Photolysis of 200 mg of Tempo in 20 mL of acetonitrile to >95% conversion followed by liquid chromatography (1:9 CH_3CN/CH_2Cl_2) gave a pure sample of **2** as a colorless oil: ^1H NMR (500 MHz, $CDCl_3$) δ 4.51 (s, 2 H), 1.6–1.3 (m, 6 H), 1.20 (s, 6 H), 1.10 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 115.89, 62.59, 60.20, 39.46, 32.89, 19.61, 16.80; mass spectrum, *m/e* (relative intensity) M^+ 196 (2.5), 181 (26.5), 156 (47.1), 41 (100); IR 2060 (w), 1740, 1690 cm^{-1}.[20] Anal. Calcd for $C_{11}H_{20}N_2O$: C, 67.31; H, 10.27; N, 14.27. Found: C, 66.92; H, 10.18; N, 14.10.

(b) Irradiation of a degassed solution of 0.05 M Tempo in toluene to ~20% conversion gave approximately equal amounts of two compounds which GC–MS indicated to be **3** and **4**. The photolysis mixture contained a large number of components if the photolysis was continued to >40% conversion. Therefore, the structure of **4** was confirmed by comparison with an authentic sample prepared by photolysis of 0.2 M dibenzyl ketone plus 0.04 M Tempo in benzene. The photolysis mixture contained ~60% **4** and ~40% of a second material probably formed by Tempo trapping of the phenylacetyl radical before decarbonylation. Flash chromatography on silica gel using 5% EtOAc/hexane as eluent gave a pure sample of **4** as a colorless oil: NMR (60 MHz, $CDCl_3$) δ 7.3 (s, 5 H), 4.8 (s, 2 H), 1.5 (s, 6 H), 1.3–1.1 (m, 12 H); mass spectrum, *m/e* (relative intensity) M^+ 247 (2.3), 156 (100).

Registry No. **1**, 2564-83-2; **2**, 102261-91-6; **3**, 7031-93-8; **4**, 102261-92-7; CH_3CN, 75-05-8; $PhCH_3$, 108-88-3.

(20) The weak band at 2060 cm^{-1} is assigned to the C≡N group and agrees well with the reported spectrum for methoxyacetonitrile,[21] although it is much weaker and somewhat shifted from the usual C≡N bands.

(21) Pouchert, C. J. *The Aldrich Library of Infrared Spectra, Edition III*, Aldrich Chemical Company Inc.: Milwaukee, WI, 1981; p 508.

Structure Elucidation of a New Neutral Macrolide Antibiotic

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The macrolide antibiotics are a class of complex natural products which may be broadly divided into two general classes, namely, basic macrolides and neutral macrolides. Although the basic macrolides comprise all of the antibacterials that are of commercial interest in the human health area, the neutral members of this family are of considerable interest as lead structures in semisynthetic programs. This report details the structure elucidation of a new, neutral, 16-membered macrolide antibacterial, which has been isolated from *Streptomyces hirsutus*.[1]

Results and Discussion

I. Molecular Formula. The antibacterial class of the natural product was readily established, since it proved to have a narrow, gram-positive in vitro spectrum with no microbiologial activity against a typical macrolide resistance marker.

The structure elucidation was initiated by assigning a molecular formula to compound **1**. This was expeditiously accomplished by ^{13}C NMR spectroscopic techniques, since electrophoresis revealed that **1** was neutral and combustion analysis established the presence of only C, H, and O. (Anal. Found: C, 61.27; H, 8.19). The standard ^{13}C NMR spectrum as well as a polarization transfer (DEPT)[2] experiment (illustrated in Figure 1) of **1** supported a carbon number of 35 with 54 nonexchangeable protons bonded directly to carbon. Furthermore, the number of exchangeable protons, and their corresponding vicinally attached carbons was ascertained by the direct observation of vicinal deuterium isotope shifts of –0.06 ppm[3] for two carbon resonances absorbing at 75.11 ppm and 72.74 ppm, respectively; thus, the total number of protons must be 56. Therefore, the ^{13}C NMR observations in conjunction with the combustion analysis support a molecular formula of $C_{35}H_{56}O_{13}$.

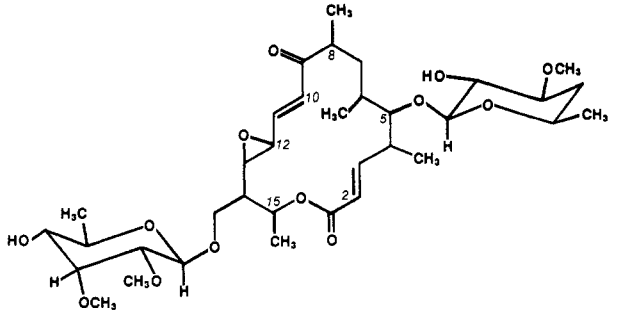
Table I. ^{13}C NMR Summary

carbohydrate carbon assignment	^1H NMR band	mycinose		^1H NMR band	chalcose	
1	IIb	100.93[a]	101.3[b]	IIc_1	103.44[a]	104.0[b]
2	IIg_1	81.92	82.1	IIf_2	75.11	74.7
3	IId	79.69	79.0	IIf_4	80.52	80.2
4	IIf_5	72.74	72.9	$IIIb/IVb_1$	36.85	37.4
5	IIe_4	70.71	70.8	IIe_5	67.82	68.0
5′	IVb_2	17.78	17.7	IVb_3	20.91	21.0
3′	IIe_2	61.69	61.4		56.81	56.9
2′	IIe_3	59.65	58.9			

[a] ^{13}C NMR chemical shifts for natural product. [b] ^{13}C NMR chemical shifts for β-methyl acetals.

Table II. ^{13}C NMR Summary

δC	proton attachments	^1H NMR band	aglycon carbon assignment
200.95	0		9
165.39	0		1
151.21	1	Ia_1	3
143.96	1	Ia_3	11
125.60	1	Ia_2	10
120.55	1	Ib	2
86.96	1	IIf_1	5
68.70	1	IIa	15
67.04	2	IIc_2,IIe_1	14′
59.04	1	IIf_3	12
58.91	1	IIg_2	13
49.48	1	IVa_1	14
44.68	1	$IIIa_2$	8
41.78	1	$IIIa_1$	4
34.14	1	IVb_5	6
32.02	2	IIIc	7
18.75	3	IVb_4	4′
18.41	3	IVa_2	15′
17.58	3	IVc	8′
17.01	3	IVd	6′



1

II. Carbohydrate Fragments. The ^{13}C NMR spectrum (Figure 1) indicates two carbon resonances with

(1) The fermentation and isolation of **1**, as well as the characterization of the producing organism, will appear elsewhere.

(2) Doddrell, D.; Pegg, D.; Bendall, M. *J. Magn. Reson.* **1982**, *48*, 323.

(3) Hauske, J. R.; Guadliana, M.; Kostek, G. *J. Org. Chem.* **1983**, *48*, 5138. Whipple, E. *J. Am. Chem. Soc.* **1980**, *102*, 4203.

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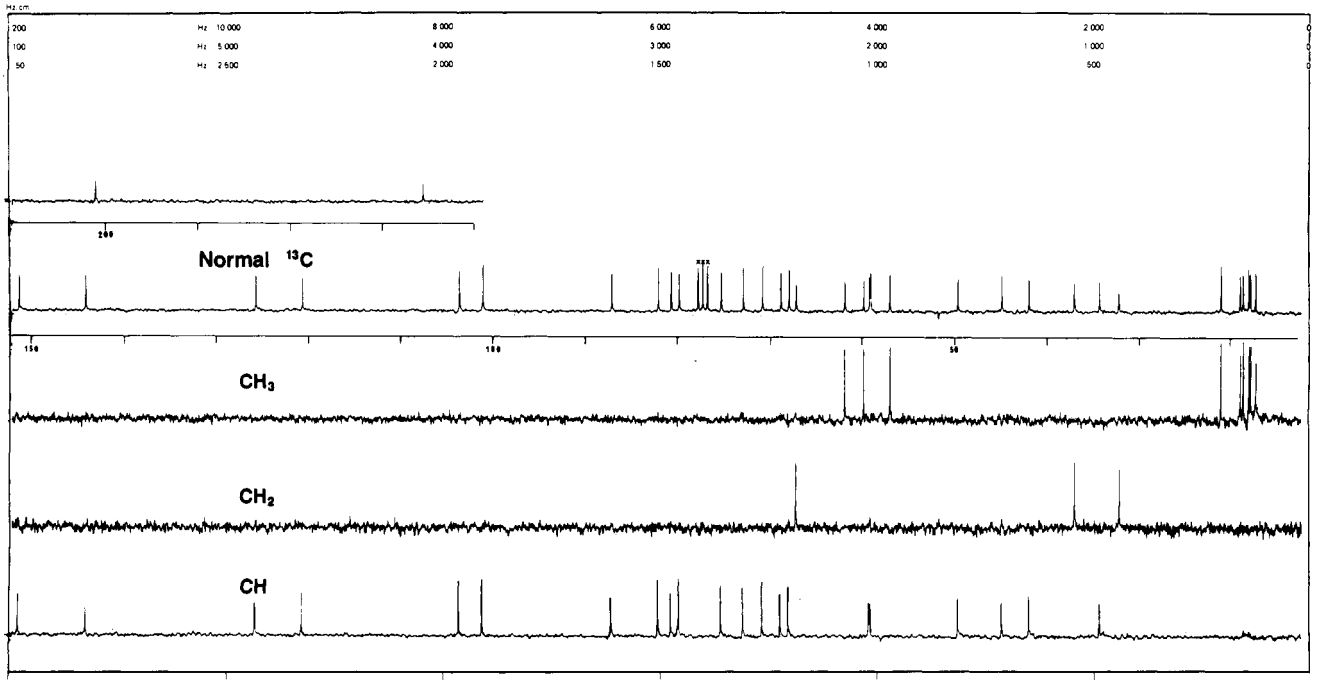


Figure 1. 62.5-MHz ^{13}C NMR spectrum and DEPT of macrolide 1 in $CDCl_3$ solution.

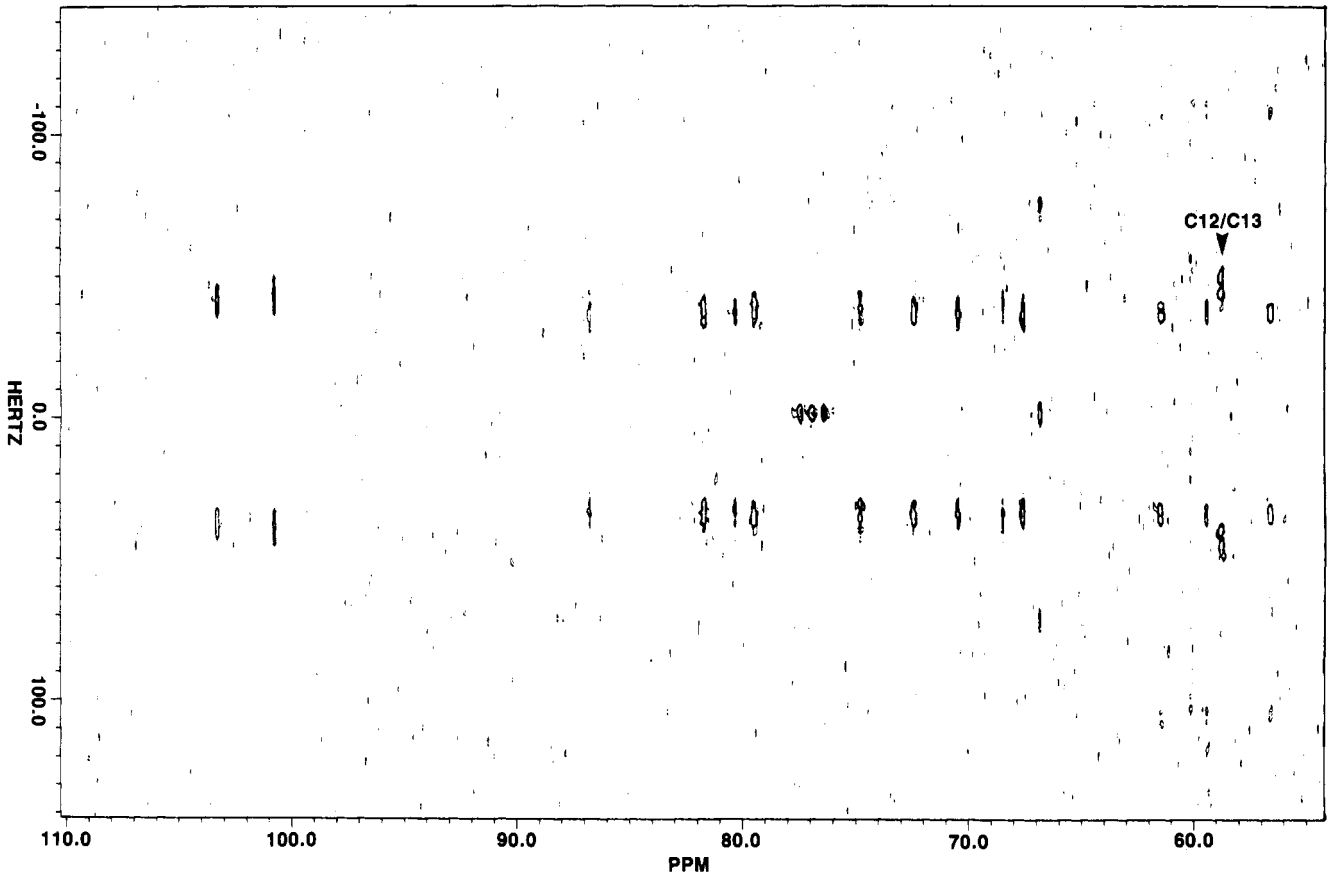


Figure 2. *J*-Resolved spectrum of macrolide 1 in $CDCl_3$ solution.

one-proton attachments that absorb in a range (105–95 ppm) consistent with the anomeric carbons of carbohydrate moieties. Additional support for the number, as well as the nature, of the carbohydrate fragments derives from high resolution mass spectrometry. The electron impact spectrum produces two mass fragments, specifically, mass 175.0986 having molecular formula $C_8H_{15}O_4$ (2) and mass 145.0869 having molecular formula $C_7H_{13}O_3$ (3), which are consistent with the nascent carbohydrate substituents. These fragments were assigned as the known neutral carbohydrates mycinose and chalcose, respectively. The NMR evidence consistent with this assignment is summarized in Table I. There is excellent agreement between the reported ^{13}C NMR values[4] of the β-methyl acetals of mycinose and chalcose and those observed for carbohy-

(4) Omura, S.; Nakagawa, A., Neszmelyi, A.; Gero, S.; Sepulchre, A.; Piriou, F.; Lukacs, G. *J. Am. Chem. Soc.* **1975**, *97*, 4001.

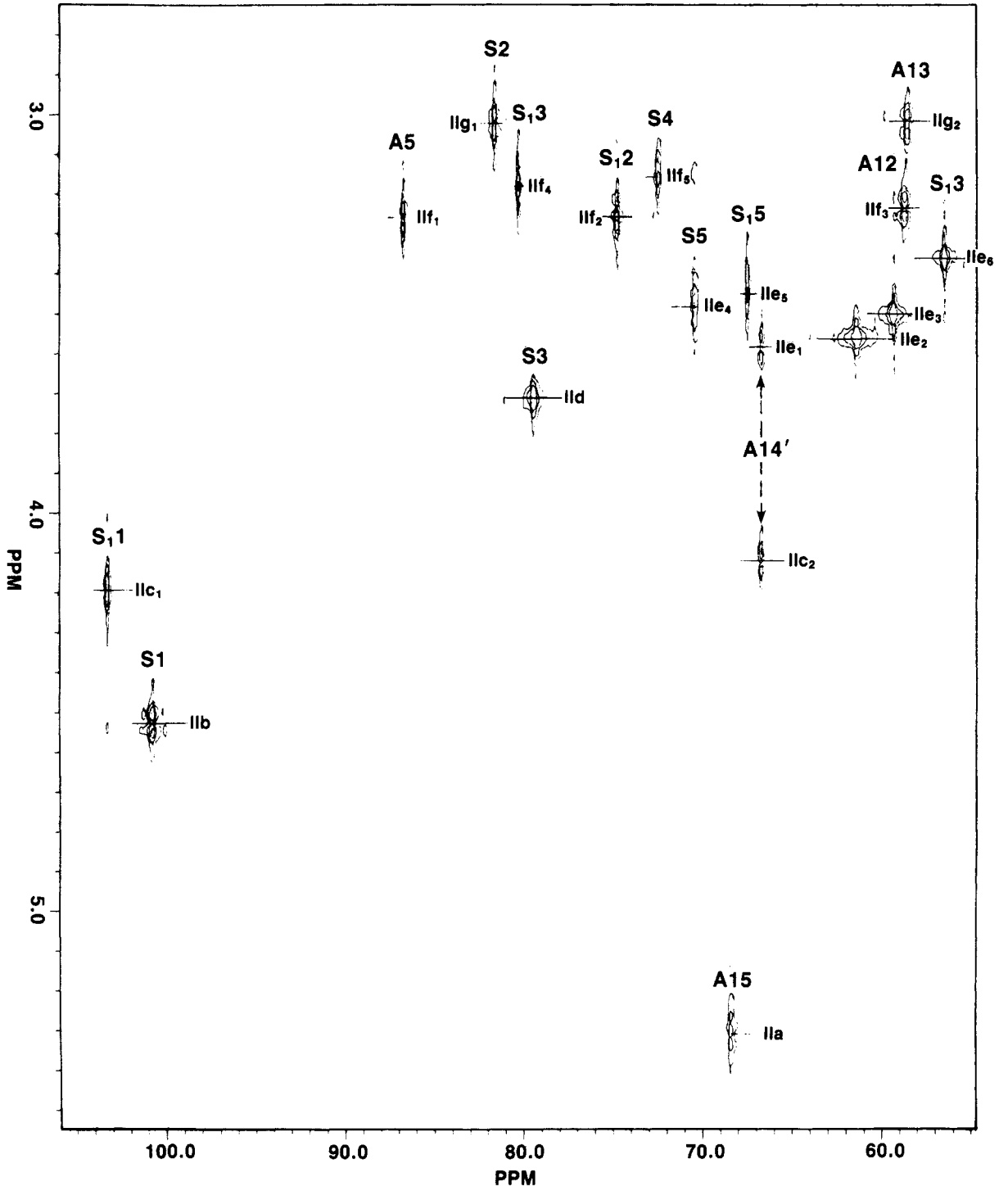
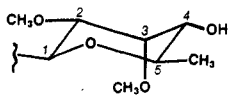


Figure 3. ^{13}C–^{1}H correlation spectrum of macrolide 1 in the range 105 to 55 ppm, where S refers to the sugar components and A refers to the aglycon moiety. The numbering system is the normal macrolide numbering.

drate fragments 2 and 3. The corresponding ^{1}H NMR bands are readily assigned by two-dimensional heteronuclear (^{13}C–^{1}H) shift correlation experiments (Figures 3 and 4).[5] Thus, macrolide 1 contains two carbohydrate fragments, namely mycinose (2) and chalcose (3).



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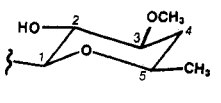

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(5) Bendall, M.; Pegg, D.; Doddrell, D.; Thomas, D. *J. Magn. Reson.* 1982, *46*, 43.

III. Aglycon Fragment. Since the assigned carbohydrate moieties accounted for only 15 carbons and 7 oxygens, the aglycon fragment must contain the remaining 20 carbons and 6 oxygens. Inspection of Figure 1 (the data summary appears in Table II) reveals that there are six sp^2-hybridized carbons downfield of 120 ppm; five sp^3-hybridized, alkoxy-like carbons that appear from 87 ppm to 58 ppm, and nine unsubstituted sp^3-hybridized carbons absorbing from 50 ppm to 17 ppm. An assessment of the ^{13}C NMR data, which is summarized in columns 1 and 2 of Table II, on the basis of chemical shift considerations permits assignment of the functionality contained within the aglycon subunit. For example, the furthest downfield carbon resonance (200.95 ppm) must be a conjugated ke-

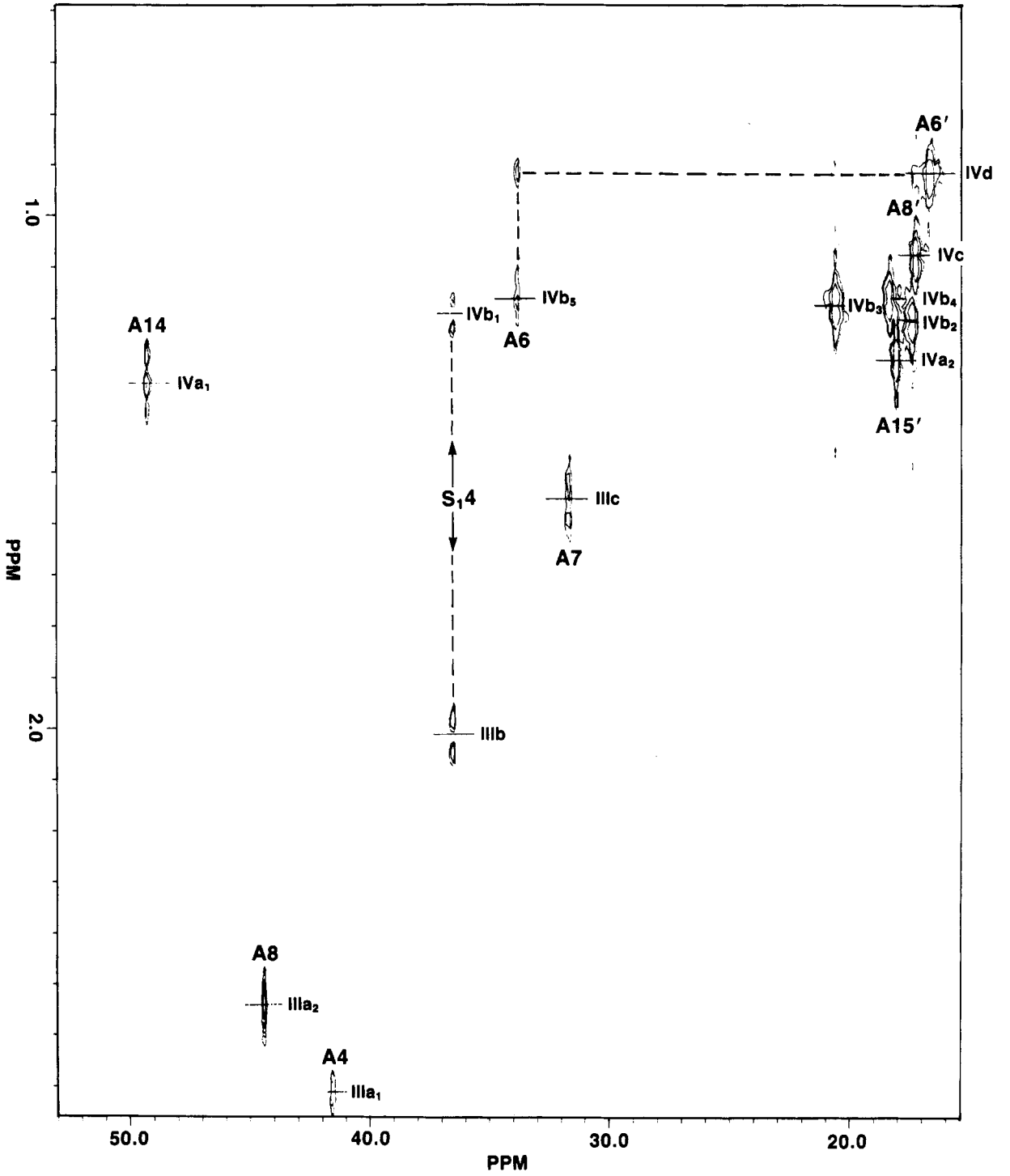

Figure 4. ^{13}C–^{1}H correlation spectrum of macrolide 1 in the range 52 to 16 ppm, where S refers to the sugar components and A refers to the aglycon moiety.

tone, since the DEPT spectrum defines it as a quaternary carbon; furthermore, the quaternary carbon resonance at 165.39 ppm is consistent with a conjugated lactone. Support for this assignment is derived from the sp^2-hybridized olefinic methinyl carbons (151.21, 143.96, 125.60, and 120.55 ppm), which are conjugated to two *different* electron-withdrawing functionalities; namely, the ketone and the lactone moieties, respectively.

The sp^3-hybridized carbons bearing oxygen must all be ethereal, since the previously detailed deuterium isotope shift experiment defined the presence of only two carbohydrate hydroxyl substituents. Interestingly, the sp^3-hybridized methinyloxy resonances at 59.04 ppm and 58.91 ppm absorb at an unusually high field chemical shift. Again, chemical shift arguments suggest assignment of the functionality as an epoxide. The proposed epoxide functionality at this position was unequivocally defined by observing the J resolved spectrum (Figure 2).[6] The direct bond (^{13}C–^{1}H) coupling constant for epoxides is J = 180 Hz, which is readily distinguished from the typical methinyloxy carbon direct bond (^{13}C–^{1}H) coupling of J = 130–140 Hz. It is clear from the results of the J resolved experiment illustrated in Figure 2 that the assignment of the resonances at 59.04 ppm and 58.91 ppm as epoxide carbons is strongly supported. Since the functionality

(6) Turner, D.; Freeman, R. *J. Magn. Reson.* 1978, *29*, 587.

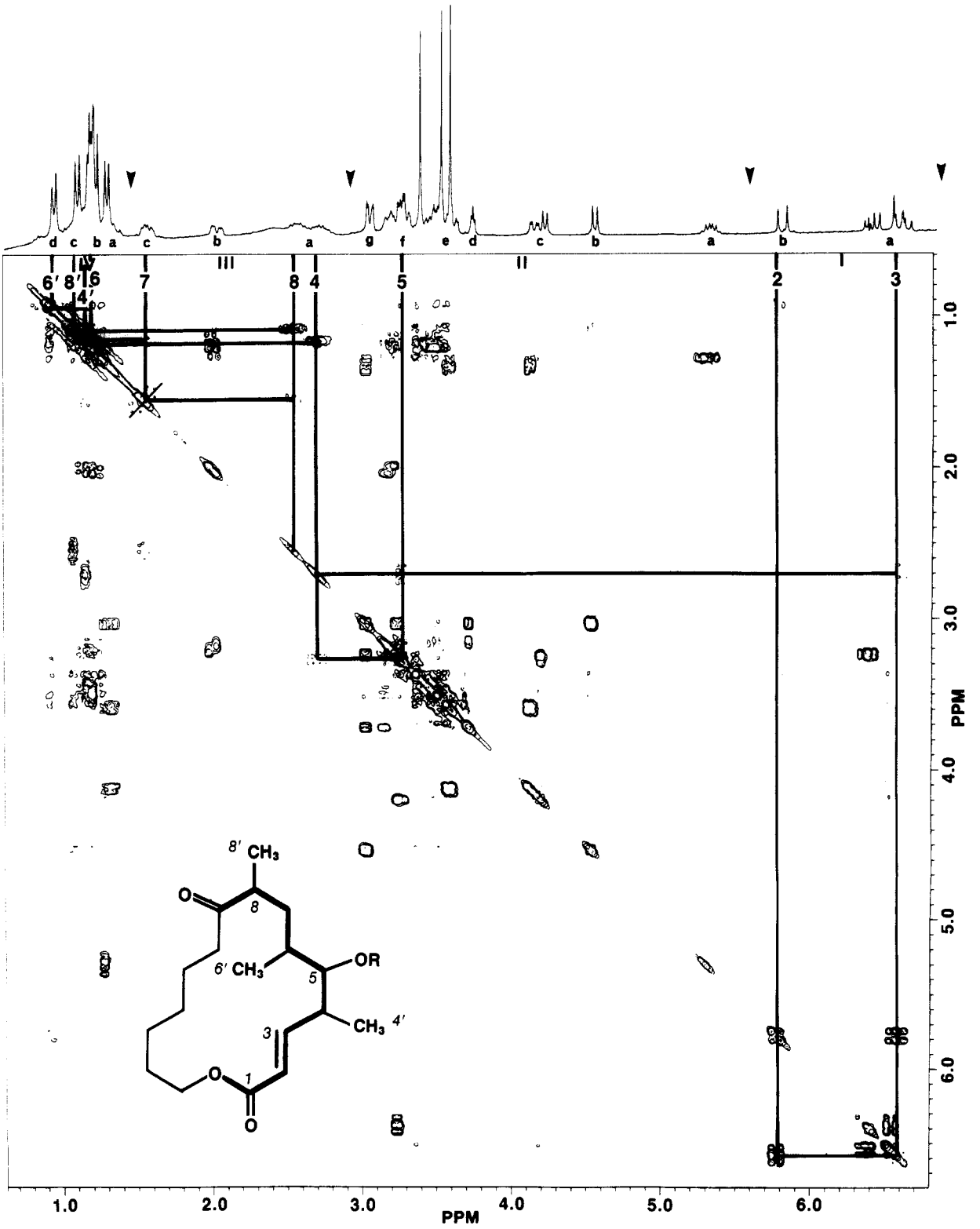

Figure 5. COSY spectrum of macrolide 1 detailing the correlation of C-1 through C-8′.

contained within the aglycon moiety is established, the next level of structural information is derived from the two-dimensional heteronuclear (^{13}C–^{1}H) shift correlation experiment which allows the direct assignment of those ^{1}H NMR bands corresponding to the aglycon carbons (Figures 3 and 4, Table II).[5]

The unequivocal assignment of the ^{1}H NMR is necessary since the carbon sequence of the aglycon moiety is determined indirectly by consideration of the homonuclear (^{1}H–^{1}H) correlated spectrum, that is, the COSY experiment.[7] Thus, the detailed assignment of the aglycon carbon segments C-1 through C-8 (Figure 5) and C-9 through C-15 (Figure 6) was inferred from the correlated vicinally coupled protons. Inspection of Figure 5 (the data is summarized in Table II) reveals that the C-2 and C-3 protons (^{1}H NMR band Ib and Ia_1, respectively) are coupled and, furthermore, that the C-3 resonance is correlated to band $IIIa_1$ (the C-4 carbon), which in turn is coupled to band IIf_1 (the C-5 carbon). Although assignment of the

(7) Wagner, G.; Kumar, A.; Wuthrich, K. *Eur. J. Biochem.* **1981**, *114*, 375.

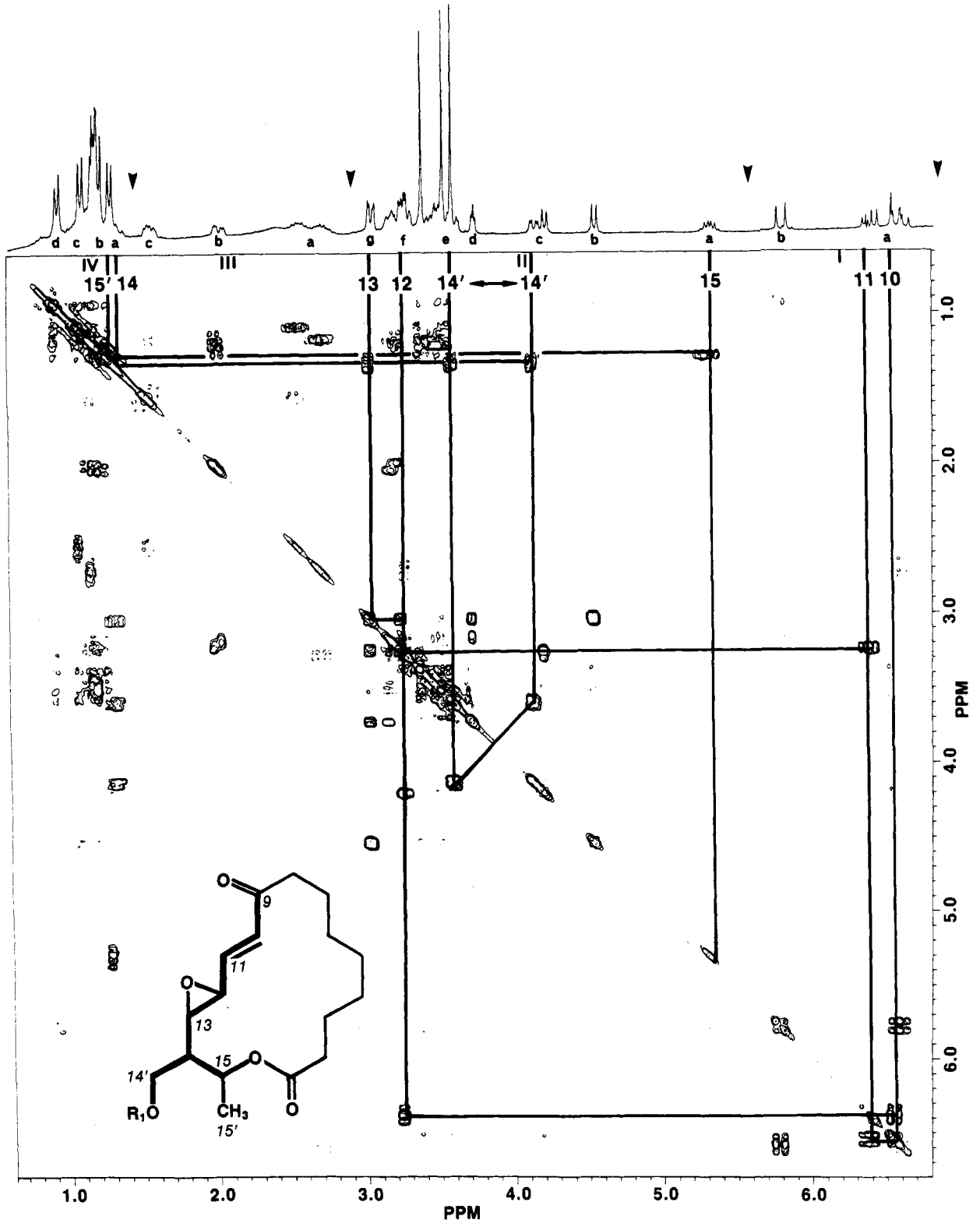

Figure 6. COSY spectrum of macrolide **1** detailing the correlations of C-9 through C-15′.

C-1 through C-5 fragment is straightforward, correlation of the ^{1}H NMR resonances beyond the C-5 position is somewhat complicated by overlap of the carbohydrate resonances in bands IIc through IIg_1. Fortunately, the well-defined chemical shift of the carbohydrate anomeric proton resonances (IIb and IIc_1) allow one to unequivocally delineate the entire carbohydrate network (see ^{1}H NMR assignments in Table I), which removes the ambiguity from the aglycon assignments and, therefore, the assignment of C-6 through C-8′ follows directly as bands IVb_5, IIIc, IIIa_2, and IVc, respectively. The C-9 through C-15′ segment (Figure 6, the data is summarized in Table II) was derived in analogous fashion.

IV. Carbohydrate Position. All that remains to complete the structural analysis of **1** is the determination of the aglycon absolute stereochemistry, as well as the substitution pattern of the carbohydrate subunits. The absolute stereochemical determination by NMR methods is quite difficult on a molecule of this complexity and was not attempted; however, the relative substitution pattern of the carbohydrate moieties may be assigned directly by NOE techniques.[8] For example, irradiation of the ^{1}H

NMR band IIb resulted in approximately a 10% nuclear Overhauser enhancement for the ^1H NMR band IIc_2. Since band IIb is the anomeric (C-1) proton of mycinose (2) and band IIc_2 is part of the diastereotopic methylene multiplet (C-14′) of the aglycon, the substitution pattern must be chalcose (3) at C-5 and mycinose (2) at C-14′.[9]

In summary, therefore, we have elucidated the molecular framework of a new, neutral macrolide (1) by two-dimensional NMR techniques, in conjunction with high resolution mass spectroscopic analysis.

Experimental Section

Structural assignments were derived from a variety of one-dimensional and two-dimensional (^1H NMR and ^{13}C NMR) NMR experiments carried out on a Bruker WH-250 spectrometer operating at ambient temperatures. The spectra were run in deuteriochloroform.

High resolution electron impact mass spectra were obtained on an AEI-MS 30 spectrometer. An analytically pure sample of macrolide 1 was obtained by HPLC on a Waters C-18 5 μm reverse phase column eluted with an acetonitrile–water gradient.

Anal. Calcd for $C_{35}H_{56}O_{13}$: C, 61.40; H, 8.23. Found: C, 61.27; H, 8.19.

Acknowledgment. We thank D. Sweet, R. Ware, and Dr. E. Whipple for assistance in obtaining spectra and invaluable discussions with regard to interpretations.

(8) Noggle, J.; Schirmer, R. *The Nuclear Overhauser Effect*, Academic Press: New York and London, 1971.
(9) Woo, P.; et al. *J. Am. Chem. Soc.* **1964**, *86*, 2726.

Synthesis of Vinylketenes. Thermolysis of 3-Azido-1,2-benzoquinones

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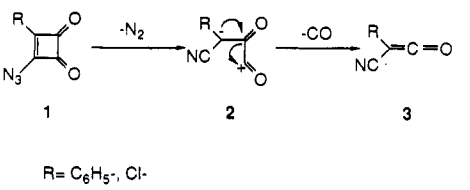
Azidocyclobutenediones 1 have previously been shown to undergo facile fragmentation to dinitrogen, carbon monoxide, and cyanoketenes 3; the zwitterionic intermediate 2 has been proposed to account for these products (Scheme I).[1-4] By analogy, it was anticipated that vinylogues of 1, i.e., 3-azido-1,2-benzoquinones 4, should cleave to the zwitterions 5 and these, in turn, suffer loss of carbon monoxide to give (2-cyanoethenyl)ketenes 6, thus providing a new entry to synthetically useful vinylketenes.[5] Reported here are the first examples of this reaction.

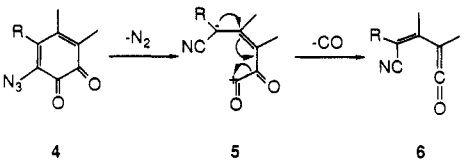
3-Azido-4,6-di-*tert*-butyl-1,2-benzoquinone (7) was subjected to thermolysis in refluxing benzene (300 mg/50 mL). Within 60 min the deep purple color of the initial solution had faded to a light yellow. The resulting residual oil was subjected to Kugelrohr distillation to give 153 mg (65%) of the ketene 9 as a light yellow oil.

The sterically hindered ketene 9 is remarkably stable. For example, at ambient temperature in methanol it requires 24 h for it to be converted to the esters 10 and 11, which are formed in 70% yield in a respective ratio of 7:3. On the other hand, the more nucleophilic cyclohexylamine reacts immediately with 9 (benzene) to give the amide 12 in 91% yield (Scheme II).

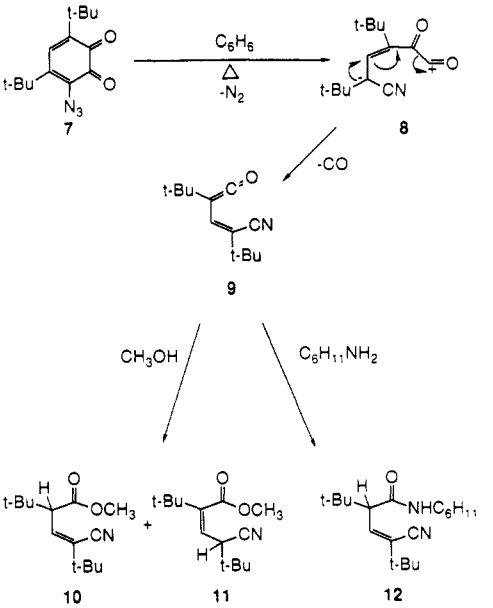
Scheme I



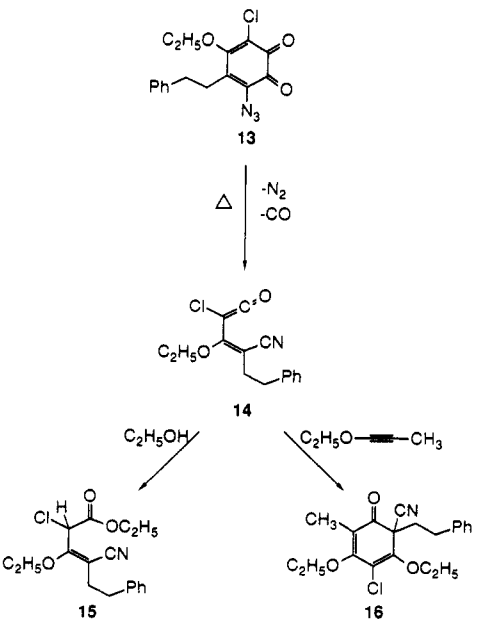
R= C_6H_5-, Cl-



Scheme II



Scheme III



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(3) Fishbein, P. L.; Moore, H. W. *J. Org. Chem.* **1984**, *49*, 2190.
(4) Moore, H. W. *Acc. Chem. Res.* **1979**, *12*, 125.
(5) See, for example: Patai, S. *The Chemistry of Ketenes, Alkenes and Related Compounds*; Wiley: New York, 1980; part 1.

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