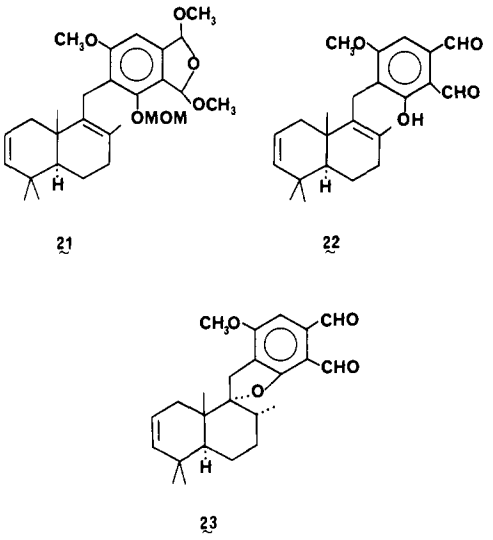
Chart IV



21 22 23

hydrochloric acid (1:1) at 23 °C for 4 h afforded 87% of (±)-K-76 (**1**) identical with an authentic sample of K-76 by ^{1}H NMR, infrared, and reversed-phase HPLC comparison.[13]

Oxidation of (±)-K-76 in 1 N sodium hydroxide with 12 equiv of silver oxide at 23 °C for 30 min afforded cleanly after acidification and workup (±)-K-76 monocarboxylic acid, which was found to be identical with an authentic sample[13] by infrared, 270-MHz ^{1}H NMR, and HPLC comparison. We have been able to show that the structure of this product is **17** rather than the isomeric **18**, which has previously been supposed.[3,4,14] The synthetic evidence for our assignment is as follows. Treatment of K-76 monocarboxylic acid with tosic acid in methanol (23 °C, 30 min) afforded the γ-methoxy lactone (97%), which was then transformed via the 2,3-acetonide (acetone, 2,2-dimethoxypropane, tosic acid at 0 °C for 1 h) into the two diastereomeric MOM ethers **19**[11] in 70% yield. These two diastereomers **19** were clearly different by ^{1}H NMR and TLC comparison with the diastereomeric pair (±)-**15** obtained by total synthesis. Structure **17**, which is firmly established for K-76 monocarboxylic acid by these results, was also favored by us a priori on mechanistic grounds. Considering that the oxidation of formyl by silver oxide under strongly basic conditions probably occurs by hydrogen atom abstraction from a hydroxide ion formyl group adduct, in the oxidation of the phenoxide ion of K-76, the formyl meta to the phenoxy oxygen should be the more reactive.

In addition to the synthetic route to K-76 described herein, we have also investigated a modification in which the aromatic–alicyclic coupling step employed the lithio derivative **20**[15] and bromide **5**. The coupling product **21** (Chart IV) (75% yield) could be converted to the dialdehyde **22** (95% with aqueous hydrochloric acid–THF). However, cyclization of **22** to **23** (tosic acid–methylene chloride at 45 °C for 30 min) afforded only 26% yield of the desired product **23**, and other reaction conditions were even less effective.

This synthesis of (±)-K-76 provides as well a useful route to a host of possibly useful structural analogues. This synthetic capability should be of value in determining the structural basis for anti-complement activity of K-76. The work has produced a number of interesting chemical findings as well as a revision of the structure of K-76 monocarboxylic acid, which has anti-complement activity at least comparable to K-76 itself.[16]

Supplementary Material Available: ^{1}H NMR and IR spectral information for **1–6, 8–13, 15–17**, and **19–23** (3 pages). Ordering information is given on any current mastehead page.

(13) We are indebted to the Otsuka Pharmaceutical Co. for their cooperation and for providing samples of K-76 and K-76 monocarboxylic acid.

(14) The same conclusion regarding the formulation of K-76 monocarboxylic acid has been reached in a reinvestigation by Dr. H. Kaise of the Otsuka Pharamaceutical Co. (personal communication) on the basis of ^{1}H NMR studies of various K-76 derivatives.

(15) The lithio derivative **20** was prepared by the hydrogen–lithium exchange reaction as indicated above for the conversion of **6** to **7**. The requisite phthalaldehyde derivative (**20** with H replacing Li) was synthesized by the sequence (1) formylation of methyl 3,5-dihydroxybenzoate (50% yield with zinc cyanide–aluminum chloride–hydrogen chloride ether at 0 °C for 30 min and 23 °C for 16 h), (2) selective etherification para to formyl (93% yield with potassium carbonate–methyl iodide in acetone at 23 °C for 20 h) to give methyl 2-formyl-3-hydroxy-5-methoxybenzoate, (3) MOM ether formation (100% with chloromethyl methyl ether–potassium carbonate–triethylamine in acetone at 0 to 23 °C for 1 h), (4) conversion to a γ-hydroxy γ-lactone (95% yield with aqueous potassium hydroxide–THF), (5) conversion to a γ-methoxy γ-lactone (100% yield with tosic acid in methanol), (6) conversion to the 3-methoxymethyl, 5-methyl ether of σ-phthalaldehyde (85% with diisobutyl aluminum hydride at −78 °C in toluene), and (7) formation of the cyclic dimethyl acetal (90% with tosic acid in methanol).

(16) We are pleased to acknowledge financial support from the National Institutes of Health and the National Science Foundation. Thanks are also due to the Otsuka Pharmaceutical Co. and in particular Drs. H. Kaise and T. Kawaguchi for their generous help.

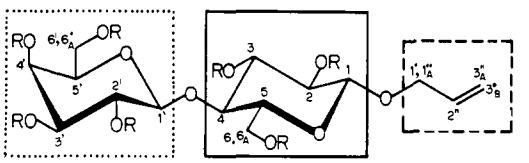
De Novo Sequencing of Oligosaccharides by Proton NMR Spectroscopy

M. A. Bernstein and L. D. Hall*

Department of Chemistry, University of British Columbia Vancouver, British Columbia, Canada V6T 1Y6

Received December 21, 1981

We report a method based on proton nuclear magnetic resonance (^{1}H NMR) for the structural elucidation of oligomeric organic molecules; we focus here on oligosaccharides[1] but believe the protocol to be generally applicable. The overall objective of the approach is to identify the monomeric units and establish their sequence in the chain; its success depends critically on the fact that the protons of each monosaccharide constitute a separate, relatively small, spin system. Identification of each monosaccharide is achieved by measurement of all the coupling constants[2] and assignment of each resonance; sequencing follows from measurement of the specific interring nuclear Overhauser enhancements.[3] All these data are obtained by a highly efficient combination of one- and two-dimensional (2-D) experiments. Results for the simple glycoside allyl β-D-galactopyranosyl-1β→4-D-glucopyranoside **1** illustrates the approach.



1 : R = H

2 : R = CO.CD_3

The first stage of the protocol requires complete resolution of all proton resonances, which is best achieved by use of the proton 2-D *J*-resolved[5] experiment. Because the spectrum of **1** in D_2O

(1) A review of Aspinall and Stephen (Aspinall, G. O.; Stephen, a. M. In "MTP International Reviews of Science. Organic Chemistry, Series One"; Aspinall, G. O., Ed.; University Park Press: Baltimore, MD, 1973; Vol. 7, pp 285–317) covers polysaccharide methodology, including analytical methods.

(2) An extensive tabulation of carbohydrate vicinal coupling constants has been compiled by Altona and Haasnoot (Altona, C.; Haasnoot, C. A. G. *Org. Magn. Reson.* **1980**, *13*, 417–429).

(3) Noggle, J. H.; Schirmer, R. E. "The Nuclear Overhauser Effect"; Academic Press: New York, 1971.

(4) The area has been reviewed by Freeman (Freeman, R. *Proc. R. Soc. London, Ser. A* **1980**, *373*, 149–178), Nagayama (Nagayama, K. *Adv. Biophys.* **1981**, *14*, 139–204), Morris (Morris, G. A. In "Fourier, Hadamard and Hilbert Transfers in Chemistry"; Marshall, A. G., Ed.; Plenum Press: New York, 1982), and Bax (Bax, A. "Two Dimensional Nuclear Magnetic Resonance in Liquids"; D. Reidel Publishing Co.; Hintham, Mass., 1982).

0002-7863/82/1504-5553$01.25/0

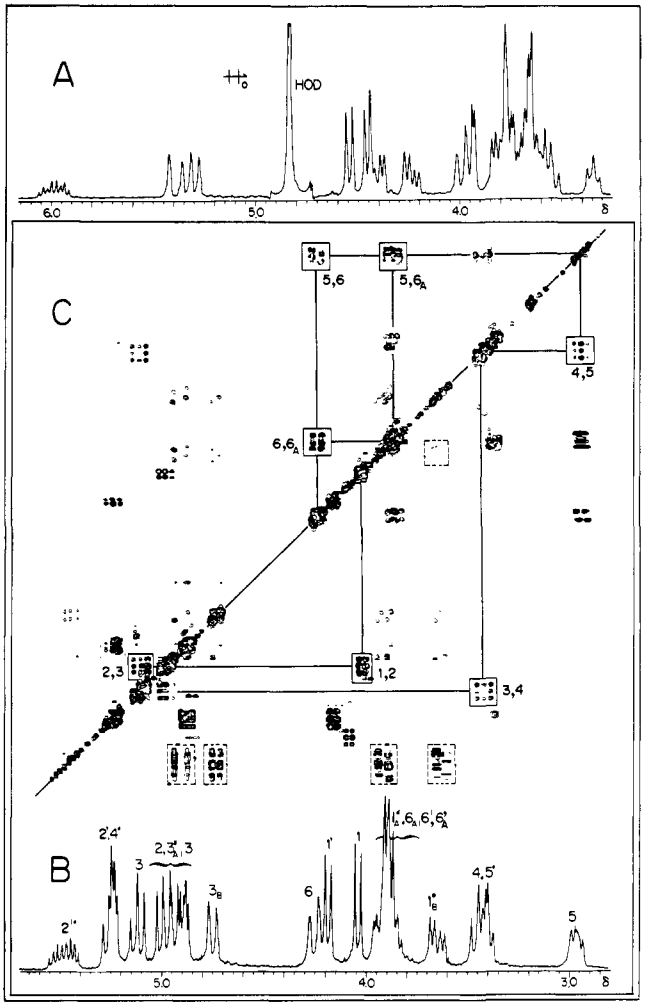


Figure 1. (A) 270-MHz ^1H spectrum of **1** in D_2O solution. (B) ^1H spectrum of **2** (0.2 M in C_6D_6). (C) Proton coupling correlation spectrum ("COSY") of **2**, viewed in the contour mode. Quadrature detection was employed in both dimensions to collect a 256 × 1K data matrix, with 96 acquisitions for each t_1 delay. Acquisition times of 256 ms for t_1 and 513 ms for t_2 were used. Both dimensions were resolution enhanced by a sine multiplication; the F_1 dimension is presented in power mode. Data were accumulated overnight (ca. 11 h), processing required 1–2 h, and the contour plot required <5 min. Cross peaks marked ··· are from the galactosyl moiety, those marked — from the glucosy, and those marked -- from the allyl moeity. Vinylic couplings were not marked for the sake of clarity. (The instrument is based on a 6.35 T Oxford Instrument magnet, Nicolet 293B pulse-programmer, and Nicolet 1180 computer with NTCFTB software; the total data acquisition time was an order of magnitude longer than necessary.)

was poorly dispersed (Figure 1A), the spectrum of the corresponding per-*O*-trideuterioacetylated derivative **2** was studied[6] in C_6D_6 solution (0.2 M, Figure 1B). Projection of the complete, tilted 2-D *J*-resolved data matrix (not shown here) onto the "chemical shift" axis (F_2) gave the equivalent of a "fully proton-decoupled proton spectrum", which consisted of one reasonably narrow line for each of the 19 protons of **2**. Besides providing direct access to all the proton shifts, the sharpness of the lines indicated that all but the two protons (6′, 6′$_A$) were weakly coupled. The magnitudes of all the ^1H–^1H couplings were obtained from the projections onto the "coupling constant" axis (F_1), of the traces corresponding to each of the individual proton shifts.

The second stage involved identifying the multiplets associated with each monosaccharide subspectrum and establishing their connectivity within them; this was best accomplished by use of ^1H–^1H 2-D chemical shift correlation spectroscopy[7] (COSY). In effect the COSY data matrix displays the equivalent of a one-dimensional spectrum along both frequency axes and shows a complex series of responses along one principal diagonal ($F_1 = F_2$). Most importantly, each pair of scalar-coupled protons gives a pair of off-diagonal responses which can be used to map out the connectivity relationship. This is illustrated in Figure 1C for the protons of the D-glucopyranose ring of **2**. The doublet of H-1 shows two off-diagonal responses, one vertically and the other horizontally displaced from the location of the H-1 resonance on the principal diagonal at $F_1 = F_2 = \delta$ 4.05. Tracking back from either response gives the intercept on the principal diagonal corresponding to the shift of H-2 (δ 5.00). In turn, vertical or horizontal excursions from that frequency on the diagonal leads to the off-diagonal responses corresponding to its scalar coupling with H-3. Continuation of this process successively locates H-4, H-5, and both H-6 resonances, at which juncture the connectivity ends. Equivalent evaluations identified the proton resonances of the galactopyranose and allyl moieties.

This combination of 2-D *J*-resolved and COSY measurements readily provided a complete assignment of all three subspectra of **2**. Subsequent comparison of the carbohydrate coupling constants with reference data from the literature[2] unequivocably identified each of the three chemical moieties and the configuration at the two anomeric centers.

The final task remaining was to determine the sequence of the three components in the chain.[8] Irradiation in turn of the H-1 and H-1′ resonances followed by measurement,[9] in the difference mode, of the resultant nuclear Overhauser enhancements showed positive nuclear Overhauser enhancements from several of the protons of the same monosaccharide (especially H-2, H-3, and H-5 or H-2′, H-3′, and H-5′) plus a unique interring nuclear Overhauser enhancement. In each case this latter response came from the resonance contiguous to the glycosidic linkage; that irradiation of H-1′ of the D-galactopyranose moiety induced a response into H-4 of the D-glucopyranose residue confirmed the sequence D-galactopyranosyl-1→4-D-glucopyranoside.

A number of obvious but important observations arise. We do not claim that the protocol is unique;[10,11] nevertheless we are attracted by both its generality and its efficiency. In particular, we note that the combination of two 2-D experiments used here not only is equivalent to an extensive array of one-dimensional experiments but does not suffer from their limitation of either frequency selectivity or spectral dispersion. Insofar that an absolutely minimal amount of reference data is required, this approach appears well suited for the de novo proof of structure of any organic molecule consisting of an array of monomeric units with no interresidue scalar coupling. In the context of oligosaccharide structure determination it is also important to note that, if necessary, the entire operation can be performed with small quantities of material and that any unusual conformation or ring

(5) (a) Aue, W. P.; Karhan, J.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 4226–4227. (b) Bodenhausen, G.; Freeman, R.; Neidermeyer, R.; Turner, D. L. *J. Magn. Reson.* **1977**, *26*, 133–164.

(6) Some oligosaccharides require no derivatization; for others per-*O*-trideuteriomethylation may be considered.

(7) (a) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229–2246. (b) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *44*, 542–561.

(8) For more complex systems additional evidence from proton spin-lattice relaxation measurements is advisable. (a) Hall, L. D.; Preston, C. M. *Carbohydr. Res.* **1973**, *29*, 522–524. (b) Hall, L. D.; Hill, H. D. W. *J. Am. Chem. Soc.* **1976**, *98*, 1269–1270. (c) Hall, L. D.; Wong, K. F.; Hull, W. E.; Stevens, J. D. *J. Chem. Soc., Chem. Commun.* **1979**, 953–955.

(9) Procedure used and references cited in: Hall, L. D.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703–5711.

(10) Studies on glycolipids in dimethyl-d_6 sulfoxide solution by Dabrowski and co-workers relate to this work: Dabrowski, J.; Hanfland, P.; Egge, H.; Dabrowski, V. *Arch. Biochem. Biophys.* **1981**, *210*, 405–411; Koerner, T. D.; Yu, R. K.; Presregard, J. H. *J. Am. Chem. Soc.*, in press. Vliegenthart, Montreuil, and co-workers (van Halbeck, H.; Dorland, L.; Vliegenthart, J. F. G.; Schmid, K.; Montreuil, J.; Fournet, B.; Hull, W. E. *FEBS Lett.* **1980**, *114*, 11–16) have studied related glycopeptides in aqueous media. Oligosaccharide conformations have been determined by Lemieux and Bock (Thøgersen, H.; Lemieux, R. U.; Bock, K.; Myer, B. *Can. J. Chem.* **1982**, *60*, 44–57).

(11) A particularly elegant study on a small protein basic bovine pancreatic trypsin inhibitor has been performed by Wüthrich and co-workers: Nagayama, K.; Wüthrich, K. *Eur. J. Biochem.* **1981**, *114*, 365–374. Wagner, G.; Kumar, A.; Wüthrich, K. *Ibid.* **1981**, *114*, 375–384.

structure is detected automatically by unexpected values for the vicinal couplings.[2,12] Given the ease with which oligosaccharides can be fully *O*-acylated,[13] we draw attention to the substantial advantages of working with such derivatives in organic solvents.[14]

Acknowledgment. This work was supported by operating grants from the Natural Sciences and Engineering Research Council of Canada (to L.D.H.).

Registry No. 1, 52211-61-7; **2**, 82892-18-0.

(12) Bothner-By, A. A. *Adv. Magn. Reson.* **1965**, *1*, 195–316.
(13) Wolfrom, M. L.; Thompson, A. *Methods Carbohydr. Chem.* **1973**, *2*, 211–215.
(14) Use may be made of solvent-induced shifts of minimize distortions arising through tight coupling.

The Endiandric Acid Cascade. Electrocyclizations in Organic Synthesis. 1. Stepwise, Stereocontrolled Total Synthesis of Endiandric Acids A and B†

K. C. Nicolaou,*‡ N. A. Petasis, R. E. Zipkin, and J. Uenishi

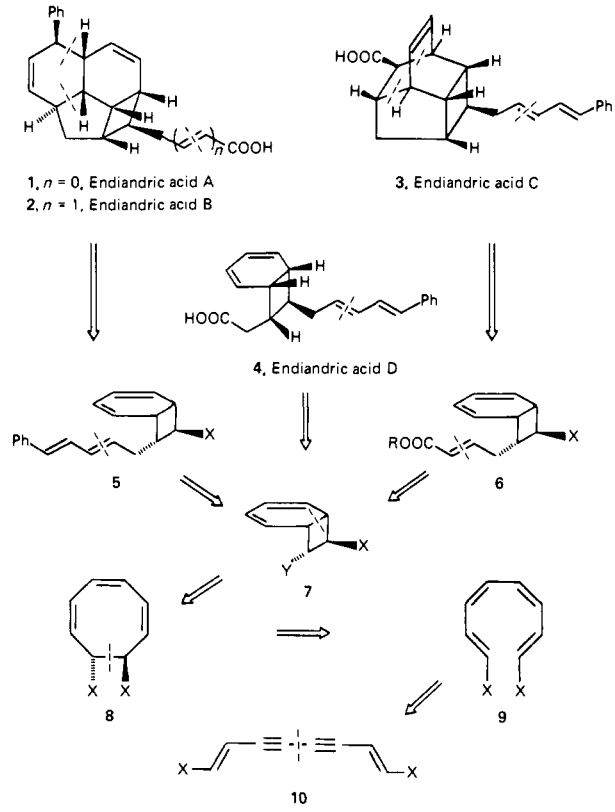
Department of Chemistry, University of Pennsylvania Philadelphia, Pennsylvania 19104

Received May 3, 1982

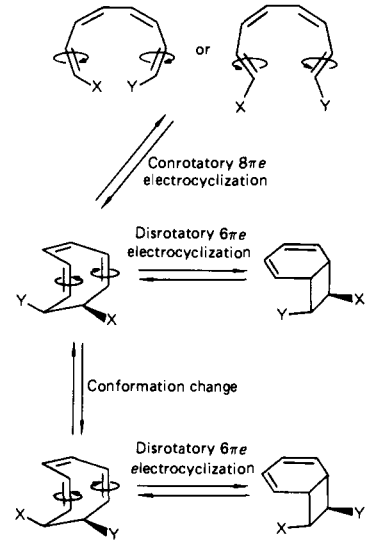
Endiandric acids A–D (**1–4**, Scheme I) were recently isolated from leaves of the Australian plant *Endiandra introrsa* (*Lauraceae*) and their structures firmly established by spectroscopic and X-ray crystallographic techniques.[1] Despite the presence of eight asymmetric centers in these novel polycyclic molecules, they occur in nature in racemic rather than enantiomeric forms, a rather unusual observation for naturally occurring compounds. This phenomenon taken together with the isolation of both types of structures (represented by A/B and C) from the same plant species led Black and his collaborators to propose a brilliant and provocative hypothesis for the "biogenesis" of these compounds in nature from achiral precursors by a series of nonenzymatic electrocyclizations.[1b] On the basis of this hypothesis and relying on well-known[2] but relatively unexplored, thermally allowed by the Woodward–Hoffmann rules,[3] electrocyclizations of the type shown in Scheme II, we devised stereocontrolled total syntheses of all four endiandric acids A–D (**1–4**, Scheme I). In this series of papers we report (a) a stepwise and stereocontrolled approach to these molecules culminating to the first total syntheses of endriandric acids A–D, (b) a "biomimetic" approach to these compounds providing an experimental test to Black's hypothesis, and (c) thermal stability studies leading to confirmation of the biogenetic hypothesis and predictions of the existence of other members of the endiandric acid cascade (endiandric acids E, F and G, see papers 2[4] and 4[6] in this series), which we have also synthesized. We begin with the retrosynthetic analysis of these complex polycyclic frameworks and the stepwise and stereocontrolled total synthesis of endiandric acids A and B (**1** and **2**, Scheme I).

In planning the synthesis of endiandric acids, we sought a general scheme that would allow the construction of each of these

Scheme I. Structures and Retrosynthetic Analysis of Endiandric Acids A–D



Scheme II. Thermally Allowed 8π e and 6π e Electrocyclizations (Woodward–Hoffmann Rules)



† This series of papers is dedicated to the memory of the late Professor Franz Sondheimer.
‡ Fellow of the A. P. Sloan Foundation, 1979–1983; recipient of a Camille and Henry Dreyfus Teacher–Scholar Award, 1980–1984.

(1) (a) Bandaranayake, W. M.; Banfield, J. E.; Black, D. St. C.; Fallon, G. D.; Gatehouse, B. M. *J. Chem. Soc., Chem. Commun.* **1980**, 162. (b) Bandaranayake, W. M.; Banfield, J. E.; Black, D. St. C. *Ibid.* **1980**, 902. (c) Bandaranayake, W. M.; Banfield, J. E.; Black, D. St. C.; Fallon, G. D.; Gatehouse, B. M. *Aust. J. Chem.* **1981**, *34*, 1655. (d) Bandaranayake, W. M.; Banfield, J. E.; Black, D. St. C. *Ibid.* **1982**, *35*, 557. (e) Bandaranayake, W. M.; Banfield, J. E.; Black, D. St. C.; Fallon, G. D.; Gatehouse, B. M. *Ibid.* **1982**, *35*, 567. (f) Endiandric acid D was predicted as a natural product in 1980,[1b] synthesized by us in 1981, and although found in *Endiandra introrsa* (*Lauraceae*) in the same year by Black's group, its structure was not determined until 1982 (personal communication); see also: Banfield, J. E.; Black, D. St. C.; Johns, S. R.; Willing, R. I. *Ibid.*, in press.
(2) See for example: (a) Meister, H. *Chem. Ber.* **1963**, *96*, 1688. (b) Huisgen, R.; Dahmen, A.; Huber, H. *J. Am. Chem. Soc.* **1967**, *89*, 7130. (c) Marvell, E. N.; Seubert, J.; Vogt, G.; Zimmer, G.; Moy, G.; Siegmann, J. R. *Tetrahedron*, **1978**, *34*, 1323. (d) Harris, S. J.; Walton, D. R. M. *Tetrahedron* **1978**, *34*, 1037.
(3) (a) Woodward, R. B.; Hoffmann, R. "The Conservation of Orbital Symmetry"; Verlag Chemie–Academic Press; New York, 1971. See also: (b) Lehr, R. E.; Marchand, A. P. "Orbital Symmetry"; Academic Press; New York, 1972. (c) Fleming, I. "Frontier Orbitals and Organic Chemical Reactions"; Wiley: New York, 1976. (d) Marchand, A. P., Lehr, R. E., Eds.; "Pericyclic Reactions"; Academic Press: New York, 1977; Vol. I, II.
(4) Paper 2: Nicolaou, K. C.; Petasis, N. A.; Uenishi, J.; Zipkin, R. E. *J. Am. Chem. Soc.*, following in this issue.
(5) Paper 3: Nicolaou, K. C.; Zipkin, R. E.; Petasis, N. A. *J. Am. Chem. Soc.*, following in this issue.
(6) Paper 4: Nicolaou, K. C.; Petasis, N. A.; Zipkin, R. E. *J. Am. Chem. Soc.*, following in this issue.

0002-7863/82/1504-5555$01.25/0