acetone as fine needles (mp 131–132 °C) and was characterized as the 7β,12α-dihydroxy ester IVa: IR 1730 (C=O), 3436, 1026, 1015, and 942 (OH) cm^{-1}; NMR δ 0.70 (3 H, s, C-18 Me), 0.93 (3 H, s, C-19 Me), 3.57 (1 H, br m, C-7 CHOH), 3.63 (3 H, s, COOMe), 3.98 (1 H, m, C-12 CHOH). Anal. Calcd for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41. Found: C, 74.11; H, 10.66.

7β,12β-Dihydroxycholanic acid (IV) was obtained from IVa by the usual hydrolysis procedure and crystallized from ethyl acetate as fine needles: mp 179–182 °C; IR 1642 (C=O), 3413, 1026, 1015, and 956 (OH) cm^{-1}; NMR ($CDCl_3$ + 10% Me_2SO-d_6) δ 0.72 (3 H, s, C-18 Me), 0.93 (3 H, s, C-19 Me), 3.41 (2 H, br m, C-7 and C-12 CHOH). Anal. Calcd for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found: C, 73.45; H, 10.23.

7β,12α-Dihydroxycholanic acid (III) was similarly obtained from the corresponding ester IIIa. The crude acid crystallized from EtOAc–hexane as fine needles: mp 174.5–176.0 °C: IR (KBr) 1689 (C=O), 3356, 1026, 1015, and 943 (OH) cm^{-1}; NMR ($CDCl_3$ + 10% Me_2SO-d_6) δ 0.67 (3 H, s, C-18 Me), 0.90 (3 H, s, C-19 Me), 3.49 (1 H, br m, C-7 CHOH), 3.88 (1 H, m, C-12 CHOH). Anal. Calcd for $C_{24}H_{40}O_4$: C, 73.43; H, 10.27. Found: C, 73.17; H, 10.14.

Reduction of the Ester VIa by $NaBH_4$. Methyl 7β-hydroxy-12-oxocholanate (VIa; 1.84 g in 160 mL of MeOH) was treated with 2.0 g of $NaBH_4$ and allowed to stand overnight at room temperature. Ice chips were added to the solution which was extracted wth CH_2Cl_2. The CH_2Cl_2 extract was washed with cold dilute–HCl and then water, dried (Drierite), and evaporated to a clear oil (1.84 g). By HPLC the oily product was seen to consist predominantly of the same two components as obtained in the amineborane complex reduction (above) but in the reversed ratio of 37:63 7β,12β/7β,12α. Column chromatographic separation as in the amine–borane reaction yielded the two expected products, IVa (581 mg) and IIIa (973 mg), shown by HPLC, NMR, and melting point comparisons to be identical with the corresponding esters prepared above.

Methyl 7α,12α-Bis(mesyloxy)cholanate (XIa). To methyl 7α,12α-dihydroxy ester Ia (500 mg in 10 mL of pyridine) was added dropwise 0.5 mL of methanesulfonyl chloride. The usual workup after overnight standing yielded product which crystallized from ethyl ether–hexane as thin plates: 567 mg (83%); mp 77.0–78.5 °C; IR 1727 (C=O), 1333 and 1170 (SO_2), 971, 943, and 901 (mesylate) cm^{-1}. NMR δ 0.78 (3 H, s, C-18 Me), 0.92 (3 H, s, C-19 Me), 3.04 and 3.08 (each 3 H, s, C-7 and C-12 OSO_2Me), 3.66 (3 H, s, COOMe), 4.91 (1 H, m, C-7, CHOMs), 5.12 (1 H, m, C-12 CHOMs). Anal. Calcd for $C_{27}H_{46}O_8S_2$: C, 57.63; H, 8.24. Found: C, 57.60; H, 8.32.

12α-(Mesyloxy)cholanic Acid (XII). Methyl 12-(mesyloxy)cholanate (XIIa,[20] 260 mg) after being subjected to the standard inversion procedure (solvent Me_2SO–1,2-dimethoxyethane)[5] and after processing yielded 201 mg of product which did not crystallize but by TLC showed a single spot, and its NMR spectrum was appropriate for acid XII: NMR δ 0.77 (3 H, s, C-18 Me), 0.91 (3 H, s, C-19 Me), 3.02 (3 H, s, C-7 SO_2Me), 5.11 (1 H, m, C-12 $CHOSO_2Me$).

Esterification of the oil (MeOH–HCl) after processing and crystallization from MeOH yielded colorless prisms, which according to mixture melting point, NMR, and HPLC comparisons was identical with the starting methyl ester XIIa.

Methyl 7α-(Mesyloxy)cholanate (XVIa). Methyl 7α-hydroxy ester XVa[27] (1.0 g) was treated with 2.0 mL of methanesulfonyl chloride in 20 mL of pyridine and was processed as described previously[5] to yield 1.1 g of oil which was crystallized from isopropyl ether as stout prisms: 940 mg (78%); mp 74–75 °C; IR 1730 (C=O), 1332 and 1170 (SO_2), 909 and 892 (mesylate) cm^{-1}; NMR δ 0.66 (3 H, s, C-18 Me), 0.91 (3 H, s, C-19 Me), 2.99 (3 H, s, SO_2Me), 3.63 (3 H, s, COOMe), 4.90 (1 H, m, C-7 (CHOMs). Anal. Calcd for $C_{26}H_{44}O_5S$: C, 66.64; H, 9.64. Found: C, 66.77; H, 9.13.

7β-Hydroxycholanic Acid (XIII). Methyl 7α-mesylate (XVIa, 1.0 g), in a 2-h reaction with the inverting solution [KO_2 (60 mg) and 18-crown-6 (33 mg) in 40 mL of Me_2SO], as described previously,[5] yielded a crude product which crystallized from EtOAc–hexane as colorless needles: 0.46 g (57%); mp 131–133.5 °C; IR (KBr) 1695 (C=O), 3401, 1015, 990 (OH) cm^{-1}; NMR δ 0.68 (3 H, d, C-18 Me), 0.93 (3 H, s, C-19 Me), 3.58 (1 H, br m, C-7 CHOH). Anal. Calcd for $C_{24}H_{40}O_3$: C, 76.55; H, 10.71. Found: C, 76.02; H, 11.12.

Methyl 7β-hydroxycholanate (XIIIa) was obtained quantatively from XIII by the usual MeOH–HCl esterification. The ester crystallized from aqueous methanol as dense prisms: mp 89.0–90.5 °C; IR 1730 (C=O), 3436, 1015, 993 (OH) cm^{-1}; NMR δ 0.84 (3 H, s, C-18 Me), 0.99 (3 H, s, C-19 Me), 3.60 (1 H, br m, C-7 CHOH), 3.68 (3 H, s, COOMe). Anal. Calcd for $C_{25}H_{42}O_3 \cdot 0.5MeOH$: C, 75.32; H, 10.90. Found: C, 75.58; H, 10.76.

Acknowledgment. This work was supported in part by a grant from the National Large Bowel Cancer Project. We thank the Chemical Research Institute of Non-Aqueous Solutions and the Pharmaceutical Institute, Tohoku University, Japan, for elemental analysis. We are indebted to Ms. Susan Brannan for valuable technical help.

Registry No. Ia, 3701-54-0; II, 84413-82-1; IIa, 84325-10-0; III, 84413-81-0; IIIa, 84895-26-1; IV, 84413-80-9; IVa, 84895-27-2; V, 84895-28-3; Va, 84895-29-4; VI, 84895-30-7; VIa, 84895-31-8; VIIa, 19684-66-3; VIIIa, 19684-67-4; Xa, 84895-32-9; XIa, 84895-33-0; XII, 84895-34-1; XIIa, 84926-46-5; XIII, 10601-78-2; XIIIa, 28050-20-6; XVa, 28050-19-3; XVIa, 84926-47-6; XVIIa, 28192-77-0; XVIIIa, 77731-11-4; methyl cholate, 1448-36-8.

Stereospecific Synthesis of Ether Phospholipids. Preparation of 1-Alkyl-2-(acylamino)-2-deoxyglycerophosphorylcholines

Nizal S. Chandrakumar and Joseph Hajdu*

Department of Chemistry, Boston College, Chestnut Hill, Massachusettes 02167

Received July 20, 1982

A novel stereospecific synthesis of biologically active ether phospholipids is reported. The synthesis is based upon (1) utilizing L-serine to provide the chiral center, (2) developing the aliphatic ether function by coupling the methanesulfonate of the fatty acid alcohol with an oxazoline-protected deoxyglyceride, and (3) introducing the phosphorylcholine moiety via the 2-chloro-2-oxo-1,3,2-dioxaphospholane–trimethylamine sequence. The synthetic alkoxyphospholipids have been shown to exhibit potent platelet activation, antihypertensive properties, and cytotoxicity against HL-60 cells. Microcalorimetric studies of one compound have revealed a unique phase-transition behavior resulting from the presence of a 1-alkyl rather than a 1-acyl substituent in the molecule. The synthetic method developed has a great deal of flexibility, providing a convenient general route to a wide range of ether phospholipids for physicochemical as well as enzymological studies.

Ether phospholipids represent an important class of exceptionally potent biologically active phospholipid derivatives.[1,2] Recent studies of a series of naturally occuring 1-*sn*-alkoxyglycerophosphorylcholines have established

0022-3263/83/1948-1197$01.50/0

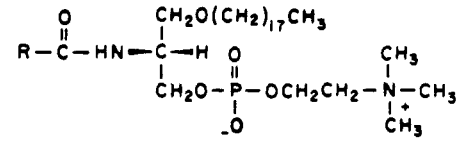
that these compounds are involved in a number of physiologically vital regulatory processes.[2-4] Specifically, ether phospholipids are potent platelet activators,[1] exhibit significant antihypertensive activity[2,5] and have been shown in function as efficient chemotactic agents as well.[4] Particular attention has been directed toward these compounds recently as it was discovered that a series of alkoxyphospholipids possess selective tumor cytotoxicity against a number of different cancer cells.[6]

Despite their well-recognized biological importance, however, the mechanistic details involved in the physiological functioning of ether phospholipids remain to be elucidated.[2] For this reason, as well as for delineation of the specific structural requirements essential for their biological activity, synthetic alkoxyphospholipids need to be prepared. Availability of convenient synthetic procedures leading to the desired compounds is therefore a prerequisite for advancing the current level of understanding of the chemistry and biochemistry of ether phospholipids.

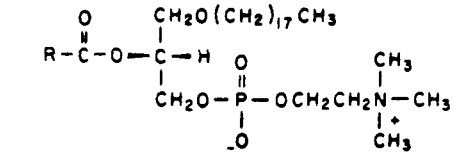
As part of our ongoing research, aimed at the development of new synthetic methods for the preparation of phospholipid derivatives,[7-10] we have begun focusing our attention on the synthesis of biologically active 1-alkyl-2-aminodeoxy-*sn*-glycerophosphorylcholines. In this context we have recently accomplished the synthesis of an isosteric amide analogue of platelet-activating factor.[10] In the present paper we describe the synthesis in detail and report an extension of the original sequence that allows the introduction of substituents at the *sn*-2-position *after* the phospholipid skeleton has been assembled. The significance of this extended flexibility of the sequence becomes apparent if one considers that according to all currently available experimental data, it is the nature of the substituent at the *sn*-2-position that controls the specificity and the potency of the compounds in biological systems.[1-5]

Results and Discussion

The structure of our target molecule (1) has been designed on the basis of the results obtained by Hanahan[1] following the isolation and characterization of platelet-activating factor (PAF, **2**, R = CH_3) and by Snyder, who discovered the enzyme responsible for the hydrolysis of the acetyl-ester linkage at the 2-position of PAF.[2] In replacing the ester moiety of platelet-activating factor by the amide function, we sought to provide a molecule resistant to enzymatic hydrolysis, while retaining all other structural features, including the stereochemistry of the naturally occurring phospholipid derivatives. Accordingly,



(1)



(2)

systematic variation of the structural components in **1** should provide a series of closely related compounds for the establishment of structure–function correlations delineating the role of individual substituents in determining the biological activities and physicochemical properties of ether phospholipids. Preliminary evidence, indicating that the acetamido derivative **1a** does exhibit potent platelet activation[11] as well as significant antihypertensive behavior,[5] strongly supports the validity of the approach here outlined.

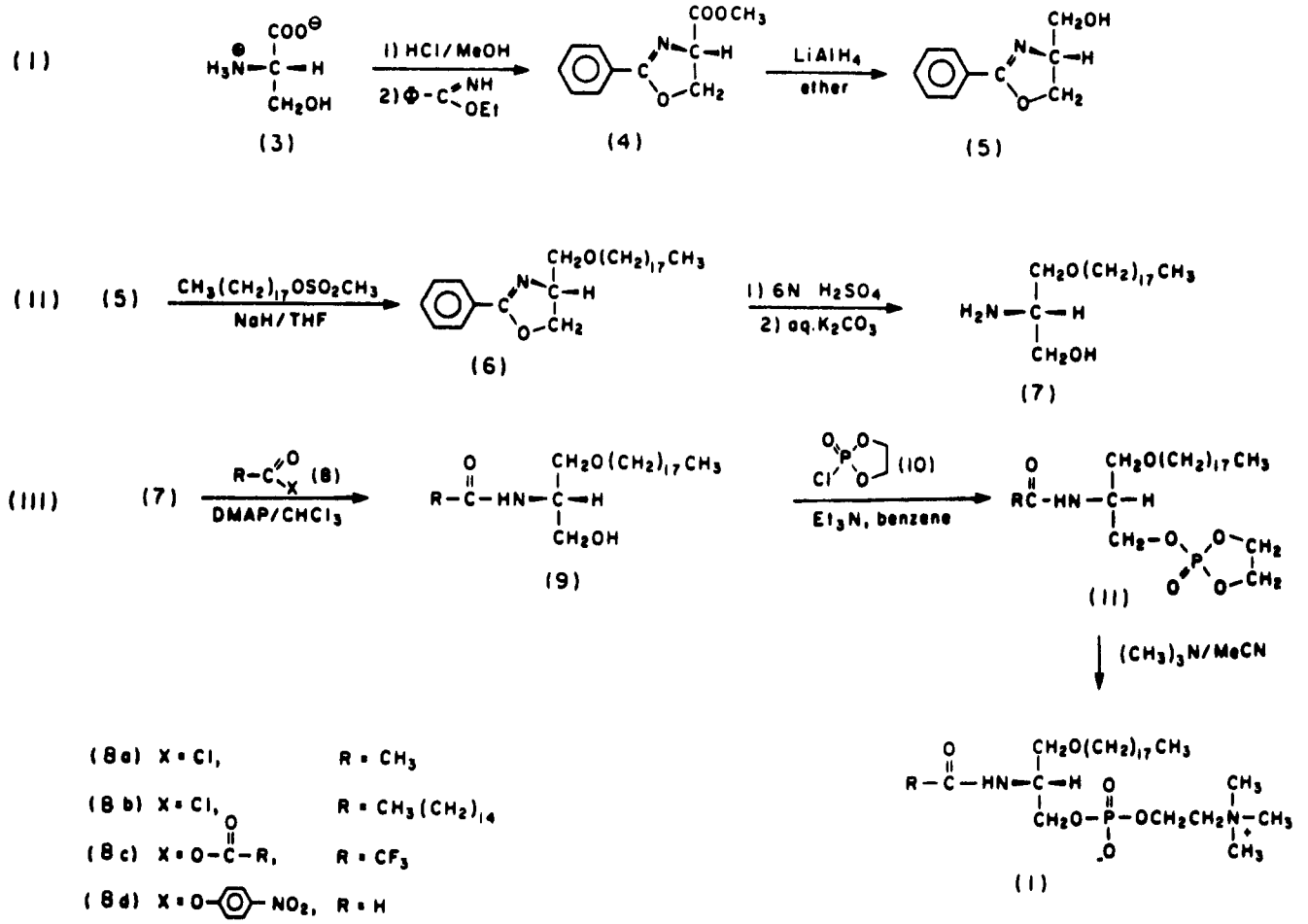
Our synthetic method for the preparation of the chiral compound **1** is outlined in Scheme I. Obvious variations in this sequence of reactions allow the preparation of a wide spectrum of related derivatives. The 2-phenyl-substituted oxazoline derivatives **4** and **5** are well suited for this synthetic scheme,[12-14] and each possesses excellent solubility properties.

Preparation of **4** was readily accomplished by condensation of L-serine methyl ester and ethyl benzimidate.[12] Reduction of 2-phenyl-4-(carbomethoxy)-2-oxazoline (**4**) with stoichiometric amounts of lithium aluminum hydride[15] led to the optically active alcohol **5** in 81% yield. The alkoxy function at the incipient *sn*-1-position of the target phospholipid (**1**) was developed in a coupling reaction between stoichiometric amounts of stearyl methanesulfonate[16] and the sodium alkoxide of alcohol **5** in tetrahydrofuran. The excellent yield (>90%) of the resulting ether (**6**) greatly exceeds the yields reported for related syntheses using potassium/benzene,[16] KOH/xylene,[17] or phase-transfer catalysis.[18]

Acid-catalyzed hydrolysis of the oxazoline ring of **6** in 6 N aqueous H_2SO_4 readily afforded the deprotected amino alcohol **7**. The optically active 1-stearyl-2-amino-2-deoxy-*sn*-glycerol **7** was easily purified and isolated as a crystalline product (93%). Although the nucleophilic reactivity of the secondary amine nitrogen in **7** is greatly diminished due to its close proximity to the long-chain aliphatic neighboring group, the compound can be effi-

(1) Demopoulos, C. A.,; Pinckard, R. N.; Hanahan, D. J. *J. Biol. Chem.* **1979**, *254*, 9355–1358.
(2) Blank, M. L.; Lee, T. C.; Fitzgerald, V.; Snyder, F. *J. Biol. Chem.* **1981**, *256*, 175–178.
(3) Blank, M. L.; Snyder, F.; Byers, L. W.; Brooks, B.; Muirhead, E. E. *Biochem. Biophys. Res. Commun.* **1979**, *90*, 1194–1200.
(4) Munder, P. G.; Weltzien, H. O.; Modolell, M. in "Immunopathology, VIIth International Symposium, Bad Schadhen"; Miescher, P. A., Ed.; Schwabe: Basel, 1976; pp 411–424.
(5) Blank, M.; Cress, E. A.; Lee, T.-C.; Malone, B.; Surles, J. R.; Piantadosi, C.; Hajdu, J.; Synder, F. *Res. Commun. Chem. Pathol. Pharmacol.*, in press.
(6) (a) Modolell, M.; Andreesen, R.; Pahlke, W.; Brugger, U.; Munder, P. G. *Cancer Res.* **1979**, *39*, 4681–4686. (b) Honma, Y.; Kasukabe, T.; Hozumi, M.; Tsushima, S.; Nomura, H. *Ibid.* **1981**, *41*, 3211–3216. (c) Berdel, W. E.; Bausert, W. R. E.; Fink, U.; Rastetter, J.; Munder, P. G. *Anticancer Res.* **1981**, *1*, 345–352.
(7) Chandrakumar, N. S., Hajdu, J. *Tetrahedron Lett.* **1981**, *22*, 2949–2952.
(8) Chandrakumar, N. S.; Boyd, V. L.; Hajdu, J. *Biochim. Biophys. Acta* **1982**, *711*, 357–360.
(9) Chandrakumar, N. S.; Hajdu, J. *J. Org. Chem.* **1982**, *47*, 2144–2147.
(10) Chandrakumar, N. S.; Hajdu, J. *Tetrahedron Lett.* **1982**, *23*, 1043–1046.
(11) These studies were conducted in Professor D. J. Hanahan's laboratory providing the first evidence for the platelet-activating behavior of **1**.
(12) Elliott, D. F. *J. Chem. Soc.* **1949**, 589–594.
(13) Bonsen, P. P. M.; Burback-Westerhuis, G. J.; de Haas, G. H.; van Deenen, L. L. M. *Chem. Phys. Lipids* **1972**, *8*, 199–220.
(14) Clearly, we wanted to exclude the possibility of carbanion formation under the strongly basic reaction conditions required for the alkylation step. For precedent see: Meyers, A. I.; Whitten, C. E. *Heterocycles* **1976**, *4*, 1687.
(15) The reducing agent must be used in stoichiometric amounts (i.e., 0.5, mol of $LiAlH_4$/mol of ester) in order to prevent reduction of the oxazoline ring.
(16) Baumann, W. J.; Mangold, H. K. *J. Org. Chem.* **1964**, 29, 3055–3057.
(17) Baumann, W. J.; Mangold, M. K. *J. Org. Chem.* **1966**, *31*, 498.
(18) Freedman, H. H.; Dubois, R.A. *Tetrahedron Lett.* **1975**, *16*, 3251–3254.

Scheme I. Synthesis of 1-Octadecyl-2-(acylamino)-2-deoxyphosphatidylcholine (1) from Serine



ciently N-acylated in the presence of 4-(dimethylamino)-pyridine. Addition of 1 equiv of acid halide or anhydride with a stoichiometric amount of the catalyst leads to nearly quantitative conversion of the amino alcohol 7 to the corresponding amide 9 with only traces of ester byproduct formed.

The absolute dependence of the acylation reaction on the nucleophilic catalyst indicates that the reactivity of 7 is a great deal lower than what is expected for a "typical sterically hindered, secondary amine",[19] yet the observed selectivity of catalytic acyl transfer to nitrogen in presence of the adjacent hydroxylic function renders the sequence 6 → 7 → 9 synthetically feasible. This turns out to be particularly important, since the alternative procedure involving oxazoline ring opening toward formation of the corresponding ester followed by base-catalyzed intramolecular O → N acyl migration proved to be much less efficient for the system here investigated.

In an effort directed at the synthesis of the formamido derivative **9d** we have discovered that the scope of acylating agents can be extended beyond the range of conventional acyl donors normally used in 4-(dimethylamino)pyridine-catalyzed acylation reactions. Specifically, we have been able to obtain catalytic acyl transfer from *p*-nitrophenyl formate[24] to the amino group of 7, resulting in the formation of the corresponding formamide (**9d**) in 70% yield. Clearly, this reaction provides a useful precedent that might become applicable for acylations involving carboxylic acid derivatives for which the acid halides or anhydrides are not readily available.

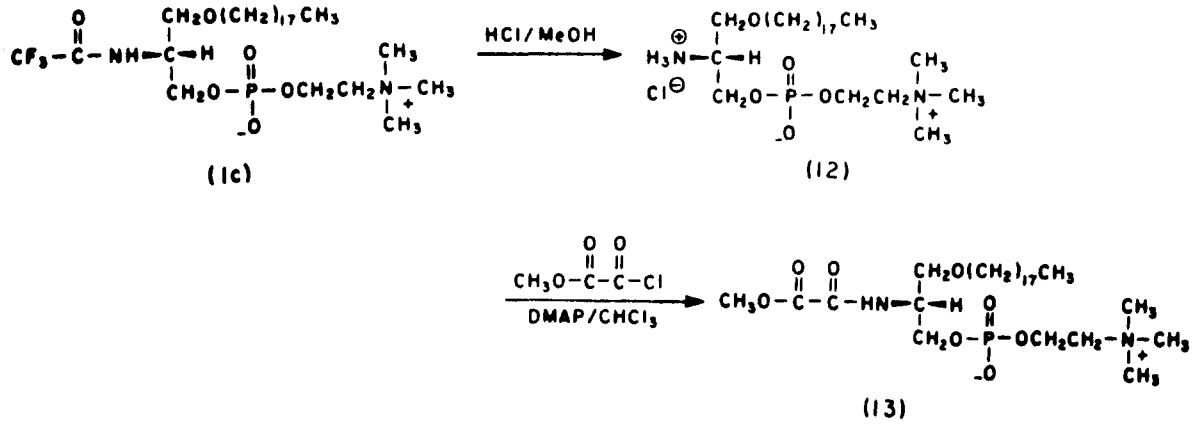
For introduction of the phosphorylcholine moiety, the alcohol 9 was allowed to react with 2-chloro-2-oxo-1,2,3-dioxaphospholane (**10**)[20] in the presence of 1 equiv of triethylamine to produce the cyclic triester 11. The five-membered ring of the phosphotriester 11 was cleaved by anhydrous trimethylamine in acetonitrile at 65 °C to form the quarternary ammonium function of the ether phospholipid 1. It should be pointed out that the cyclic phosphochloridate **10** proved to be a much better synthon for developing the phosphorylcholine function of the target molecule 1 than the more cnventional alternative β-bromoethyl phosphodichloridate. Although the latter reagent has been extensively used in syntheses of ester phospholipids,[13,22] it leads to poor yields and numerous byproducts when employed for phosphorylation of hydroxylic groups which are part of β-amido alcohols (such as compound 9).[13]

In addition to providing a facile and efficient scheme for the preparation of the chiral phosphodiester 1, a number of useful synthetic strategies have emerged from the sequence. The first one concerns the use of the oxazoline ring for protection of the amino alcohol moiety of the substrate, both in reduction as well as in alkylation. It is remarkable that while this heterocyclic function

(19) Nuzzo, R. G.; Haynie, S. L.; Wilson, M. E.; Whitesides, G. M. *J. Org. Chem.* 1981, *46*, 2861–2867.

(20) Edmundson, R. N. *Chem. Ind. (London)* 1962, 1828.
(21) Dittmer, J. C.; Lester, R. L. *J. Lipid Res.* 1964, *5*, 126–127.
(22) Heymans, F.; Michel, E.; Borrel, M.-C.; Wichrowski, B.; Godfried, E.; Tence, M.; Benveniste, J. *Biochim. Biophys. Acta* 1981, *666*, 230.
(23) Compounds in this series are likely to be important as isosteric N-analogues of naturally occuring triglycerides for inhibition studies of lipid-hydrolyzing enzymes. See: Brockerhoff, H.; Jensen, R. G. "Lipolytic Enzymes"; Academic Press: New York, 1974; pp 25–176.
(24) Okawa, K.; Hase, S. *Bull. Chem. Soc. Jpn.* 1963, *36*, 754.

Scheme II. Further Functionalization of the *sn*-2-Position



continues to be the subject of intensive investigations, its synthetic utility is being explored almost entirely as a protecting group for functionalization of carboxylic acids.[25] We have recently shown that oxazolines can be equally useful when derivatization of the amino alcohol component of the heterocycle is desired.[9] The present synthesis provides a further example in this context, affording a new stereospecific method for the preparation of fatty acid ether derivatives of 2-amino-2-deoxyglycerols.[23] The second principal contribution of general synthetic significance emerging from the sequence involves the development of a new, catalytic acyl-transfer reaction for specific N-acylation of sterically hindered amino groups using *p*-nitrophenyl esters (**7d** → **9d**). This reaction is likely to be useful for functionalization of amino acid derivatives, with particular emphasis on active-ester dependent peptide syntheses.[26] Finally our success in employing 2-chloro-1,3,2-dioxaphospholane for the synthesis of ether phospholipids should open the way for the preparation of a new series of alkoxyphospholipids, incorporating various different polar headgroups (i.e., ethanolamine, serine). These compounds should be available by variation of the nucleophilic reagent used for the ring-opening of the cyclic phosphotriester **11**.[27]

Further Functionalization of the Ether Phospholipids 1. Because of the important role attributed to the nature of the group substituting at the *sn*-2-position of ether phospholipids,[1-5] we developed a method (shown in Scheme II) that allows incorporation of the desired substituent *after* the phospholipid skeleton has been assembled. Thus, the amine analogue (**12**) of 1-octadecyllysophosphatidylcholine was prepared by acid-catalyzed alcoholysis of the corresponding *sn*-2-trifluoroacetamide (**1c**) in quantitative yield as the hydrochloride salt. Compounds **12** can be readily reacylated, for example, by methyloxalyl chloride in the presence of 4-(dimethylamino)pyridine to give the phospholipid **13**, which in this case contains an additional ester function carried by the *sn*-2-amido substituent.

Obviously, a similar sequence of steps should allow incorporation of additional types of side chains, including positive and negative charge-carrying substituents as well as photoactivable and covalently reactive functional groups. Such functionalized phospholipid derivatives are likely to find important applications as structural probes in physicochemical as well as biochemical studies focusing on phospholipid–phospholipid and phospholipid–protein interactions.[28]

Biological Activities and Physicochemical Properties of 1. We have been able to obtain evidence demonstrating that the synthetic ether phospholipids exhibit potent biological activities in (1) activating radioactively labeled rabbit platelets,[11] (2) eliciting hypotensive responses from spontaneous hypertensive rats,[5] and (3) displaying effective cytotoxicity against human leukemia cells in vitro.[29] Preliminary experiments have shown that the 2-acetamido compound **1a** appears to have the highest platelet-stimulating potency in the series, causing 50% serotonin secretion from ^{14}C-labeled rabbit platelets in 1 min at a 1.5×10^{-8} M concentration in vitro.[11] For achievement of the same response with the *sn*-2-trifluoroacetamido (**1c**) and the *sn*-2-formamido (**1d**) ether phospholipids, the required concentrations are 4.06×10^{-7} and 9.7×10^{-6} M, respectively. A similar trend is observed in comparing the in vivo antihypertensive activities of the compounds, **1a** being the most effective in the series.[5] In contrast, the *sn*-2-palmitoyl derivative (**1b**) has shown consistently low activities in these tests.

Finally, we have observed that introduction of the alkyl substituent in place of the 1-acyl group dramatically alters the physicochemical properties of the phospholipid. The phase-transition behavior of **1b**, for example, is very much different from that of the corresponding diacyl derivative.[30] Specifically, microcalorimetric measurements have shown that **1b** exhibits (1) a complex phase-transition curve that can be resolved into five state components, (2) an overall calorimetric enthalpy of 27.9 kcal mol^{-1}, more than 3 times higher than that of a diacyl phosphatidylcholine, and (3) a temperature range of 17 °C for the overall excess heat capacity vs. temperature profile.[30] Clearly, an understanding of the phase-transition behavior of **1** requires a great deal of further investigation. However, it should be pointed out that the transition curves and the microcalorimetric parameters obtained for diacyl phosphatidylcholine and the corresponding 2-deoxyaminoacyl analogue are quite similar,[30] and it is likely that the unique thermotropic behavior exhibited by the 1-*O*-alkyl derivative

(25) Levin, J. I.; Weinreb, S. M. *Tetrahedron Lett.* **1982**, *23*, 2347–2350.
(26) Bodanszky, M.; du Vigneaud, V. *J. Am. Chem. Soc.* **1959**, *81*, 5688–5691.
(27) Thuong, N. T.; Chabrier, P. *Bull. Soc. Chim. Fr.* **1974**, 667. These authors have provided evidence for ring-opening of the cyclic phosphodiester moiety by a number of different nucleophilic reagents.
(28) (a) Gupta, C. M.; Radhakrishnan, R.; Khorana, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 4315. (b) Verger, R.; de Haas, G. H. *Annu. Rev. Biophys. Bioeng.* **1976**, *5*, 77.
(29) The cytotoxicity studies were carried out in collaboration with Professor F. Snyder, Oak Ridge Association Universities, Oak Ridge, TN, by using serum-free HL-60 cells and will be published elsewhere.
(30) Chowdhry, B. Z.; Hajdu, J.; Lipka, G.; Sturtevant, J. M., submitted for publication in *Nature (London)*.

might result from a more hydrophobic microenvironment created by the presence of the alkoxy group near the polar head of the molecule, which changes the hydration properties of the phospholipid.

In conclusion it might be noted that the synthesis that we have described provides a facile and efficient route to a wide range of ether phospholipids. Its strength lies in its simplicity and flexibility, and the methods that we have developed are likely to be applicable for the synthesis of additional types of phospholipid derivatives. Synthetic work toward this goal is currently underway in our laboratory.

Experimental Section

General Methods. Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 599B spectrophotometer. ^1H NMR (internal Me_4Si) spectra were taken on a Hitachi Perkin-Elmer R-24 60-MHz instrument. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. L-Serine and octadecanol were obtained from Sigma Chemical Co. 4-(Dimethylamino)-pyridine, dicyclohexylcarbodiimide, palmitoyl chloride, methyloxalyl chloride, and trifluoroacetic anhydride (gold label) were obtained from Aldrich and used as received. Acetyl chloride, benzonitrile, and anhydrous trimethylamine were purchased from Eastman. Acetonitrile (Burdick and Jackson) and triethylamine (Eastman) were dried over Linde 4A molecular sieves (Ventron). Benzene was distilled from calcium hydride. Chloroform and methylene chloride were distilled from phosphorus pentoxide prior to use. Tetrahydrofuran was freshly distilled from lithium aluminum hydride. 2-Chloro-2-oxo-1,3,2-dioxaphospholane,[20] 2-phenyl-4-(carbomethoxy)-2-oxazoline,[12] *p*-nitrophenyl formate,[24] and octadecyl methanesulfonate[16] were prepared by literature procedures. Column chromatography was carried out by using silica gel 60 (70–230-mesh ASTM), Sephadex LH-20 (25–100-μm beads), and Rexyn I-300 mixed-bed resin obtained from EM Laboratories, Pharmacia and Fisher, respectively. Thin-layer chromatography was carried out on Whatman K6F plates. The phospholipids were visualized by molybdic acid spray.[21] Amine-containing compounds were spotted by use of ninhydrin, and all other compounds were detected by charring (50% sulfuric acid) or by iodine vapor. Elemental analyses were performed by Galbraith Laboratories, Inc.

2-Phenyl-4-(hydroxymethyl)-2-oxazoline (5). To a stirred solution of ester **4** (10.1 g, 0.0493 mol) in 500 mL of anhydrous ether at 0 °C was added in one portion lithium aluminum hydride (0.995 g, 0.0263 mol). The reaction mixture was allowed to come to room temperature and then stirred for 2 h. Moist ether was added to decompose excess hydride followed by 50 mL of water. The aqueous layer was separated and extracted with ether (3 × 50 mL). The combined etheral extract was washed with saturated NaCl solution and dried over $MgSO_4$. Evaporation of the solvent gave 7.75 g (89%) of **5** as a white solid. Recrystallization from ether gave an analytical sample of the alcohol: mp 99.5 °C; IR (Nujol) 3275 (br), 1650 cm^{-1}; NMR ($CDCl_3$) δ 3.55–4.55 (m, 6 H), 7.15–7.9 (m, 5 H). Anal. Calcd for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.81; H, 6.47; N, 7.90.

2-Phenyl-4-[(octadecyloxy)methyl]-2-oxazoline (6). To a suspension of sodium hydride [0.0386 mol, 1.544 g of 60% dispersion in mineral oil, washed with petroleum ether (3 × 25 mL)] in 50 mL of tetrahydrofuran cooled to 0 °C was added dropwise the alcohol **5** (5.96 g, 0.0336 mol) in 30 mL of THF. The bright yellow-colored mixture was stirred at room temperature for 1 h and at 55 °C for 2 h. The mixture was then cooled to 0 °C, and octadecyl methanesulfonate (12.6 g, 0.0362 mol) in 50 mL of tetrahydrofuran was added dropwise. The resulting mixture was stirred at room temperature for 1 h, refluxed for 20 h, further stirred at room temperature for 10 h, and poured into 100 mL of ice-cold water. The solid precipitate was filtered (12.5 g), and the filtrate was extracted with ether (3 × 100 mL). The etheral extract was washed with saturated NaCl solution and dried over $MgSO_4$. Evaporation of the solvent left an additional 2.0 g of the product. Recrystallization of the combined solid from methanol gave 13.7 g (91%) of the aliphatic ether **6**: mp 49–50 °C; IR (Nujol) 3040, 1645 cm^{-1}; NMR ($CDCl_3$) δ 0.9 (t, 3 H), 1.25 (br s, 32 H), 3.5 (m, 4 H), 4.4 (m, 3 H), 7.25–8.25 (m, 5 H); $[\alpha]^{25}_D$ +23.62° (*c* 1.38, 4:1 $CHCl_3$–CH_3CH_3OH). Anal. Calcd for: $C_{28}H_{47}NO_2$: C, 78.27; H, 11.01; N, 3.13. Found: C, 78.46; H, 11.03; N, 3.26.

1-Octadecyl-2-amino-2-deoxy-*sn*-glycerol (7). A solution of the ether **6** in 10 mL of 6 N sulfuric acid was refluxed for 10 h. The solid obtained on cooling was filtered and suspended, with vigorous stirring, in a mixture of 100 mL of aqueous saturated K_2CO_3 and 150 mL of ether. After continuous stirring for 1 h the etheral layer was separated, and the aqueous portion was extracted with ether (2 × 100 mL). The combined organic phase was washed with 100 mL of saturated K_2CO_3 solution followed by 100 mL of saturated NaCl solution and dried ($MgSO_4$). The solvent was evaporated, and the crude product was flash chromatographed on silica gel (5% MeOH/$CHCl_3$) to give 4.7 g (93%) of the pure amino alcohol **7**. Recrystallization from ether gave an analytical sample: mp 75 °C; IR (Nujol) 3340, 3295 cm^{-1}; NMR ($CDCl_3$–CD_3OD) δ 0.9 (t, 3 H), 1.25 (br s, 32 H), 2.8–4.1 (m, 10 H); $[\alpha]^{25}_D$ +1.37° (*c* 1.25, 4:1 $CHCl_3$–CH_3OH). Anal. Calcd for $C_{21}H_{45}NO_2$: C, 73.41; H, 13.20; N, 4.08. Found: C, 73.60; H, 13.15; N, 4.01.

1-Octadecyl-2-acetamido-*sn*-glycerol (9a). A solution of amino alcohol **7** (0.765 g, 2.23 mmol), acetyl chloride (0.175 g 2.23 mmol), and 4-(dimethylamino)pyridine (0.272 g, 2.23 mmol) in 30 mL of chloroform was kept at room temperature for 48 h. The solvent was then removed in vacuo, and the residue was flash chromatographed on silica gel with 4.5% methanol in chloroform to give 0.825 g (96%) of pure acetamide (**9a**). Recrystallization from chloroform provided an analytical sample of **9a**: mp 84 °C; IR (Nujol) 3410, 3295, 1630 cm^{-1}; NMR ($CDCl_3$) δ 0.8 (m, 3 H), 1.2 (br s, 32 H), 2.0 (s, 3 H), 3.0–4.05 (m, 9 H); $[\alpha]^{25}_D$ −11.6° (*c* 1.33, 1:4 CH_3OH–$CHCl_3$). Anal. Calcd for $C_{23}H_{47}NO_3$: C, 71.64; H, 12.29; N, 3.63. Found: C, 71.86; H, 12.50; N, 3.60.

1-Octadecyl-2-deoxy-2-(hexadecanoylamino)-*sn*-glycerol (9b). A mixture of **7** (2.87 g, 8.38 mmol), palmitoyl chloride (2.307 g, 8.39 mmol), and 4-(dimethylamino)pyridine (1.024 g, 8.39 mmol) in 200 mL of chloroform was stirred at room temperature for 48 h. The solvent was then evaporated, and the solid residue was washed several times with water, filtered, and finally flash chromatographed on silica gel with 4.5% methanol in chloroform (to remove traces of the corresponding ester and the unreacted amino alcohol) to give 4.35 g (90%) of **9b**. An analytical sample was obtained by recrystallization from chloroform: mp 95 °C; IR (Nujol) 3480, 3300, 1645 cm^{-1}; NMR ($CDCl_3$–CD_3OD) δ 0.85 (br t, 6 H), 1.20 (br s, 58 H), 2.15 (br t, 2 H), 3.4 (m, 9 H) $[\alpha]^{25}_D$ −8.54° (*c* 1.04, 1:4 CH_3OH–$CHCl_3$). Anal. Calcd for $C_{37}H_{75}NO_3$: C, 76.36; H, 12.99; N, 2.41. Found: C, 76.53; H, 12.76; N, 2.37.

1-Octadecyl-2-deoxy-2-(trifluoroacetamido)-*sn*-glycerol (9c). A solution of amino alcohol **7** (1.040 g, 3.0 mmol), trifluoroacetic anhydride (0.640 g, 3.0 mmol), and 4-(dimethylamino)pyridine (0.373 g, 3.0 mmol) in 50 mL of chloroform was allowed to stand at room temperature for 48 h. The solvent was evaporated, and the solid residue was rapidly flash chromatographed (silica gel; methanol–chloroform, 4:96) to give 1.2 g (90%) of the trifluoroacetamide **9c** as an analytically pure white crystalline solid: mp 75.5 °C; IR (Nujol) 3280, 1695 cm^{-1}; NMR ($CDCl_3$) δ 0.8 (br t, 3 H), 1.2 (br s, 32 H), 2.65 (m, 1 H), 3.2–3.95 (m, 7 H). Anal. Calcd for $C_{23}H_{44}F_3NO_3$: C, 62.84; H, 10.09; N, 3.19. Found: C, 63.01; H, 9.80; N, 3.20.

1-Octadecyl-2-deoxy-2-formamido-*sn*-glycerol (9d). A solution of amino alcohol **7** (0.9366 g, 2.7 mmol), *p*-nitrophenyl formate (0.456 g, 2.7 mmol), and 4-(dimethylamino)pyridine (0.3331 g, 2.7 mmol) in 50 mL of chloroform was stirred at room temperature for 48 h. The solution was then diluted by addition of 50 mL of chloroform and washed with 5% aqueous K_2CO_3 until the solution remained colorless. The organic layer was dried over $MgSO_4$, and the solvent was removed in vacuo to give the crude formamide as an off-white solid. The product was then chromatographed on silica gel (20 g) with methanol–chloroform (4:96), yielding 0.710 g (70%) of formamide **9d** as analytically pure white crystals: mp 76–77 °C; IR (Nujol) 3340, 3275, 3080, 1665, 1645 cm^{-1}; NMR ($CDCl_3$) δ 0.85 (br t, 3 H), 1.2 (br s, 32 H) 3.1–3.9 (m, 9 H), 8.15 (s, 1 H). Anal. Calcd for $C_{22}H_{45}NO_3$: C, 71.11; H, 12.21; N, 3.77. Found: C, 71.34; H, 12.27; N, 3.68.

2-(1-Octadecyl-2-acetamido-2-deoxy-*sn*-glycero)-2-oxo-1,3,2-dioxaphospholane (11a). Alcohol **9a** (0.5727 g, 1.48 mmol)

was suspended in 60 mL of benzene. To this suspension was added triethylamine (0.15 g, 1.48 mmol), and the mixture was cooled to 5 °C. To this was added 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.2112 g, 1.48 mmol) in 2 mL of benzene in one portion. The mixture was stirred at room tempeature for 50 h. The crystalline Et_3N·HCl that precipitated was filtered off, and the solvent was removed in vacuo to give the phosphate triester **11a** as a white solid (0.725 g): NMR ($CDCl_3$) δ 0.9 (t, 3 H), 1.2 (br s, 32 H) 2.05 (s, 3 H), 3.0–3.85 (m, 4 H) 4.05–4.65 (m, 7 H), consistent with the structure. This material was used for the next step without purification.

Compounds **11b–d** were prepared in a similar procedure. Each of the triester intermediates gave confirmatory NMR spectra and were used for the subsequent ring-opening step without further treatment.

1-Octadecyl-2-acetamido-2-deoxy-*sn*-glycero-3-phosphorylcholine (1a). Phosphate triester **11a** (0.7 g) was transferred into a pressure bottle with dry acetonitrile, and 1 mL of anhydrous trimethylamine was added to it. The bottle was kept in an oil bath at 65 °C for 48 h. Cooling and subsequent filtration yielded 0.69 g of **11a** as a white crystalline solid. A 0.39-g sample of this product was chromatographed on silica gel with chloroform–methanol–aqueous NH_3 (1:9:1 v/v) to give 0.28 g (71%) of analytically pure ether phospholipid **1a**: $[\alpha]^{25}_D$ −9.87° (*c* 1.15, 1:4 CH_3OH–$CHCl_3$); IR (Nujol) 3290, 1655 cm^{-1}; NMR ($CDCl_3$–CD_3OD) δ 0.9 (br t, 3 H), 1.25 (br s, 32 H), 2.0 (s, 3 H), 3.1–4.0 (m, 20H with a singlet at 3.25); R_f ($CHCl_3$–MeOH–aqueous NH_3, 1:9:1) 0.27, R_f ($CHCl_3$–MeOH–water, 65:25:4) 0.32. Anal. Calcd for $C_{28}H_{59}N_2O_6P·H_2O$: C, 59.15; H, 10.98; N, 4.92; P, 5.53. Found: C, 59.13; H, 10.81; N, 4.93; P, 5.45.

1-Octadecyl-2-deoxy-2-(hexadecanoylamino)-*sn*-glycero-3-phosphorylcholine (1b). A solution of the phosphate triester **11b** (1.720 g) and 1 mL of anhydrous trimethylamine in 50 mL of dry acetonitrile was kept at 65 °C for 48 h. The white precipitate that formed was filtered, giving 0.72 g of crude product. This was chromatographed on silica gel with chloroform–methanol–water (65:25:4) as the eluent and gave 0.47 g (65%) of analytically pure white crystalline phospholipid **1b**: $[\alpha]^{25}_D$ −8.12° (*c* 1.23, 1:4 CH_3OH–$CHCl_3$); IR (Nujol) 3270 (br), 1645 cm^{-1}; NMR ($CDCl_3$–CD_3OD) δ 0.85 (br t, 6 H), 1.25 (br s, 58 H), 2.1 (br t, 2 H), 3.0–4.4 (m, 20 H, with a singlet at 3.2). Anal. Calcd for $C_{42}H_{87}N_2O_6P·2H_2O$: C, 64.41; H, 11.71; N, 3.58; P, 3.96. Found: C, 64.40; H, 11.55; N, 3.59; P, 4.15.

1-Octadecyl-2-deoxy-2-(trifluoroacetamido)-*sn*-glycero-3-phosphorylcholine (1c). Phosphate triester **11c** (1.1 g) was treated with trimethylamine in acetonitrile as described for **11b**. The phosphorylcholine obtained was chromatographed on 6.0 g of silica gel with chloroform–methanol–water (65:25:4) to give 0.61 g (45%)[31] of **1c** as an analytically pure white crystalline solid: $[\alpha]^{25}_D$ −6.08° (*c* 0.97, 1:4 CH_3OH–$CHCl_3$); IR (Nujol) 3300 (br), 1710 cm^{-1}; NMR ($CDCl_3$–CD_3OD) δ 0.8 (br t, 3 H), 1.2 (br s, 32 H), 3.1 (s, 9 H), 3.15–4.2 (m), 8.0 (s, 1 H); R_f ($CHCl_3$–MeOH–water, 65:25:4) 0.21. Anal. Calcd for $C_{28}H_{56}F_3N_2O_6P·H_2O$: C, 54.00; H, 9.06; N, 4.50; P, 4.97. Found: C, 53.90; H, 9.07; N, 4.57; P, 4.97.

1-Octadecyl-2-deoxy-2-formamido-*sn*-glycero-3-phosphorylcholine (1d). A solution of 0.6 g of phosphate triester **11d** in 50 mL of anhydrous acetonitrile was treated with 1 mL of trimethylamine and heated in a pressure bottle at 65 °C for 48 h. The solvent was then evaporated in vacuo, and the resulting solid was purified by chromatography on silica gel (6.0 g) with chloroform–methanol–water (65:25:4 v/v) to give 310 mg (44%)[31] of analytically pure ether phospholipid **1d** as a strongly hydroscopic white crystalline sample: $[\alpha]^{25}_D$ −6.35° (*c* 0.95, 1:4 CH_3OH–$CHCl_3$); IR (Nujol) 3300 (br), 1675 (br) cm^{-1}; NMR ($CDCl_3$–CD_3OD) δ 0.8 (br t, 3 H), 1.2 (br s, 32 H), 3.15 (s, 9 H), 3.1–4.2 (m), 8.0 (s, 1 H); R_f ($CHCl_3$–MeOH–water, 65:25:4) 0.12. Anal. Calcd for $C_{27}H_{57}N_2O_6P·3.5H_2O$: C, 54.07; H, 10.76; N, 4.67; P, 5.16. Found: C, 54.10; H, 10.23; N, 4.56; P, 5.22.

1-Octadecyl-2-amino-2-deoxy-*sn*-glycero-3-phosphorylcholine Hydrochloride (12). A solution of 0.250 g of phospholipid **1c** in 30 mL of anhydrous methanol was saturated with HCl gas at room temperature. This reaction mixture was allowed to stand for an additional 48 h, and then the solvent was evaporated. The resulting white solid residue (**12**) exhibited a single ninhydrin- and phosphate-positive[21] spot on thin-layer chromatography [R_f ($CHCl_3$–MeOH–aqueous NH_3, 1:9:1) 0.22] and had no carbonyl absorption in its IR spectrum. It was used for the next step without further treatment.

1-Octadecyl-2-(*O*-methyloxalamido)-2-deoxy-*sn*-glycero-3-phosphorylcholine (13). The amine hydrochloride **12** (0.140 g, 0.258 mmol) was suspended in 25 mL of chloroform, and 0.075 g (0.614 mmol) of 4-(dimethylamino)pyridine was added to it. The solution was cooled to 0 °C, and 0.040 g (0.330 mmol) of methyl oxalyl chloride in 5 mL of chloroform was added. The reaction mixture was kept at room temperature for 48 h. The solvent was then evaporated, and the residue was dissolved in 30 mL of chloroform–methanol–water (4:5:1 v/v) and passed through a column of Rexyn 1-300 ion-exchange resin (20-mL bed volume). The resin washed with 20 mL of the same solvent. The product from the combined effluent was dried and subsequently chromatographed on silica gel (4.0 g) with chloroform–methanol–water (65:25:4 v/v) as the solvent to give the analytically pure phospholipid: 0.060 g (37%);[31] semisolid; $[\alpha]^{25}_D$ −8.68° (*c* 1.1, 1:4 CH_3OH–$CHCl_3$); IR (Nujol) 3320 br, 1740, 1685 cm^{-1}; NMR ($CDCl_3$–CD_3OD) δ 0.85 (br t, 3 H), 1.15 (br s, 32 H), 3.1–4.5 (m) 3.15 (s, 9 H), 3.8 (s, 3 H); R_f ($CHCl_3$–MeOH–water, 65:25:4) 0.18. Anal. Calcd for $C_{29}H_{59}N_2O_8P·H_2O$: C, 56.84; H, 10.03; N, 4.57; P, 5.05. Found: C, 56.84; H, 10.41; N, 4.40; P, 5.06.

Acknowledgment. We are grateful to Professor D. J. Hanahan of the University of Texas, San Antonio, for testing the ether phospholipids with ^{14}C-labeled rabbit platelets, to Professor F. Snyder of Oak Ridge Associated Universities for the in vivo experiments, to Professor J. M. Sturtevant and Dr. B. Z. Chowdhry of Yale University for determination of the phase-transition properties of the synthetic phospholipids, and to Professor Raymond Panzica of the University of Rhode Island for access to the polarimeter in his laboratory. It is a pleasure to acknowledge the financial support of the Research Corp., of the American Heart Association, Greater Boston Massachusetts Division and American Heart Association Massachusetts Affiliate, Inc. (Grant No. 13-503-798), and the National Institutes of Health (Grant No. AM26165).

Registry No. **1a**, 82936-54-7; **1b**, 82936-55-8; **1c**, 83922-26-3; **1d**, 83922-25-2; **4**, 78715-83-0; **5**, 82936-49-0; **6**, 82936-50-3; **7**, 82936-51-4; **9a**, 82936-52-5; **9b**, 82936-56-9; **9c**, 84895-20-5; **9d**, 84895-21-6; **11a**, 82936-53-6; **11b**, 82936-57-0; **11c**, 84895-22-7; **11d**, 84895-23-8; **12**, 84895-24-9; **13**, 84895-25-0; octadecyl methanesulfonate, 31081-59-1; palmitoyl chloride, 112-67-4; 2-chloro-2-oxo-1,3,2-dioxophospholane, 6609-64-9; trimethylamine, 75-50-3; methyl oxalyl chloride, 5781-53-3.

(31) We believe that the apparent low yield is due to incomplete recovery of the phospholipid product from the silica gel chromatography. Efforts are underway to improve this purification step.