IR (film, as a mixture of C-2 epimers) 2470, 2940, 2860, 1595, 1470, 1430, 1385, 1205, 1110, 740, 705 cm^{-1}; NMR (360 MHz, $CDCl_3$) δ 7.66 (m, 4 H, aromatic), 7.37 (m, 11 H, aromatic), 4.50 (AB q, J = 12.0 Hz, $\Delta\nu$ = 19.7 Hz, 2 H, benzylic), 3.92 (dd, J_1 = 2.4 Hz, J_2 = 9.6 Hz, 1 H), 3.71 (m, 2 H), 3.58 (m, 3 H), 3.55 (m, 1 H), 3.44 (s, 3 H, OCH_3), 3.33 (d, J = 6.0 Hz, 1 H), 2.92 (br s, 1 H, H-5'), 2.00 (m, 1 H), 1.84 (m, 1 H), 1.70 (m, 1 H), 1.65-1.20 (m, 6 H), 1.36 (s, 3 H, acetonide methyl), 1.32 (s, 3 H, acetonide methyl), 1.04 (s, 9 H, *tert*-butyl), 0.81 (d, J = 6.7 Hz, 3 H, CH_3); MS, m/z (relative intensity, 70 eV) 633 (0.01, $M^+ - CH_3$), 409 (0.03, $M^+ - SiPh_2t\text{-}Bu$), 393 (0.43), 393 (0.43), 369 (0.53), 340 (0.31), 339 (1.32), 323 (0.15), 315 (0.30), 285 (0.99), 263 (2.06), 213 (2.07), 200 (1.94), 199 (11.40), 183 (2.42), 135 (6.01), 125 (25.85), 107 (13.32), 91 (base, $PhCH_2$), 71 (41.03), 58 (24.88). Anal. Calcd for $C_{39}H_{56}O_6Si$: C, 72.18; H, 8.70; Si, 4.33. Found (as a mixture of C-2 epimers): C, 72.48; H, 8.80; Si, 4.18. Acetonide-alcohol **42**: R_f 0.26 (silica, 50% EtOAc/hexane); NMR (360 MHz, $CDCl_3$) δ 7.66 (m, 4 H, aromatic), 7.38 (m, 11 H, aromatic), 4.52 (AB q, J = 11.5 Hz, $\Delta\nu$ = 14.6 Hz, 2 H, benzylic), 3.95 (m, 1 H), 3.73 (m, 3 H), 3.60 (m, 3 H), 3.50 (m, 1 H), 3.45 (s, 3 H, OCH_3), 3.32 (dd, J_1 = 4.3 Hz, J_2 = 11.0 Hz, 1 H), 2.97 (br s, 1 H, H-5'), 2.00 (m, 2 H), 1.83 (m, 2 H), 1.57-0.88 (m, 4 H), 1.42 (s, 3 H, acetonide methyl), 1.37 (s, 3 H, acetonide methyl), 1.05 (s, 9 H, *tert*-butyl), 0.81 (d, J = 5.3 Hz, 3 H, CH_3).

Acknowledgment. We express our appreciation to the NIH and the NSF for support of this program and to Prof. K. C. Nicolaou for providing the copies of comparison spectra.

Note Added in Proof. It has recently come to our attention that A. J. Pearson and T. Ray (*Tetrahedron Lett.* **1986**, *27*, 3111) have developed an alternative approach to carbomycin B.

Stereospecific Synthesis of Ether and Thioether Phospholipids. The Use of L-Glyceric Acid as a Chiral Phospholipid Precursor

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A novel stereospecific synthesis of biologically active ether phospholipids is reported. The synthesis is based on (1) the use of L-glyceric acid as the chiral center for the construction of the optically active phospholipid molecule, (2) the introduction of the *sn*-2-short chain alkyl substituent via silver tetrafluoroborate catalyzed alkylation reaction that leaves the neighboring carbomethoxy group unaffected, and then elaboration of the lipophilic alkoxy function at the *sn*-1-position, and (3) the development of the *sn*-3-phosphorylcholine moiety through the 2-chloro-2-oxo-1,3,2-dioxaphospholane-trimethylamine sequence. The entire scheme involves the use of a single protecting group. Through the use of intermediates that became available from the sequence, a scheme for the preparation of antitumor active *sn*-1-thio ether phospholipids has been developed. The synthetic methods have a great deal of flexibility, providing convenient routes to a wide range of ether and thioether phospholipids for physicochemical as well as enzymological studies.

The synthesis of biologically active phospholipid compounds is one of the most timely problems in membrane chemistry today.[1-4] Ether phospholipids occupy a particularly important position in this regard as they have been shown to possess high levels of activity in a wide range of physiologically vital regulatory events.[5,6] Specifically, 1-alkylglycerophosphocholines are potent platelet-activators,[1,7] exhibit strong antihypertensive activity[1,8] and function as effective immunomodulating agents.[9] In addition, recent evidence has established that a series of structural analogues of platelet-activating factor (PAF) show selective tumor cytotoxicity against a number of different human cancer cells.[10] These observations have recently initiated vigorous activity in an attempt to explore the use of alkylphospholipids as potential drugs in antileukemic chemotherapy.[11]

Despite the well-recognized biological importance of these compounds, relatively little progress has been made toward elucidation of their mechanism of action. In order to achieve this goal and to delineate the structural requirements associated with the respective biological activities, synthetic phospholipid derivatives need to be prepared. Furthermore, availability of facile and efficient synthetic procedures leading to the desired compounds represents not only a prerequisite to the establishment of structure-function correlations but also a basis for the

(1) Venuti, M. C. *Annu. Rev. Med. Chem.* **1985**, *20*, 193-202.
(2) Weber, N.; Benning, H. *Eur. J. Biochem.* **1985**, *146*, 323-328.
(3) Horrocks, L. A.; Sharma, M. In *Phospholipids*; Hawthorn, J. N., Ansell, G. B., Eds.; Elsevier: Amsterdam, 1982; pp 51-93.
(4) Lee, T.-c.; Malone, B.; Snyder, F. *J. Biol. Chem.* **1986**, *261*, 5373-5377.
(5) Lee, T.-c.; Snyder, F. In *Phospholipids and Cellular Regulation*; Kuo, J. F., Ed.; CRC: Boca Raton, FL, 1985; Vol. 2, pp 1-39.
(6) O'Flaherty, J. T.; Wykle, R. L. *Clin. Rev. Allergy* **1983**, *1*, 353-367.
(7) (a) Snyder, F. *Annu. Rev. Med. Chem.* **1982**, *17*, 243-252. (b) Snyder, F. In *Platelet Activating Factor and Related Lipid Mediators*; Snyder, F., Ed.; Plenum: New York, 1987; pp 89-114.
(8) Blank, M. L.; Snyder, F.; Byers, L. W.; Brooks, B.; Muirhead, E. E. *Biochem. Biophys. Res. Commun.* **1979**, *90*, 1194-1200.
(9) Munder, P. G.; Weltzien, H. O.; Modollel, M. In *Immunopathology, VIIth International Symposium, Bad Schadhe*; Miescher, P. A., Ed.; Schwabe: Basel, 1976; pp 411-424.
(10) (a) Hoffman, D. R.; Hoffman, L. H.; Snyder, F. *Cancer Res.* **1986**, *46*, 5803-5809. (b) Berdel, W. E.; Andreesen, R.; Munder, P. G. In *Phospholipids and Cellular Regulation*; Kuo, J. F., Ed.; CRC: Boca Raton, FL, 1985; Vol. 2, pp 42-72. (c) Hoffman, D. R.; Hajdu, J.; Snyder, F. *Blood* **1984**, *63*, 545-552.
(11) (a) Berdel, W. E. *Blut* **1982**, *44*, 71-78. (b) Berdel, W. E.; Bausert, W. R. E.; Fink, U.; Rastetter, J.; Munder, P. G. *Anticancer Res.* **1981**, *1*, 345-352. (c) Berdel, W. E.; Munder, P. In *Platelet-Activating Factor and Related Lipid Mediators*; Snyder, F., Ed.; Plenum: New York, 1987; pp 449-467.

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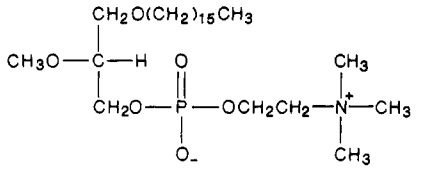
design and development of new derivatives with targeted activity and potency.

As part of our research in this area,[12-16] we recently introduced a new approach to phospholipid synthesis using L-glyceric acid as a chiral precursor for the synthesis of the antitumor active 1-hexadecyl-2-methyl-*sn*-glycero-3-phosphocholine (1).[16] In this article we describe the



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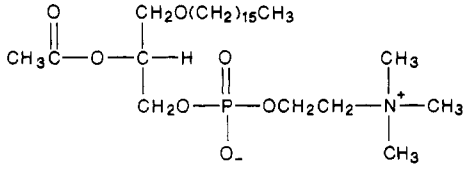
synthesis in detail, and demonstrate that the sequence can readily be extended for the preparation of other related phospholipid derivatives, yielding structurally modified analogues, substituted at the 2-position as well as in the quaternary nitrogen function of the phospholipid molecule. In addition, we now report an extention of the sequence for the stereospecific synthesis of *sn*-1-thioether analogues.

Results and Discussion

The structural design of 1 as our target compound was based on recent biochemical studies demonstrating that "nonhydrolyzable analogues"[10c,17] of platelet-activating factor (PAF, 2) showed selective tumor cytotoxicity against



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a number of human cancer cells.[11] The lead compound (1) appeared to fulfill the criteria attributed to effective antitumor activity[10c] in possessing (1) an alkyl moiety at the *sn*-1-position, (2) a PAF-hydrolase resistant short-chain substituent at the *sn*-2-position,[17] and (3) a phosphocholine function at the *sn*-3-position of glycerol.

Although most previously conducted in vitro as well as in vivo studies had to rely on racemic mixtures of ether–phospholipids, the few cases where enantiomerically pure compounds were available clearly indicated that the synthetic D stereoisomers were either inactive[18] or exhibited very different activities from those shown by the naturally occurring L enantiomers.[10c] Consequently, an approach employing stereospecific syntheses of structurally variable phospholipid derivatives should allow (1) determination of the role of the substituents and (2) assignment of the biological activity in terms of stereochemical identity.

Our synthetic method for the preparation of the chiral ether–phospholipid 1 is outlined in Scheme I. Obvious variations of this sequence of reactions allow preparation of a wide range of related analogues. Significantly, in contrast to previous stereospecific phospholipid syntheses that relied almost exclusively on derivatization of D-mannitol,[19] we have employed L-glyceric acid as a chiral educt to optically active lipids. Although the corresponding methyl glycerate can be prepared via esterification of the parent acid with methanolic HCl,[16] availability of the acetonide 3 in high optical purity[20] makes it a more convenient source of compound 4. Specifically, deprotection of the isopropylidene 3 is readily achieved in anhydrous methanolic HCl at room temperature for 1 h. The resulting product (4) is obtained as a strongly hygroscopic glassy residue that must be thoroughly dried (first in vacuo and then in acetonitrile solution over activated molecular sieves[21]) before further derivatization.

L-Methyl glycerate 4 was readily converted to triphenylmethyl ether 5 in reaction with tritylpyridinium tetrafluoroborate in acetonitrile.[22] The use of this reagent alleviated the need for a base, assuring that no racemization could occur in the course of the reaction. For introduction of the short-chain alkyl substituent at the *sn*-2-position we have developed a mild yet very efficient alkylation reaction using silver tetrafluoroborate/methyl iodide in the presence of excess solid Ag_2CO_3. The reaction readily proceeds at room temperature. Silver carbonate is added in order to neutralize the HBF_4 formed as a by-product of the reaction that otherwise would detritylate the neighboring *sn*-3-function. Significantly, under these alkylation conditions the short-chain *sn*-2-moiety could be developed *prior* to incorporation of the lipophilic *sn*-1 hexadecyl ether function, without affecting the stereochemistry of the chiral center. The resulting methyl ether (6), purified on silica gel chromatography, was obtained as a cystalline solid (mp 103 °C, 84% isolated yield).

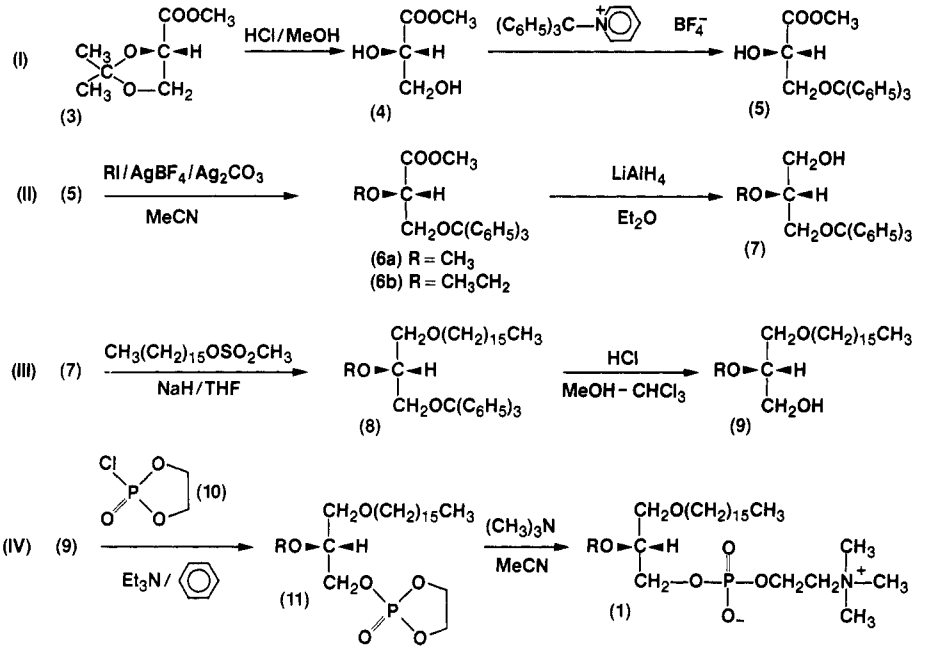
Reduction of the carbomethoxy group of 6 with lithium aluminum hydride gave the corresponding primary alcohol (7) in good yield (82%). The long-chain alkoxy moiety at the incipient *sn*-1-position of the target phospholipid (1) was next developed in a coupling reaction between equimolar hexadecyl methanesulfonate and the sodium alkoxide of 7 in refluxing tetrahydrofuran.[15] The resulting triether 8 was chromatographed on activated silica gel, yielding a low-melting waxy product (mp 42–43 °C, from petroleum ether).

Acid-catalyzed deprotection of the *sn*-3-hydroxy group was accomplished in anhydrous methanol–chloroform (1:1) solution with HCl. The purified alcohol 9, obtained as a strongly hygroscopic low-melting solid (90%), had to be dried in two stages: first in vacuo over phosphorus pentoxide and then in benzene solution over 3A molecular sieves to render it suitable for phosphorylation.

For elaboration of the phosphodiester function at the *sn*-3-position, the alcohol 9 was first allowed to react with 2-chloro-2-oxo-1,3,2-dioxaphospholane (10)[24] in the presence of 1 equiv of triethylamine in benzene to produce the cyclic triester 11. Formation of compound 11 was complete in 2 h at room temperature in quantitative yield. Ring

(12) Chandrakumar, N. S.; Hajdu, J. *Tetrahedron Lett.* **1981**, *22*, 2949–2952.
(13) Chandrakumar, N. S.; Hajdu, J. *J. Org. Chem.* **1982**, *47*, 2144–2147.
(14) Chandrakumar, N. S.; Hajdu, J. *Tetrahedron Lett.* **1982**, *23*, 1043–1046.
(15) Chandrakumar, N. S.; Hajdu, J. *J. Org. Chem.* **1983**, *48*, 1197–1202.
(16) Bhatia, S. K.; Hajdu, J. *Tetrahedron Lett.* **1987**, *28*, 271–274.
(17) Blank, M. L.; Lee, T.-c.; Fitzgerald, V.; Snyder, F. *J. Biol. Chem.* **1981**, *256*, 175–178.
(18) Blank, M. L.; Cress, E. A.; Lee, T.-c.; Malone, B.; Surles, J. R.; Piantadosi, C.; Hajdu, J.; Snyder, F. *Res. Comm. Chem. Pathol. Pharmacol.* **1982**, *38*, 3–31.
(19) Eibl, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 257–271.
(20) The compound is now available from Fluka Chemical Corp., Ronkonkoma, NY.
(21) The use of Linde 3A molecular sieves is preferred for this step since the large pore size 4A type sieves causes substantial losses in recovery, probably due to adsorption of methyl glycerate by the drying agent.
(22) Hanessian, S.; Staub, A. P. A. *Tetrahedron Lett.* **1973**, 3555–3558.
(23) Arnap, J.; Lonngren, J. *Acta Chem. Scand., Ser. B* **1978**, *B32*, 465–467.
(24) (a) Berdel, W. E.; Fromm, M.; Fink, U.; Pahlke, W.; Bicker, U.; Reicher, A.; Rasteter, J. *Cancer Res.* **1983**, *43*, 5538–5541. (b) Hermann, D. B. J. In *Abstracts of the Second International Conference on Platelet-Activating Factor and Structurally Related Alkyl Ether Lipids*; Gatlinburg, TN, October 26–29, 1986; p 53.

Scheme I. Synthesis of 1-Hexadecyl-2-methyl-*sn*-glycero-3-phosphocholine

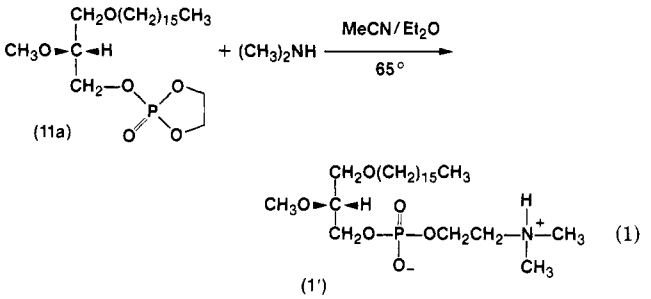


opening with anhydrous trimethylamine in acetonitrile at 65 °C readily yielded the target phospholipid **1**. As it has been previously observed,[12-16] the cyclic phosphochloridate **10** appears to be an excellent reagent for the preparation of glycerophosphorylcholine derivatives. Although the reaction times vary significantly depending on the nature of the neighboring *sn*-2-function,[12,14] with respect to the synthesis of 1,2-dialkylglycerophosphocholines such as compound **1**, the phosphorylation proceeds rapidly and in quantitative yields. It should be noted, however, that because of its high reactivity toward nucleophilic/hydrolytic cleavage, the cyclic intermediate **11** should not be stored but used for the ring-opening step directly, as obtained.

In addition to developing a facile and efficient scheme for the preparation of 1,2-dialkylglycerophosphocholines, a number of useful synthetic strategies have emerged from the sequence. The first one involves the use of L-glyceric acid methyl ester as a chiral precursor for the synthesis of optically active phospholipids. Specifically, with respect to more traditional alternatives, 1,2-isopropylidene-*sn*-glycerol has been reported to undergo racemization on storage,[19] and 3,4-isopropylidene-D-mannitol must be oxidatively cleaved in order to obtain the three-carbon glycerol skeleton.[19] L-Methyl glycerate, on the other hand, converted to its *sn*-3-trityl derivative **4**, yields an intermediate in which each glycerol position can be independently derivatized while the stereochemistry of the chiral center is securely fixed. Thus, depending on the methods employed and the sequence in which the substituents are introduced, a wide range of *sn*-1 as well as *sn*-3 phospholipids should become readily available. Furthermore, this synthetic flexibility is achieved by the introduction of a single protecting group in the entire scheme.

The alkylation conditions employed for introduction of the short-chain *sn*-2-substituent in the sequence represents a highly efficient yet exceptionally mild methylation method. Specifically, electrophilic catalysis via silver tetrafluoroborate activation of the alkylating agent at ambient temperatures provides significant improvement over the alternative method, relying on hindered-base substrate activation using methyl trifluoromethanesulfonate that requires reflux conditions for several hours.[23] Thus, the reaction here described should be particularly useful for O-methylation of compounds incorporating base-sensitive substituents. The fact that the corresponding ethyl derivative **6b** could be prepared in a similar fashion indicates that the reaction is not restricted to methylation and it might be used for introduction of other alkyl substituents as well.

Synthesis of Related Analogues. Because of the well-known important role of the hydrophilic component of the phosphodiester function in determining the physicochemical and biological properties of phospholipids,[10,19] we have examined the feasibility of extending the scope of the synthesis for the preparation of related derivatives, incorporating other nitrogen bases at the cationic portion of the molecule. Along these lines we have prepared the corresponding dimethylamino compound (**1'**), in the reaction between the cyclic phosphodiester **11** and anhydrous dimethylamine in acetonitrile/ether (eq 1) and obtained

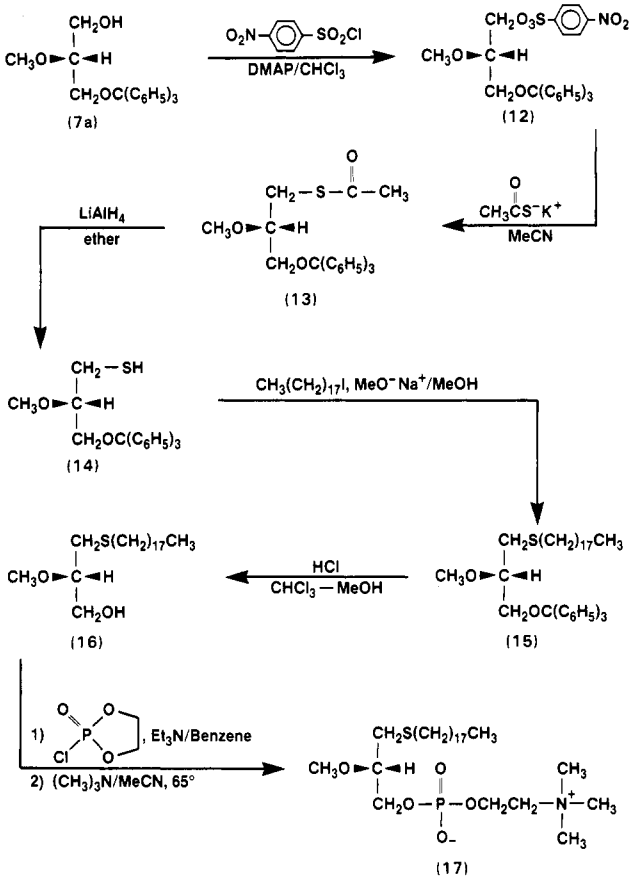


the phospholipid as a single reaction product. The synthesis of compound **1'** indicates that analogous ring-opening reactions with other nitrogen nucleophiles are likely to yield a series of related phospholipid derivatives including the corresponding ethanolamines.

Because of recent developments in the area of antitumor active phospholipids, demonstrating that introduction of an *sn*-1 thioether substituent in place of the *O*-alkyl function significantly improves the chemotherapeutic properties of these derivatives,[24,25] we set out to develop

(25) Morris-Natschke, S.; Surles, J. R.; Daniel, L. W.; Berens, M. E.; Modest, E. J.; Piantadosi, C. *J. Med. Chem.* **1986**, *29*, 2114-2117.

Scheme II. Synthesis of 1-*S*-Octadecyl-2-methyl-*sn*-thioglycero-3-phosphocholine



a stereospecific sequence for the preparation of thioether analogues of compound **1**. In order to achieve this we have used intermediate **7a** from Schene I to provide a suitable precursor for elaboration of the *sn*-1-thioether function. Specifically, activation of the primary alcohol moiety of **7a** through the corresponding *p*-nitrobenzenesulfonate **12** allows introduction of the sulfur atom with potassium thioacetate in dipolar aprotic media such as acetonitrile in high yield (93%).[26] The resulting thioester **13** can then be reductively cleaved with lithium aluminum hydride to produce the thiol **14**. Alkylation of the sulfhydryl function by octadecyl iodide in methanol in the presence of stoichiometric amounts of sodium methoxide at room temperature yields the required thioether derivative **15**. The sequence used for elaboration of the thioalkyl function also provides an intermediate (**14**) applicable for introduction of a wide range of thio functions at the *sn*-1-position of phospholipids. In contrast, attempts to achieve direct displacement of *p*-nitrobenzoate by octadecylmercaptan turned out to be much less efficient. Specifically, the long-chain thiolate seems quite unreactive toward compound **12** under the reaction conditions, instead it affords dialkyl disulfide, which is isolated as the main product from the reaction mixture. Finally, deprotection of the *sn*-1-glycerol position followed by elaboration of the phosphodiester function as in the *O*-alkyl series leads to the target compound **17** in good yield (Scheme II).

In conclusion, it might be noted that the strengths of the synthetic methods that have been described are their (1) simplicity and efficiency, (2) flexibility with respect to the substituent groups that can be introduced, and (3) applicability to the development of specific ether and thioether phospholipid analogues with the desired target structures for biological and physicochemical studies. Synthetic work along these lines is currently under way 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 Beckman AccuLab 2 spectrophotometer. ^{1}H NMR (internal Me_4Si) spectra were taken on an IBM NR-80 instrument. Optical rotations were determined either on a Perkin-Elmer 141 or 241MC polarimeter. Methyl-α,β-isopropylidene-L-glycerate, *p*-nitrobenzenesulfonyl chloride, potassium thioacetate, and trityl pyridinium tetrafluoroborate were obtained from Fluka Chemical Corp. Silver carbonate and silver tetrafluoroborate were obtained from Johnson Matthey, Inc.; hexadecyl methanesulfonate was purchased from Nu-Chek Prep., Elysian, MN. Octadecyl iodide was obtained from Aldrich. Anhydrous trimethylamine (Kodak) and anhydrous etheral dimethylamine (Alfa) were used as received. Acetonitrile (Burdick and Jackson) and triethylamine (Fluka) were dried over activated Linde 4A molecular sieves (Fluka). Benzene (Burdick and Jackson) was kept on sodium wire, chloroform (Mallinckrodt) was distilled from phosphorus pentoxide, and tetrahydrofuran was distilled from sodium benzophenone just prior to use. 2-Chloro-2-oxo-1,3,2-dioxaphospholane was prepared by literature procedures.[27] Column chromatography was carried out with silica gel 60 (70–230 mesh ASTM, E.M. Laboratories). The silica gel support was constantly kept at 120 °C and was cooled to room temperature only prior to use in a dessicator over P_2O_5. Thin-layer chromatography was carried out on Whatman MK6F plates. The phospholipids were visualized by molybdic acid spray[28] and by charring (50% aqueous sulfuric acid). Trityl compounds were visualized by UV light, and all other compounds were detected either by charring or by iodine vapor. Elemental analyses were performed by Galbraith Laboratories, Inc.

L-Glyceric Acid Methyl Ester (4). Dry HCl gas was bubbled through absolute methanol (100 mL) for 45 min. To this was added 2,3-isopropylidene-L-methyl glycerate (**3**, 6.4 g, 40 mmol), and the resulting solution was stirred at room temperature for 1 h. The methanol was then removed in vacuo, and the hygroscopic product was dried over KOH in a vacuum dessicator for 3 h and kept in dry acetonitrile (100 mL) on molecular sieves (Linde 3A) overnight. Evaporation of the solvent gave 4.8 g (100%) of **4** as colorless viscous liquid. It was used as quickly as possible for the next reaction.

3-(Triphenylmethyl)glyceric Acid Methyl Ester (5). To a stirred solution of ester **4** (4.6 g, 38.33 mmol) in 200 mL of dry acetonitrile was added pyridinium triphenylmethyl tetrafluoroborate (22 g, 53.79 mmol) and the resulting mixture was stirred at room temperature for 24 h. Acetonitrile was then evaporated and replaced by chloroform (200 mL). The pyridinium tetrafluoroborate that precipitated was filtered and washed with chloroform, and the combined filtrate was evaporated to dryness. The residue was chromatographed on freshly activated silica gel to give 9.85 g (71%) of product **5** that solidified on storage at 0–4 °C: mp 72–73 °C; IR (Nujol) 3405, 1740, 1705 cm^{-1}; NMR ($CDCl_3$) δ 3.09–3.42 (m, 3 H), 3.76 (s, 3 H), 4.21 (m, 1 H), 7.24–7.31 (m, 15 H); $[\alpha]^{23}_D$ +6.8° (*c* 1.66, 1:4 CH_3OH–$CHCl_3$). Anal. Calcd for $C_{23}H_{22}O_4$: C, 76.22; H, 6.12. Found: C, 76.21; H, 6.00.

2-Methyl-3-(triphenylmethyl)glyceric Acid Methyl Ester (6a). A solution of the ester **5** (5.6 g, 15.47 mmol) in 125 mL of dry acetonitrile containing silver tetrafluoroborate (3.315 g, 17 mmol) and silver carbonate (8.28 g, 30 mmol) was stirred with methyl iodide (4.26 g, 30 mmol) at room temperature for a period of 60–72 h. The insoluble inorganic material was then filtered off, and solvent was evaporated. Chromatography of the crude residue on freshly activated silica gel yielded 4.88 g (84%) of the methyl ether **6a**. An analytical sample was obtained by trituration with petroleum ether: mp 103 °C; IR (Nujol) 1740, 1090, 695 cm^{-1};

(26) The use of *p*-nitrobenzenesulfonate rather than methanesulfonate provides a much better leaving group for displacement by potassium thioacetate. When methanesulfonate must be used, the reaction takes 48 h at room temperature (cf. Bhatia, S. K.; Hajdu, J. *Tetrahedron Lett.* **1988**, *29*, 31–34).

(27) Edmundson, R. N. *Chem. Ind. (London)* **1962**, 1828–1829.

(28) Dittmer, J. C.; Lester, R. L. *J. Lipid Res.* **1964**, *5*, 126–127.

NMR ($CDCl_3$) δ 3.36 (br s, 2 H), 3.44 (s, 3 H), 3.75 (s, 3 H), 3.87–3.98 (m, 1 H), 7.24–7.38 (m, 15 H); $[\alpha]^{23}_D$ −5.1° (*c* 1.44, 1:4 CH_3OH–$CHCl_3$). Anal. Calcd for $C_{24}H_{24}O_4$: C, 76.57; H, 6.43. Found: C, 76.66; H, 6.49.

2-Methyl-3-(triphenylmethyl)glycerol (7a). To a stirred solution of compound **6a** (4.136 g, 11 mmol) in 200 mL of anhydrous ether at 0 °C was added lithium aluminum hydride (0.3 g, 7.89 mmol) in one portion. The reaction mixture was stirred at 0 °C for 30 min followed by stirring at room temperature for 2 h. Excess hydride was then decomposed by cautious addition of water at 0 °C (50 mL added in small portions), and the reaction mixture was stirred for 15 min. The insoluble material was filtered over a Celite pad, and the filtrate was extracted with ether (3 × 75 mL). The combined etheral extract was washed with saline and dried over anhydrous $MgSO_4$. The crude material, obtained after evaporation of the solvent, was chromatographed rapidly over freshly activated silica gel to give 3.14 g (82%) of alcohol **7a** as a colorless viscous oil. It was dried over P_2O_5 in vacuo for 4 h before being used for the next step: IR (neat) 3180, 2920, 1435, 1105 cm^{-1}; NMR ($CDCl_3$) δ 3.24 (m, 4 H), 3.40 (s, 3 H), 3.67 (m, 2 H), 7.24–7.41 (m, 15 H); $[\alpha]^{23}_D$ +15.6° (*c* 1.87, 1:4 CH_3OH–$CHCl_3$).

1-Hexadecyl-2-methyl-3-(triphenylmethyl)-*sn*-glycerol (8a). To a suspension of NaH (21 mmol, 0.84 g of 60% dispersion in mineral oil, washed with petroleum ether (3 × 25 mL) in an atmosphere of N_2) in 20 mL of anhydrous tetrahydrofuran cooled to 0 °C was added dropwise the alcohol **7a** (2.4 g, 6.9 mmol) in 50 mL of THF. The mixture was stirred at room temperature for 30 min and heated at 60 °C for 1 h. The suspension was then cooled to 0 °C, and hexadecyl methanesulfonate (2.24 g, 7 mmol) in 40 mL of tetrahydrofuran was added dropwise. The resulting mixture was heated at reflux for 36 h and diluted with 100 mL of ice-cold water, and most of the solvents were removed in vacuo. The residue was then extracted with ether (3 × 50 mL). The combined etheral extract was washed with saline and dried over anhydrous $MgSO_4$. The solvent was then evaporated in vacuo, and the residue was chromatographed on freshly activated silica gel to give 2.68 g (68%) of triether (**8a**). Recrystallization from petroleum ether provided an analytical sample of **8a**: mp 42–43 °C; IR (Nujol) 1100, 705 cm^{-1}; NMR ($CDCl_3$) δ 0.88 (br t, 3 H), 1.25 (br s, 28 H), 3.21–3.52 (m, 10 H with a singlet at 3.40), 7.23–7.40 (m, 15 H); $[\alpha]^{23}_D$ −6.75° (*c* 1.23, 1:4 CH_3OH–$CHCl_3$). Anal. Calcd for $C_{39}H_{56}O_3$: C, 81.77; H, 9.85. Found: C, 81.72; H, 9.77.

1-Hexadecyl-2-methyl-*sn*-glycerol (9a). Dry HCl gas was passed through 90 mL of chloroform–methanol (1:1) at a gentle rate for a period of 40 min. To this triether was added **8a** (1.8 g, 3.15 mmol), and the resulting solution was stirred at room temperature for 45 min. The solvents were then evaporated in vacuo, and the crude residue was chromatographed on silica gel ($CHCl_3$–EtOAc, 95:5) to give 0.935 g (90%) of **9a** as a low-melting solid: mp 35–36 °C; IR (Nujol) 3320, 1095, 1055 cm^{-1}; NMR ($CDCl_3$) δ 0.88 (br t, 3 H), 1.25 (br s, 28 H), 3.36–3.80 (m, 11 H with a singlet at 3.46); $[\alpha]^{23}_D$ −7.65° (*c* 1.28, 1:4 CH_3OH–$CHCl_3$). Anal. Calcd for $C_{20}H_{42}O_3$: C, 72.67; H, 12.81. Found: C, 72.52; H, 12.78.

2-(1-Hexadecyl-2-methyl-*sn*-glyceroyl)-2-oxo-1,3,2-dioxaphospholane (11a). To a solution of alcohol **9a** (0.726 g, 2.2 mmol) in 50 mL of dry benzene was added triethylamine (0.253 g, 2.5 mmol), and the mixture was cooled to 0 °C. To this was added 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.355 g, 2.5 mmol) in 4 mL of benzene in one portion. The mixture was stirred at room temperature for 2 h. The crystalline $(C_2H_5)_3N$·HCl that precipitated was filtered off, and the solvent was removed in vacuo to give phosphate triester **11a** as a colorless smeisolid (0.95 g): NMR ($CDCl_3$) δ 0.88 (br s, 3 H), 1.25 (br s, 28 H), 2.96–3.71 (m, 10 H with a singlet at 3.46), 4.32–4.48 (m, 4 H), consistent with the structure. This compound should not be stored and was used as early as possible for the next reaction without further treatment.

1-Hexadecyl-2-methyl-*sn*-glycero-3-phosphocholine (1a). Phosphate triester **11a** (0.95 g) was transferred into a pressure bottle with 30 mL of dry acetonitrile. It was cooled in a dry ice bath, and to this was added 1.5 mL of anhydrous trimethylamine. The bottle was sealed and then heated in an oil bath at 65 °C for 24 h. Cooling and subsequent filtration yielded 1 g (92% from alcohol **9**) of **1a** as a white hygroscopic solid. A 0.5-g sample of this product was chromatographed on activated silica gel with chloroform–methanol–aqueous NH_3 (1:9:1, v/v/v) to give 0.29 g (58%) of analytically pure phospholipid: IR (Nujol) 3380, 1225, 1040 cm^{-1}; NMR ($CDCl_3$ + CD_3OD) δ 0.88 (br t, 3 H), 1.25 (br s, 28 H), 3.23 (s, 9 H), 3.46–3.66 (m, 10 H with singlet at 3.46), 3.90–4.32 (m, 4 H); $[\alpha]^{23}_D$ −0.74°[16] (*c* 2.43, 1:4 CH_3OH–$CHCl_3$); R_f ($CHCl_3$–CH_3OH–water, 65:25:4) 0.17. Anal. Calcd for $C_{25}H_{54}NO_6P·2H_2O$: C, 56.47; H, 10.99; N, 2.63; P, 5.82. Found: C, 56.18; H, 11.07; N, 2.62; P, 6.06.

***N*-Demethyl-1-hexadecyl-2-methyl-*sn*-glycero-3-phosphocholine (1′).** A solution of phosphate triester **11a** (0.436 g, 1 mmol) in 20 mL of dry acetonitrile was cooled in dry ice bath. To this was added 3.75 mL (5 mmol) of 1.3 M solution of dimethylamine in ether. The pressure bottle was kept in an oil bath at 65 °C for 24 h. It was allowed to attain room temperature and then cooled in a dry ice bath followed by addition of 1.5 mL more of dimethylamine solution in ether (1.3 M). The reaction mixture was further heated at 65 °C for 16 h and cooled to room temperature, and solvent was evaporated in vacuo. The crude product was chromatographed on activated silica gel with $CHCl_3$·MeOH·H_2O (65:25:4) to give 0.26 g (54%) of analytically pure phospholipid **1′** as a hygroscopic white solid: IR (Nujol) 3375, 1230, 1045 cm^{-1}; NMR ($CDCl_3$ + CD_3OD) δ 0.88 (br t, 3 H), 1.26 (br s, 28 H), 2.89 (s, 6 H), 3.34–3.52 (m, 1 H with a singlet at 3.45), 3.99–4.32 (m, 4 H); $[\alpha]^{23}_D$ −0.84° (*c* 1.42, 1:4 CH_3OH–$CHCl_3$); R_f ($CHCl_3$–CH_3OH–water, 65:25:4) 0.52. Anal. Calcd for $C_{24}H_{52}NO_6P·H_2O$: C, 57.69; H, 10.89; N, 2.80; P, 6.20. Found: C, 57.87; H, 10.72; N, 2.58; P, 6.39.

2-Ethyl-3-(triphenylmethyl)glyceric Acid Methyl Ester (6b). A solution of ester (**5**) (2.5 g, 6.9 mmol) in 60 mL of dry acetonitrile was stirred with silver tetrafluoroborate (1.365 g, 7 mmol), silver carbonate (3.312 g, 12 mmol), and ethyl iodide (1.872 g, 12 mmol) at room temperature for 7 days. TLC of the reaction mixture showed that most of the starting material had reacted.[29] The reaction mixture was then worked up via the same procedure as described for compound **6a**. Chromatography on freshly activated silica gel afforded the ether **6b** (1.7 g, 63%) as an oil: IR (neat) 3000, 1750, 1445 cm^{-1}; NMR ($CDCl_3$) δ 1.25 (t, 3 H), 3.34–3.58 (m, 3 H), 3.73 (s, 3 H), 4.04–4.31 (m, 2 H), 7.25–7.39 (m, 15 H); $[\alpha]^{23}_D$ −2.53° (*c* 1.62, 1:4 CH_3OH–$CHCl_3$).

Compounds **7b**, **8b**, and **9b** were prepared by employing the same experimental conditions as described for corresponding *sn*-2 methyl ethers **6a**, **7a**, and **8a**.

2-Ethyl-3-(triphenylmethyl)-*sn*-glycerol (7b) was obtained as an oil in 75% yield: IR (neat) 3400, 2880, 1430, 1100 cm^{-1}; NMR ($CDCl_3$) δ 1.18 (t, 3 H), 3.17–3.22 (m, 2 H), 3.44–3.66 (m, 6 H), 7.24–7.39 (m, 15 H); $[\alpha]^{23}_D$ +16.12° (*c* 1.16, 1:4 CH_3OH–$CHCl_3$).

1-Hexadecyl-2-ethyl-3-(triphenylmethyl)-*sn*-glycerol (8b) was isolated as a colorless oil in 66% yield: IR (neat) 2920, 2850, 1465, 1075 cm^{-1}; NMR ($CDCl_3$) δ 0.88 (br t, 3 H), 1.25 (br s, 31 H), 3.14–3.64 (m, 9 H), 7.24–7.41 (m, 15 H). Anal. Calcd for $C_{40}H_{58}O_3$: C, 81.86; H, 9.96. Found: C, 82.26; H, 10.08.

1-Hexadecyl-2-ethyl-*sn*-glycerol (9b) was obtained as a low-melting solid in 90% yield: mp 31–32 °C; IR (Nujol) 3430, 1100 cm^{-1}; NMR ($CDCl_3$) δ 0.88 (br t, 3 H), 1.25 (br s, 31 H), 3.36–3.66 (m, 10 H); $[\alpha]^{23}_D$ −6.08° (*c* 2.17, 1:4 CH_3OH–$CHCl_3$). Anal. Calcd for $C_{21}H_{44}O_3$: C, 73.20; H, 12.87. Found: C, 73.22; H, 12.95.

2-(1-Hexadecyl-2-ethyl-*sn*-glyceroyl)-2-oxo-1,3,2-dioxaphospholane (11b). To a cooled solution of alcohol **9b** (0.2 g, 0.58 mmol) in 15 mL of dry benzene containing triethylamine (0.06 g, 0.6 mmol) was added 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.085 g, 0.6 mmol). The reaction mixture was stirred at room temperature for 2 h, the crystalline Et_3N·HCl salt was filtered off, and solvent was evaporated in vacuo to give phosphate triester **11b** as a semisolid. This was used for the next reaction without further treatment.

1-Hexadecyl-2-ethyl-*sn*-glycero-3-phosphocholine (1b). Phosphate triester **11b** obtained in the previous step was transferred into a pressure bottle with 15 mL of dry acetonitrile. This was cooled in a dry ice bath, 1 mL of anhydrous trimethylamine was added, and the pressure bottle was heated in an oil bath at 65 °C for 24 h. Subsequent cooling precipitated

(29) It should be noted that we have not yet optimized the yield of this reaction.

out the phospholipid (**1b**), which was filtered off. The hygroscopic solid (0.22 g) was chromatographed on activated silica gel with chloroform–methanol–aqueous NH_3 (1:9:1, v/v/v) to give 0.115 g of pure phospholipid **1b**: IR (Nujol) 3380, 1230, 1045, 955 cm^{-1}; NMR ($CDCl_3$) δ 0.88 (br t, 3 H), 1.25 (br s, 31 H), 3.24–4.30 (m, 22 H with a singlet at 3.37); $[\alpha]^{23}_D$ –0.19 (*c* 1.3, 1:4 CH_3OH–$CHCl_3$); R_f ($CHCl_3$–CH_3OH–water, 65:25:4) 0.19. Anal. Calcd for $C_{26}H_{56}NO_6P \cdot H_2O$: C, 59.18; H, 11.08; N, 2.65; P, 5.87. Found: C, 58.80; H, 10.86; N, 2.67; P, 5.97.

1-[(4-Nitrophenyl)sulfonyl]-2-methyl-3-(triphenylmethyl)-*sn*-glycerol (12). A solution of alcohol **7a** (2.3 g, 6.61 mmol) in 100 mL of anhydrous $CHCl_3$ was stirred with 4-(dimethylamino)pyridine (1.22 g, 10 mmol) and 4-nitrobenzenesulfonyl chloride (2 g, 9 mmol) for 16 h at room temperature. It was then diluted with water (50 mL), and the product was extracted with $CHCl_3$ (3 × 60 mL). The combined organic extract was washed with saline (50 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent in vacuo afforded the crude residue, which was chromatographed on freshly activated silica gel ($CHCl_3$) to give 2.92 g (83%) of 4-nitrobenzenesulfonate. The product **12**, which solidified on standing, was taken into petroleum ether and filtered to provide an analytically pure sample: mp 97 °C; IR (Nujol) 1710, 1605, 1530, 1160 cm^{-1}; NMR ($CDCl_3$) δ 3.24 (s, 3 H), 3.14–3.50 (m, 3 H), 4.28 (m, 2 H), 7.24–7.37 (m, 1k H), 7.97–8.36 (dd, 4 H); $[\alpha]^{23}_D$ +21.8° (*c*, 1.5, 1:4 CH_3OH–$CHCl_3$). Anal. Calcd for $C_{29}H_{27}NO_7S$: C, 65.28; H, 5.10; N, 2.62; S, 6.01. Found: C, 65.01; H, 5.18; N, 2.34; H, 6.19.

1-*S*-(Methylcarbonyl)-2-methyl-3-(triphenylmethyl)-*sn*-thioglycerol (13). A solution of 4-nitrobenzenesulfonate **12** (2.4 g, 4.5 mmol) in 50 mL of dry acetonitrile was stirred with potassium thioacetate (0.9 g, 7.9 mmol) in N_2 atmosphere at room temperature for 6 h. The precipitated potassium *p*-nitrobenzoate was filtered off, and the acetonitrile was evaporated in vacuo. The residue was then diluted with 50 mL of $CHCl_3$, and the additional precipitate was filtered off. The solvent was then evaporated to afford light brown oily product **13** (1.7 g, 93%). This was used as obtained for the next step without further purification: IR (neat) 1605, 1405, 1440, 1100 cm^{-1}; NMR ($CDCl_3$) δ 2.29 (s, 3 H), 3.10–3.21 (m, 5 H), 3.38 (s, 3 H), 7.24–7.40 (m, 15 H); R_f ($CHCl_3$) 0.64.

2-Methyl-3-(triphenylmethyl)-*sn*-thioglycerol (14). Reduction of compound **13** was carried out with $LiAlH_4$ by employing the same experimental conditions as described earlier except that the thiol **14**, obtained as a colorless oil (85%), was used directly without chromatography for the next reaction: IR (neat) 2930, 1490, 1450, 1070, 760, 705 cm^{-1}; NMR ($CDCl_3$) δ 1.51 (s, 1 H, exchangeable with D_2O), 2.70 (m, 2 H), 3.26 (m, 3 H), 3.38 (s, 3 H), 7.24–7.39 (m, 15 H); R_f ($CHCl_3$–hexane) 0.5.

1-*S*-Octadecyl-2-methyl-3-(triphenylmethyl)-*sn*-thioglycerol (15). A suspension of thiol **14** (1.2 g, 3.3 mmol) in 60 mL of absolute methanol was stirred with sodium methoxide (0.3 g, 5.5 mmol) and octadecyl iodide (1.7 g, 4.47 mmol) in a N_2 atmosphere at room temperature for 24 h. The methanol was evaporated in vacuo, and the residue was diluted with water (50 mL) and extracted with $CHCl_3$ (3 × 50 mL). The organic extract was washed with saline and dried over anhydrous $MgSO_4$, and solvent was removed. The crude product was then chromatographed on freshly activated silica gel to give 1.25 g (61.5%) of compound **15** as a semisolid,[25] $[\alpha]^{23}_D$ +4.25° (*c* 1.2, 1:4 CH_3OH–$CHCl_3$).

1-*S*-Octadecyl-2-methyl-*sn*-thioglycerol (16). This compound was obtained by the same deprotection procedure as described earlier for compound **9a**. The product **16** was obtained in 92% yield as a waxy solid,[25] $[\alpha]^{23}_D$ –14.95° (*c* 1.05, 1:4 CH_3OH–$CHCl_3$).

1-*S*-Octadecyl-2-methyl-*sn*-thioglycero-3-phosphocholine (17). The phospholipid **17** was prepared from alcohol **16** in a two-step sequence as described for phospholipid **1a**. The formation of cyclic triester was complete in 4 h for this reaction, and then similar ring opening with anhydrous trimethylamine provided the crude phospholipid. This was finally chromatographed on activated silica gel with $CHCl_3$–MeOH–H_2O (65:25:4) to obtain analytically phospholipid (**17**) in 55% isolated yield: IR (Nujol) 3360, 1235, 1080, 965 cm^{-1}; NMR ($CDCl_3$) δ 0.89 (t, 3 H), 1.26 (s, 32 H), 2.45–2.71 (m, 4 H), 3.22 (m, 3 H), 3.38 (s, 9 H), 3.42 (s, 3 H), 3.83–4.32 (m, 4 H); $[\alpha]^{23}_D$ –5.49° (*c* 1.22, 1:4 CH_3OH–$CHCl_3$). Anal. Calcd for $C_{27}H_{58}NO_5PS \cdot 0.25H_2O$: C, 59.58; H, 10.83; N, 2.57; P, 5.69; S, 5.89. Found: C, 59.53; H, 10.85; N, 2.58; P, 5.39; S, 6.25.

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Registry No. **1a**, 78858-44-3; **1b**, 78858-42-1; **1'**, 116149-96-3; **3**, 60456-21-5; **4**, 10303-88-5; **5**, 111247-96-2; **6a**, 111247-97-3; **6b**, 116149-97-4; **7a**, 111247-98-4; **7b**, 116149-98-5; **8a**, 111247-99-5; **8b**, 116149-99-6; **9a**, 96960-92-8; **9b**, 116257-47-7; **10**, 6609-64-9; **11a**, 111248-00-1; **11b**, 116150-00-6; **12**, 116150-01-7; **13**, 116150-02-8; **14**, 116150-03-9; **15**, 116257-48-8; **16**, 116257-49-9; **17**, 116257-50-2; $CH_3(CH_2)_{15}OSO_2Me$, 20779-14-0; Me_3N, 75-50-3; Me_2NH, 124-40-3; 4-$(NO_2)C_6H_4SO_2Cl$, 98-74-8; AcSK, 10387-40-3; $CH_3(CH_2)_{17}I$, 629-93-6; L-glyceric acid, 28305-26-2.