^{1}H NMR (250 MHz, $CDCl_3$) δ 1.34 (1 H, d, J = 9), 1.92 (2 H, d, J = 11), 2.10 (1 H, d, J = 9), 2.28 (3 H, s), 3.88 (2 H, centroid of AB q, J = 13) 7.1–7.7 (5 H, m); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 35.0, 35.4, 45.4, 57.0, 57.1, 58.4, 60.8, 126.6, 128.1, 128.5, 140.0; HRMS, found 216.1588, calcd for $C_{14}H_{20}N_2$ 216.1626.

6-Methyl-3,6-diazabicyclo[3.2.1]octane Dihydrochloride (4c). A mixture of 3.00 g of 3-benzyl-6-methyl-3,6-diazabicyclo[3.2.1]octane (**4a**), 75 mL of methanol, and 5 mL of concentrated aqueous hydrochloric acid was hydrogenated at 50 psi in the presence of 1 g of 10% Pd/C for 7 h. The catalyst was removed by filtration and the filtrate was evaporated to a solid which was triturated in *i*-PrOH and ether to afford 2.43 g (88%) of 6-methyl-3,6-diazabicyclo[3.2.1]octane dihydrochloride (**4c**), mp 266–269 °C: ^{1}H NMR (D_2O, 300 MHz) δ 2.22 (1 H, d, J = 13), 2.3–2.5 (1 H, m), 3.06 (3 H, s), 3.45 (1 H, s), 3.53 (1 H, d, J = 13), 3.77 (1 H, d, J = 13), 4.18 (1 H, s); ^{13}C NMR (D_2O + NaOD,[11] 63 MHz) δ 35.1, 37.0, 41.3, 48.4, 50.4, 58.3; 61.0; HRMS, found 126.1162, calcd for $C_7H_{14}N_2$ 126.1157.

3-Methyl-3,6-diazabicyclo[3.2.1]octane Dihydrochloride (4d). Following the same procedure for the synthesis of **4c**, 1.01 g of 6-benzyl-3-methyl-3,6-diazabicyclo[3.2.1]octane (**4b**) was hydrogenated to provide 0.78 g (84%) of 3-methyl-3,6-diazabicyclo[3.2.1]octane dihydrochloride (**4d**), mp 259–261 °C: ^{1}H NMR (D_2O, 250 MHz) δ 2.05–2.2 (1 H, m), 2.28 (1 H, d, J = 13), 2.98 (3 H, s), 3.04 (1 H, s), 3.35–3.8 (5 H, m), 3.88 (1 H, d, J = 13), 4.38 (1 H, s); ^{13}C NMR (D_2O, 63 MHz) δ 33.0, 33.6, 44.8, 48.2, 55.0, 55.5, 58.6.

Anal. Calcd for $C_7H_{14}N_4 \cdot 2HCl \cdot 0.5\ H_2O$: C, 40.40; H, 8.23; N, 13.46. Found: C, 40.20, H, 7.89; N, 13.32.

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Registry No. 1, 3693-69-4; **2a**, 95019-15-1; **2b**, 112375-05-0; **3a**, 112375-06-1; **3b**, 112375-07-2; **3c**, 112375-08-3; **3d**, 112375-09-4; **3e**, 112375-10-7; **4a**, 112375-11-8; **4b**, 112375-12-9; **4c**, 112375-13-0; **4d**, 112375-14-1; methylene bisurethane, 3693-53-6; 1,3-cyclohexadiene, 592-57-4; methylamine, 74-89-5; cyclopentadiene, 542-92-7; benzylamine hydrochloride, 3287-99-8.

(11) The ^{13}C NMR of the dihydrochloride salt was poorly resolved due to the equilibrium between the various protonated species. A resolved spectrum was obtained on the free diamine by in situ neutralization with NaOD.

Synthesis and Evidence for the Stability of a Glycerophosphochloridate: *rac*-1-*O*-Hexadecyl-2-*O*-(methylcarbamyl)-*sn*-glycero-3-phosphorochloridocholine

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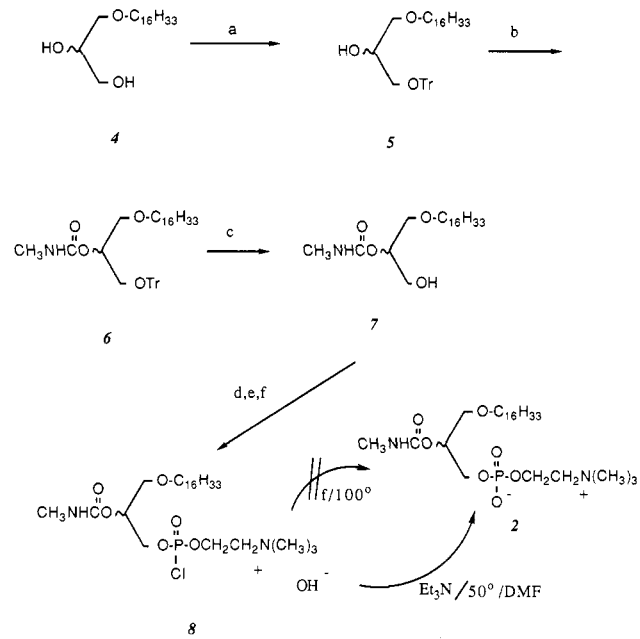
The carbamyl moiety has been reported[1] to be resistant to phospholipase hydrolysis. As part of our ongoing study[2]

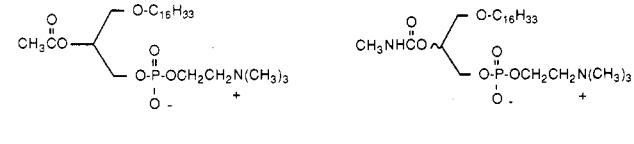
(1) Gupta, C. M.; Bali, A. *Biochim. Biophys. Acta* **1981**, *663*, 506.

Scheme I[a]



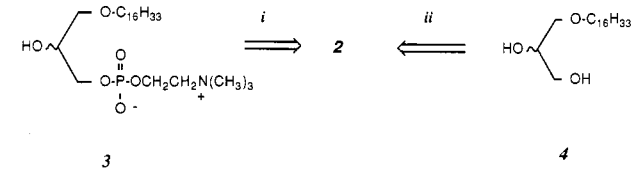
[a] (a) TrCl/hexanes/reflux; (b) CH_3NCO/DMAP/CH_2Cl_2; (c) $BF_3 \cdot 2MeOH/CH_2Cl_2$; (d) $POCl_3/CHCl_3/Et_3N$; (e) C_5H_5N/choline tosylate; (f) H_2O.

of the diverse properties of alkylglycerolipids such as platelet-activating factor **1** (PAF), the 2-methylcarbamyl analogue **2** was envisioned as meeting the strict size re-



quirements[2a-c,3] for activity imposed on moieties at the glycerol *sn*-2 position.[4] It also has the potential to be a biologically nonhydrolyzable PAF analogue, since analogue **2** might prove to be stable to hydrolysis by the acetylhydrolase[5] that degrades PAF.

Two synthetic routes to the desired methylcarbamyl analogue **2** are (i) directly from the lyso phospholipid **3** and (ii) de novo from chimyl alcohol **4**. Reaction of the lyso



phospholipid with methyl isocyanate[1,6] initially resulted

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(3) Reviews on PAF: (a) Snyder, F. *Ann. Rep. Med. Chem.* **1982**, *17*, 243. (b) Venuti, M. C. *Ibid.* **1985**, *20*, 193.

(4) Glycerolipid stereospecific numbering (*sn*) nomenclature is covered in Hirschmann, H. *J. Biol. Chem.* **1960**, *235*, 2762.

(5) Blank, M. L.; Lee, T.-C.; Fitzgerald, V.; Snyder, F. *J. Biol. Chem.* **1981**, *256*, 175.

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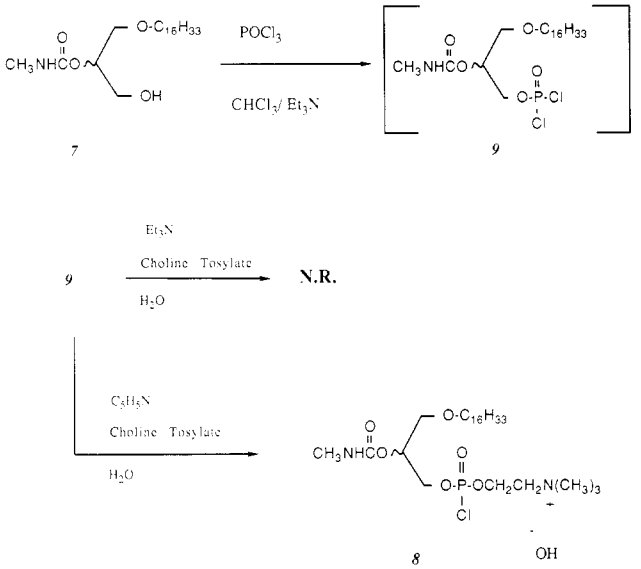
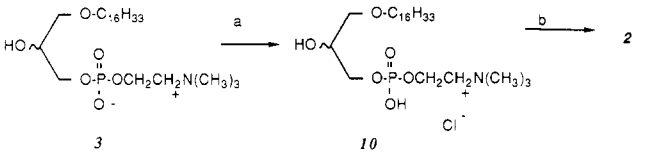
in a product of variable purity and biological activity;[7] thus, the de novo synthetic route ii was pursued as shown in Scheme I. The product of this synthetic scheme was not the expected methylcarbamyl analogue **2**. Instead, compound **8** was reproducibly isolated from the final reaction mixture after silica gel column chromatography.

The structure of compound **8** was consistent with elemental analysis, FAB-MS, and 250-MHz ^{1}H NMR spectroscopy data (see Experimental Section). However, the elemental analysis data were not conclusive for compound **8** (i.e., the counterion could be either chloride or hydroxide) nor was the carbamyl methyl doublet at 3.1 ppm in the ^{1}H NMR spectrum. The FAB-MS of compound **8** showed a parent ion peak at m/z 557 (M + 1) and a peak at 559 m/z (M + 3) in a 3:1 ratio, indicative of one constituent chlorine atom in the molecule. The isolation of compound **8** was at variance with the known ease of hydrolysis of phosphochloridates[8,9] and our previous experience with the Brockerhoff procedure for introduction of the phosphocholine moiety.[2a,b,d] A literature serach revealed no known glycerophosphochloridates stable to treatment with water, but some stable sterol phosphodichloridates are known.[10]

The synthesis of compound **8** was monitored by ^{31}P NMR in order to gather more spectroscopic evidence for the proposed structure and to gain more insight into the mechanism of the Brockerhoff reaction. Racemic 1-*O*-hexadecyl-2-*O*-(methylcarbamyl)-*sn*-glycerol (**7**) was reacted with $POCl_3$ in the presence of $CDCl_3$ and Et_3N. The initial ^{31}P NMR spectra were taken at five 1-h intervals. No change in the ^{31}P NMR spectrum was found over this time period and no signal corresponding to product **8** was observed. Addition of pyridine resulted in an immediate shift of the ^{31}P NMR signal to a set of multiplets at −10 to −12 ppm and −21 to −24 ppm. After reaction for 1 h, a new signal (−0.72 ppm) consistent with that expected for the product **8** began to appear. The ratio of the new signal to the multiplet signals increased as the reaction proceeded overnight. The chemical shift of the ^{31}P NMR signal for **8** did not change when H_2O was added. Our interpretation of this experiment is as follows: (1) alcohol **7** reacts with the $POCl_3$ to give the phosphodichloridate monoester intermediate **9** (−4.2 ppm); Et_3N does not necessarily catalyze this reaction but merely neutralizes the HCl generated, resulting in unreactive Et_3N·HCl salt; (2) the phosphodichloridate monoester is not as reactive as $POCl_3$, as evidenced by the lack of reactivity with choline tosylate in the presence of Et_3N; (3) addition of pyridine results in the formation of reactive phosphopyridine complexes that react with choline tosylate to form compound **8**; (4) phosphochloridate **8** is stable to mild hydrolysis, since the ^{31}P NMR signal does not shift to +0.8 ppm (a reference value for PC phospholipids[12]) upon addition of H_2O. PAF exhibits a ^{31}P NMR signal at 0.37 ppm in MeOH-d_4. Phosphochloridate **8** can be converted to the corresponding phosphocholine **2** [(M + 1) = m/z 539] by heating at 50 °C/8 h in Et_3N. The ^{1}H NMR signal for the carbamyl methyl group in compound **2** appears as a doublet at 2.75 ppm, compared to 3.1 ppm for compound **8**.



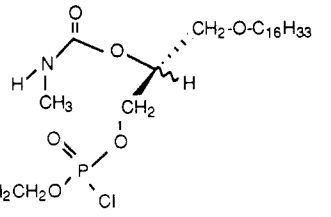
Scheme II[a]



[a] (a) $CHCl_3$/MeOH/5% HOAc/H_2O; (b) CH_3NCO/DMF/5 h.

A reexamination of the synthesis of compound **2** from the lyso phospholipid (Scheme II) indicated that the reaction proceeded in 70% yield if two conditions were met: (1) the zwitterionic form of the phospholipid was converted to the acid form **10** prior to reaction with CH_3NCO; (2) the reaction proceeded better in warm DMF than in CH_2Cl_2 or $CHCl_3$.

The unusual stability of phosphochloridate **8** appears to be linked to the methylcarbamate moiety at the glycerol 2-position. Replacement of the carbamyl moiety of compound **7** with benzyl, substituted-benzyl, methyl, ethyl, or TBDMS ethers gives compounds which, when treated with $POCl_3$ and choline tosylate, react normally with water to yield the corresponding phosphocholines directly. This suggests that the methylcarbamate moiety at the glycerol *sn*-2 position is shielding the intermediate phosphochloridate from attack by water. Also, the downfield shift of the *N*-methyl doublet in the ^{1}H NMR spectrum (3.1 ppm for **8**, compared to 2.75 ppm for **2**) is consistent with the argument that the carbamyl methyl group may lie in the deshielding cone of the phosphate moiety. Strain-free conformations of **8** can be made with CPK and Dreiding models in which the carbamyl moiety is able to shield the P–Cl bond from hydrolysis by approaching water molecules. Thus, the carbamyl moiety can intramolecularly



shield the phosphochloridate from hydrolysis. However,

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(9) Brockerhoff, H.; Ayengar, N. K. N. *Lipids* **1978**, *14*, 88.
(10) (a) Cremlyn, R. J. W.; Dewhurst, B. B.; Wakeford, D. H. *Synthesis* **1971**, 648. (b) Cremlyn, R. J. W.; Dewhurst, B. B.; Wakeford, D. H.; Raja, R. A. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1171. (c) Sosnovsky, G.; Konieczny, M. *Z. Naturforsch.* **1976**, *31b*, 1379.
(11) Gorenstein, D. G. *Phosphorus-31 NMR Principles and Applications*; Academic Press: Orlando, FL, 1984; p 563. ^{31}P NMR chemical shifts are notoriously solvent-dependent.
(12) Reference 11, p 427.

intermolecular shielding cannot be ruled out. This possible association between carbamyl and phosphoryl moieties of compound 8 can be disrupted only by relatively drastic reaction conditions (anhydrous Et_3N, 50 °C, 8 h). Compound 8 is stable to treatment with water (reflux, 1 h) but decomposes on heating in dilute aqueous base.

In summary, we present evidence for the isolation of a relatively stable glycerophosphochloridate (8). What at first glance appears to be a relatively straightforward reaction, on closer examination yields some rather interesting structural and mechanistic results. Elemental analysis, ^1H NMR, and ^{31}P NMR data are consistent with the proposed structure. The stability of the P–Cl bond appears to be intimately connected to shielding of the phosphochloridate by the methylcarbamyl moiety on the adjacent *sn*-2 glycerol carbon atom. The phosphochloridate can be converted to the corresponding phosphate by heating with anhydrous Et_3N.

Experimental Section

General Methods. All chemicals were used as supplied without further purification unless indicated. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ. FAB-MS were obtained on a VG-7070EQ spectrometer in a glycerol matrix. IR spectra were obtained on a Perkin-Elmer 1320 infrared spectrometer. ^1H NMR spectra were taken at ambient temperature with chemical shifts expressed as ppm downfield from Me_4Si as an internal standard on either a JEOL-FX-60 or Bruker 250-MHz spectrometer. The ^{31}P NMR experiment was performed on a Varian XL-400 spectrometer with 85% H_3PO_4 as an external standard. TLC data were determined on either Bakerflex I-BF or Analtech silica gel chromatographic plates in the indicated solvent systems. Column chromatography was performed with Merck silica gel, 230–400 mesh, in the indicated solvent systems.

***rac*-1-*O*-Hexadecyl-3-*O*-tritylglycerol (5).** The procedure of Baumann and Mangold[13] was employed to provide 4 in 92% yield (mp 64–65 °C; lit. mp 65.5 °C). Compound 4 was tritylated according to Heymans et al.[14] to produce 5 in 56% yield as a white solid, mp 56–58 °C.

***rac*-1-*O*-Hexadecyl-2-*O*-(methylcarbamyl)glycerol (7).** The procedure of Gupta and Bali[1] was employed to introduce the methylcarbamate moiety. To a solution of 5 (9.0 g, 16 mmol) and 4-(dimethylamino)pyridine (2.07 g, 17 mmol) in CH_2Cl_2 (50 mL) was added the methyl isocyanate (4.85 g, 85 mmol). The reaction vessel was flushed with N_2 and sealed and the contents were stirred in the dark at room temperature. After 72 h, the volatiles were removed with a rotary evaporator, resulting in 15.2 g of crude product as an orange oil. Chromatography on 150 g of silica gel (Et_2O) resulted in 10.1 g of slightly impure yellow oil [R_f (Et_2O) 0.44]. The trityl group was removed[15] and the viscous yellow oil purified by chromatography (95/5 $CHCl_3$/MeOH), resulting in 3.7 g of product 7 (62% from 5) as a white solid [R_f (95/5 $CHCl_3$/MeOH) 0.59], mp 59–60 °C]: IR 1690 cm^{-1}, 3450, 3330; FAB-MS, m/z (M + 1) 374.

***rac*-1-*O*-Hexadecyl-2-*O*-(methylcarbamyl)glycero-3-phosphorochloridocholine (8).** A solution of alcohol 7 (1.5 g, 4 mmol) and Et_3N (0.5 g, 5 mmol) in 40 mL of CH_2Cl_2 was added to freshly distilled $POCl_3$ (0.77 g, 5 mmol) cooled to 4 °C in an ice bath under an atmosphere of nitrogen. The resulting solution was stirred for 0.5 h, at which time it was warmed to room temperature. After the addition of 2.0 mL of pyridine and solid choline tosylate[15,16] (2.43 g, 8.8 mmol), the solution was stirred at room temperature for 5 h followed by the addition of 2.0 mL of H_2O and continued stirring for 2 h. The volatiles were removed with a rotary evaporator, and the resulting semisolid was taken up in CH_2Cl_2 (50 mL) and washed with 2 × 20 mL of H_2O, 5 × 25 mL of 5% HCl, and 3 × 25 mL of H_2O, using MeOH to break the emulsions. The nonaqueous layer was dried ($MgSO_4$) and filtered, and volatiles were removed with a rotary evaporator to give 1.6 g of crude material. Chromatography on 10 g of silica gel (50/25/8/4 $CHCl_3$/MeOH/HOAc/H_2O) followed by acetone precipitation and drying of the resulting solid under high vacuum/KOH resulted in 617 mg (28%) of pure product (C, H, N):[17] FAB-MS, m/z (M + 1) 557, (M + 3) 559; IR 1697 cm^{-1}; ^1H NMR ($CDCl_3$) 0.88 (3 H, t, CH_3), 1.26 (26 H, t, $-CH_2-$), 1.52 (2 H, dt, β-CH_2), 3.1 (3 H, d, CH_3N), 3.35 (9 H, s, $N(CH_3)_3$), 3.42 (4 H, m, $-CH_2-$, 1-CH_2O), 3.52 (m, 2 H, 3-CH_2O), 3.65 (1 H, m, -NH-), 3.85 (2 H, m, $-CH_2N$), 4.32 (2 H, dm, $POCH_2$), 4.98 (1 H, m, CH).

Synthesis of 2 from the Lyso Phospholipid 3. The lyso phospholipid 3 (100 mg, 0.2 mmol) was converted to the acid form 10 by a modified Bligh and Dyer[18] extraction procedure. After removal of solvent under a N_2 stream and drying under vacuum/P_2O_5, the lyso phospholipid was taken up in 4 mL of DMF. Methyl isocyanate (0.5 mL) was added and the reaction mixture stirred at 50 °C for 5 h. Volatiles and solvent were removed under vacuum and chromatography on silica gel (70/35/7 $CHCl_3$/MeOH/NH_4OH) resulted in 75.5 mg (70%) of 2 as a white powder after acetone precipitation: FAB-MS, m/z (M + 1) 539; ^1H NMR 2.75 (3 H, d, CH_3N).

^{31}P NMR Experiment. To a 10-mm NMR tube was added 12.3 μL (1.25 equiv) of $POCl_3$ and 3 mL of $CDCl_3$. A reference spectrum was obtained. A solution of 50 mg of 7 (1.0 equiv, 0.13 mmol) and 19 μL (1.25 equiv) of Et_3N in 2 mL of $CDCl_3$ was added to the NMR tube and the shift in ^{31}P NMR signal monitored for 1.0 h. Et_3N (0.1 mL) and choline tosylate (0.1 g, 2.75 equiv) were added to the reaction tube and spectra were obtained over a 5-h period. Pyridine (0.1 mL) was added to the reaction tube and spectra were obtained at 5-min intervals for 30 min. The reaction was allowed to proceed at ambient temperature overnight. Another spectrum of the reaction product was obtained and H_2O (0.3 mL) was added to the reaction tube. A final spectrum was obtained after 30 min.

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Registry No. 2, 111057-91-1; 3, 17364-21-5; 4, 6145-69-3; 5, 82002-20-8; 6, 112247-71-9; 7, 112247-72-0; 8, 112247-73-1; 10, 112247-74-2; $HOCH_2CH_2N^+Me_3 \cdot TsO^-$, 55357-38-5.

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(15) The choline tosylate was prepared from *N,N*-dimethylethanolamine and methyl tosylate by the procedure of Rosenthal, A. F. *J. Lipid Res.* **1966**, *7*, 779.
(16) The choline tosylate was dried overnight under high vacuum over P_2O_5.
(17) Phospholipids commonly contain variable amounts of water of hydration; therefore, elemental analyses are viewed as being consistent with proposed structure, not diagnostic.
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Intermolecular Diels–Alder Reactions of 3-Vinylcyclohex-2-en-1-ol and a Silyl Ether Derivative[1]

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The dienol system 1 and related derivatives have proven to be valuable building blocks in the total synthesis of

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