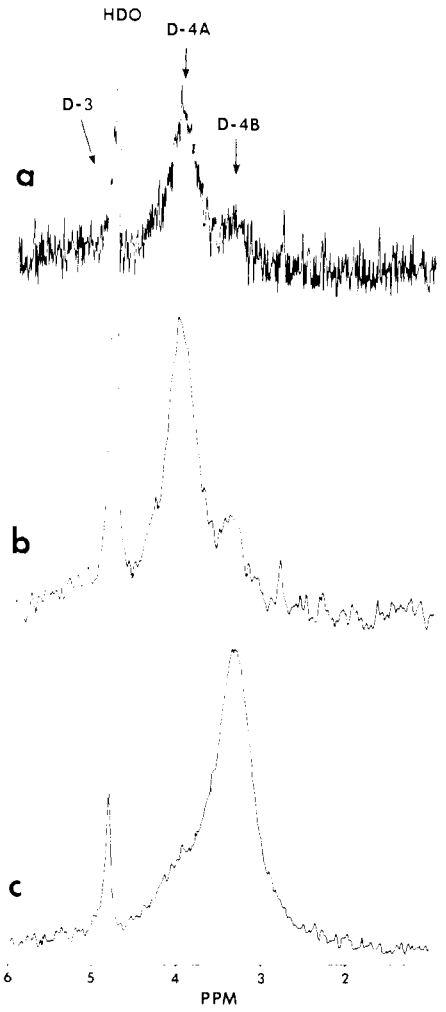


Figure 1. ^2H NMR spectra of nocardicin A (**3**) obtained from incorporations of diastereotopically ^2H labeled serines **8/9** and **10/11** acquired under the following conditions: Bruker WM-300, 46.1 MHz; spectral width 2000 Hz, 4K points, acquistion time 4.096 s; 90° pulse. (a) A 55-mg sample in 2.5 mL of deuterium-depleted water;[20] 52250 transients, zero filling the FID by successive transfers into 8K and 16K of zeros prior to Fourier transformation. (b) As in (a), 51715 transients, sensitivity enhancement achieved by treatment of FID with 1.5-Hz line broadening. (c) A 150 mg sample in 2.5 mL of deuterium-depleted water;[20] 23505 transients, FID treated as in (a) and (b) but with 1.0-Hz line broadening.

at 46.1 MHz in deuterium-depleted water (Aldrich) at 45 °C, pH 7.6.[20] The spectral data obtained under conditions of broad-band proton decoupling are depicted in Figure 1.

A sample of nocardicin A derived from fermentation in the presence of the (2*S*,3*R*)/(2*R*,3*S*)-serines **8/9** gave spectrum a. As was hoped for (cf. Table I), a degree of deuterium enrichment at C-3 was detectable as a weak signal on the downfield side of the HDO resonance. Application of 1.5-Hz line broadening to the FID that gave rise to spectrum a generated spectrum b. The D-3 resonance was now merged with the comparatively intense HDO signal but the distribution of deuterium label at C-4 was clearly discernible, the A position bearing approximately 85% of the heavy isotope. An entirely complementary result was obtained from a second specimen of nocardicin A derived from the diastereomeric (2*S*,3*S*)/(2*R*,3*R*)-serines **10/11**, whose ^2H NMR analysis is displayed as spectrum c. In summary, therefore, L-serine bearing a label at the (3*R*)-locus gave rise to enhanced ^2H content at position 4A while a (3*S*)-label specifically enriched position 4B; that is, within the accuracy of the method, the stereochemical course of β-lactam formation in vivo is substantially, if not exclusively, inversion.

The most direct interpretation of the stereochemical results is a simple nucleophilic displacement of presumably activated seryl hydroxyl by amide nitrogen in an at present hypothetical peptide intermediate to form the critical β-lactam ring. Alternative mechanisms involving intermediate participation of an enzyme nucleophile, whether by displacement or elimination/addition (loss of serine α hydrogen) are of diminished likelihood.

Acknowledgment. We thank Y.-F. Cheung (Merck) and S. J. Benkovic (Penn State) for generously providing experimental details for the serine syntheses, S. B. Christensen of these laboratories for obtaining the ^2H NMR spectra, W. G. Egan (Bureau of Biologics, FDA) and D. E. Cane (Brown University) for helpful technical suggestions regarding ^2H NMR spectroscopy, and M. Koreeda (University of Michigan) for mass spectral analyses. The National Institutes of Health are gratefully acknowledged for financial support (Grants A1 14937 and RR 07041) 07041) and for providing partial funding to acquire the Bruker WM-300 used (Grant GM 27512).

Registry No. 3, 39391-39-4; (±)-**8**, 80612-42-6; (±)-**10**, 80612-43-7; L-serine, 56-45-1.

(20) Line widths at half height for the C-4 deuterons were approximately 30 Hz at 25 °C, broader than expected presumably owing to aggregation (spectrum c was recorded in a solution close to saturation at 25 °C, 0.12 M). So that the rotational correlation times could be reduced, the samples were heated to 45 °C, giving line widths of about 15 Hz. For a demonstrated case of intermolecular association in a species of similar molecular weight investigated by ^2H NMR see: Egan, W. *J. Am. Chem. Soc.* **1976**, *98*, 4091-93. The deuterium-depleted water had a ^2H content 3.3×10^{-3} times natural abundance.

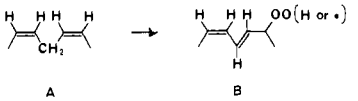
Irreversible Inhibition of the Enzymic Oxidation of Arachidonic Acid to 15-(Hydroperoxy)-5,8,11(*Z*),13(*E*)-eicosatetraenoic Acid (15-HPETE) by 14,15-Dehydroarachidonic Acid

E. J. Corey* and Hokoon Park

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

Received November 18, 1981

The biosynthesis of physiologically important eicosanoids such as prostaglandins and leukotrienes from arachidonic acid involves an initial oxidation of the lipoxygenase type in which a *cis,cis*-1,4-pentadiene unit (**A**) is transformed into a 1-oxygenated *cis,trans*-2,4-pentadiene moiety (**B**).



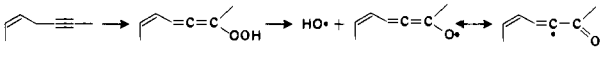
Aspirin, indomethacin, and a number of other nonsteroidal antiinflammatory agents (NSIA's) have been found to inhibit the biosynthesis of prostaglandins (PG's) by blocking the initial step, a lipoxygenase reaction of arachidonic acid at C-11 catalyzed by the so-called cyclooxygenase (CO) enzyme.[1,2] Although the mechanism of action of these empirically discovered and therapeutically valuable PG biosynthesis inhibitors is unclear, they seem to block only the 11-lipoxygenase and to be ineffective as inhibitors of other biooxidation modes of arachidonic acid, for example, the 5-lipoxygenase (leukotriene) pathway.[3] In contrast, eicosa-5,8,11,14-tetraynoic acid (ETYNA) inhibits 11- and 12- but not 5-lipoxygenase reactions of arachidonic acid when used at sufficiently high concentration.[2] Because of the significant biological role of eicosanoids formed by the lipoxygenation of arachidonic acid at different positions (5, 11, 12, and 15) we have been attempting to develop position-selective inhibitors that can selectively block any of the arachidonate oxidation pathways. This com-

(1) Schaaf, T. K. *Annu. Rep. Med. Chem.* **1977**, *12*, 182.
(2) Bailey, D. M.; Chakrin, L. W. *Annu. Rep. Med. Chem.* **1981**, *16*, 213.
(3) Borgeat, P.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 3213.

0002-7863/82/1504-1750$01.25/0

munication and the following one report on our initial studies in this area which have resulted in the rational development of a group of inhibitors that irreversibly block the oxidation of arachidonic acid by either the 15-, 11-, or 5-lipoxygenase (LO) pathways.

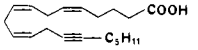
The observation that ETYNA is a good competitive inhibitor of arachidonate oxidation, which is itself relatively resistant to enzymic oxidation, suggested that a dehydroarachidonic acid (DHA) in which only one double bond is replaced by a triple bond might be more strongly bound by a lipoxygenase enzyme and might be much more susceptible to lipoxygenation. Of great interest with regard to lipoxygenation at the acetylenic bond is the fate of the resulting allenic hydroperoxide (C), which we



C D

estimate could undergo O—O bond homolysis with an activation energy as low as 13 kcal/mol to form HO radical and D.[4] Obviously homolysis of **C** at or near the catalytic site could result in irreversible deactivation of the lipoxygenase. On the basis of these considerations 5,6-, 8,9-, 11,12-, and 14,15-DHA's have been synthesized and tested as lipoxygenase inhibitors. Described herein are the results with soybean lipoxygenase, a 15-lipoxygenase of arachidonic acid (15-LO(SB)), which demonstrate that 14,15-DHA (**1**) is a potent and irreversible inhibitor in comparison with 5,6-, 8,9-, and 11,12-DHA's.

14,15-DHA (**1**) was synthesized from arachidonic acid with the first step being conversion to the peroxy acid and internal oxygen transfer to give 14,15-oxidoarachidonic acid (98%).[5] Esterification (diazomethane in ether) and subsequent exposure to saturated potassium bromide in acetic acid–water–tetrahydrofuran (20:3:4) at 4 °C for 4 h under argon gave a mixture of 14,15-bromohydrins of methyl arachidonate (94%),[6a] which was oxidized by using Jones' reagent in acetone at −20 °C for 20 min to afford a mixture of corresponding 14-bromo-15-keto ester and 15-bromo-14-keto ester (82%).[6a] Treatment of the mixture of keto esters with 2 equiv of *p*-toluenesulfonylhydrazine in methylene chloride–acetic acid (2:1) in the presence of 0.1 equiv of hydroquinone at 23 °C for 32 h gave after extractive isolation and purification by thin-layer chromatography on silica gel methyl 14,15-dehydroarachidonate (52%),[6] which was saponified to 14,15-DHA (**1**)[6a] with 1 N lithium hydroxide in dimethoxyethane–water (5:2) at 23 °C for 11 h under argon. After acidification and extractive isolation, **1** was obtained as a colorless



oil, which was stored in frozen benzene at −20 °C under argon prior to use. 5,6-, 8,9-, and 11,12-DHA's were synthesized as described elsewhere.[7-9]

Biosynthesis of 15-HPETE[10] from arachidonic acid (99+% purity) was studied by using type I soybean lipoxygenase of ca. 85% purity[11] (Sigma Chemical Co.) in 0.2 M sodium borate buffer (pH 9.0) as medium[12] at 23 °C assaying for the product, 15-HPETE (λ_{max} 236 nm, ϵ 27 200),[13] by absorbance measurements at 236 nm. With standard conditions being 1.2 μg (9.35 pmol) of 15-LO(SB) enzyme and 40 μg (0.132 μmol) of arachidonic acid in 2 mL (total volume) of aerated buffer at 23 °C, a maximum conversion to 15-HPETE of 47% of theoretical was observed 90 min of incubation time. Since the enzyme is relatively stable under the same conditions but in the absence of arachidonic acid, it is evident that the catalytic process itself leads to loss of enzymic activity. A total turnover number of 6.6×10^3 follows from the data for the 15-LO(SB) used in these experiments.[14]

Aerobic incubation of the enzyme 15-LO(SB) with 1.2 nmol of 14,15-DHA (0.6 μM) under the above detailed standard conditions, but in the absence of arachidonic acid, resulted in ca. 50% deactivation of the enzyme as shown by the reduced ability of the resulting solution to convert added arachidonic acid to 15-HPETE, both with regard to rate and maximum conversion to 15-HETE. Under these conditions the half-life for pre-incubation, time-dependent deactivation was roughly 2 min, and no increase in deactivation of enzyme was observed upon prolonging the preincubation time with 14,15-DHA and enzyme beyond 10 min. With a 10-min preincubation time of enzyme with varying amounts of 14,15-DHA, a linear relationship was observed between the molar ratio of 14,15-DHA and the degree of deactivation of the enzyme, complete deactivation requiring a DHA/enzyme molar ratio of ca. 260 (giving a deactivation efficiency of 0.38%).

Anaerobic incubation of 15-LO(SB) with 130 nmol of 14,15-DHA (molar ratio 1:13,900) under argon at 23 °C for several hours resulted in no detectable deactivation of the enzyme as measured by the initial ability of the solution to catalyze formation of 15-HPETE when arachidonic acid and oxygen are introduced. Thus deactivation is not due to the presence of 14,15-DHA per se but is intimately connected with its oxidation.

Results paralleling those described above for type I 15-LO(SB) were obtained also for type V soybean lipoxygenase (Sigma Chemical Co., 99+% purity, purified by affinity chromatography).

The efficiency of inhibition of the enzyme 15-LO(SB) by 14,15-DHA was the same in the presence of sodium borohydride (160 equiv relative to 14,15-DHA) as without, consistent with the notion that the inactivating species (e.g., **C**) is short-lived and perhaps functions just after its formation at the active site. Sodium borohydride is known to reduce 15-HPETE to 15-HETE without deactivation of the enzyme 15-LO(SB) and also to increase the maximum conversion of arachidonic acid to 15-HETE by a fixed amount of enzyme.[13]

Use of 5,6-, 8,9-, or 11,12-DHA as test inhibitors of 15-LO(SB) oxidation of arachidonic acid to 15-HPETE in place of 14,15-DHA resulted in no detectable deactivation of the enzyme at concentrations 100 times that at which 14,15-DHA shows unambiguous deactivation when the standard preincubation techniques are used.

Experiments on the deactivation of 15-LO(SB) by radiolabeled (2-tritio) 14,15-dehydroarachidonic acid suggest that the irreversible inhibition is accompanied by chemical binding of 14,15-DHA to the enzyme, although the exact nature of the linkage has yet to be determined. Tritiated **1** (2.3 Ci/mol) was prepared from the methyl ester by α-deprotonation with lithium diisopropylamide in THF at −78 °C for 5 min followed by quenching with tritium oxide, chromatographic purification of the resulting radiolabeled ester, and saponification. Aerobic incubation of 0.14 nmol of 15-LO(SB) with just sufficient tritiated 14,15-DHA (33 nmol, 17 430 dpm) to effect complete deactivation afforded a solution containing inactivated enzyme, from which 16 510 dpm of tritiated fatty acid could be recovered by acidification to pH 3 and seven extractions with ether (further extraction was essentially ineffective). The aqueous layer from the extractions was then passed through a column containing 50 mg of C_{18}-silanized silica gel (10 μ), and the column was washed with water (1.5 mL) until no more radioactive material could be removed. A total of 768 dpm of labeled material was contained

(4) This estimate has been made from the delocalization energy of an allylic radical (ca. 15 kcal/mol) augmented to 20 kcal/mol to reflect the greater strength of the carbonyl π bond (ca. 70 kcal/mol) relative to the olefinic π bond (ca. 60 kcal/mol), with the RO–OH bond dissociation energy taken as ca. 33 kcal/mol.

(5) Corey, E. J.; Niwa, H.; Falck, J. R. *J. Am. Chem. Soc.* **1979**, *101*, 1586.

(6) Satisfactory (a) infrared and proton magnetic resonance spectra and (b) mass spectra were obtained for this compound by using chromatographically purified and homogeneous samples.

(7) Corey, E. J.; Munroe, J. *J. Am. Chem. Soc.* **1982**, *104*, 0000.

(8) Corey, E. J.; Kang, J. *Tetrahedron Lett.* in press.

(9) Compare the synthesis of 5,6-dehydroarachidonic acid: Corey, E. J.; Park, H.; Barton, A.; Nii, Y. *Tetrahedron Lett.* **1980**, *21*, 4243.

(10) Hamberg, M.; Samuelsson, B. *J. Biol. Chem.* **1967**, *242*, 5329.

(11) Verified by gel electrophoretic analysis.

(12) Pistorius, E. K.; Axelrod, B. *J. Biol. Chem.* **1974**, *249*, 3183.

(13) Baldwin, J. E.; Davies, D. I.; Hughes, L.; Gutteridge, N. A. *J. Chem. Soc., Perkin Trans. 1* **1979**, 115.

(14) A total turnover number of 15.8×10^3 has been reported previously for the oxidation of linoleic acid by type I soybean lipoxygenase: Christopher, J.; Pistorius, E.; Axelrod, B. *Biochim. Biophys. Acta* **1970**, *198*, 12.

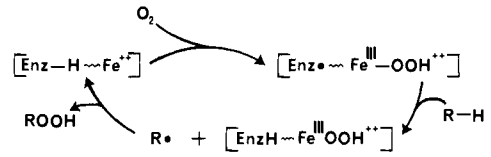


Figure 1.

in the eluent and 65 dpm remained on the C_{18} column (total recovery of radioactivity 99.5%). The radioactivity remaining on the column corresponds to 0.122 nmol of tritiated 14,15-DHA, that is, 0.87 molecule of 14,15-DHA per molecule of enzyme. It was demonstrated in a separate experiment that the lipoxygenase enzyme itself is not eluted from a C_{18} column under these conditions whereas labeled 14,15-DHA is. In a parallel experiment with 15-LO(SB) and tritiated 11,12-DHA, conducted exactly as described above for the enzyme and labeled 14,15-DHA, it was shown that by starting with 13 980 dpm of 11,12-DHA, 12 850 dpm were recovered by extraction and 718 dpm by elution of the C_{18} column, with essentially no radioactivity (0–10 dpm) remaining on the column. Taken together these results show that ca. 1 molecule of 14,15-DHA becomes tightly bound to each molecule of deactivated 15-LO(SB) enzyme, whereas the isomeric 11,12-DHA is neither a strong irreversible inhibitor nor tightly bound. Since covalent binding of 14,15-DHA to deactivated 15-LO(SB) seems a logical possibility, degradative studies on the inactivated enzyme are in order.

Paralleling the findings reported above on the deactivation of soybean lipoxygenase by 14,15-DHA are the results presented in the following communication, which demonstrate a similar irreversible inactivation of arachidonate cyclooxygenase by 11,12-DHA. Further, preliminary data indicate irreversible blockage of the 5-lipoxygenase (leukotriene) pathway by 5,6-DHA. Thus it appears that the presence of a single acetylenic unit in place of a *Z*-ethylenic unit at a site of lipoxygenation of arachidonic acid leads to potent and irreversible inhibition of the corresponding lipoxygenase pathway.

Despite the heuristic value of the hypothesis leading to the discovery of DHA's as lipoxygenase inhibitors, the mechanism by which enzyme deactivation occurs still remains to be established, as in fact is true also for the mechanism of the lipoxygenase reaction itself. Present thinking favors the intermediacy of pentadienyl free radicals[15] and a catalytic scheme of the general type shown in Figure 1,[16,17] in which RH is the polyunsaturated fatty acid and [Enz–H⋯Fe^{2+}] is the lipoxygenase enzyme. Autodestruction of the lipoxygenase enzyme during catalysis could be the result of any of the following: (1) reaction of Enz· with oxygen, ROOH, R·, or radicals produced from ROOH; (2) reaction of Enz–H at one or more vulnerable sites other than the group holding the explicit donor H with oxygen, ROOH, R·, or radicals produced from ROOH. Radical formation from ROOH might be promoted by the enzyme itself (e.g., at the Fe^{2+} site) or might be spontaneous. Clearly 14,15-DHA may function by masquerading as arachidonic acid and by enhancing one or more of these catalytically destructive processes.[18]

Registry No. 1, 80738-21-2; 5,6-DHA, 58688-54-3; 8,9-DHA, 80738-22-3; 11,12-DHA, 80738-23-4; 15-HPETE, 69371-38-6; arachidonic acid, 506-32-1; 14,15-oxidoarachidonic acid, 74868-37-4; methyl 14-bromo-15-hydroxy-5,8,11-eicosatrienoate, 80738-24-5; methyl 14-hydroxy-15-bromo-5,8,11-eicosatrienato, 80738-25-6; methyl (*Z*,*Z*,*Z*)-14-bromo-15-keto-5,8,11-eicosatrienoato, 80738-26-7; methyl (*Z*,*Z*,-*Z*)-15-bromo-14-keto-5,8,11-eicosatrienoate, 80738-27-8; methyl 14,15-dehydroarachidonate, 80738-28-9.

(15) DeGroot, J. J. M. C.; Garssen, G. J.; Vliegenthart, J. F. G.; Boldingh, J. *Biochim. Biophys. Acta* **1973**, *326*, 279.
(16) DeGroot, J. J. M. C.; Veldink, G. A.; Vliegenthart, J. F. G.; Boldingh, J.; Wever, R.; van Gelder, B. F. *Biochim. Biophys. Acta* **1975**, *377*, 71.
(17) "Plant Lipid Biochemistry"; Hitchcock, C., Nichols, B. W., Eds.; Academic Press: New York, 1971; p 226.
(18) This research was assisted financially by grants from the National Science Foundation and the National Institutes of Health. We are indebted to Dr. Jahyo Kang and John Munroe for providing tritiated 11,12- and 14,15-DHA.

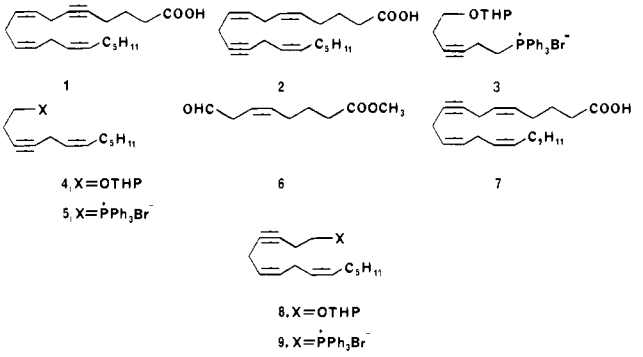
Irreversible Inhibition of Prostaglandin and Leukotriene Biosynthesis from Arachidonic Acid by 11,12-Dehydro- and 5,6-Dehydroarachidonic Acids, Respectively

E. J. Corey* and John E. Munroe

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138
Received November 18, 1981

Four oxidative pathways are now known for the biosynthesis of physiologically active eicosanoids from arachidonic acid, each commencing with an enzymic lipoxygenase (LO) type reaction.[1] The 5-LO pathway leading to leukotrienes and the 11-LO pathway leading to prostaglandins (PG's) and thromboxanes are of special interest currently because of their relevance to a number of disease states.[1] Effective and selective inhibitors of specific LO pathways could be of service in unraveling the biological effects of the numerous naturally occurring eicosanoids. Nonsteroidal antiinflammatory agents such as aspirin inhibit the 11-LO (cyclooxygenase, CO) pathway only. In the preceding communication[2] a rationale was outlined for the use of monoacetylenic, dehydroarachidonic acids as position-selective lipoxygenase inhibitors and evidence was presented that the arachidonic 15-LO from soybean is subject to potent and irreversible inhibition by 14,15-dehydroarachidonic acid (14,15-DHA) but not by 5,6-, 8,9-, or 11,12-dehydroarachidonic acids. Described herein are the effects of these DHA's on prostaglandin biosynthesis and also initial studies on the inhibition of leukotriene biosynthesis by 5,6-DHA.

Synthesis of 5,6-, 8,9-, 11,12-, and 14,15-DHA's. 5,6-DHA (**1**)



was available from arachidonic acid as recently described[3] and also by an independent total synthesis.[4] 14,15-DHA was prepared as described in the foregoing paper.[2] 8,9- and 11,12-DHA's were made by short and efficient total syntheses utilizing as a common intermediate the readily available acetylenic phosphonium bromide **3**.[5] For the synthesis of 11,12-DHA (**2**) the ylide derived from **3** (1 equiv of *n*-BuLi in THF[6] at −78 to −30 °C, for 30 min) in 9:1 THF–HMPT at −78 °C was treated with hexanal (0.9 equiv) to afford after reaction at −78 °C for 30 min and at −78 to 0 °C

(1) For reviews see: (a) Bailey, D. M.; Chakrin, L. W. *Annu. Rep. Med. Chem.* **1981**, *16*, 213. (b) Schaaf, T. K. *Ibid.* **1977**, *12*, 182.
(2) Corey, E. J.; Park, H. *J. Am. Chem. Soc.* **1982**, *104*, 0000.
(3) Corey, E. J.; Park, H.; Barton, A.; Nii, Y. *Tetrahedron Lett.* **1980**, *21*, 4243.
(4) Corey, E. J.; Kang, J. *Tetrahedron Lett.*, in press. Credit is due to a referee for suggesting that this paper be published separately.
(5) The phosphonium bromide **3** was prepared from the known corresponding alcohol, the monotetrahydropyranyl ether of 3-hexyne-1,6-diol [Raphael, R. A.; Roxburgh, C. M. *J. Chem. Soc.* **1952**, 3875] by the following sequence: (1) reaction with 1.5 equiv of carbon tetrabromide, 1.1 equiv of pyridine, and 1.0 equiv of triphenylphosphine in methylene chloride (ca. 1 M in alcohol) at 23 °C for 2.5 h to form the bromide tetrahydropyranyl ether (90% isolated yield after nonaqueous isolation and passage through a column of silica gel); (2) reaction of the bromide with 1.5 equiv of triphenylphosphine in toluene (or acetonitrile where indicated) at reflux for 26 h and treatment of the resulting crude product with 0.003 equiv of *p*-toluenesulfonic acid and 1.1 equiv of dihydropyran in methylene chloride at 0 °C for 30 min (to replace any of the THP group lost in the previous step) to afford after nonaqueous workup the crystalline phosphonium salt **3** (93% overall yield), mp 111–115 °C.
(6) Chemical abbreviations: THF, tetrahydrofuran; HMPT, hexamethylphosphoric triamide.

0002-7863/82/1504-1752$01.25/0