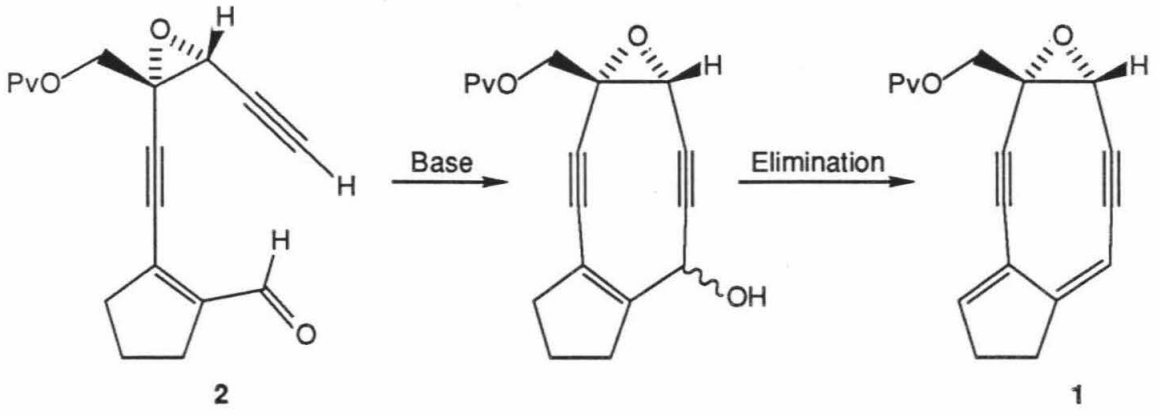
11

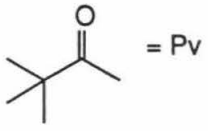
The synthetic studies of neocarzinostatin chromophore presented here are an extension of work already done in the Myers' labs.[1] Model compound **1**, which lacks the oxygen functionality at C10 and C11 and has a pivaloate ester instead of the carbonate moiety, is the target. It will be of interest to determine if **1** undergoes an analogous activation reaction with thiol as the parent chromophore, as outlined in Scheme 1. This model system may provide insight into the mechanistic roles of the sugar and naphthoate subunits of NCS chromophore.



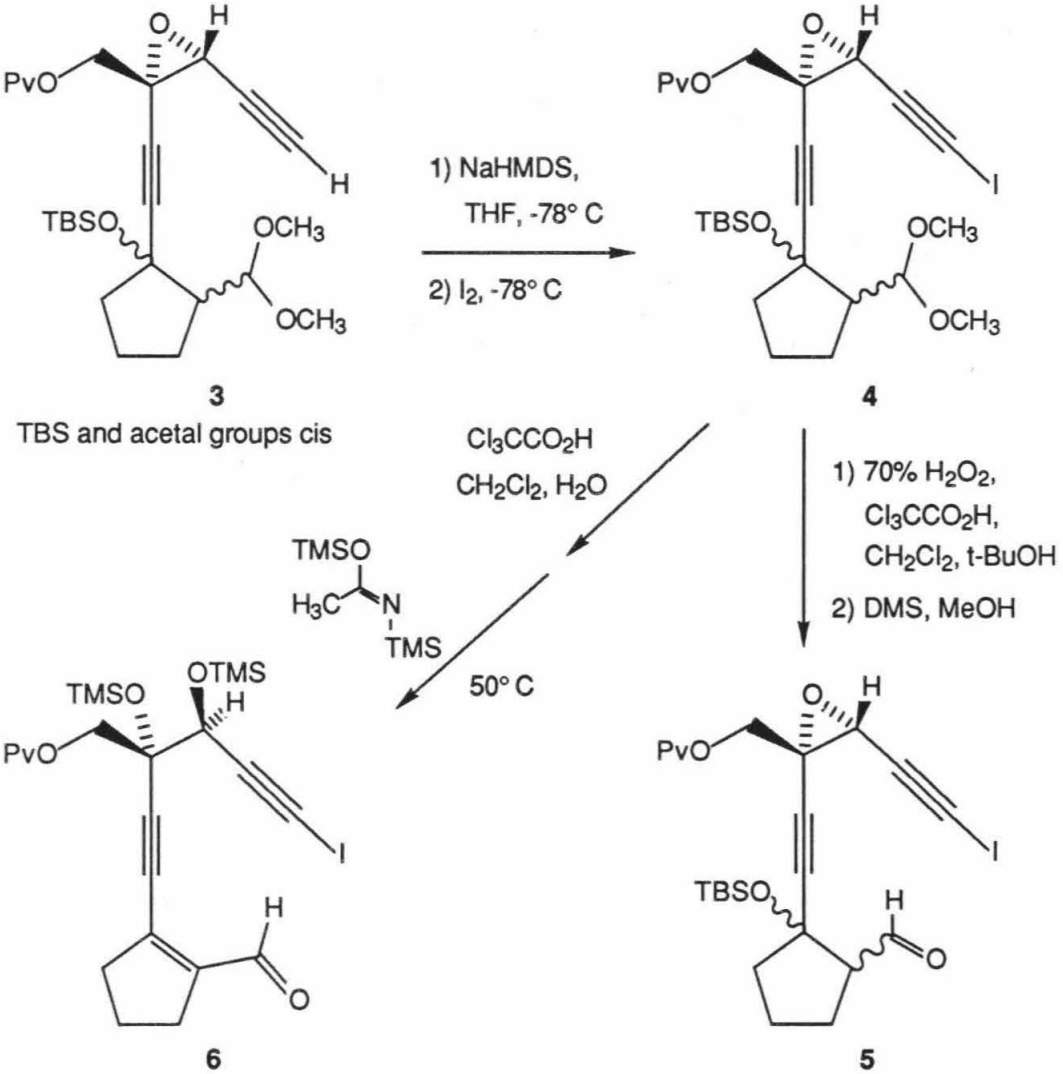
Scheme 1

A series of cyclization reactions have been conducted on the epoxy aldehyde substrate **2** and related epoxy compounds. The basis for the cyclization reaction is intramolecular acetylide addition to an aldehyde. The resulting alcohol can be transformed into the desired product by elimination of water (Scheme 2). A variant of this cyclization scheme is the formation of a chromium acetylide species from an acetylenic iodide and intramolecular addition into the aldehyde.[2]



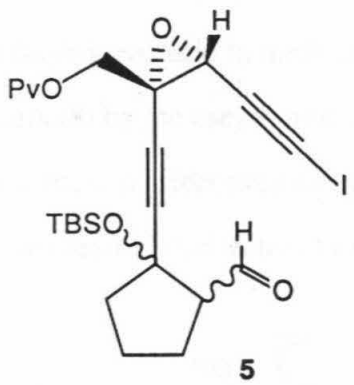


Scheme 2



Scheme 3

Two chromium acetylide addition reactions were attempted. The general procedure followed was Kishi's requiring catalytic nickel (II).[3] Compound **3**[4] is converted into the acetylenic iodide **4** by deprotonation with sodium bis(trimethylsilyl)amide followed by treatment with iodine. The epoxy aldehyde **5** was obtained by cleavage of the dimethyl-acetal with hydrogen peroxide in methylene chloride/*tert*-butyl alcohol with trichloroacetic acid present[5]. Cyclization of this substrate led to isolated products which appeared to be deoxygenated epoxides. To avoid this problem, the epoxide **4** was transformed to the diol α,β-unsaturated aldehyde with aqueous trichloroacetic acid in methylene chloride. The diol was doubly protected as trimethylsilyl ethers (**6**) by using neat bis(trimethylsilyl)acetamide at 50° C (Scheme 3). The attempted cyclization of **6** led mainly to the reduced acetylene and some minor reduction of the unsaturated system in the lower half of the molecule.

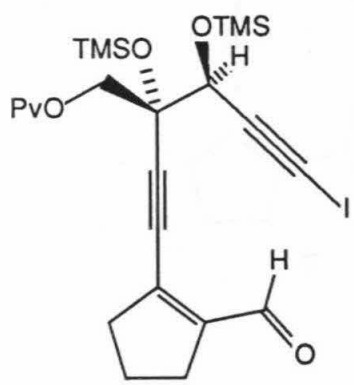


5

TBS and aldehyde cis

$Cr(II)Cl_2$
cat. $NiAcac_2$
DMSO

Mainly deoxygenated epoxide species; possibly minor reduced acetylenic iodide saturated and unsaturated aldehydes



6

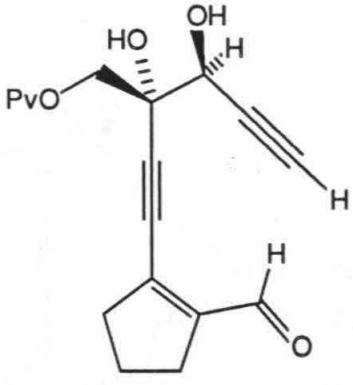
$Cr(II)Cl_2$
cat. $NiAcac_2$
DMF

Mainly reduced acetylenic iodide; minor isolated products had no aldehyde peaks and NMR signals for the 5-membered ring were altered

Figure 1

The opening of the epoxide to the diol also created a new series of terminal acetylene compounds for cyclization. The desired cyclized product will have considerable strain energy, most of which will be manifested in the transition state. The inherently strained epoxide may increase the transition state energy above that for a precursor that does not contain an epoxide. With this thought in mind, diol **7** and the bis(trimethylsilyl)-protected diol **8** were selected as cyclization precursors (Figure 2). A protected diol cyclization product could be converted to the desired epoxide model compound by cleavage of the trimethylsilyl ethers, mono-tosylation, and then base induced epoxide formation (Scheme 4).

The starting compound for the synthetic studies is the *tert*-butyldimethylsilyl-protected epoxy dimethylacetal **3**[4]. Conversion to the desired diol α,β-unsaturated aldehyde **7** is accomplished in 67% yield in one pot by treatment with aqueous trichloroacetic acid in methylene chloride. The diol is doubly protected as trimethylsilyl ethers (**8**) by the use of neat bis(trimethylsilyl)acetamide at 50° C. Various conditions were tried to affect cyclization of both **7** and **8**. These reactions are summarized in Table 1. No desired cyclization products were obtained.



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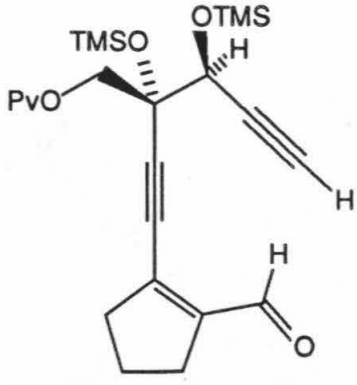
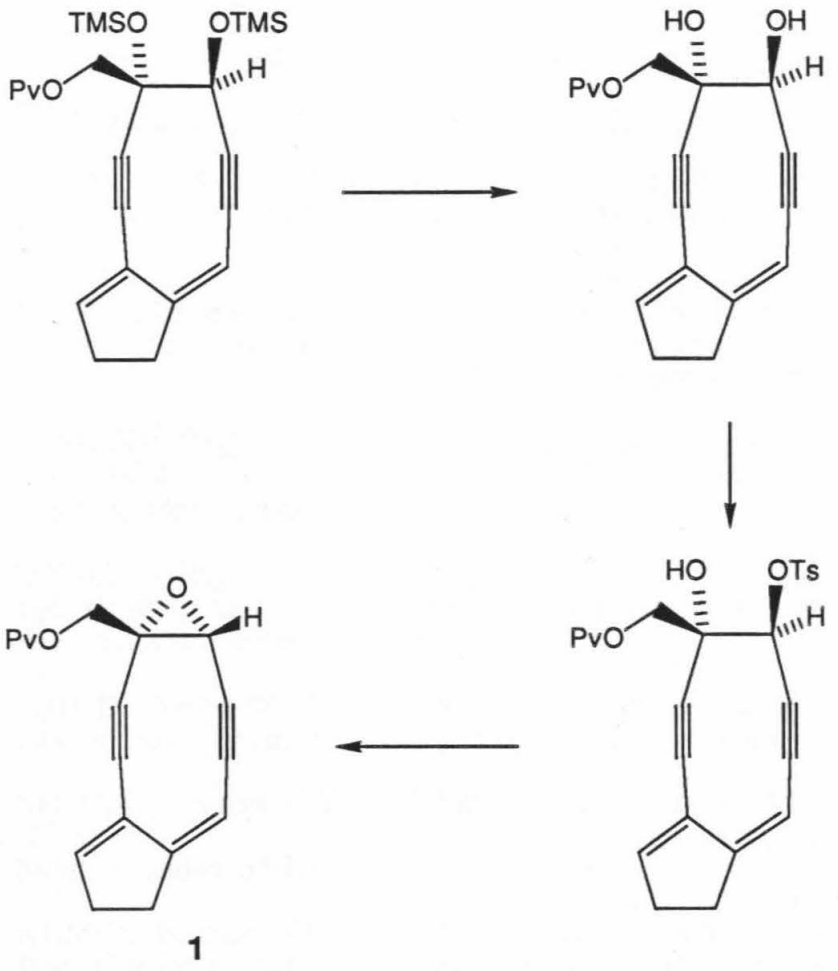
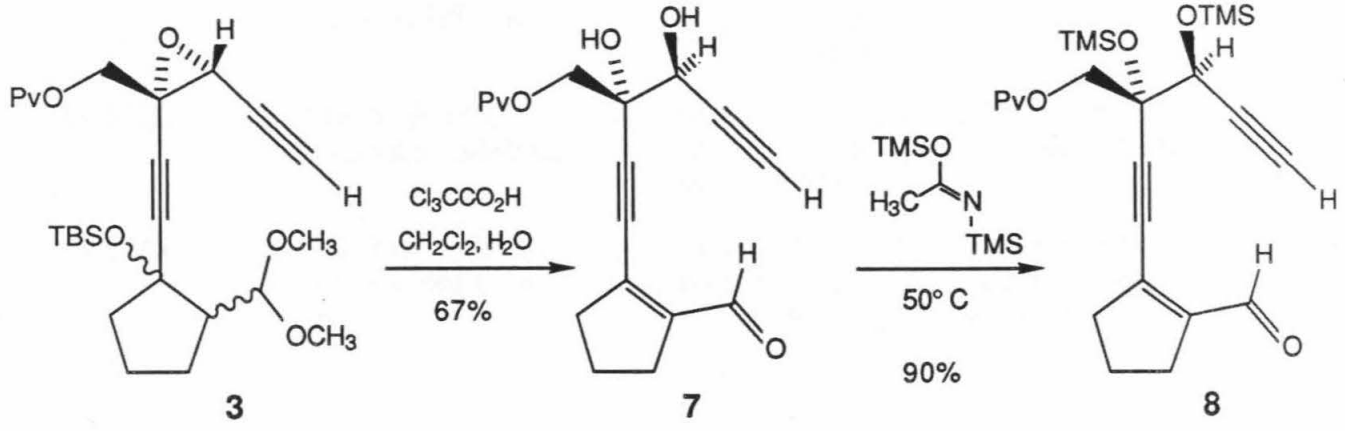


8

Figure 2



Scheme 4



TBS and acetal groups cis

Scheme 5

Table 1

Substrate	Conditions	Results
Diol	LiHMDS, THF, -78° C Base added to substrate	Mainly recovered S.M.; two minor isolated products contained aldehyde peaks by NMR.
Diol	KHMDS, Toluene, -78° C Substrate added to base	Mainly recovered S.M.; minor isolated product contained an aldehyde peak by by NMR; poor mass recovery.
Diol	$LiN[(CH_3)_2Ph]_2$ THF, -78° C Substrate added to base	Mainly S.M.; several minor uncharacterized products
Diol	$LiN[(CH_3)_2Ph]_2$ THF:DMPU, -78° C Substrate added to base	Mainly S.M.; several minor uncharacterized products.
Bis-TMS	LiHMDS, THF, -78° C Base added to substrate	Mainly S.M.; several minor uncharacterized products.
Bis-TMS	NaHMDS, Toluene:THF -78° C Substrate added to base	Non-aqueous work-up; TMS imine was main product; poor mass recovery.
Bis-TMS	KHMDS, Toluene, -78° C Base added to substrate	Mainly recovered S.M.; minor isolated products contain aldehyde peaks.
Bis-TMS	$LiN[(CH_3)_2Ph]_2$ THF, -78° C Substrate added to base	Mainly S.M.; several minor uncharacterized products; products decomposed on PTLC
Bis-TMS	LiTMP, THF, -78° C Substrate added to base	Mainly recovered S.M.; minor products contained aldehyde peaks; apparent THF derivative also present.
Bis-TMS	LiTMP, THF, 0° C Base added to substrate	Mainly recovered S.M.; several minor uncharacterized products; apparent THF derivative present.
Bis-TMS	LiTMP, Et_2O, -78° C Substrate added to base	Mainly recovered S.M.; minor isolated products had no aldehyde peaks, but an acetylene signal was present.

Two other cyclization precursors were investigated. The dimethylhydrazone **9** was prepared from **8** by reaction with 1,1-dimethylhydrazine in the presence of sodium sulfate. Conditions to affect intramolecular acetylide addition into the hydrazone failed to produce the desired cyclized hydrazine (Figure 3). Reaction of **8** with triflic anhydride in the presence of 2,6-di-*tert*-butyl-4-methylpyridine at ~-60° C provided the enol triflate **10**. Treatment of this substrate with potassium *tert*-butoxide should give the vinylidene carbene[6] which could insert intramolecularly into the acetylenic C-H bond.[7] Attempted cyclizations in either dimethoxyethane or tetrahydrofuran led to decomposition (Figure 3).

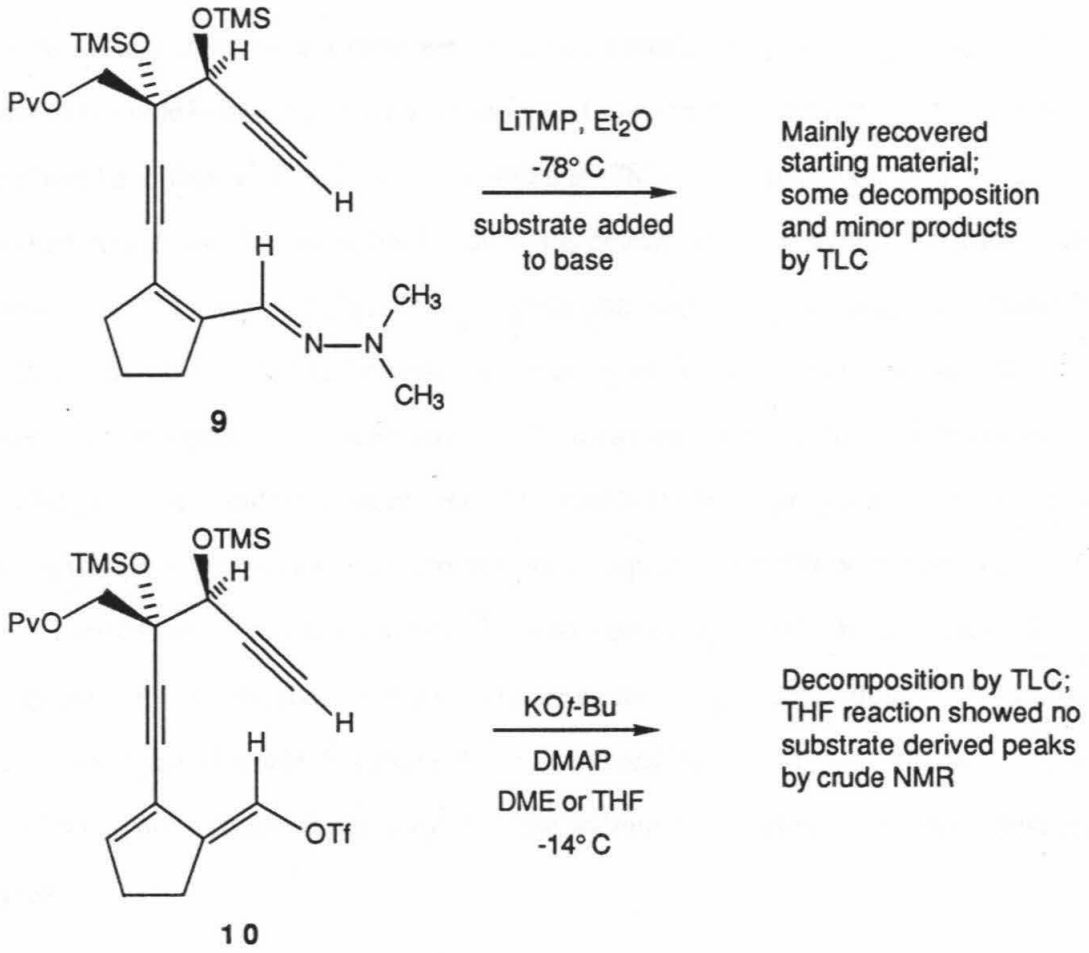
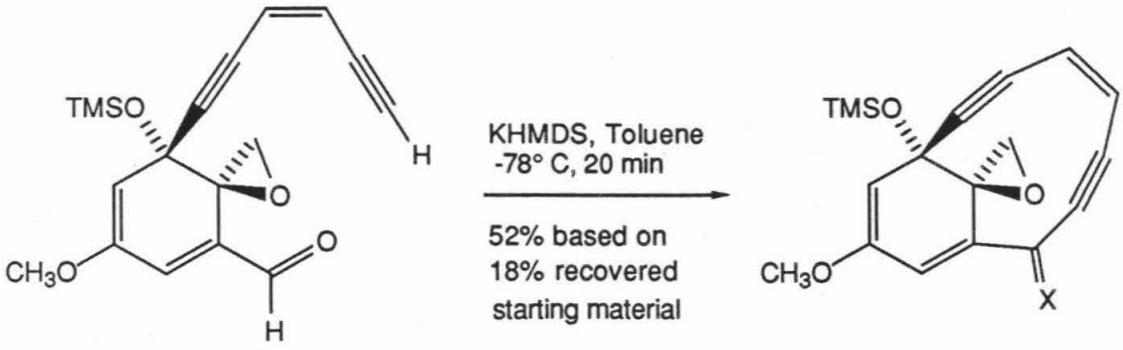


Figure 3

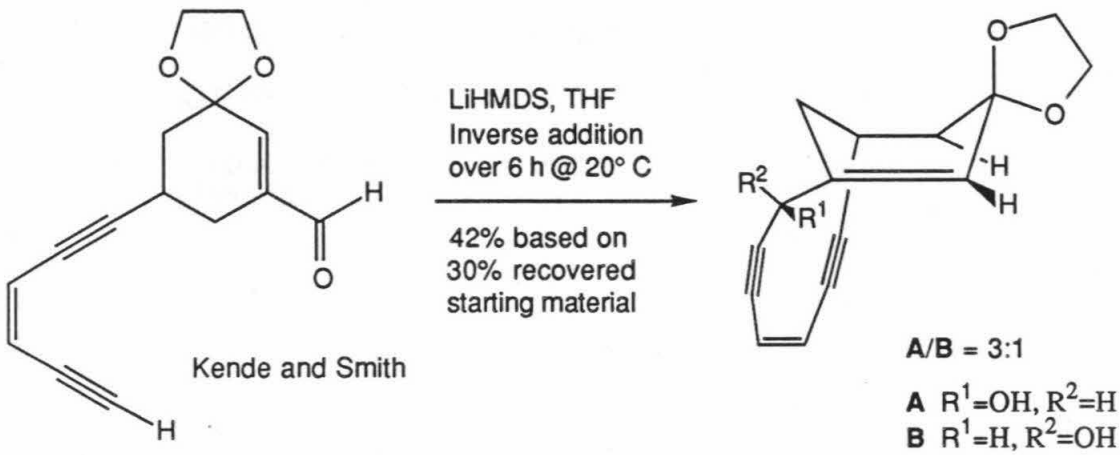
Two potential problems exist for the intramolecular addition of an acetylide ion to an α,β-unsaturated aldehyde: competitive base addition into the electrophilic aldehyde center and γ-deprotonation to form the extended enolate. Three examples of intramolecular acetylide addition into aldehydes are known (Figure 4).[8-10] In the first two cases, there are no γ-protons so enolate formation is not a problem. In the third (Tius), extended enolate formation is possible and could play a role in reducing the yield. The large amount of recovered starting material in the latter case (60%) relative to Danishefsky's (18%) and Kende's (30%) may indicate that such a process is occurring. In all cases yields are low to moderate and starting material is recovered. In an attempt to find the best conditions for intramolecular acetylide addition, we undertook a model study using cyclohexene-1-carboxaldehyde[11] and 5-phenyl-1-pentyne. At low concentration, the two substrates were combined and then various bases were added at -78° C. The reaction mixture was then worked up and the yield of desired adduct was determined. Recovery of starting aldehyde was hampered by its volatility. The best yield obtained was 48% using either NaHMDS or KHMDS in toluene. Table 2 contains a summary of bases and solvents used. Toluene appears to be superior to ethereal solvents for these reactions. Although lithium *tert*-octyl-*tert*-butyl amide[12] did not give encouraging results at -78° C (only a trace of adduct by TLC), it is possible that a higher temperature is required for facile acetylide formation. Potassium *tert*-octyl-*tert*-butyl amide[12] is also a potentially useful base. Other hindered bases that may be effective for this system are tri-*tert*-butylphenyl lithium[13], lithium *tert*-butyltrimethylsilyl amide[14], lithium N-*tert*-butyl anilide[15], and Masamune's base (lithium 1,3-diphenyltetra-methyldisilazide)[16] or the sodium or potassium analogs of these lithium bases.



Danishefsky, et al.

A/B = 10:1

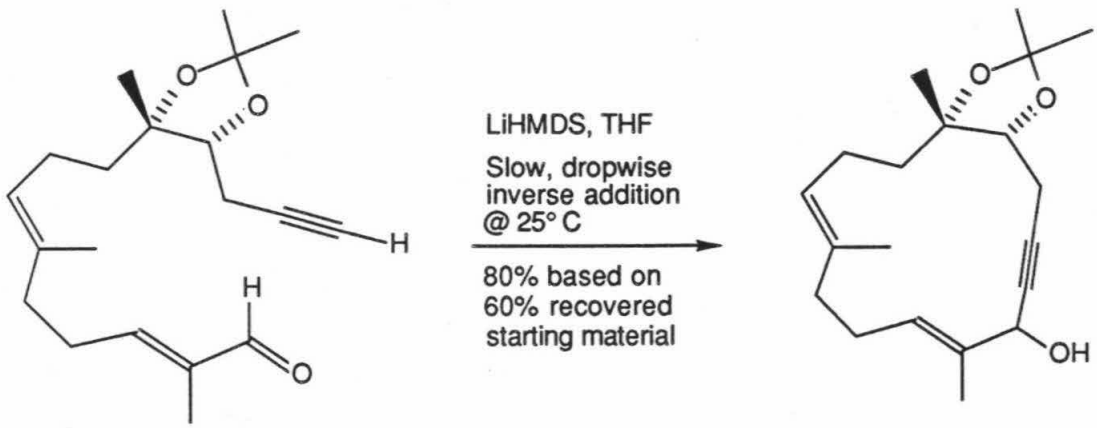
A X=βH, αOH
B X=αH, βOH



Kende and Smith

A/B = 3:1

A R^1=OH, R^2=H
B R^1=H, R^2=OH



Tius and Cullingham

One diastereomer

Figure 4

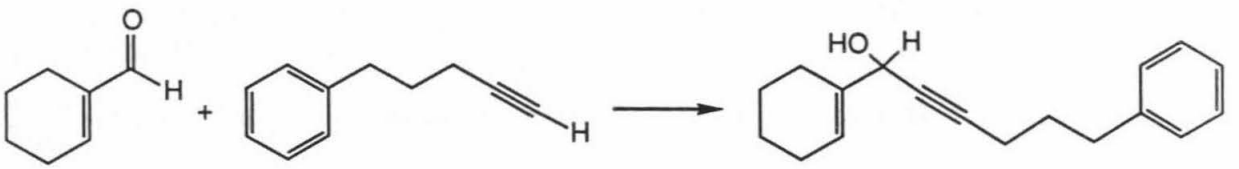


Table 2

Base	Solvent	Yield (%)
LTMP	Diethyl ether	13
NaHMDS	THF	32
NaHMDS	Toluene	48
LiHMDS	Toluene	20
KHMDS	Toluene	48
Lithium *t*-octyl-*t*-butyl amide	THF	<5

References

1. Fuhry, M.A.M., M.S. Thesis, California Institute of Technology, Pasadena, California, 1989.

2. Takai, K.; Kuroda, T.; Nakatsukasa, S.; Oshima, K.; Nozaki, H. *Tetrahedron Lett.* **1985**, *26*, 5585.

3. Jin, H.; Uenishi, J.; Christ, W.J.; Kishi, Y. *J. Am. Chem. Soc.* **1986**, *108*, 5644.

4. The synthesis of **3** is described in detail in the Master's Thesis of Mary Ann M. Fuhry. The only exception is that (-)-DET was used in the Sharpless oxidation reaction. The substrate used in these studies was prepared by Dr. Mian M. Alauddin and Dr. Paul Kamer.

5. Myers, A.G.; Fundy, M.A.M.; Lindstrom, P.A., Jr.; *Tetrahedron Lett.* **1988**, *29*, 5609.

6. (a) Stang, P.J.; Learned, A.E. *J. Am. Chem. Soc.* **1987**, *109*, 5019 and references therein. (b) Gilbert, J.C.; Giamalva, D.H.; Baze, M.E. *J. Org. Chem.* **1985**, *50*, 2557.

7. An alternative mechanistic explanation would be concurrent carbene and acetylide formation, followed by acetylide attack on the carbene providing a vinyl anion, which could then be protonated.

8. Danishefsky, S.J.; Mantlo, N.B.; Yamashita, D.S.; Schulte, G. *J. Am. Chem Soc.* **1988**, *110*, 6895.

9. Kende, A.S.; Smith, C.A. *Tetrahedron Lett.* **1988**, *29*, 4217.

10. Tius, M.A.; Cullingham, J.M. *Tetrahedron Lett.* **1989**, *30*, 3749.

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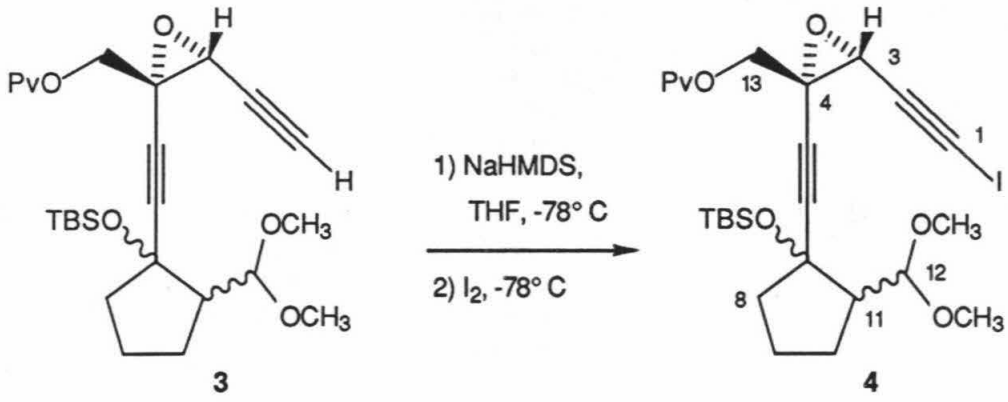
Experimental Section

All reaction solvents were distilled prior to use. Air sensitive reactions were performed under an argon atmosphere or in a nitrogen-filled glove box. Tetrahydrofuran (THF), diethyl ether, dimethoxyethane (DME), and toluene were distilled from sodium and benzophenone. Methylene chloride, 2,2,6,6-tetramethylpiperidine, and hexamethyldisilazane were distilled from calcium hydride. Sodium hexamethyldisilazide was obtained as a 1.0 M solution in THF from Aldrich. Anhydrous chromium(II) chloride was from Strem Chemicals Inc.1,3-diphenyltetramethyldisilazane (Petrarch Systems Inc.) was distilled at reduced pressure from calcium hydride. Triflic anhydride was distilled from a mixture of triflic acid and phosphorus pentoxide. 5-Phenyl-1-pentyne (Farchan Laboratories, Inc.) was distilled from calcium hydride prior to use. Cyclohexene-1-carboxaldehyde was prepared according to the method of Heilbron, et al.[11]

Analytical and preparative thin layer chromatography were performed on Merck pre-coated silica gel 60 F-254 plates (0.25 mm, glass-backed). Merck Silica Gel 60 (230-400 mesh ASTM) was used for flash chromatography. HPLC grade ethyl acetate and hexanes were used as received for extractions and chromatography.

NMR spectra were recorded on a Jeol JNM-GX400 instrument using residual chloroform (∂ 7.26) as a reference. FTIR spectra were recorded on a Perkin-Elmer 1600 Series FTIR instrument. The intensity of IR peaks are abbreviated as follows: v.w.= very weak, w = weak, m = medium, s = strong, v.s.= very strong, brd = broad.



3 **4**

TBS and acetal groups cis

Epoxy Acetylenic Iodide Dimethyl Acetal **4**

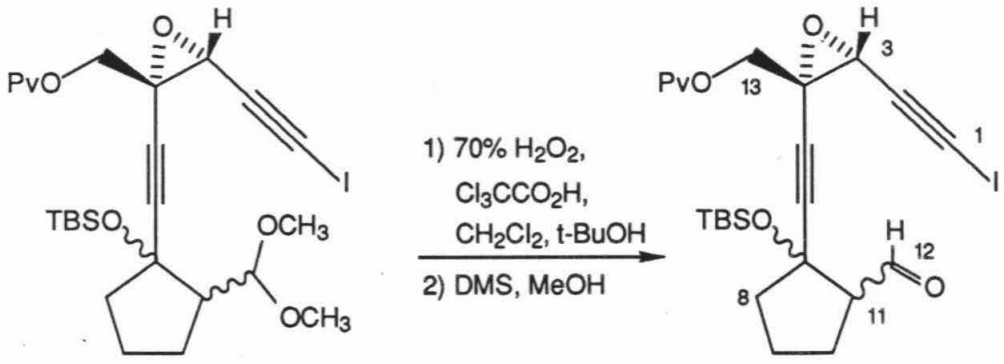
A Schlenk flask with magnetic stirring bar was flame-dried under vacuum and then flushed with argon. The substrate (272.8 mg, 0.57 mmol, 1 equiv) was added to the flask as a solution in toluene. The sample was dried by azeotropic removal of water (2 x 1 ml toluene). The dried substrate was dissolved in tetrahydrofuran (THF, 5 ml), then cooled to -78° C. Sodium bis(trimethylsilyl)amide solution (1.0 M in THF, 1.25 ml, 1.25 mmol, 2.2 equiv) was added dropwise via syringe. The resulting solution was stirred for 10 min before dropwise cannula addition of iodine (289.3 mg, 1.14 mmol, 2.0 equiv) in THF (2 ml). The solution was initially yellow-orange, but approximately halfway through the addition of the iodine solution, the solution turned green. The flask was wrapped with foil and after 30 minutes the reaction was quenched cold with several drops of water. The solution changed to dark orange. When the mixture had warmed to room temperature, 1M $Na_2S_2O_3$ (10 ml) was added to quench the excess iodine. The solution was extracted with hexanes and the organic layer was dried over sodium sulfate. Concentration *in vacuo* provided a viscous yellow oil in essentially quantitative yield. The product was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) : (mixture of diastereomers)	4.49, 4.46 (d, 1H, *J*=6.6 Hz, H12), 4.43, 4.40 (d, 1H, *J*=12.2 Hz, H13), 4.21, 4.20 (d, 1H, *J*=12.2 Hz, H13), 3.70 (s, 1H, H3), 3.37, 3.36, 3.35 (s, 6H,

-OCH_3s), 2.34-2.29 (m, 1H, H11), 2.06-1.99 (m, 1H, H8 or 10), 1.90-1.79 (m, 2H, H8 or 10), 1.73-1.63 (m, 3H, H9 and H8 or 10), 1.20 (s, 9H, *t*-butyl ester), 0.87 (s, 9H, silyl *t*-butyl), 0.22, 0.21, 0.19 (s, 6H, silyl methyls).

FTIR (neat film, cm^{-1}) : 2957 (s), 2936 (s), 2857 (m), 2243 (v.w.), 2195 (w), 1740 (s), 1141 (v.s.).

TLC (7% EtOAc/Toluene) : S.M. **3** $R_f = 0.36$
Product **4** $R_f = 0.41$



4
TBS and acetal groups cis

5

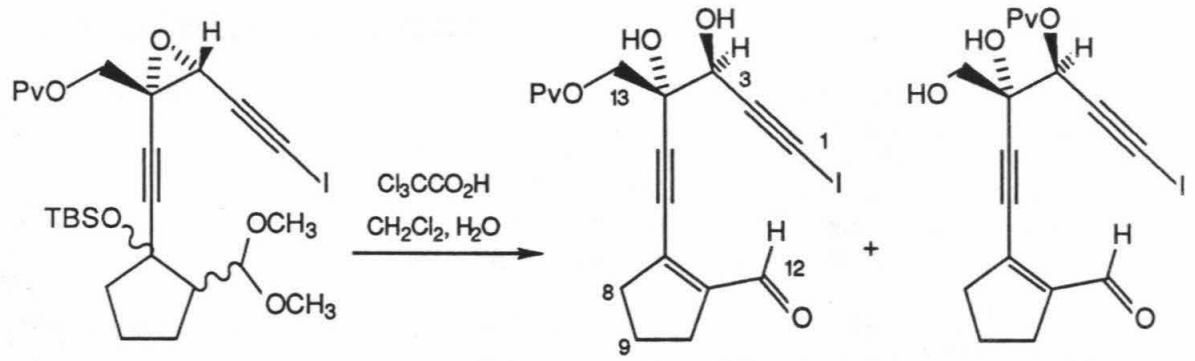
Epoxy Acetylenic Iodide Aldehyde **5**

Epoxy iodide **4** (81 mg, 0.134 mmol, 1 equiv) was dissolved in methylene chloride (2 ml) and then *tert*-butyl alcohol (2 ml) was added. The solution was cooled in an ice-water bath. Hydrogen peroxide (70%, 0.5 ml) and trichloroacetic acid solution (130 µl of a 0.51 g/ml solution in methylene chloride) were then introduced. The cooling bath was removed, the flask was wrapped with foil, and a blast shield was placed in front of the flask. After 28 h more 70% hydrogen peroxide (200 µl) and trichloroacetic acid solution (80 µl) were added. The reaction was complete after 87.5 h. The reaction mixture was poured into 10 ml ice-cold brine solution and the flask was rinsed with 50% ethyl acetate/hexanes (15 ml). Wash the organic layer with ice-cold brine solution (10 ml), then filter through a plug of sodium sulfate. To this solution was added dimethyl sulfide/methanol (5:1, 12 ml). Stir in the dark. The reaction was complete in 75 min. The reaction mixture was concentrated *in vacuo* to a yellow oil. Purification by flash chromatography (3:97 triethylamine:toluene) afforded semi-pure **5** (48.4 mg, 64% crude yield, >90% pure by NMR). Compound **5** is a mixture of diastereomers.

^{1}H NMR (400 MHz, $CDCl_3$) : (mixture of diastereomers)	9.84, 9.83 (d, 1H, *J*=2.2 Hz, H12), 4.36, 4.34 (d, 1H, *J*=12.4 Hz, H13), 4.15 (d, 1H, *J*=12.4 Hz,H13), 3.68 (s, 1H, H3), 2.83-2.79 (m, 1H, H11), 2.04-1.79 (m, 6H, H8, 9, 10), 1.20 (s, 9H, *t*-butyl ester), 0.88(s, 9H, silyl *t*-butyl), 0.24, 0.21 (s, 6H, silyl methyls).

FTIR (neat film, cm^{-1}) : 2956 (m), 2933 (m), 2856 (m), 2733 (w), 2195 (w), 1725 (s, multiplet), 1480 (m), 1472 (m), 1463 (m), 1280 (m), 1253 (m), 1141 (v.s.), 859 (s), 839 (s), 779 (s).

TLC (20% EtOAc/Hexanes) : Product **5** $R_f = 0.39$
(7% EtOAc/Toluene) : " $R_f = 0.59$



4

TBS and acetal groups cis

11

40%

12

4%

Diol Acetylenic Iodide **11**

Trichloroacetic acid solution (1 ml, 3.12 mmol, 47 equiv; 0.51 g/ml CH_2Cl_2) was added to the substrate (40 mg, 0.066 mmol, 1 equiv) and the flask was wrapped with foil. Water (150 µl) was then introduced. The bright yellow solution turned orange-brown with time. After 2 days the reaction mixture was poured into ice-cold, saturated, aqueous sodium bicarbonate solution (10 ml). The mixture was diluted with ethyl acetate (15 ml) and the layers were separated after vigorous agitation. The aqueous layer was back-extracted with ethyl acetate (10 ml). The combined organics were dried with sodium sulfate and concentrated *in vacuo*. Purification by flash chromatography (35% ethyl acetate/hexanes) afforded 11.8 mg (40%) of desired diol and 1.2 mg (4%) of the rearranged diol, both as pale yellow oils.

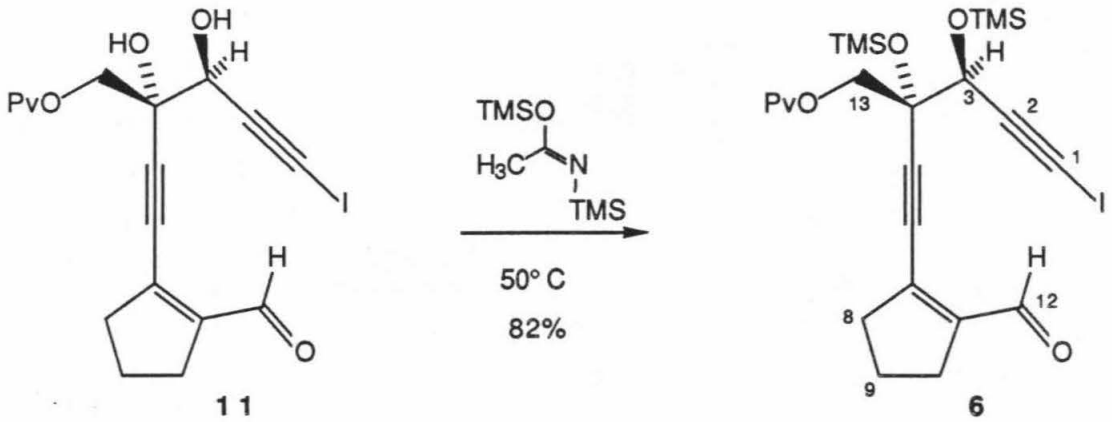
^{1}H NMR (400 MHz, $CDCl_3$) : 10.04 (s, 1H, H12), 4.66 (d, 1H, *J*=6.7 Hz, H3), 4.44 (AB quartet, 2H, *J*=11.6 Hz, H13), 3.45 (s, 1H, 3°-OH), 2.90 (d, 1H, *J*=6.7 Hz, 2°-OH), 2.73-2.69 (m, 2H, H8 or 10), 2.65-2.60 (m, 2H, H8 or 10), 1.98 (quintet, 2H, *J*=7.8 Hz, H9), 1.24 (s, 9H, *t*-butyl ester).

FTIR (neat film, cm^{-1}) : 3429 (m, brd), 2972 (m), 2873 (w), 2185 (w), 1716 (s), 1661 (v.s.), 1652 (v.s.), 1284 (s), 1160 (s), 1067 (s).

TLC (50% EtOAc/Hexanes) : Product **11** $R_f = 0.40$

Rearranged Diol Acetylenic Iodide **12**

^{1}H NMR (400 MHz, $CDCl_3$) :	10.06 (s, 1H, H12), 5.72 (s, 1H, H3), 3.84 (dd, 1H, *J*=6.2, 11.8 Hz, H13), 3.73 (dd, 1H, *J*=6.2, 11.8 Hz, H13), 3.03 (br. s, 1H, 3°-OH), 2.76-2.71 (m, 1H, H8 or 10), 2.66-2.61 (m, 1H, H8 or 10), 2.32 (br. t, 1H, 1°-OH), 1.99 (quintet, 2H, *J*=7.8, 7.5 Hz, H9), 1.24 (s, 9H, *t*-butyl ester).
FTIR (neat film, cm^{-1}) :	3437 (m,brd), 2971 (m), 2188 (w), 1733 (s, multiplet), 1661 (v.s., multiplet), 1595 (m), 1145 (v.s), 1053 (s).
TLC (50% EtOAc/Hexanes) :	Product **12** R_f = 0.28



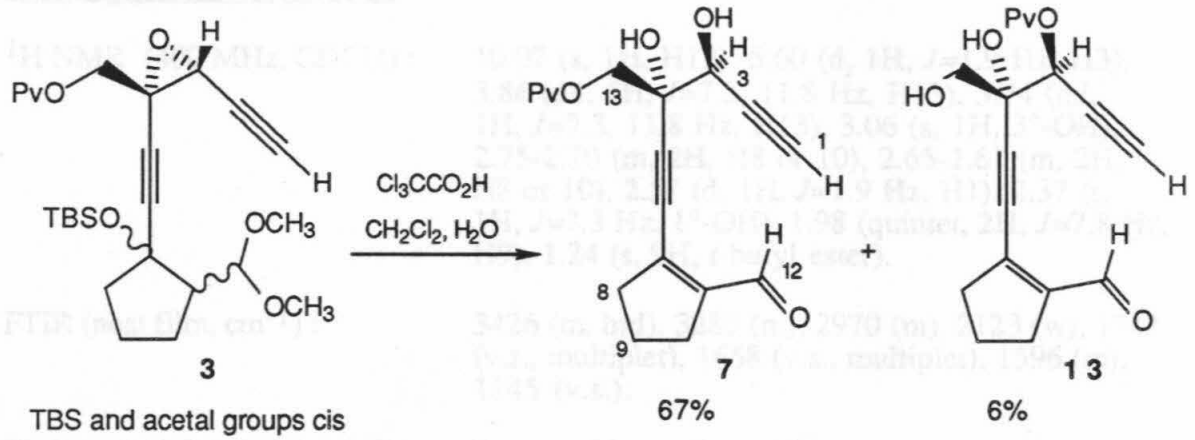
11 6

Bis-TMS Acetylenic Iodide **6**

The substrate (11.8 mg, 27 μmol, 1 equiv) was dried by azeotropic removal of water using toluene. An argon balloon was attached to the flask and then bis(trimethylsilyl) acetamide (1 ml, 4.0 mmol, 148 equiv) was added. The flask was immersed in a 50° C oil bath for 11.2 h. After cooling to room temperature, the reaction mixture was diluted with 15% ethyl acetate/hexanes (15 ml) and then washed with water (10 ml). The organic layer was filtered and concentrated *in vacuo*. Removal of solvent provided a crystalline material, probably acetamide. The polar impurities were removed by flash chromatography (10% ethyl acetate/hexanes) through a short silica plug to provide a clear oil (13.1 mg, 82%). Contact with silica is minimized to avoid significant hydrolysis of the TMS ethers. The product was stored wrapped in foil to avoid exposure to light; acetylenic iodides are in general light sensitive.

^{1}H NMR (400 MHz, $CDCl_3$) :	10.07 (s, 1H, H12), 4.56 (d, 1H, *J*=11.0 Hz, H13), 4.56 (s, 1H, H3), 4.03 (d, 1H, *J*=11.0 Hz, H13), 2.76-2.71 (m, 2H, H8 or 10), 2.67-2.62 (m, 2H, H8 or 10), 1.99 (quintet, 2H, *J*=7.8 Hz, H9), 1.21 (s, 9H, *t*-butyl ester), 0.21 (s, 9H, TMS), 0.18 (s, 9H, TMS).
FTIR (neat film, cm^{-1}) :	2965 (m), 2906 (w), 2183 (v.w.), 1740 (m), 1676 (s), 1251 (s), 1158 (s), 1106 (s), 853 (v.s.), 849 (v.s.).

TLC	(30% EtOAc/Hexanes) :	Product **6**	R_f = 0.55
	(7% EtOAc/Toluene) :	"	R_f = 0.56



3

TBS and acetal groups cis

7

67%

13

6%

Diol Acetylene **7**

A methylene chloride solution of trichloroacetic acid (5 ml, 15.6 mmol, 29.4 equiv; 0.51 g/ml solution) was added to the substrate (253.7 mg, 0.53 mmol, 1 equiv) and then water (750 µl) was introduced. The yellow solution turned golden brown with time. After 2 days, the reaction mixture was poured into an ice-cold solution of saturated aqueous sodium bicarbonate (50 ml). The mixture was diluted with 75% ethyl acetate/hexanes (E/H, 50 ml) and the layers were separated after agitation in a separatory funnel. The aqueous phase was back-extracted with 75% E/H (25 ml). The organics were dried (Na_2SO_4) and concentrated *in vacuo* to give a crude orange-brown oil. Purification by flash chromatography (35% E/H) provided the desired diol (113.0 mg, 67%) as a viscous yellow oil and the rearranged diol (10.7 mg, 6%) as a clear oil.

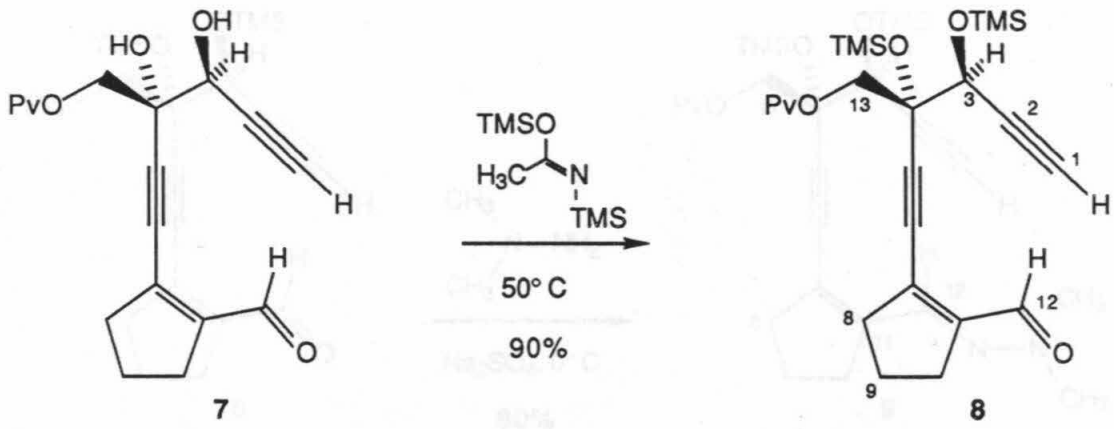
^{1}H NMR (400 MHz, $CDCl_3$) : 10.05 (s, 1H, H12), 4.54 (dd, 1H, *J*=2.2, 7.0 Hz, H3), 4.47 (AB quartet, 2H, *J*=11.6 Hz, H13), 3.46 (s, 1H, 3°-OH), 2.83 (d, 1H, *J*=7.0 Hz, 2°-OH), 2.73-2.69 (m, 2H, H8 or 10), 2.65-2.60 (m, 2H, H8 or 10), 2.62 (d, 1H, *J*=2.2 Hz, H1), 1.98 (quintet, 2H, *J*=7.8 Hz, H9), 1.24 (s, 9H, *t*-butyl ester).

FTIR (neat film, cm^{-1}) : 3435 (s, brd), 3282 (s), 2971 (s), 2120 (w), 1733 (v.s., multiplet), 1652 (v.s., multiplet), 1596 (s), 1398 (s), 1357 (s), 1286 (s), 1158 (s), 1070 (s).

TLC (50% EtOAc/Hexanes) : Product **7** R_f = 0.36

Rearranged Diol Acetylene **13**

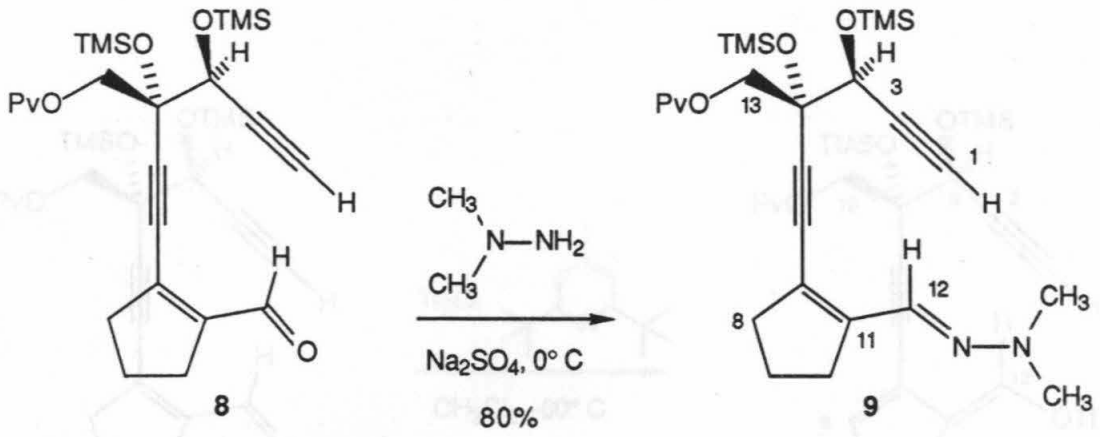
^{1}H NMR (400 MHz, $CDCl_3$) :	10.07 (s, 1H, H12), 5.60 (d, 1H, *J*=1.9 Hz, H3), 3.86 (dd, 1H, *J*=7.3, 11.8 Hz, H13), 3.74 (dd, 1H, *J*=7.3, 11.8 Hz, H13), 3.06 (s, 1H, 3°-OH), 2.75-2.70 (m, 2H, H8 or 10), 2.65-2.61 (m, 2H, H8 or 10), 2.57 (d, 1H, *J*=1.9 Hz, H1), 2.37 (t, 1H, *J*=7.3 Hz, 1°-OH), 1.98 (quintet, 2H, *J*=7.8 Hz, H9), 1.24 (s, 9H, *t*-butyl ester).
FTIR (neat film, cm^{-1}) :	3426 (m, brd), 3285 (m), 2970 (m), 2123 (w), 1742 (v.s., multiplet), 1668 (v.s., multiplet), 1596 (m), 1145 (v.s.).
TLC (50% EtOAc/Hexanes) :	Product **13** R_f = 0.23



Bis-TMS α,β-Unsaturated Aldehyde **8**

The diol substrate (113 mg, 0.355 mmol, 1 equiv) was azeotroped from toluene to remove traces of water. The flask was equipped with an argon balloon and then bis(trimethylsilyl)acetamide (2 ml, 8.09 mmol, 22.8 equiv) was added via syringe. The flask was immersed in a 50° C oil bath for 11.5 h and then cooled to room temperature. The reaction mixture was diluted with 15% ethyl acetate/hexanes (E/H, 25 ml) and then washed rapidly with water to avoid trimethylsilyl ether hydrolysis. The organics were dried with sodium sulfate and then concentrated *in vacuo*. Purification was done by flash chromatography through a short silica column (10% E/H) to provide 148.4 mg of a light yellow oil (90%). Contact with silica is kept to a minimum to avoid significant hydrolysis of the TMS ethers.

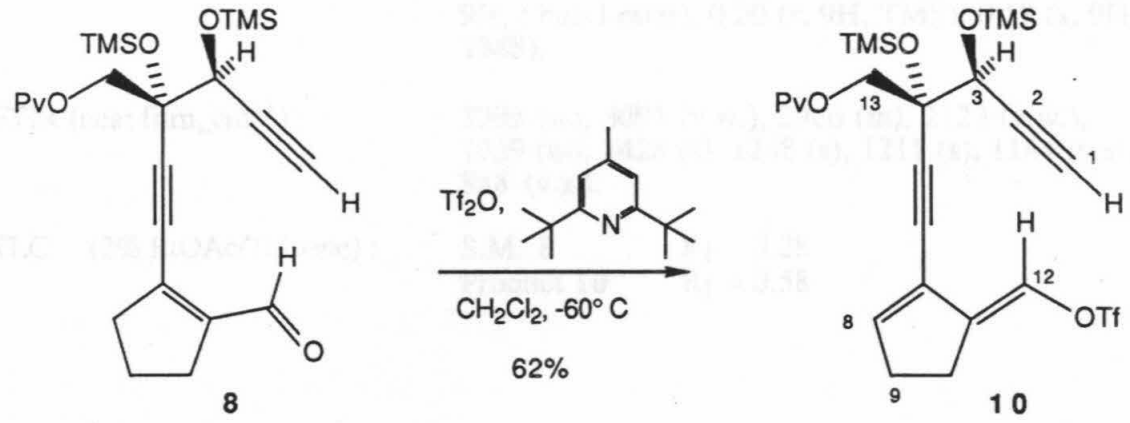
^{1}H NMR (400 MHz, $CDCl_3$) :	10.09 (s, 1H, H12), 4.57 (d, 1H, *J*=11.3 Hz, H13), 4.45 (d, 1H, *J*=2.2 Hz, H3), 4.06 (d, 1H, *J*=11.3 Hz, H13), 2.75-2.70 (m, 2H, H8 or 10), 2.65-2.61 (m, 2H, H8 or 10), 2.40 (d, 1H, *J*=2.2 Hz, 1.99 (quintet, 2H, *J*=7.8 Hz, H9), 1.22 (s, 9H, *t*-butyl ester), 0.21 (s, 9H, TMS), 0.19 (s, 9H, TMS).
FTIR (neat film, cm^{-1}) :	3309 (w), 3281 (w, brd), 2959 (m), 2872 (w), 2120 (v.w.), 1739 (s), 1674 (s), 1251 (s), 1158 (s), 1102 (s), 848 (v.s.).
TLC (30% EtOAc/Hexanes) :	Product **8** R_f = 0.59
(7% EtOAc/Toluene) :	" R_f = 0.55



Bis-TMS Dimethyl Hydrazone **9**

Bis-TMS aldehyde **8** (30 mg, 0.065 mmol, 1 equiv) was concentrated from a toluene solution *in vacuo*. Anhydrous sodium sulfate (two small spatula tipfuls) was added and the flask was cooled in an ice-water bath. Dimethylhydrazine (1 ml, 13 mmol, 200 equiv) was added neat. After 2 h the reaction mixture was filtered thru a Celite plug, rinsing with 50% ethyl acetate/hexanes (E/H). Concentration *in vacuo* produced a light yellow oil which was determined to be a 60:40 mixture of starting material:product by NMR. The crude product mixture was re-submitted to the reaction conditions, except at ambient temperature. The reaction was complete in 2 h as determined by FTIR. The reaction mixture was filtered through a Celite plug, rinsing with 50% E/H. Concentration *in vacuo* provided a yellow oil which was purified by flash chromatography (15% E/H) to obtain a pale yellow oil (26.2 mg, 80% yield).

^{1}H NMR (400 MHz, $CDCl_3$) :	7.40 (s, 1H, H12), 4.56 (d, 1H, *J*=11.0 Hz, H13), 4.44 (d, 1H, *J*=2.2 Hz, H3), 4.06 (d, 1H, *J*=11.0 Hz, H13), 2.91 (s, 6H, N-CH_3's), 2.66 (t, 2H, *J*=7.5 Hz, H8 or 10), 2.57 (t, 2H, *J*=7.5 Hz, H8 or 10), 2.34 (d, 1H, *J*=2.2 Hz, H1), 1.89 (quintet, 2H, *J*= 7.5 Hz, H9), 1.21 (s, 9H, *t*-butyl ester), 0.21 (s, 9H, TMS), 0.18 (s, 9H, TMS).
FTIR (neat film, cm^{-1}) :	3312 (w), 3279 (w, brd), 2959 (s), 2851 (m), 2787 (w), 2207 (w), 2118 (v.w.), 1742 (s), 1739 (s), 1733 (s), 1251 (s), 1143 (s), 1106 (s), 1054 (s), 848 (v.s.).
TLC (5% EtOAc/Toluene) :	Product **9** R_f = 0.46



Bis-TMS Enol Triflate **10**

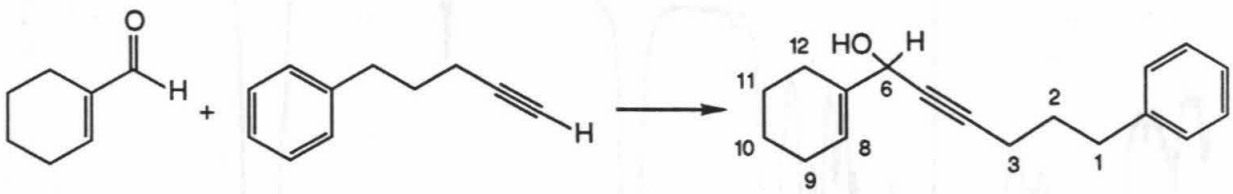
A 10-ml Schlenk flask with magnetic stirring bar was flame-dried under vacuum, then flushed with argon. The aldehyde substrate (30 mg, 64.8 µmol, 1 equiv) was added as a solution in toluene and then concentrated *in vacuo*. Methylene chloride (2 ml) and 2,6-di-*tert*-butyl-4-methylpyridine (300 µl, 1.3 mmol, 20 equiv) were then introduced. The solution was cooled to -78° C prior to syringe addition of triflic anhydride (109 µl, 648 µmol, 10 equiv). The flask was immersed in a -62° C cooling bath; "milky" yellow solution. Within 0.5 h the solution had turned dark green. After 2 h (bath cooled to -70° C) the reaction was quenched at low temperature with 5% aqueous sodium bicarbonate solution. The solution turned yellow on warming. The reaction mixture was diluted with 20% ethyl acetate/hexanes (E/H, 15 ml) and washed with water (10 ml). The aqueous layer was back-extracted with 20% E/H (15 ml). The combined organics were dried with anhydrous sodium sulfate and concentrated *in vacuo*. The crude enol triflate was purified by flash silica chromatography (initially 50% toluene/hexanes, then 100% toluene) to a pale yellow oil (24.0 mg, 62%). The sample was kept neat only briefly; it was stored as a solution in toluene containing a few drops of triethylamine. Acidic conditions cause rapid decomposition.

^{1}H NMR (400 MHz, $CDCl_3$) : 7.07 (br. s, 1H, H12), 6.50 (br. t., 1H, *J*=2.7 Hz, H12), 4.57 (d, 1H, *J*=11.3 Hz, H13), 4.43 (d, 1H,

J=2.2 Hz, H3), 4.05 (d, 1H, J=11.3 Hz, H13), 2.78-2.74 (m, 2H, H9 or 10), 2.68-2.64 (m, 2H, H9 or 10), 2.44 (d, 1H, J=2.2 Hz, H1), 1.22 (s, 9H, *t*-butyl ester), 0.20 (s, 9H, TMS), 0.19 (s, 9H, TMS).

FTIR (neat film, cm^{-1}) : 3305 (w), 3095 (v.w.), 2966 (m), 2123 (v.w.), 1739 (m), 1428 (s), 1248 (s), 1211 (s), 1144 (v.s), 848 (v.s.).

TLC (2% EtOAc/Toluene) : S.M. **8** $R_f = 0.28$
Product **10** $R_f = 0.58$



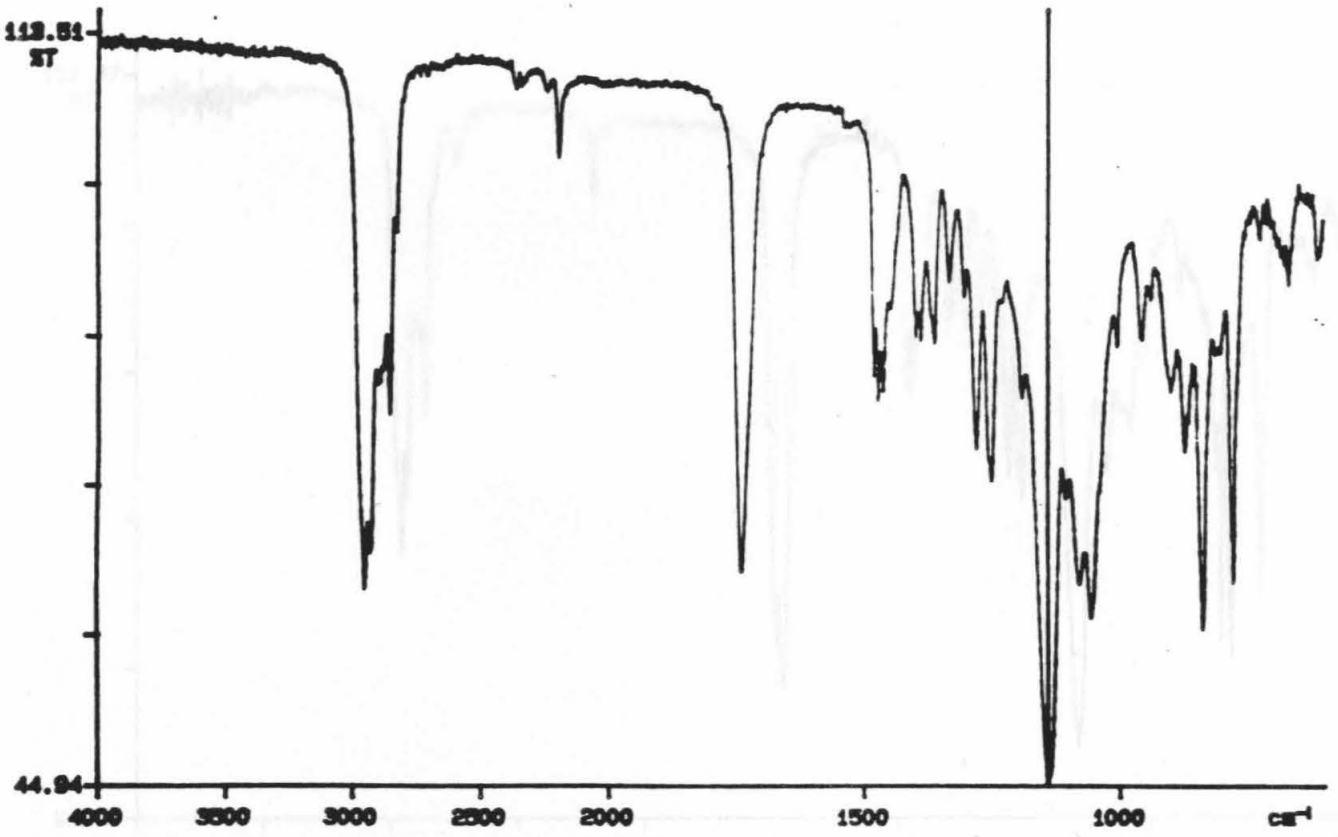
Acetylenic Alcohol

General procedure. Cyclohexene-1-carboxaldehyde (22.7 µl, 200 µmol, 1 equiv) and 5-phenyl-1-pentyne (31.8 µl, 200 µmol, 1 equiv) were combined and then dissolved in the desired solvent (19 ml). The solution was cooled to -78° C and then cold base solution was added via cannula. After 15-20 minutes the reaction was quenched at low temperature with methanol. The cooling bath was removed and a few drops of saturated aqueous ammonium chloride solution were introduced. After warming to room temperature, the reaction mixture was diluted with 30% ethyl acetate/hexanes (E/H, 15 ml) and then washed with half-saturated brine (20 ml). The organic layer was dried (sodium sulfate) and then concentrated *in vacuo*. Purification was done by flash chromatography (10% E/H).

^{1}H NMR (400 MHz, $CDCl_3$) : 7.31-7.18 (m, 5H, phenyl), 5.90 (br. m, 1H, H8), 4.74 (br. d, 1H, J=4.6 Hz, H6), 2.72 (t, 2H, *J*=7.6 Hz, H1), 2.26 (dt, 2H, *J*=1.7, 7.1 Hz, H3), 2.22-2.18 (br. m, 1H, H12), 2.15-2.11 (br. m, 1H, H12), 2.07 (br. m, 2H, H9), 1.85 (quintet, 2H, *J*=7.3 Hz, H2), 1.75 (d, 1H, *J*=5.6 Hz, -OH), 1.71-1.65 (m, 2H, H10 or 11), 1.62-1.56 (m, 2H, H10 or 11).

FTIR (neat film, cm^{-1}) : 3372 (m, brd), 3026 (m), 2927 (v.s.), 2858 (m), 2837 (m), 2257 (v.w.), 2220 (v.w.), 1603 (w), 1496 (m), 1455 (m), 1436 (m), 992 (s, brd), 746 (s), 699 (v.s.).

TLC (20% EtOAc/Hexanes) : Product R_f = 0.30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3500
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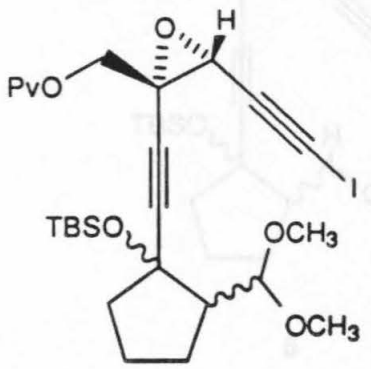
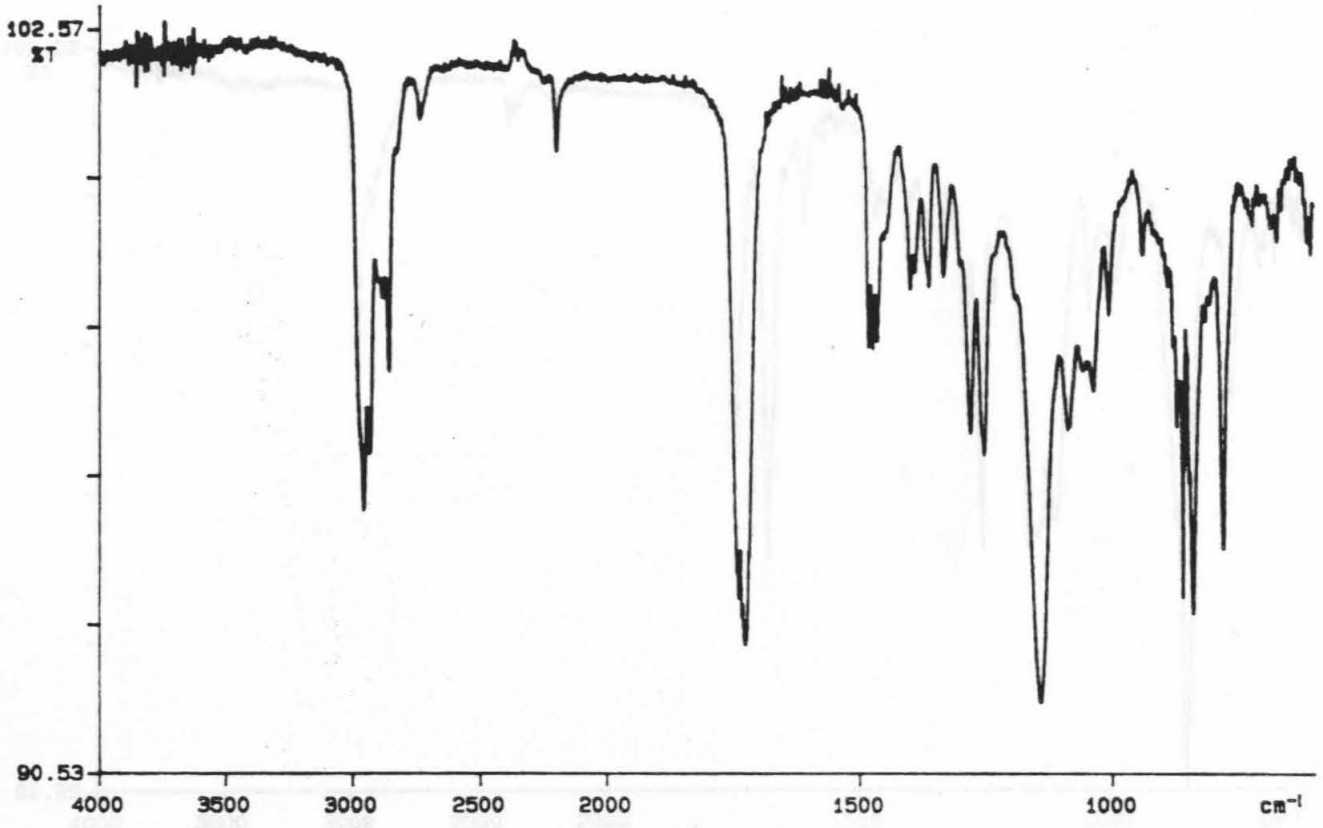
1500
1000
cm-1


O
H
PvO
I
TBSO
OCH3
OCH3

4

ppm
8
7
6
5
4
3
2
1
0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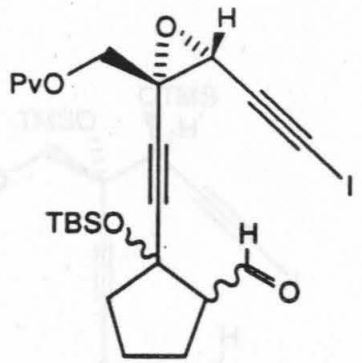
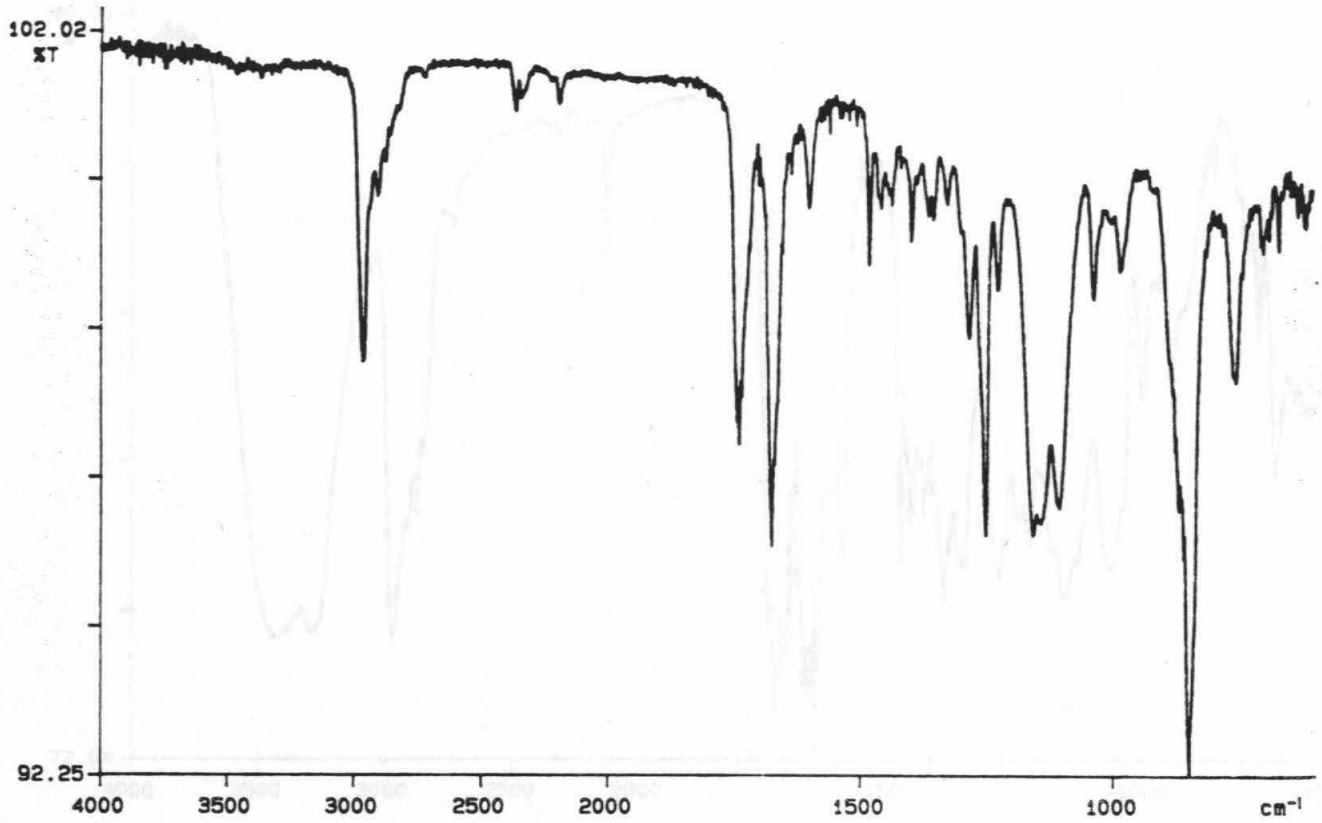
2000
1500
1000
cm-1


O
H
PvO
I
TBSO
H
O

5

PPM
10
9
8
7
6
5
4
3
2
1
0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92.25
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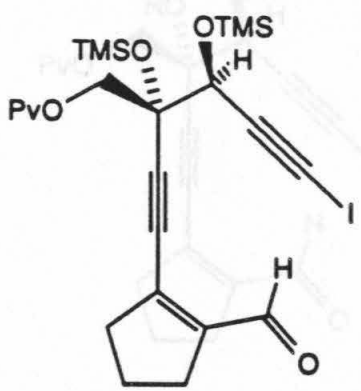
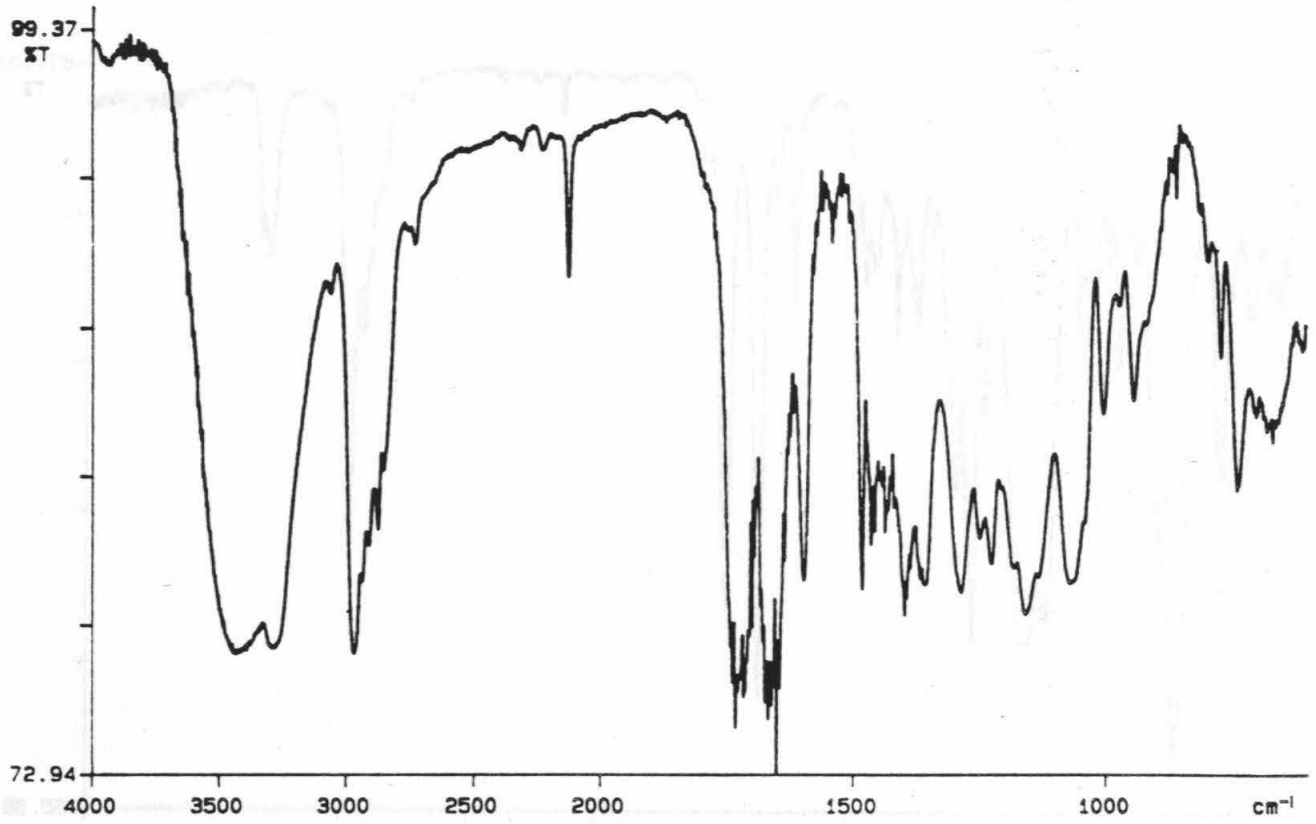
1500
1000
cm-1


TMSO
OTMS
H
PvO
I
H
O

6

PPM
10
9
8
7
6
5
4
3
2
1
0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%T
72.94
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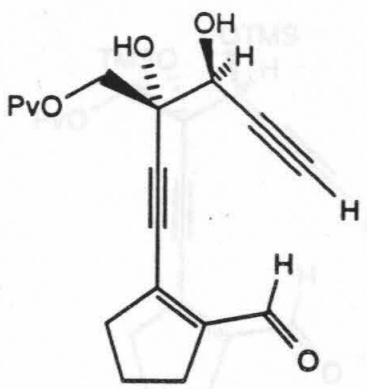
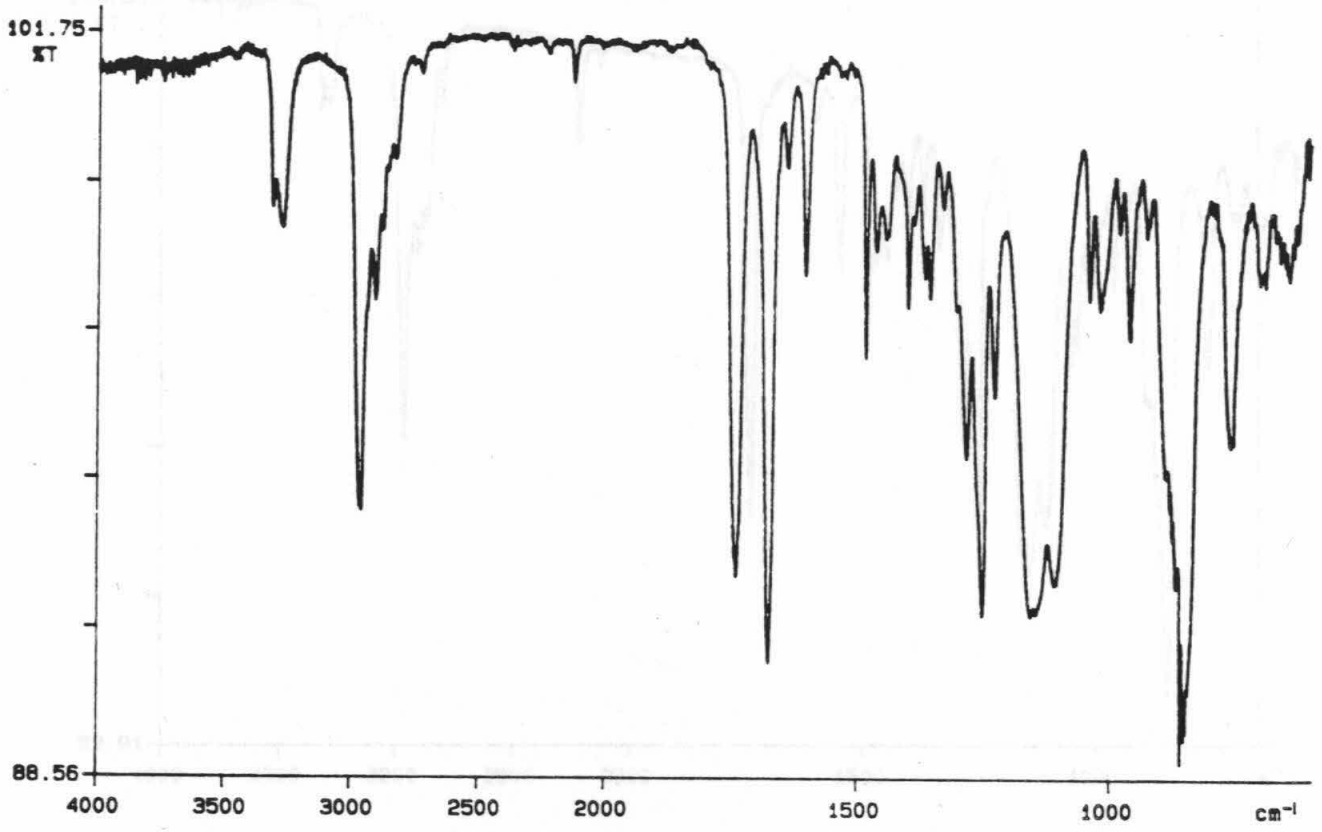
3500
3000
2500
2000
1500
1000
cm⁻¹


HO
OH
H
PvO
H
H
O

7

10
9
8
7
6
5
4
3
2
1
0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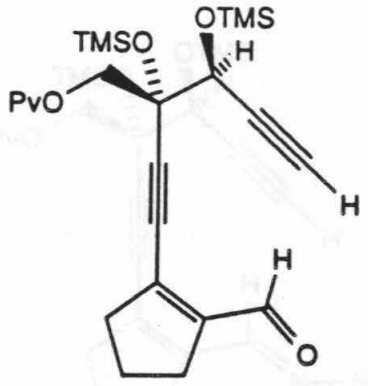
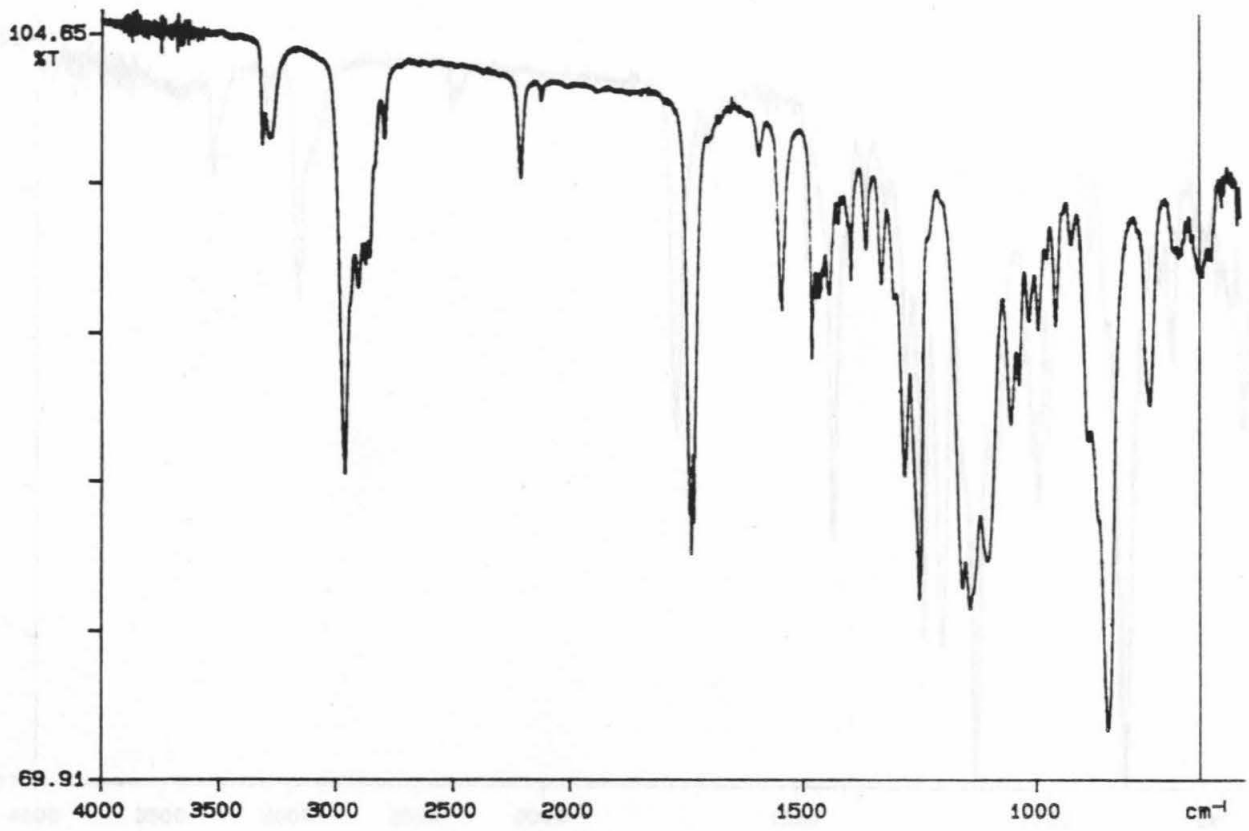
2500
2000
1500
1000
cm⁻¹


OTMS
TMSO
H
PvO
H
H
O

8

10
9
8
7
6
5
4
3
2
1
0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69.91
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3500
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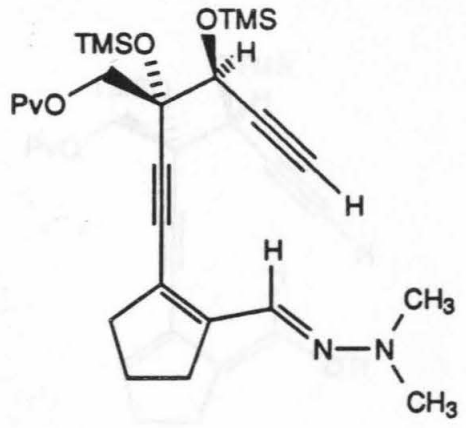
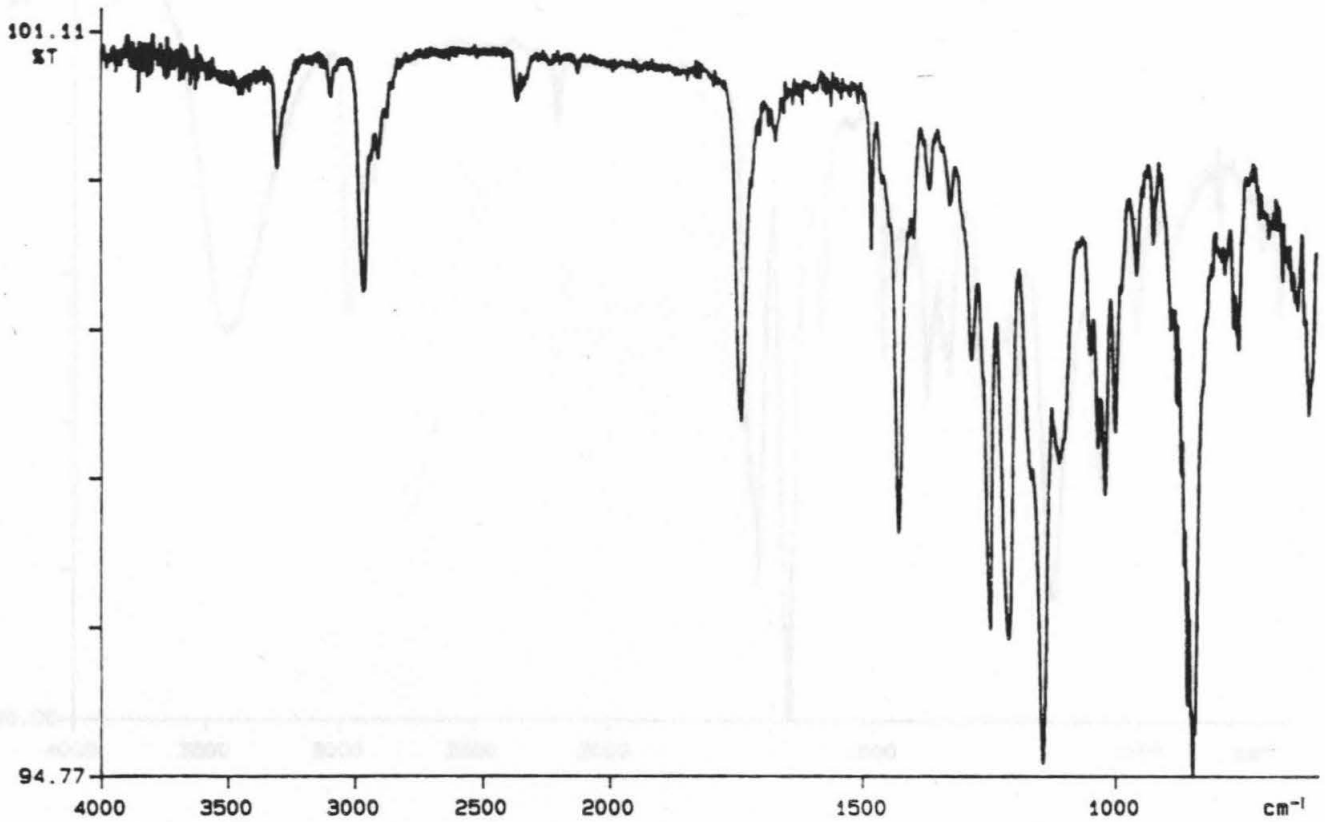
2500
2000
1500
1000
cm⁻¹


TMSO
OTMS
H
PvO
H
H
CH3
N
N
CH3

9

PPM
9
8
7
6
5
4
3
2
1
0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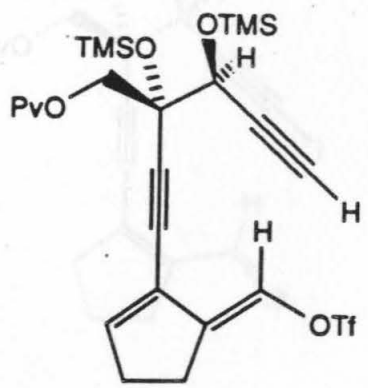
1500
1000
cm-1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OTMS
H
PvO
H
H
OTf

10

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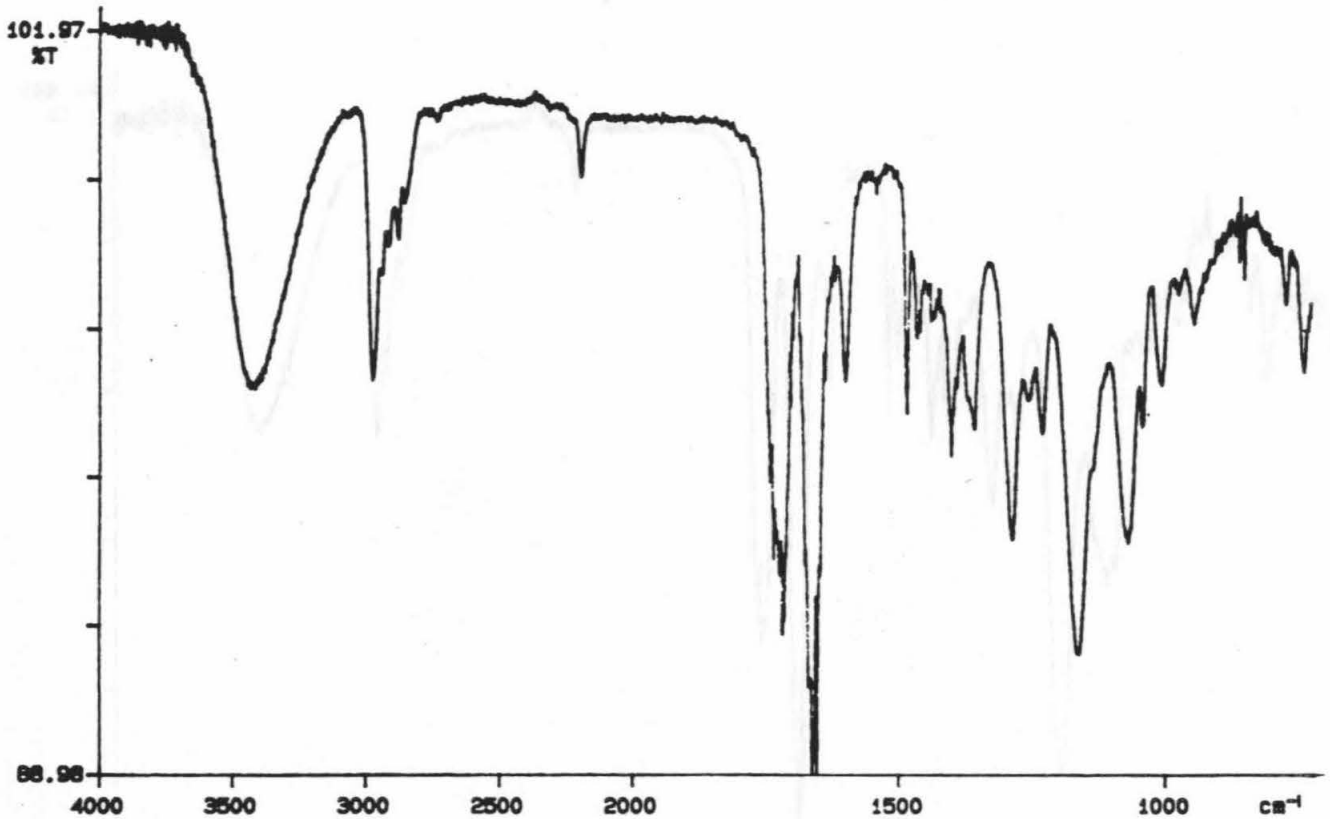
6
5
4
3
2
1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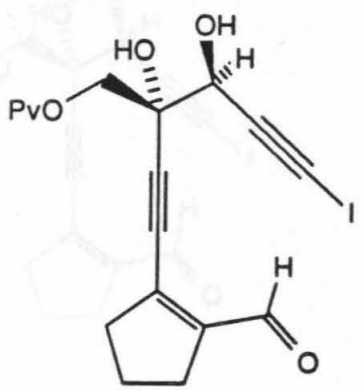
cm⁻¹


HO
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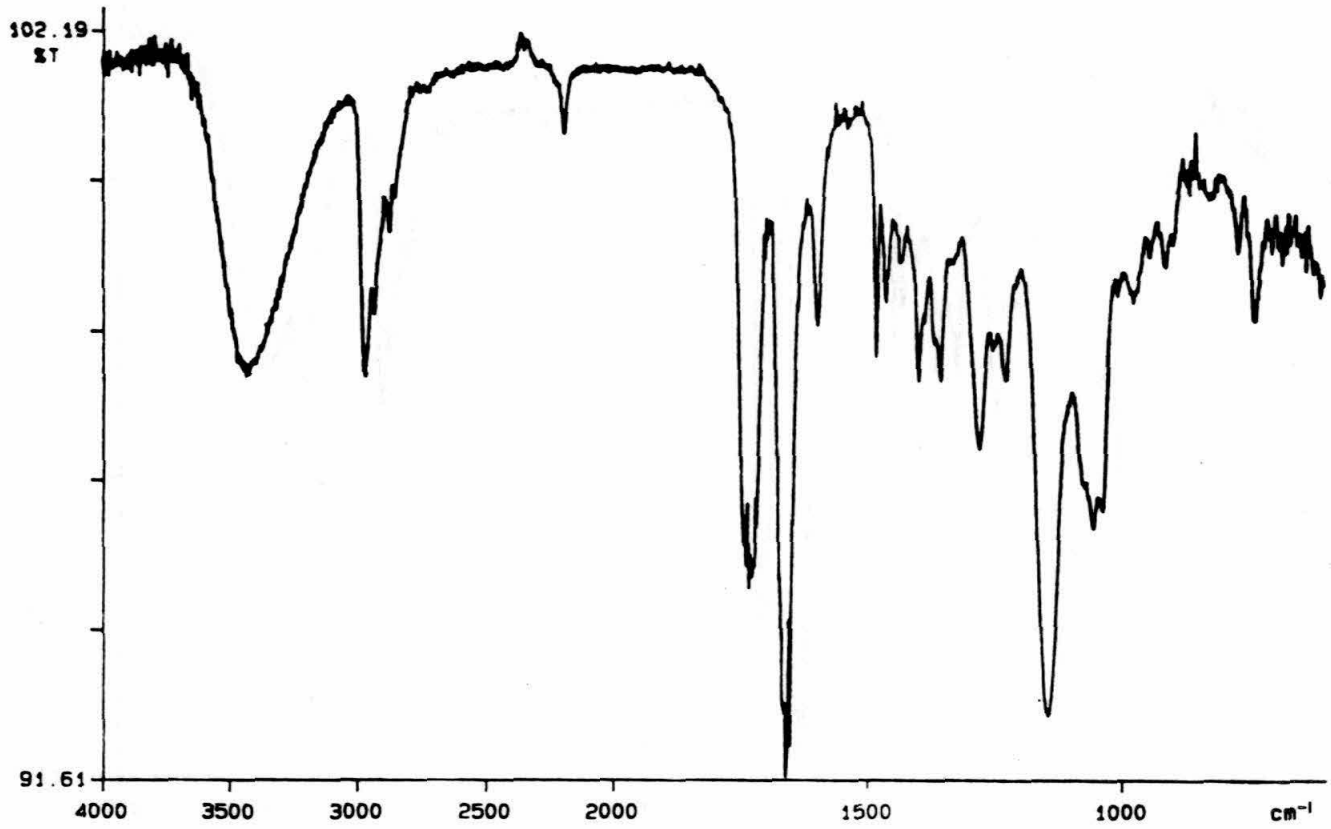
H
PvO
I
H
O

11

PP
10
9
8
7
6
5
4
3
2
1
0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2500
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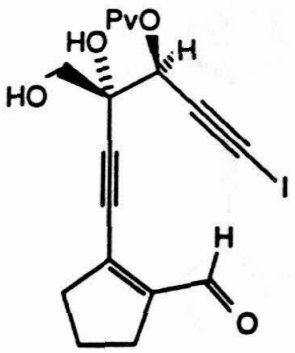
1500
1000
cm⁻¹


PvO
HO
HO
H
I
H
O

12

PPM
10
9
8
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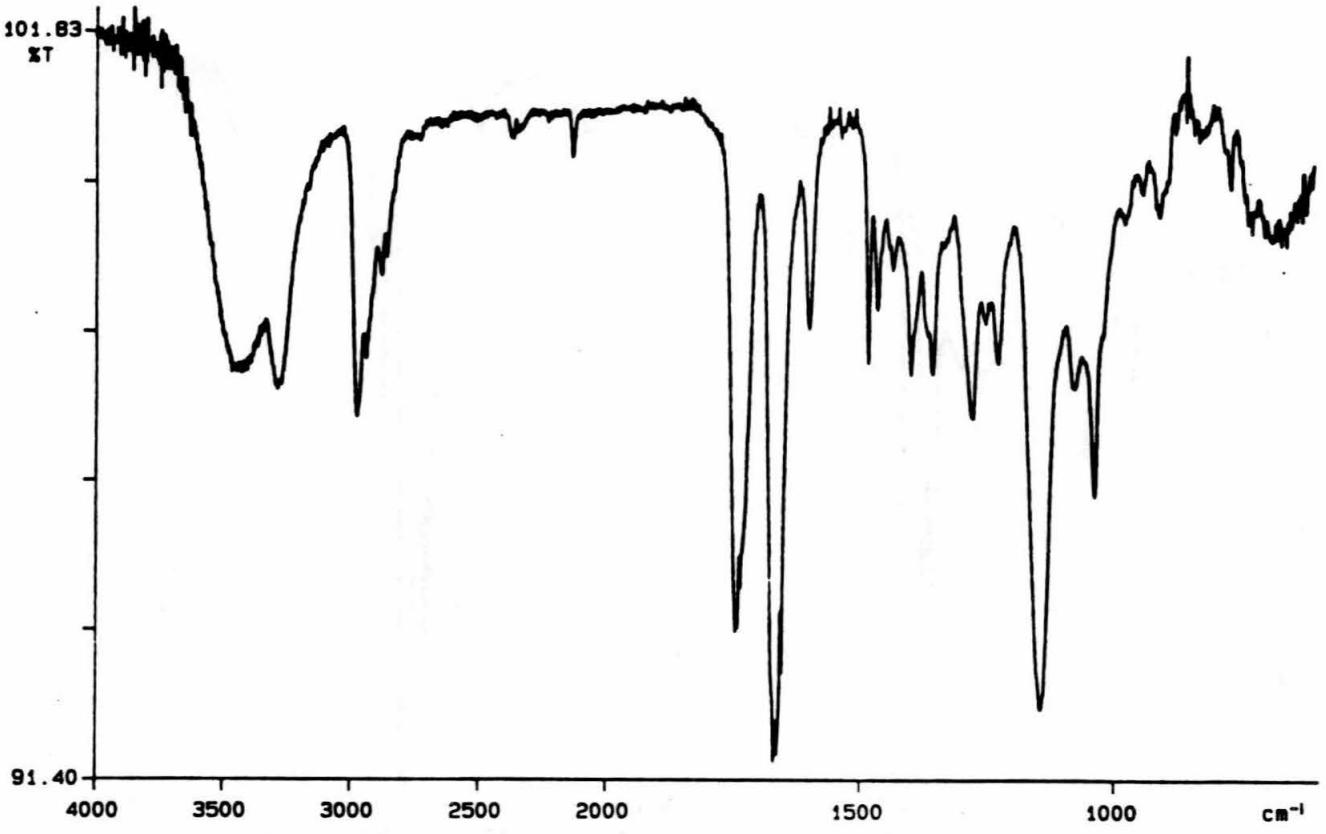
6
5
4
3
2
1
0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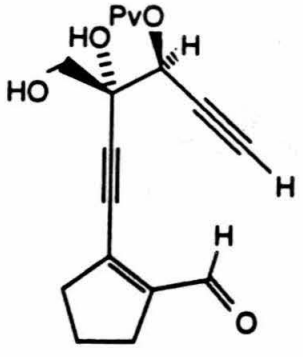
cm⁻¹


HO
PvO
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HO
H
H
O

13

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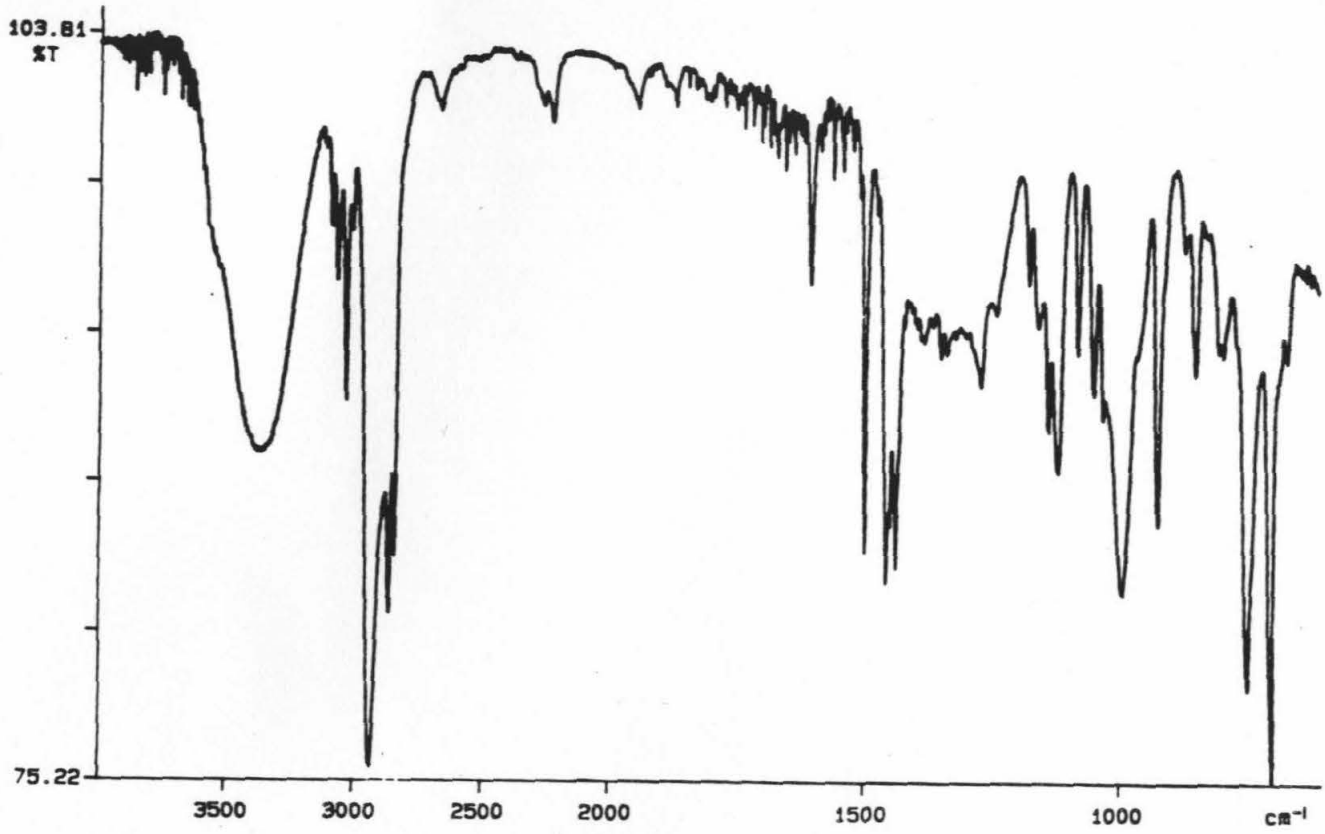
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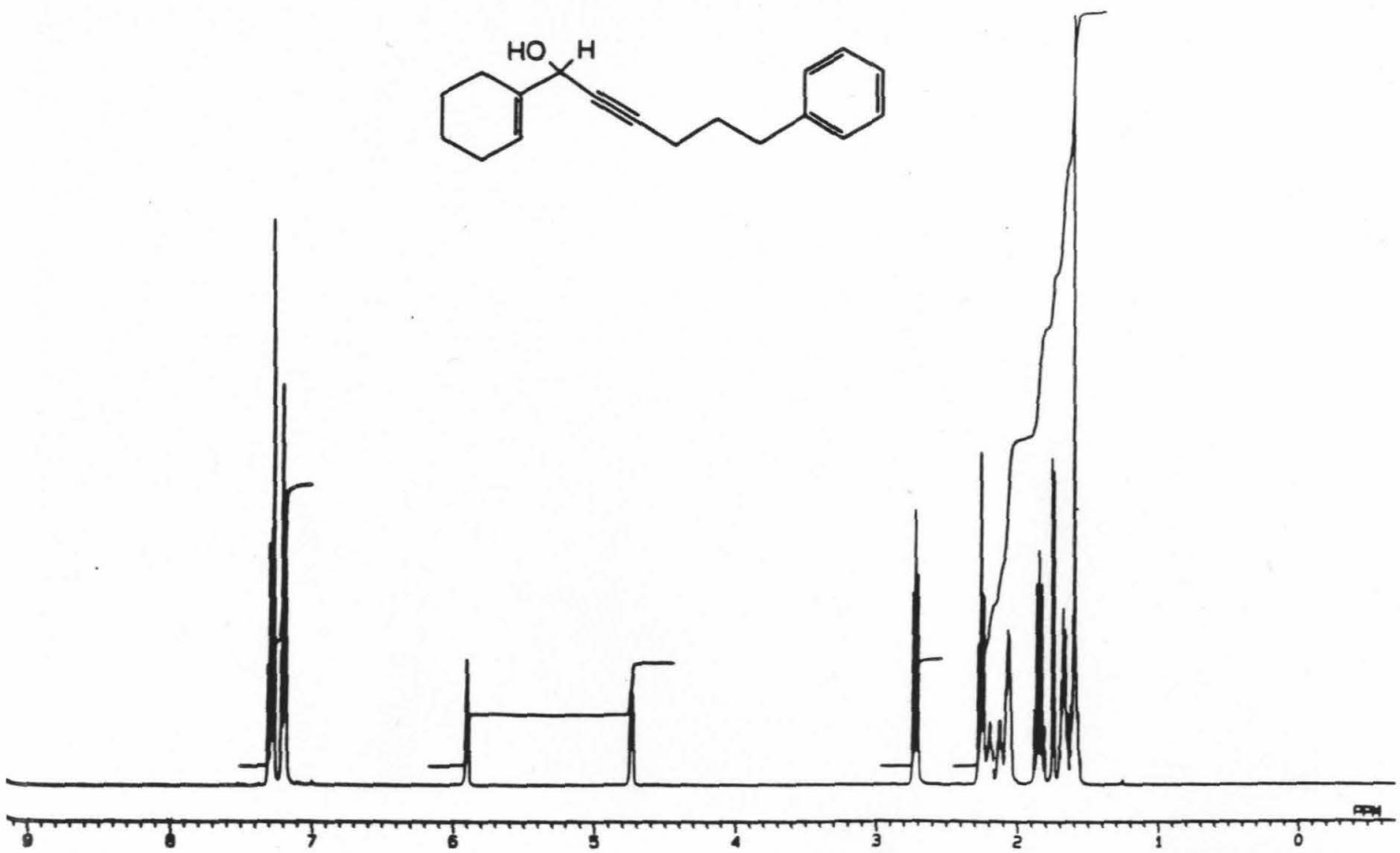
1000
cm⁻¹


HO
H
9
8
7
6
5
4
3
2
1
0
PPM