Phosphonolipids 4. A phosphonic acid analogue of platelet activating factor[1,2]

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A phosphonic acid analogue of platelet activating factor (PAF) bearing a backbone that is isosteric with and has stereochemistry corresponding to that of natural PAF has been synthesized. The synthesis uses a route maintaining stereochemical integrity.

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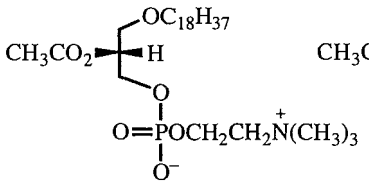
On a synthétisé un analogue du facteur activant des plaquettes (FAP) contenant de l'acide phosphonique et comportant un squelette isostère et de la même stéréochimie que le FAP naturel. La synthèse fait appel à une route qui préserve l'intégrité stéréochimique.

[Traduit par la redáction]

Introduction

Since the discovery (3) of the series of natural ether lipids 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphorylcholine (**1**), also known by a name describing one of its biological functions, platelet activating factor (PAF), the natural material and structural analogues thereof have been targets of numerous laboratory syntheses (for a review of this area of effort, see ref. 4). These efforts have been stimulated by the particularly intriguing range of biological activities associated with natural PAF (5).

In the continuing efforts of our laboratory toward the preparation and investigation of phosphonic acid analogues of natural phosphate esters, we herein describe the synthesis of a new analogue of **1** which is anticipated to serve as a particularly useful probe of PAF activity. This analogue is one in which are incorporated fundamental structural similarities with **1** while rendering the backbone resistant to hydrolysis at the phosphorus by the incorporation of a carbon–phosphorus bond (6). Specifically, this analogue, illustrated as **2**, bears a backbone that is isosteric with that of **1** and a stereogenic center of the same absolute configuration as found in **1**.



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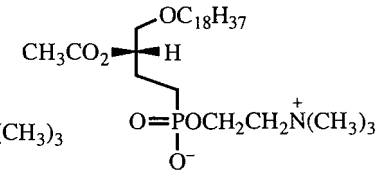

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Results and discussion

The synthesis of **2** is accomplished starting with (*S*)-malic acid as the source of the stereogenic carbon site. The conversion of (*S*)-malic acid into (*S*)-4-benzyloxy-1,2-butanediol is accomplished in five steps as previously reported (7). Continued conversion of the (*S*)-4-benzyloxy-1,2-butanediol into the target material **2** has been accomplished as illustrated in Scheme 1.

The regiospecificity of the tosylation step, and of the alcohol attack of the epoxide by the alcohol in the presence of boron trifluoride etherate, have been established by prior work of this and other laboratories (2, 8, 9). Once acetylation is accomplished, the remainder of the route involves standard procedures for deprotection of the primary hydroxyl group, conversion to the alkyl chloride, phosphonylation via the Arbuzov reaction, and final functionalization. The selective cleavage of the phosphonate methyl ester groups is accomplished under very mild conditions using bromotrimethylsilane followed by work-up with aqueous tetrahydrofuran (10). Esterification of **10** to generate the target choline derivative **2** was accomplished using a technique previously applied for the preparation of choline derivatives (11).

Experimental

General

All chemicals were of reagent quality and were used without further purification with the following exceptions: chloroform was distilled over phosphorus pentoxide; pyridine was dried over calcium hydride and distilled; dimethylformamide (DMF) and methanol were dried over molecular sieves 4A prior to use. (*S*)-4-Benzyloxy-1,2-butanediol and its precursors were prepared as previously described (7). Thin-layer chromatography (TLC) was performed using Polygram Sil N-HR sheets. Silica gel for flash chromatography was from EM Science (230–300 mesh). Infrared spectra were measured using a Perkin–Elmer 1600 FTIR instrument, and ^{1}H and ^{31}P NMR spectra were measured using an IBM-Bruker WP200SY instrument. Optical rotations were measured using a Jasco DIP-140 digital polarimeter. Elemental analyses were performed by Desert Analytics of Tucson, Arizona, and by Schwarzkopf Microanalytical Laboratories of Woodside, New York.

Preparation of (S)-4-benzyloxy-2-hydroxy-1-tosyloxybutane (3)

In a reaction system under a nitrogen atmosphere the (*S*)-4-benzyloxy-1,2-butanediol (4.20 g, 21.4 mmol) was dissolved in a mixture of chloroform (50 mL) and pyridine (3.6 mL) and the resultant mixture was stirred at 0°C for 5 min. After this time there was added *p*-toluenesulfonyl chloride (4.10 g, 21.4 mmol) in chloroform (5 mL) solution over a period of 2 min. The mixture was stirred at 0°C for 2.5 h, after which time the cooling bath was removed and the mixture was stirred for an additional 2 h at ambient temperature. After this time diethyl ether (200 mL) was added with stirring and the mixture was washed with water (2 × 15 mL). The organic layer was dried over anhydrous calcium chloride, fil-

[1]A preliminary report of this work has been presented, see ref. 1.

[2]Paper 3 in this series is ref. 2.

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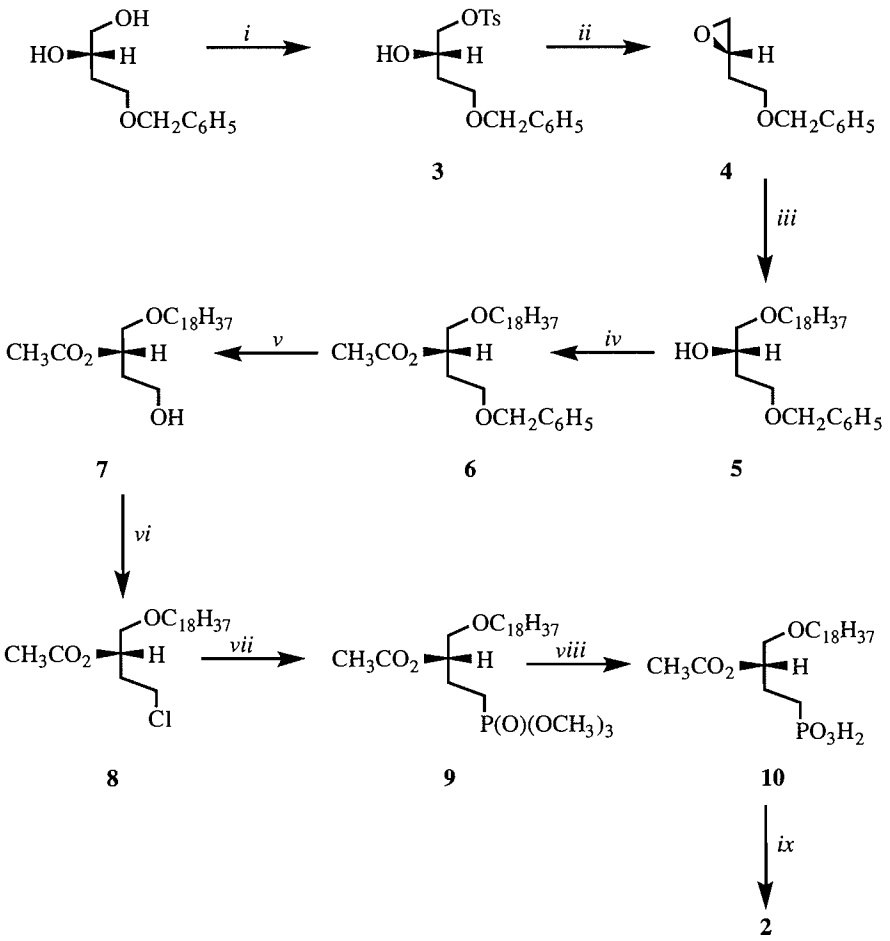

i. TsCl, $CHCl_3$, pyridine, 0°C (~94% yield); *ii*. methanol, anh. K_2CO_3, −10°C (98% yield); *iii*. BF_3 etherate, methylene chloride, 1-octadecanol, 0°C (~95% yield); *iv*. acetic anhydride, methylene chloride, DMAP, 0°C (97% yield); *v*. 95% ethanol, H_2, 5% Pd/C, ambient temp. (97% yield); *vi*. methylene chloride, tetrachloromethane, triphenyl phosphine, ambient temp. (89% yield); *vii*. DMF, trimethyl phosphite, 135°C (59% yield); *viii*. methylene chloride, TMSBr, −10°C; THF/water, ambient temp. (90% yield); *ix*. pyridine, choline tosylate, trichloroacetonitrile, 50°C (39% yield).

SCHEME 1

tered, and the volatile materials were evaporated under reduced pressure to give the desired material (7.00 g, 94%), which exhibited a major spot on TLC (R_f 0.80, hexane ethyl acetate 2:1) with a trace of starting material, and IR and ^{1}H NMR spectra in accord with the proposed structure **3**. Owing to its highly reactive nature, the material was not further purified, but used immediately in the continuing reaction. The IR spectrum (CCl_4) exhibited the following signals (partial) (cm^{-1}): 3500 (broad), 3050–3150, 2900–2990, 1510, 1470, 1400, 1270, 1180, 1100. The ^{1}H NMR spectrum ($CDCl_3$) exhibited the following signals: δ 1.87, multiplet, 2H; δ 2.34, singlet, 3H; δ 2.98, broad singlet, 1H; δ 3.35–3.85, broad multiplet, 4H; δ 4.00, multiplet, 1H; δ 4.51, doublet, 2H, J = 5 Hz; δ 7.25, broad singlet, 5H; δ 7.60, AA'BB', 4H.

Preparation of (S)-4-benzyloxy-1,2-epoxybutane (4)

(*S*)-4-Benzyloxy-2-hydroxy-1-tosyloxybutane (**3**) (3.5 g, 10.0 mmol) was dissolved in dry methanol (30 mL) and cooled to −10°C, and anhydrous potassium carbonate (1.70 g, 12.3 mmol) was added. The reaction mixture was stirred at −10°C for 3 h and allowed to come to room temperature with stirring for an additional 2 h. After this time diethyl ether (50 mL) was added and the resultant precipitate was removed by filtration through a pad of silica gel. The precipitate was washed with diethyl ether (100 mL) and the combined filtrates were evaporated under reduced pressure. The residue was purified by flash chromatography (20 g, 2 cm diameter) eluted using hexane:chloroform mixtures. The fractions exhibiting R_f 0.70 on TLC (hexane:chloroform 2:1) were combined and evaporated under reduced pressure to give the pure desired material (1.65 g, 98.0%), which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure **4**. The IR spectrum (CCl_4) exhibited the following signals (partial) (cm^{-1}): 3010–3050, 2850–2990, 1510, 1480, 1450, 1410, 1350, 1130. The ^{1}H NMR spectrum ($CDCl_3$) exhibited the following signals: δ 1.85, multiplet, 2H; δ 2.51, multiplet, 1H; δ 2.76, multiplet, 1H; δ 3.10, multiplet, 1H; δ 3.62, multiplet, 2H; δ 4.58, singlet, 2H; δ 7.38, broad singlet, 5H. Anal. calcd for $C_{11}H_{14}O_2$: C 74.13%, H 7.92%; found: C 74.49%, H 7.89%. Specific rotation: $[\alpha]_{25}$ = −14.8 (0.08 M, ethanol).

Preparation of (S)-4-benzyloxy-2-hydroxy-1-octadecyloxybutane (5)

In a reaction system under a nitrogen atmosphere a 10% solution of boron trifluoride in methylene chloride (0.5 mL) was added to a mixture of (*S*)-4-benzyloxy-1,2-epoxybutane (**4**) (1.60 g, 9.0 mmol) and 1-octadecanol (2.43 g, 9.0 mmol) in methylene chloride (25 mL) at 0°C. After stirring for 10 min, the reaction mixture was allowed to warm to room temperature and to stir for 24 h. After this time volatile materials were removed under reduced pressure and the residue was purified by flash chromatography (20 g, 2 cm diameter) eluted using hexane:chloroform

mixtures. The fractions exhibiting R_f 0.60 (chloroform:hexane 3:1) were combined and evaporated under reduced pressure to give the pure desired material (3.90 g, 97%), which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure **5**. The IR spectrum (CCl_4) exhibited the following signals (partial) (cm^{-1}): 3450 (broad), 3010–3050, 2850–2990, 1480, 1400, 1150. The ^{1}H NMR spectrum ($CDCl_3$) exhibited the following signals: δ 0.91, triplet, 3H, J = 6 Hz; δ 1.12–1.52, broad singlet, 32H; δ 1.80, triplet, J = 6 Hz, 2H; δ 3.25–4.02, broad multiplet, 8H; δ 4.52, singlet, 2H; δ 7.29, singlet 5H. Anal. calcd. for $C_{29}H_{52}O_3$: C 77.62%, H 11.68%; found: C 77.91%, H 11.83%. Specific rotation: $[\alpha]_{25} = -15.7$ (0.022 M, ethanol).

Preparation of (S)-2-acetyloxy-4-benzyloxy-1-octadecyloxybutane (6)

(*S*)-4-Benzyloxy-2-hydroxy-1-octadecyloxybutane (**5**) (3.00 g, 6.68 mmol) was added to a mixture of methylene chloride (15 mL) and acetic anhydride (15 mL). The mixture was stirred at 0°C for 10 min, after which time *p*-(dimethylamino)pyridine (20 mg) was added. The mixture was allowed to warm to room temperature and stirring was continued for 24 h. After this time volatile materials were evaporated under reduced pressure and the liquid residue was purified by flash chromatography (20 g, 2 cm diameter) eluted using hexane: chloroform mixtures. Fractions exhibiting R_f 0.50 (hexane:chloroform 1:1) were combined and evaporated to give the pure desired material (3.20 g, 97%), which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure **6**. The IR spectrum (CCl_4) exhibited the following signals (partial) (cm^{-1}): 3010–3050, 2850–2990, 1740, 1480, 1380, 1290, 1100, 1030. The ^{1}H NMR spectrum ($CDCl_3$) exhibited the following signals: δ 0.90, triplet, 3H, J = 6 Hz; δ 1.15–1.55, broad singlet, 32H; δ 1.82, multiplet, 2H; δ 2.09, singlet, 3H; δ 3.25–3.82, broad multiplet, 7H; δ 4.52, singlet, 2H; δ 7.38, singlet, 5H. Anal. calcd. for $C_{31}H_{54}O_4$: C 75.87%, H 11.09%; found: C 75.61%, H 11.12%. Specific rotation: $[\alpha]_{25} = -14.9$ (0.029 M, ethanol)

Preparation of (S)-3-acetyloxy-1-hydroxy-4-octadecyloxybutane (7)

A mixture of (S)-2-acetyloxy-4-benzyloxy-1-octadecyloxybutane (**6**) (2.02 g, 4.11 mmol) was stirred with 95% ethanol (25 mL) and 5% palladium on charcoal (0.51 g) under a hydrogen atmosphere (1 atm (101.3 kPa)) for 20 h at ambient temperature. After this time the mixture was filtered through a pad of silica gel and the solid was washed with ethanol (75 mL). The combined filtrates were evaporated under reduced pressure and the residue was purified by flash chromatography (20 g, 2 cm diameter) eluted using hexane:ethyl acetate mixtures. The fractions exhibiting R_f 0.72 (hexane:ethyl acetate 3:1) were combined and evaporated under reduced pressure to give the pure desired material (1.60 g, 97%), which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure **7**. The IR spectrum (CCl_4) exhibited the following signals (partial) (cm^{-1}): 3580 (sharp), 3450 (broad), 2850–2990, 1830, 1740, 1480, 1380, 1110. The ^{1}H NMR spectrum ($CDCl_3$) exhibited the following signals: δ 0.89, triplet, 3H, J = 6 Hz; δ 1.28–1.40, broad singlet, 32H: δ 1.71, multiplet, 2H; δ 2.08, singlet, 3H; δ 2.50, broad singlet, 1H; δ 3.30–3.50, broad multiplet, 5H; δ 4.24, multiplet, 2H. Anal. calcd. for $C_{24}H_{48}O_4$: C 71.95%, H 12.08%; found: C 71.63%, H 12.00%. Specific rotation: $[\alpha]_{25} = -9.5$ (0.26 M, ethanol).

Preparation of (S)-3-acetyloxy-1-chloro-4-octadecyloxybutane (8)

To a solution of (*S*)-3-acetyloxy-1-hydroxy-4-octadecyloxybutane (3.90 g, 9.73 mmol) in methylene chloride (5 mL) and tetrachloromethane (3.24 g, 21.1 mmol) was added dropwise a solution of triphenylphosphine (3.57 g, 9.73 mmol) in methylene chloride (6 mL) over a period of 5 h. After completion of the addition, the reaction mixture was stirred at ambient temperature for an additional 2 h, at which time pentane (40 mL) was added, resulting in the formation of a white precipitate. The precipitate was removed by filtration, washed with pentane (50 mL), and the filtrates were combined and volatile materials were evaporated under reduced pressure. The residue was purified by flash chromatography (20 g, 2 cm diameter) eluted using hexane:ethyl acetate mixtures. The fractions exhibiting R_f 0.80 (hexane:ethyl acetate 5:1) were combined and evaporated under reduced pressure to give the pure desired material (3.62 g, 89%), which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure **8**. The IR spectrum (CCl_4) exhibited the following signals (partial) (cm^{-1}): 2850–2990, 1730, 1480, 1360, 1240, 1100, 1040. The ^{1}H NMR spectrum ($CDCl_3$) exhibited the following signals: δ 0.89, triplet, 3H, J = 6 Hz; δ 1.28–1.40, broad singlet, 32H; δ 1.52, multiplet, 2H; δ 2.08, singlet, 3H; δ 3.40–3.60, multiplet, 7H. Anal. calcd. for $C_{24}H_{47}O_3Cl$: C 68.78%, H 11.30%; found: C 68.60%, H 11.20%. Specific rotation: $[\alpha]_{25} = -11.8$ (0.026 M, ethanol).

Preparation of dimethyl (S)-3-acetyloxy-4-octadecyloxybutyl-1-phosphonate (9)

A mixture of (*S*)-3-acetyloxy-1-chloro-4-octadecyloxybutane (**8**) (3.62 g, 8.63 mmol), DMF (10 mL), and trimethyl phosphite (15 g, 120 mmol) was heated at 140°C for 48 h with continual stirring. After that time the volatile materials were evaporated under reduced pressure and the residue was purified by flash chromatography (20 g, 2 cm diameter) eluting with chloroform. In addition to the recovery of unchanged **8** (1.40 g), fractions exhibiting R_f 0.40 (chloroform) were combined and the volatile materials were evaporated to give the pure desired material (2.50 g, 59%), which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure **9**. The IR spectrum (CCl_4) exhibited the following signals (partial) (cm^{-1}): 2850–2990, 1740, 1480, 1230, 1040. The ^{1}H NMR spectrum ($CDCl_3$) exhibited the following signals: δ 0.88, triplet, 3H, J = 6 Hz; δ 1.28–1.40, broad singlet, 32H; δ 1.49–1.65, multiplet, 4H; δ 2.08, singlet, 3H; δ 3.35–3.60, multiplet, 5H; δ 3.80, doublet, 6H, J = 12 Hz. Anal. calcd. for $C_{26}H_{53}O_6P$: C 63.39%, H 10.84%; found: C 63.44%, H 10.84%. Specific rotation: $[\alpha]_{25} = -2.7$ (0.021 M, ethanol).

Preparation of (S)-3-acetyloxy-4-octadecyloxybutyl-1-phosphonic acid (10)

To dimethyl (*S*-3-acetyloxy-4-octadecyloxybutyl-1-phosphonate (**9**) (1.50 g, 3.04 mmol) in methylene chloride (5 mL) solution at −10°C under a nitrogen atmosphere was added bromotrimethylsilane (4.0 g, 26 mmol) and the reaction mixture was stirred for 15 min at −10°C and allowed to come to room temperature for 5 min. After that time the volatile materials were evaporated under reduced pressure, a 9:1 THF:water mixture (15 mL) was added, and the mixture was stirred for 20 min. Again the volatile materials were evaporated under reduced pressure. There was thus isolated the pure material (1.28 g, 90%) of mp 65°C, which exhibited IR and ^{1}H NMR spectra in accord with the proposed structure **10**. The IR spectrum (CCl_4) exhibited the following signals (partial) (cm^{-1}): 3400 (broad), 2850–2990, 1730, 1480, 1380, 1230, 1040 (broad). The ^{1}H NMR spectrum ($CDCl_3$) exhibited the following signals: δ 0.89, triplet, 3H, J = 6 Hz; δ 1.28–140, broad singlet, 32H; δ 1.49–1.65, multiplet, 4H; δ 2.08, singlet, 3H; δ 3.35–3.60, multiplet, 5H; δ 4.74, broad singlet, 2H. Anal. calcd. for $C_{24}H_{49}O_6P$: C 62.04%, H 10.63%; found: C 62.01%, H 10.85%. Specific rotation: $[\alpha]_{25} = -5.0$ (0.011 M, ethanol).

Preparation of (S)-3-acetyloxy-4-octadecyloxybutyl-1-phosphonylcholine (2)

To a solution of (*S*)-3-acetyloxy-4-octadecyloxybutyl-1-phosphonic acid (**10**) (110 mg, 0.237 mmol) in pyridine (5 mL) under a nitrogen atmosphere was added dry choline tosylate (500 mg, 1.82 mmol) and trichloroacetonitrile (1.00 g, 6.92 mmol). The reaction mixture was stirred for 72 h at 50°C, after which time the volatile materials were evaporated under reduced pressure. Acetonitrile (30 mL) was added to the residue and the solid material was isolated by filtration, purified by dissolution in 9:1 THF:water (10 mL), and passed through an ion exchange column (Amberlite MB-3 resin). From the eluent was isolated the pure target material (50.0 mg, 39%), which exhibited IR and ^{1}H and ^{31}P NMR spectra

in accord with the proposed structure **2**. The IR spectrum (CCl_4) exhibited the following signals (partial) (cm^{-1}): 3400 (broad), 2850–2990, 1730, 1480, 1260 (broad), 1080 (broad). The ^1H NMR spectrum ($CDCl_3$) exhibited the following signals: δ 0.88, triplet, 3H, J = 6 Hz; δ 1.18–1.30, broad singlet, 32H; δ 1.36, singlet, 9H; δ 1.49–1.65, multiplet, 4H; δ 2.08, singlet, 3H; δ 3.20–3.65, multiplet, 9H. The ^{31}P NMR spectrum ($CDCl_3$) exhibited a signal at −30 ppm relative to external 85% H_3PO_4. Anal. calcd. for $C_{29}H_{60}NO_6P$: C 63.36%, H 11.00%; found: C 63.71%, H 11.35%. Specific rotation: $[\alpha]_{25}$ = −6.2 (0.01 M, ethanol).

Acknowledgement

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1. Y.-j. Liu, B. E. Tropp, and R. Engel, Paper No. 75. Organic Division, 200th National Meeting of the American Chemical Society, Washington, D.C. August 1990.
2. C. Pratt, Y.-j. Liu, T.-y. Chu, K. Melkonian, B. E. Tropp, and R. Engel. Can. J. Chem. **70**, 2135 (1992).
3. (*a*) P. M. Henson. J. Exp. Med. **131**, 287 (1970); (*b*) J. Benveniste, P. M. Henson, and C. G. Cochrane. J. Exp. Med. **136**, 1356 (1972); (*c*) C. A. Demopoulos, R. N. Pinckard, and D. J. Hanahan. J. Biol. Chem. **254**, 9355 (1979); (*d*) D. J. Hanahan, C. A. Demopoulos, J. Liehr, and R. N. Pinckard. J. Biol. Chem. **255**, 5514 (1980); (*e*) J. Polonsky, M. Tence, P. Varenne, B. C. Das, J. Lunel, and J. Benveniste. Proc. Natl. Acad. Sci. U.S.A. **77**, 7019 (1980).
4. L. A. Vargas. *In* Handbook of organophosphorus chemistry. *Edited by* R. Engel. Marcel Dekker, Inc., New York. 1992.
5. (*a*) M. L. Blank, F. Snyder, L. W. Byers, B. Brooks, and E. E. Muirhead. Biochem. Biophys. Res. Commun. **90**, 1194 (1979); (*b*) F. Snyder. Platelet activating factors and related lipid mediators. Plenum Press, New York. 1987; (*c*) M. C. Venuti. Annu. Rep. Med. Chem. **20**, 193 (1985); (*d*) F. Heymans, E. Michel, M.-C. Borrel, B. Wichrowski, J.-J. Godfroid, O. Convert, E. Coeffier, M. Tence, and J. Benveniste. Biochim. Biophys. Acta, **666**, 230 (1981); (*e*) R. L. Wykle, C. H. Miller, J. C. Lewis, J. D. Schmitt, J. A. Smith, J. R. Surles, C. Piantadosi, and J. T. O'Flaherty. Biochem. Biophys. Res. Commun. **100**, 1651 (1981).
6. (*a*) R. Engel. Chem. Rev. **77**, 349 (1977); (*b*) *In* The role of phosphonates in living systems. *Edited by* R. L. Hilderbrand. CRC Press, Inc., Boca Raton, Fla. 1982; (*c*) *In* Handbook of organophosphorus chemistry. *Edited by* R. Engel. Marcel Dekker, Inc., New York. 1992.
7. (*a*) H. Hayashi, K. Nakanishi, C. Brandon, and C. Marmur. J. Am. Chem. Soc. **95**, 8749 (1973); (*b*) K.-C. Tang, B. E. Tropp, and R. Engel. Tetrahedron, **34**, 2873 (1978).
8. S. Ali and R. Bittman. Chem. Phys. Lipids, **50**, 11 (1989).
9. Y.-j. Liu, T.-y. Chu, and R. Engel. Synth. Commun. **22**, 2367 (1992).
10. C. E. McKenna, M. T. Higa, N. H. Cheung, and M.-C. McKenna. Tetrahedron Lett. 155 (1977).
11. (*a*) F. Cramer and G. Weimann. Chem. Ber. **94**, 996 (1961); (*b*) A. F. Rosenthal. J. Lipid Res. **7**, 779 (1966); (*c*) D. Braksmayer, B. E. Tropp, and R. Engel. Chem. Phys. Lipids, **19**, 93 (1977); (*d*) P. W. Schwartz, B. E. Tropp, and R. Engel. Chem. Phys. Lipids, **48**, 1 (1988).