Stereospecific Synthesis of Functionalized Ether Phospholipids

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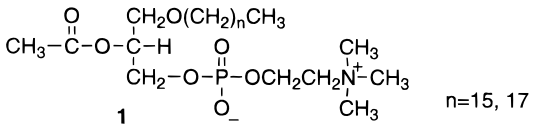
A new stereospecific synthesis of functionalized alkyl ether phospholipids is reported. The synthesis is based upon the following: (1) the use of (*R*)-glycidyl tosylate as a chiral glycerol precursor; (2) the opening of a boron trifluoride catalyzed epoxide ring to introduce the functionalized *sn*-1-alkyl substituents; (3) the role of tetrahydropyranyl in protecting the *sn*-2-glycerol position; and (4) the elaboration of the *sn*-3-carbinol function, via the base hydrolysis of the acetoxy intermediate, obtained from the displacement of the toluenesulfonyl group of the substrate in dipolar aprotic media. Phosphorylation, using two different methods, has led to the development of two major classes of alkyllysophospholipids. For preparation of "modulator-phospholipid" analogues, the substituted glycerol is coupled with 2,2,2-trichloro-*tert*-butyl phosphodichloridite and an N-protected amino acid ester, while elaboration of the phosphocholine headgroup of the target platelet-activating factor (PAF) analogues is achieved via the 2-chloro-2-oxo-1,3,2-dioxaphospholane/trimethylamine sequence. The synthesis provides rapid and efficient access to both types of phospholipids: (1) construction of the functionalized/substituted glycerol skeleton is achieved in a straightforward four-step sequence in better than 50% overall yield, and (2) phosphitylation or phosphorylation of the respective glycerol intermediates relies on reagents that require minimal use of protecting groups. The phospholipid compounds prepared include (1) the first synthetic analogue exhibiting modulator activity in conjunction with the glucocorticoid-receptor complex and (2) an *sn*-1-(*ω*-amino)alkyl derivative of PAF, suitable for introduction of chain-terminal spectroscopic labels for biological and physicochemical studies to elucidate the mechanism of action of this highly potent alkyl ether phospholipid. The synthetic methods described herein have a great deal of flexibility, thus providing convenient general routes to a wide range of alkyl ether phospholipids.

Structurally modified analogues of platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF, **1**) represent a series of synthetic phospholipid compounds that exhibit high levels of activity in a wide spectrum of biological systems.[1−4] Included among these are platelet-activating[1] antihypertensive[2] and antitumor active[3] alkyl ether phospholipids and immunological response modifiers.[4] PAF analogues have been shown to inhibit phosphatidylcholine biosynthesis,[5] protein kinase C activity,[6] and phorbol ester stimulation of protein kinase C.[7] Despite ongoing vigorous investigation of the biochemistry and cell biology of ether phospholipids, their physiological functioning and their mechanism of action remain unclear.[8,9]



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Amino acid derivatives of ether phospholipids are recent additions to the family of highly potent PAF analogues.[10−15] Among these compounds, particular attention has been directed toward a series of polar carboxyalkyl phospholipid derivatives (**2**) that carry amino acid substituents as a part of their *sn*-3-phosphodiester function; two such amino acid derivatives of ether phospholipids, isolated from rat liver cytosol, have shown potent inhibition of glucocorticoid receptor complex activation.[11−15] In addition, lyso-PAF **3** has been shown to be a key intermediate in the metabolic conversion of platelet-activating factor **1** to 1-alkyl-2-acyl-*sn*-

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(1) (a) Hanahan, D. J. *Annu. Rev. Biochem.* **1986**, *55*, 483. (b) Snyder, F. *Med. Res. Rev.* **1985**, *5*, 107.
(2) Blank, M. L.; Snyder, F.; Byers, L. W.; Brooks, B.; Muirhead, E. E. *Biochem. Biophys. Res. Commun.* **1979**, *90*, 1194.
(3) (a) Berdel, W. E.; Andreesen, R.; Munder, P. G. In *Phospholipid and Cellular Regulation*; Kuo, F. J., Ed.; CRC Press: Boca Raton, FL, 1985; Vol. 2, pp 42−77. (b) Berdel, W. E.; Munder, P. *Platelet-Activating Factor and Related Lipid Mediators*; Snyder, F., Ed.; Plenum Press: New York, 1987, pp 449−467.
(4) (a) Munder, P. G.; Weltzien, H. O.; Modolell, M. *Immunopathology*, VIIth Int. Symposium, Bad Schachen; Niescher, P. A., Ed.; Schwabe: Basel, **1976**, pp 411−424. (b) O'Flaherty, J. T.; Wykle, R. L. *Clin. Rev. Allergy* **1983**, *1*, 353.
(5) Vogler, W. R.; Whigham, E.; Bennett, W.; Olsen, A. C. *Exp. Hematol. (Charlottesville, Va)* **1985**, *13*, 629.
(6) (a) Helfman, D. M.; Barnes, K. C.; Kinkade, J. M., Jr.; Vogler, W. R.; Shoji, M.; Kuo, J. F. *Cancer Res.* **1983**, *43*, 2955. (b) Zheng, B.; Ioshi, K.; Shoji, M.; Eibl, H.; Berdel, W. E.; Hajdu, J.; Vogler, W. R.; Kuo, J. F. *Cancer Res.* **1990**, *50*, 3025.
(7) Kiss, Z.; Deli, E.; Vogler, W. R.; Kuo, J. F. *Biochem. Biophys. Res. Commun.* **1987**, *142*, 661.
(8) (a) Snyder, F. *Biochem. J.* **1995**, *305*, 689. (b) Snyder, F.; Lee, T.-C.; Blank, M. L. *Prog. Lipid Res.* **1992**, *31*, 65.
(9) Kleuser, B.; Meister, A.; Sternfeld, L.; Gercken, G. *Chem. Phys. Lipids* **1996**, *79*, 29.
(10) Brachwitz, H.; Langen, P.; Dube, G.; Schildt, J.; Paltauf, F.; Hermetter, A. *Chem. Phys. Lipids* **1990**, *54*, 89.
(11) Bodine, P. V.; Litwack, G. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1462.
(12) Bodine, P. V.; Litwack, G. *J. Biol. Chem.* **1988**, *263*, 1462.
(13) Bodine, P. V.; Litwack, G. *Receptor* **1990**, *1*, 83.
(14) (a) Bodine, P. V.; Litwack, G. *Mol. Cell. Endocrinol.* **1990**, *74*, C77. (b) Schulman, G.; Bodine, P. V.; Litwack, G. *Biochemistry* **1992**, *31*, 1734.
(15) Bodine, P. V.; Litwack, G. *J. Biol. Chem.* **1990**, *265*, 9544.

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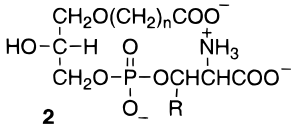
glycero-3-phosphocholine.[8,16] Furthermore, compound **3** also serves as a substrate of the enzyme alkylglycerophosphocholine acetyltransferase,[8,9] which catalyzes formation of PAF **1** after the release of arachidonic acid, showing that lyso-PAF **3** is central to the biosynthesis of eicosanoids and platelet-activating factor from the same lipid pool.[8,9] Because the production of these compounds is known to play an important role in transmembrane signaling, there is great interest in elucidating the mechanism of the catalytic reactions and the function of lysophospholipid intermediates in the PAF cycle.[9]



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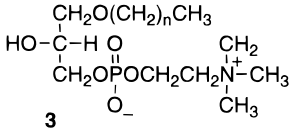

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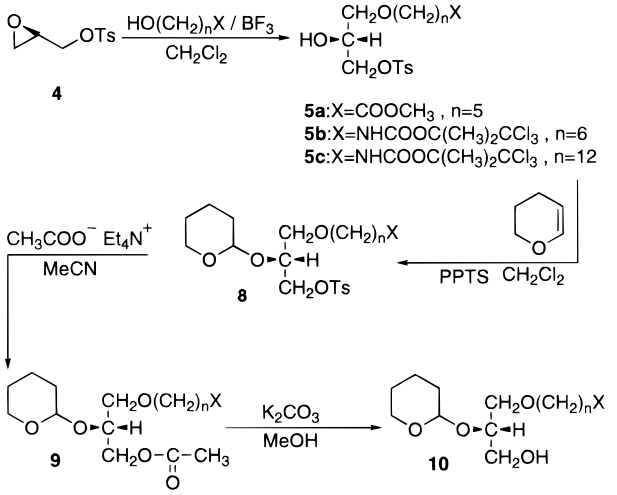
The development of new synthetic methods for preparation of ether-lysophospholipids, in a convergent pathway leading to PAF analogues and modulator-phospholipid analogues, is an important prerequisite to the elucidation of the chemical−structural basis of their biological mechanism of action. Availability of the compounds should not only contribute to advancement in the level of understanding of the chemistry and biology of alkyllysophospholipids, but also provide important insight into the design of new target molecules with the desired activity and potency.[8,17]

As part of our research in this area, we now report stereospecific syntheses of functionalized ether lysophospholipids by two closely related sequences that should be applicable to the preparation of a wide range of synthetic-modulator and PAF analogues. Specifically, both syntheses rely on (1) the use of (R)-glycidyl tosylate as a chiral glycerol precursor, (2) the opening of a BF_3-catalyzed epoxide ring to introduce the functionalized *sn*-1-alkyl substituents, (3) the role of tetrahydropyranyl in protecting the *sn*-2-glycerol position, and (4) the elaboration of the *sn*-3-carbinol function via base hydrolysis of the acetoxy intermediate obtained from the displacement of the toluenesulfonyl group of the substrate in a dipolar aprotic media. The sequences diverge at the phosphorylation step. For the preparation of modulator analogues, the substituted glycerol is coupled with 2,2,2-trichloro-*tert*-butyl phosphodichloridite and an N-protected amino acid ester, while elaboration of the phosphocholine headgroup of the target PAF analogues is accomplished via the 2-chloro-2-oxo-1,3,2-dioxaphospholane/trimethylamine sequence.

Results and Discussion

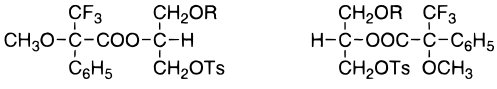
Our synthetic approach to the preparation of ether lysophospholipid targets involved three strategic components. The first phase of the synthesis, shown in Scheme

Scheme 1



1 , started with the Lewis acid catalyzed ring opening of (*R*)-glycidyl tosylate **4**[18] by a series of medium- and long-chain primary alcohols carrying either an N-protected *ω*-amino group or a chain-terminal carbomethoxy function. The resulting products (**5**) were obtained in good yield (73−95%), high enantiomeric purity, and complete regiospecificity. Significantly, both chain-terminal 2,2,2-trichloro-*tert*-butoxycarbonyl and carbomethoxy groups remained stable under the acidic conditions that existed during the catalytic ring-opening reaction.

The stereochemistry of the 1-*O*-alkyl-*sn*-glycerol products **5a**,**b** obtained in the ring-opening reaction was determined through preparation and ^{1}H-NMR spectroscopic characterization of their Mosher esters[19] **6a**,**b** in comparison with the spectra of the corresponding diastereoisomeric compounds **7a**,**b**, prepared from (*S*)-glycidyl tosylate in a similar series of reactions. The chemical shifts recorded for the series **6a**,**b** versus **7a**,**b** turned out to be sufficiently different from each other as to allow clear distinction between the respective diastereoisomers. Specifically, the 360 MHz ^{1}H-NMR spectrum of **6a**[20] in $CDCl_3$ exhibited a multiplet for the *sn*-2-glycerol proton centered at *δ* 5.341, showing no traces of absorption except at baselines of 5.399 and 5.413 ppm, which are part of the multiplet centered at *δ* 5.833 and, therefore, assigned to the corresponding CH-proton of the Mosher ester prepared from (*S*)-glycidyl tosylate.



6a: R=$(CH_2)_5COOCH_3$
6b: R=$(CH_2)_{12}NHCO_2C(CH_3)_2CCl_3$

7a: R=$(CH_2)_5COOCH_3$
7b: R=$(CH_2)_{12}NHCO_2C(CH_3)_2CCl_3$

Similarly, the 500 MHz ^{1}H-NMR spectrum of compound **6b** shows a glycerol CH-multiplet in the *δ* 5.351−5.392 range with a baseline between *δ* 5.399 and *δ* 5.429, which includes six broad peaks of the 5.410 ppm centered multiplet assigned to the CH-proton of Mosher ester **7b**. In addition, the high-field proton of the *sn*-3-CH_2 group in compound **6b** shows a four-line multiplet (doublet of

(16) Malone, B.; Lee, T.-C.; Snyder, F. *J. Biol. Chem.* **1985**, *260*, 1531.

(17) Kini, G. D.; Beadle, J. R.; Aldern, K. A.; Hostetler, K. Y. Presented at the 211th National Meeting of the American Chemical Society, New Orleans, LA, March 24−28, 1996; Abstr. MEDI 172.

(18) (a) Guivisdalsky, P. N.; Bittman, R. *Tetrahedron Lett.* **1988**, *29*, 4393. (b) The availability of this compound in high enantiomeric excess (>99%) makes it preferable to other glycidol derivatives for the stereospecific synthesis of phospholipids.

(19) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 3543.

(20) Kazi, A. B.; Hajdu, J. *Tetrahedron Lett.* **1992**, *33*, 2291.

doublets) at δ 4.10, while the corresponding proton in the spectrum of **7b** occurs 0.10 ppm higher. Absence of the latter in the spectrum of **6b** at baseline level separation confirms the stereochemical purity of product **6b** (and consequently that of the corresponding glycerol compound **5b**) at the NMR detection level (>99%).

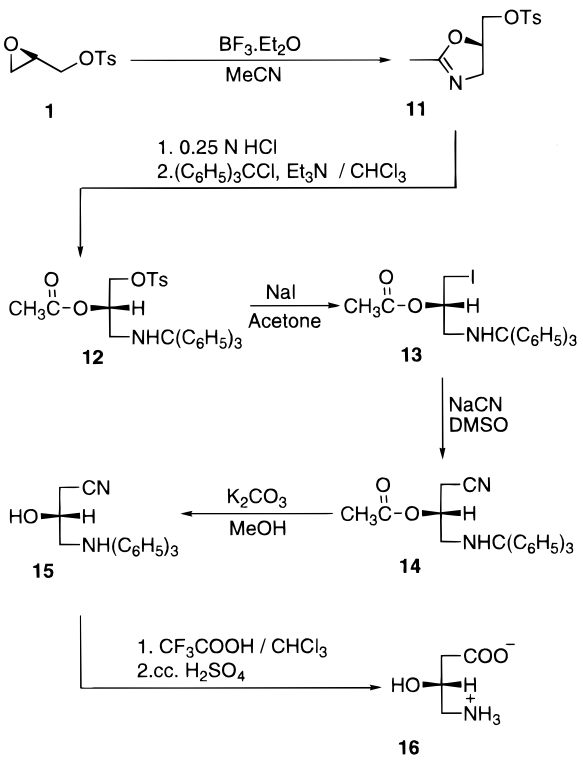
Tetrahydropyranylation of the secondary hydroxyl group of **5** was readily accomplished with pyridinium-*p*-toluenesulfonate catalysis in methylene chloride at ambient temperature. The products **8a**−**c**, purified by silica gel chromatography, were isolated as analytically pure mixtures of stereoisomers in the form of colorless viscous liquids, in 69−95% yield. Next, the *p*-toluenesulfonate function of **8** was displaced by "naked" acetate using anhydrous tetraethylammonium acetate in acetonitrile (68−90% yield). The yield and duration of this reaction was greatly dependent upon successful drying of the reagent and the dipolar aprotic reaction medium, as the presence of even small amounts of water or other hydroxylic solvents slows the reaction down a great deal and decreases the yields of the products drastically, due to the formation of numerous byproducts.[21]

The base-catalyzed hydrolytic cleavage of the *sn*-3-acetoxy group with methanolic potassium carbonate provided the deprotected carbinol function in the final step of this sequence (Scheme 1). The hydrolysis occurred with high selectivity, as both the methyl ester (**10a**) and the 2,2,2-trichloro-*tert*-butoxycarbonyl function (**10b**,**c**) remained unchanged in the course of the reaction. Purification of the products by silica gel chromatography followed by freeze-drying with benzene gave the substituted glycerol **10** in anhydrous form, suitable for phosphorylation (68−85% yield).

In the second phase of the synthesis, we turned to our attention to the preparation of protected amino acid derivatives to be used for elaboration of the polar headgroup of modulator phospholipids (**2**). As we had previously observed, N-triphenylmethylation, rather than *tert*-butoxycarbonylation, of the amino group seemed to be a better choice to provide protection during phosphorylation, because the former provides a more hydrophobic substrate and lacks the electron rich carbonyl oxygen present in the *N*-*t*-Boc function. The latter has been known to interfere with the phosphorylation of the hydroxyl group, resulting in longer reaction times and decreased yields, due to the formation of numerous byproducts.[22] Thus, both serine methyl ester and its corresponding threonine analogue were treated with 1 equiv of triphenylmethyl chloride/triethylamine in chloroform at room temperature to give their respective *N*-trityl compounds in high yield (89−91%).[23]

Considering that the authentic structure of the naturally occurring modulator phospholipids **(2)** has not yet been elucidated and the scope of structural variation of the amino acid component in the polar phosphodiester headgroup has not yet been determined, it seemed necessary to develop a sequence for preparation of hydroxy amino acids that are not readily commercially

Scheme 2



available yet are likely candidates to serve as part of the target compounds. In this context, we focused on the synthesis of γ-amino-β-hydroxybutyric acid (GABOB).[24] Our approach, as shown in Scheme 2, relied on development of the amino alcohol function by converting epoxide **1** to the corresponding oxazoline **11** by reacting it with acetonitrile, using boron trifluoride as catalyst.[25a] Acidolytic cleavage of the oxazoline heterocycle **11** followed by tritylation of the amino group yielded intermediate **12**, whose primary toluenesulfonyl function could subsequently be used to accomplish the desired chain extension. To that end, tosylate **12** was first converted to the corresponding iodide **13**, followed by nucleophilic displacement of the latter with cyanide.[25b] Next, compound **14** was allowed to react with methanol in the presence of potassium carbonate to produce cyanohydrin **15** which was then treated with trifluoroacetic acid in chloroform and concentrated sulfuric acid on a steam bath to give the γ-amino-β-hydroxybutyric acid target **16**. The characteristic physical parameters of compound **16**, such as its ^{1}H-NMR spectrum and optical rotation, were found to be in agreement with the literature values reported (see the Experimental Section).

The third phase of the synthesis focused on assembling the target phospholipid **(2)** via phosphite coupling of the substituted glycerol **10** with a series of N-protected hydroxy amino acid methyl esters, as shown in Scheme 3. We chose 2,2,2-trichloro-*tert*-butyl phosphodichloridite as a coupling agent because of its high reactivity and ready availability. It was also selected because of the ease with which the phosphitylated products can be manipu-

(21) In some cases, the addition of activated molecular sieves seemed to be helpful in removing traces of moisture from the reaction mixture, thereby increasing the reactivity of the anionic nucleophile (see the Experimental Section).

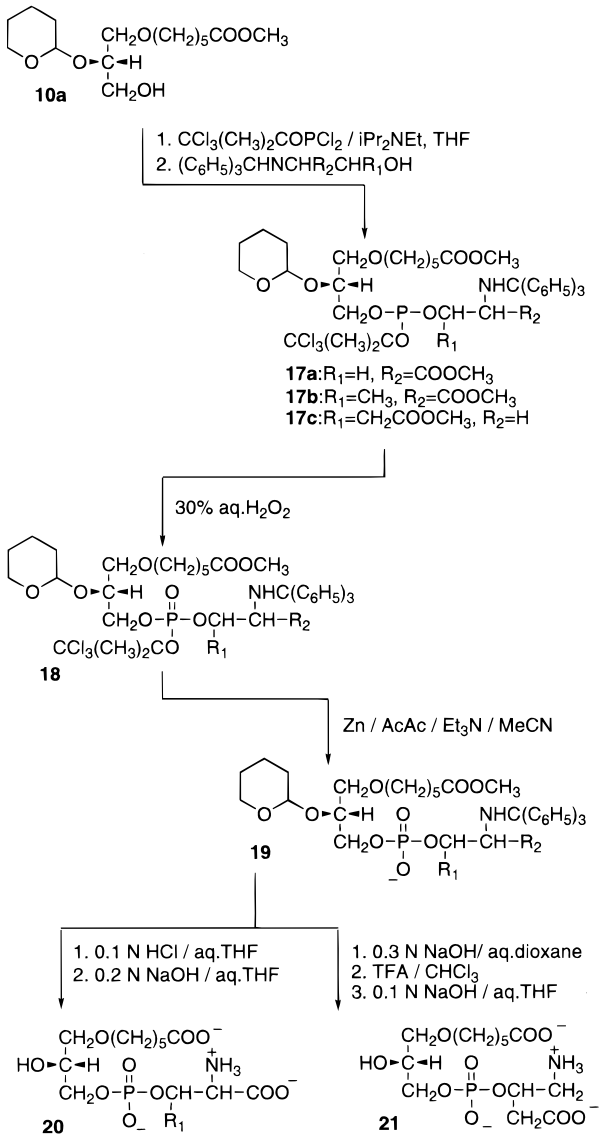
(22) (a) Bhatia, S. K.; Hajdu, J. *Lipids* **1991**, *26*, 1424. (b) Bhatia, S. K.; Hajdu, J. *Tetrahedron Lett.* **1988**, *29*, 31.

(23) The *N*-triphenylmethyl derivatives of serine methyl ester and threonine methyl ester were prepared by the method described in ref **22a**; they were obtained in 91 and 89% yields, respectively.

(24) Jung, M. J.; Shaw, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 6304.

(25) (a) Klunder, J.; Onami, T.; Sharpless, K. B. *J. Org. Chem.* **1989**, *54*, 1295. We found that, contrary to the previous report, the pure oxazoline **11** is quite stable at room temperature (for at least several weeks). (b) Preparation of iodide **13** turned out to be necessary, because direct displacement of the tosylate with sodium iodide gave poor yields of compound **14**.

Scheme 3



lated, upon subsequent oxidation and deprotection, to produce the desired amino acid substituted phosphodiesters **20** and **21**. Along these lines, alcohol **10** was coupled with *N*-tritylserine methyl ester using stoichiometric amounts of 2,2,2-trichloro-*tert*-butyl phosphodichloridite in the presence of 3 equiv of diisopropylethylamine in tetrahydrofuran at −78 °C to give the trialkyl phosphite (**17)** (69%). This compound was then reacted with 30% aqueous hydrogen peroxide in a biphasic system, including methylene chloride, at room temperature for 2 h, to obtain phosphotriester **18a** in 95% yield. The corresponding threonine derivative (**18b)** and the phosphotriester derived from γ-amino-β-hydroxybutyric acid (**18c)** were prepared in a similar way, except that in those reactions, the secondary alcohol derived from the corresponding amino acid was first phosphorylated and then followed by addition of glycerol-alcohol **10** to the reaction mixture. Phosphotriesters **18b** and **18c** were obtained from the respective alcohols in 45% and 42.5% overall yields.

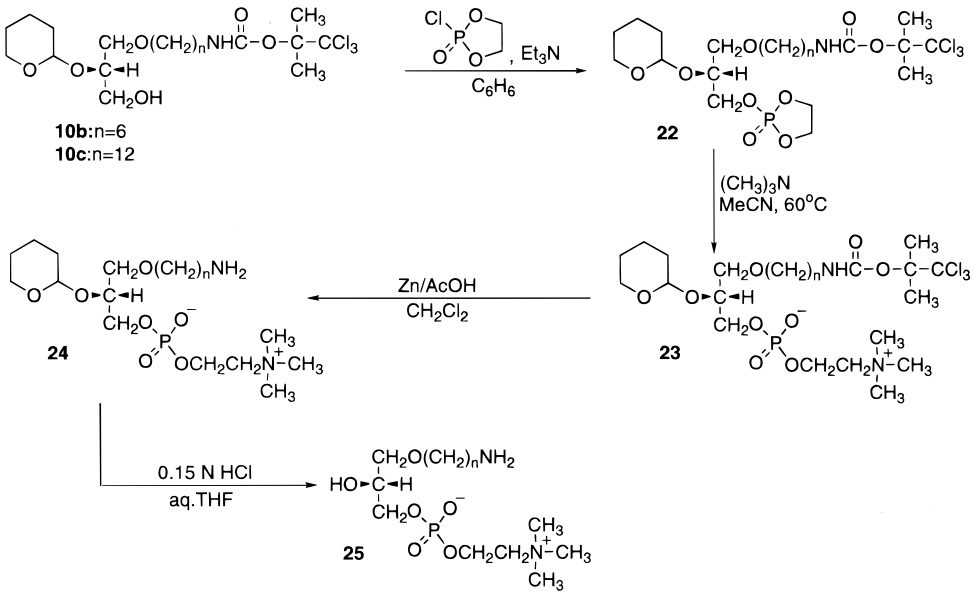
Reductive cleavage of **18a** by zinc powder in the presence of acetylacetone/triethylamine in anhydrous acetonitrile at room temperature gave phosphodiester **19a** as the single major product, which was isolated following silica gel chromatography in an analytically pure form (61% yield). Sequential hydrolysis of the protecting groups was accomplished by addition of 0.1 N HCl, to remove the acid labile *N*-trityl and *O*-tetrahydropyranyl groups and treatment with 0.2 N NaOH in aqueous tetrahydrofuran to obtain the deprotected target phospholipid **20a** as its trisodium salt in 62% yield. The corresponding threonine derivative **20b** was obtained in a similar series of reactions in 59% overall yield. Deprotection of the phospholipid derived from γ-amino-β-hydroxybutyric acid **21** was achieved by treating compound **19c** first with 0.3 N NaOH in aqueous dioxane, followed by trifluoroacetic acid in chloroform, and finally with 0.1 N HCl in aqueous tetrahydrofuran. Deprotected phospholipid compound **21** was isolated as the trisodium salt following silica gel chromatography in 61% overall yield.

Biological Activity of the Synthetic Modulator Analogues. Compounds **20a**,**b** and **21** have been tested for their ability to inhibit in vitro glucocorticoid-receptor complex activation, in conjunction with a series of structurally related synthetic analogues and compared to the naturally occurring modulator.[26] Of the series tested, **20b** was the first synthetic compound possessing modulator activity. Specifically, in the 10^{-3} M concentration range, 1-*O*-(5′-carboxypentyl)-*sn*-glycero-3-phosphothreonine **20b** was found to inhibit glucocorticoid-receptor activation, as well as unoccupied glucocorticoid-receptor hormone binding in a dose-dependent manner. The inhibition was stereospecific, as evidenced by the fact that the stereoisomer of **20b**, prepared from (*S*)-glycidyl tosylate in a similar series of reactions, did not show any activity in either one of the systems tested. Furthermore, since the degree of steroid-binding inhibition by **20b** was found to be the same as its ability to inhibit steroid-receptor complex activation (40%, at $(3-4) \times 10^{-3}$M concentration), it has been suggested that the synthetic modulator may interact with the steroid-binding domain of the glucocorticoid receptor.[26] Although the potency of **20b** is ∼2−3 orders of magnitude lower than that of the naturally occurring modulator, it is the first and only one of the 26 synthetic phosphoglycerides examined thus far that exhibited any modulator-like effects.[12,26,27] Hence, although the activity of the natural modulator is not exactly mimicked by **20b**, it provides a useful lead compound for the design of analogues, including compounds that aim to confirm the exact structure of the naturally occurring modulator.

Synthesis of Functionalized PAF-Analogue Precursors. Because of the well-recognized importance of phospholipid hydrolyzing enzymes in cellular signaling, as well as the importance of the enzyme alkylglycerophosphocholine acetyltransferase in the formation of platelet-activating factor, functionalized alkyllysophosphocholines have been shown to be valuable synthetic analogues in studying the enzymatic reactions involved in the interconversion of biologically active ether phospholipids, with a particular emphasis on the elucidation of the PAF cycle.[8,9,28] However, spectroscopically labeled metabolic PAF intermediates have only been available

(26) Bodine, P. V.; Hajdu, J.; Litwack, G. *Biochem. Biophys. Res. Commun.* **1994**, *203*, 408.
(27) Bodine, P. V.; Garcia, M. L.; Pascual, J.; Bastida, E.; Carganico, G.; Litwack, G. *Receptor* **1991**, *1*, 167.
(28) Baker, R. R.; Chang, H. *Biochim. Biophys. Acta* **1996**, *1302*, 257.

Scheme 4



through "total synthesis" rather than via the derivatization of appropriately functionalized synthetic alkyllysophospholipid precursors.[9] Thus, in extending our synthetic method to the preparation of such derivatives, we focused on the synthesis of ω-NH_2-substituted analogues of 1-*O*-alkyl-*sn*-glycero-3-phosphocholine **3**, to provide a chain-terminal site for the introduction of spectroscopic labels. Specifically, it has recently been shown that ether phospholipid analogues, functionalized at a position remote from the catalytic site, are highly suitable substrates for the study of PAF-metabolizing enzymes, with a view toward mechanistic elucidation of the PAF cycle.[9,28]

For the synthesis of chain-terminal amino-substituted analogues of compound **3**, we first prepared 2,2,2-trichloro-*tert*-butoxycarbonyl-protected 12-aminododecanol via the diborane reduction of commercially available 12-aminododecanoic acid, followed by triethylamine catalyzed carbamoylation using 2,2,2-trichloro-*tert*-butyl chloroformate in 79% overall yield. The corresponding six-carbon analogue was prepared similarly, from 6-aminohexanol (91%). These N-protected amino alcohols were then subjected to the four-step synthetic sequence outlined in Scheme 1, which gave rise to the alkylated/protected glycerols **10b**,**c** in ~52% overall yield. Next, the *sn*-3-hydroxyl group of **10c** was phosphorylated using 2-chloro-2-oxo-1,3,2-dioxaphospholane in benzene at room temperature for 3 h (Scheme 4). The cyclic dioxaphospholane intermediate **22c**, which formed as a single phosphate-positive product, was immediately transferred into a pressure bottle and allowed to react with anhydrous trimethylamine at 60 °C for 16 h. The phosphocholine product **23c** was chromatographed on silica gel to afford the analytically pure phospholipid in 74% yield. Finally, deprotection of the amino group using zinc/acetic acid/methylene chloride (78%) followed by acid-catalyzed cleavage of the *sn*-2-tetrahydropyranyl function gave the target compound **25c** in 66% yield.

In addition to developing an efficient method for the preparation of functionalized ether lysophospholipids, a number of useful synthetic strategies have emerged from the sequences herein presented. The first strategy concerns the introduction of the N-protected aminoalkyl function for the synthesis of ether phospholipids. Specifically, the use of 2,2,2-trichloro-*tert*-butoxycarbonyl function provides an acid- and base-resistant protection strategy that is more stable and more convenient to handle than the corresponding 2,2,2-trichloroethylcarbamoyl alternative previously used in phospholipid synthesis.[29] Moreover, because the trichloro-*t*-BOC group is stable under both acidic and basic conditions, it provides orthogonal protection in the presence of both *t*-BOC[30] and FMOC[31] derivatives of amino groups, such that it is likely to receive further attention for side-chain protection in peptide chemistry as well.

Preparation of an *O*-tetrahydropyranyl protected substituted glycerol, such as compound **5**, in a rapid four-step sequence from readily available starting materials should provide a convenient general method for the synthesis of mixed-chain glycerolipids and phospholipids that incorporate a wide spectrum of different substituents. Despite the temporary presence of a second chiral center that is introduced on the tetrahydropyranylation of the secondary alcohol function, the resulting mixture of stereoisomers is readily purified. This provides a hydrophobic neighboring group that promotes efficient phosphorylation/acylation and allows convenient deprotection, under very mild reaction conditions, to obtain the desired target compound.

Finally, the third element of general synthetic significance involves the use of trichloro-*tert*-butyl phosphodichloridite for coupling the two alcohols en route to the desired phosphodiesters. This reagent, which has been shown to be useful for the construction of internucleotide linkages between sugar moieties in nucleic acid chemistry,[32] has now been applied to the synthesis of lipid phosphodiesters as well. Specifically, the compound was shown to be reactive toward both primary and secondary alcohols, even at low temperatures, allowing simple

(29) Srisiri, W.; Lamparski, H. G.; O'Brien, D. F. *J. Org. Chem.* **1996**, *61*, 5911.

(30) Kocienski, P. *Protecting Groups*; Verlag: New York, 1994, pp 192−195.

(31) Carpino, L. A. *Acc. Chem. Res.* **1987**, *20*, 401.

(32) (a) Letsinger, R. L.; Bach, S. A.; Eadie, J. S. *Nucleic Acids Res.* **1986**, *14*, 3487. (b) Letsinger, R. L.; Groody, E. P.; Tanaka, T. *J. Am. Chem. Soc.* **1982**, *104*, 6806.

sequential addition of the alcohols to be coupled. Phosphite triesters are easily purified, readily oxidized, and reductively deprotected to afford the desired phosphodiesters. Such features should make this reagent an attractive choice for the phosphorylation of glycerol derivatives for cases in which the nature of the polar headgroup precludes the use of the conventional phosphorylating agents that are commonly used in lipid chemistry.

In conclusion, the syntheses that we have presented provide rapid and efficient routes to a wide range of functionalized alkyllysophospholipid compounds. The strength of the methods lie in their simplicity and flexibility. They are likely to become applicable to the preparation of additional types of phospholipid derivatives for biological and physicochemical studies. Work toward this goal is underway in our laboratory.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded at 200, 360, and 500 MHz. (2*R*)-(−)-Glycidyl tosylate, (2*S*)-(+)-glycidyl tosylate, boron trifluoride etherate, 3,4-dihydro-2*H*-pyran, 2,2,2-trichloro-1,1-dimethylethyl chloroformate, 6-aminohexanol, and 2 M lithium borohydride in tetrahydrofuran were purchased from Aldrich. 12-Aminolauric acid 2-chloro-2-oxo-1,3,2-dioxaphospholane, pyridinium *p*-toluenesulfonate, 2,2,2-trichloro-*tert*-butylphosphochloridite, triphenylmethyl chloride, and *S*-(+)-α-methoxy-α-trifluoromethylacetyl chloride were purchased from Fluka. L-Serine methyl ester and L-threonine methyl ester were obtained from Sigma, and anhydrous trimethylamine (Kodak) were all used as received. Zinc powder (−100 mesh, Aldrich) was used without treatment for phosphotriester deprotection. Zinc dust (<10 μm, 98+ %, Aldrich) was activated using 6% aqueous HCl followed by washing with water, ethanol, and anhydrous acetonitrile for reductive cleavage of the 2,2,2-trichloro-*tert*-butoxycarbamoyl group. Alternatively, zinc powder (7 μm, Aesar) was used as received. Acetonitrile (spectrograde, Burdick & Jackson) and triethylamine (spectrograde, Fluka) were dried over activated molecular sieves (3 Å). Reagent grade chloroform and dichloromethane (Fisher) were freshly distilled from phosphorus pentoxide. Benzene (HPLC grade, Aldrich) was kept over sodium wire and distilled from calcium hydride prior to use. Diethyl ether (reagent grade, Fisher) was kept over sodium wire. Dioxane (J. T. Baker), ethyl acetate, hexane (reagent grade, Fisher), and tetrahydrofuran (spectrograde, Burdick & Jackson) were used without further treatment. Tetraethylammonium acetate tetrahydrate (Aldrich) was dried in an anhydrous acetonitrile solution over activated molecular sieves (3 Å) for at least 2 days prior to use. Methyl 6-hydroxyhexanoate was prepared by esterification from 6-hydroxyhexanoic acid (Sapon Laboratories, Aurora, OH) in anhydrous HCl/methanol. Commercially available reagent grade, or better, inorganic reagents were used as received. Regular column chromatography was carried out with silica gel 60 (70−230 mesh ASTM, E. M. Science), and flash column chromatography was carried out with silica gel 60 (230−400 mesh ASTM, E. M. Science). The silica gel was activated at 120 °C and then cooled to room temperature prior to its use in a desiccator over P_2O_5. Thin-layer chromatography was carried out on Whatman diamond MK6F silica gel 60 Å plates. AG 50W-X8 ion-exchange resin (100−200 mesh) was obtained from Bio-Rad Laboratories. Sephadex LH-20 and Sephadex G-10 were obtained from Pharmacia. The TLC plates were visualized by iodine vapor and UV light, where appropriate. The phospholipids were visualized by molybdenum spray,[33] and the primary amines were sprayed by 0.25% ninhydrin in acetone solution. Trityl compounds were visualized by using concentrated hydrochloric acid solution vapor. Elemental analyses were performed by Desert Analytics, Tucson, AZ; Oneida Research Services (ORS), Whitesboro, NY; and Galbraith Laboratories, Inc., Knoxville, TN. Fast atom bombardment (FAB) mass spectra were obtained at the University of California Riverside Mass Spectrometry Facility.

1-*O*-(5′-Carbomethoxypentyl)-*sn*-glyceryl-3-tosylate (5a). To a solution of 6-hydroxyhexanoic acid methyl ester (8.05 g, 55 mmol) and (2*R*)-(−)-glycidyl tosylate **4** (8.4 g, 36.6 mmol) in 200 mL dry of dichloromethane was slowly added boron trifluoride etherate (1.2 mL, 9.76 mmol), at room temperature, and the resulting solution was stirred for 4 h. The reaction mixture was then washed with saturated brine (2 × 50 mL), the organic phase was dried over anhydrous $MgSO_4$, and the solvent was removed with a rotary evaporator. The residue was chromatographed (SiO_2, hexanes/EtOAc 2:3) to give 12.3 g (89%) of the product **5a** as a colorless liquid: IR (Nujol) 3510, 1732 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ) 0.98−1.89 (m, 6 H), 2.37 (t, J = 7.5 Hz, 2 H,), 3.44 (m, 4 H), 3.67 (s, 3 H), 4.04 (m, 3 H), 7.35 (d, J = 8.3 Hz, 2 H), 7.80 (d, J = 8.3 Hz, 2 H); $[\alpha]^{25}_D$ −7.5 ± 0.2 (*c* 1.00, MeOH); R_f (hexanes/EtOAc 2:3) 0.50; FAB-MS [M + H]$^+$ calcd for $C_{17}H_{27}O_7S$ 375.1477, found 375.1488.

1-*O*-[6′-(*N*-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)hexyl]-s*n*-glyceryl-3-tosylate (5b). (i) 6-(*N*-2′,2′,2-Trichloro-*tert*-butoxycarbonylamino)hexanol. To a solution of 6-amino-1-hexanol (2.0 g, 17.1 mmol) in 50 mL of dry chloroform was added 4-(dimethylamino)pyridine (2.09 g, 17.1 mmol) followed by 2,2,2-trichloro-1,1-dimethylethyl chloroformate (4.09 g, 17.1 mmol) at 0 °C. The yellow reaction mixture was stirred at room temperature for 24 h. After complete conversion, as shown by TLC, the reaction mixture was washed with 50 mL of water. The organic layer was dried over sodium sulfate. The solvent was removed by a rotary evaporator, and the light yellow oil, which solidified upon standing, was purified by column chromatography on silica gel using chloroform/methanol (93:7) for elution. The resulting white solid was isolated as an analytically pure product (4.99 g, 91%): IR ($CHCl_3$) 1722 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ) 1.36−1.60 (m, 8 H), 1.92 (s, 6 H), 3.12−3.18 (m, 2 H), 3.65 (t, J = 6.4 Hz, 2 H), 4.85 (br. m, 1 H); R_f ($CHCl_3$/MeOH 93:7) 0.49. Anal. Calcd for $C_{11}H_{20}NO_3Cl_3$: C, 41.20; H, 6.29; N, 4.37. Found: C, 41.10; H, 6.26; N, 4.35.

(ii) 5b. To a solution of **4** (1.03 g, 4.51 mmol) in 20 mL of dichloromethane were added 6-(*N*-TCBOC)aminohexanol (2.20 g, 6.86 mmol) and boron trifluoride etherate (75 μL, 0.083 equiv) at room temperature. The reaction mixture was then refluxed at 45 °C for 6 h, followed by stirring at room temperature for 20 h. The solvent was evaporated, and the resulting viscous oil was purified by silica gel chromatography with a dichloromethane/methanol (95:5) solvent system to afford pure **5b** (2.34 g, 95%) as an oil: IR (neat) 1724 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ) 1.35−1.60 (m, 8 H), 1.93 (s, 6 H), 2.48 (s, 3 H), 3.15 (m, 2 H), 3.44 (m, 4 H), 4.03 (m, 3 H), 7.36 (d, J = 8.4 Hz, 2 H), 7.82 (d, J = 8.4 Hz, 2 H); R_f (CH_2Cl_2/CH_3OH 95:5) 0.45; FAB−MS, [M + H]$^+$, calcd 548.1043, found 548.1046. Anal. Calcd for $C_{21}H_{33}NO_7SCl_3C0.5\ H_2O$: C, 45.28; H, 6.15; N, 2.51. Found: C, 45.15; H, 6.06; N, 2.46.

1-*O*-[12′-(*N*-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)dodecyl]-*sn*-glycero-3-tosylate (5c). (i) 12-Aminododecanol Hydrochloride. To a suspension of 12-aminododecanoic acid (2.0 g, 9.29 mmol) in 13 mL of tetrahydrofuran was added 37 mL of 1 M BH_3·THF (37 mmol) under N_2 atmosphere at room temperature. The reaction mixture was refluxed overnight and then cooled. Next, 30 mL of 10% AcOH in methanol was added dropwise, followed by stirring at room temperature for 30 min. The solvent was removed in vacuo, and the solid was dried by subsequent addition and evaporation of 3 × 30 mL of methanol. The resulting white solid was suspended in 20 mL of water, followed by addition of 40 mL of 9 M aqueous HCl. The suspension was stirred for 48 h. The white solid was isolated by filtration to give 12-aminododecanol hydrochloride (1.86 g, 99%): ^{1}H NMR ($CDCl_3$/CD_3OD 9:1, δ) 1.26−1.67 (m, 20 H), 2.84 (t, J = 7.7 Hz, 2 H), 3.52 (t, J = 6.6 Hz, 2 H).

(ii) 12-(*N*-2′,2′,2′-Trichloro-*tert*-butoxycarbonyl)aminododecanol. To a suspension of 12-aminododecanol hydrochlo-

(33) Dittmer, J. C.; Lester, R. L. *J. Lipid Res.* **1964**, *4*, 126.

ride (2.03 g, 8.54 mmol) in 75 mL of freshly distilled chloroform, was added triethylamine (2.38 mL, 17.1 mmol) under nitrogen. After the mixture was stirred at room temperature for 30 min, 2,2,2-trichloro-*tert*-butyl chloroformate (1.92 g, 8.00 mmol) was added. The reaction mixture was stirred at room temperature for an additional 30 min, and the precipitate that formed was gravity filtered. The chloroform filtrate was washed with water (3 × 50 mL), and the combined aqueous layer was extracted with chloroform (2 × 30 mL). The combined chloroform solution was dried with magnesium sulfate, the solvent was evaporated, and the resulting white solid was dried in a vacuum desiccator over P_2O_5 to give 2.80 g (86%) of analytically pure product: IR ($CHCl_3$) 1724 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.28 (br s, 16 H), 1.40−1.62 (br m, 4 H), 1.92 (s, 6 H), 3.15 (m, 2 H), 3.65 (t, J = 6.5 Hz, 2 H), 4.85 (br m, 1 H); R_f ($CHCl_3$/MeOH 94:6) 0.61; FAB-MS $[M + H]^+$ calcd 404.1526, found 404.1528. Anal. Calcd for $C_{17}H_{32}NO_3Cl_3$: C, 50.44; H, 7.97; N, 3.46. Found: C, 50.37; H, 7.98; N, 3.43.

(iii) 5c. To a solution of **4** (0.42 g, 1.84 mmol) in 20 mL of freshly distilled dichloromethane was added 12-(*N*-2′,2′,2′-trichloro-*tert*-butoxycarbonylamino)dodecanol (1.12 g, 2.77 mmol), followed by boron trifluoride etherate (0.72 mL, 5.85 mmol) under N_2 at room temperature. The reaction mixture was refluxed at 45 °C for 5 h. Most of the glycidyl tosylate had been converted to the product, as shown by TLC. The solution was cooled to room temperature, added to methylene chloride (30 mL), and washed with 50 mL of water. The resulting emulsion was broken with 50 mL of methanol. The organic layer was separated, and the aqueous layer was extracted with 50 mL of CH_2Cl_2. The combined organic layer was washed with 50 mL of water; the combined aqueous layer was washed with 30 mL of CH_2Cl_2. The organic solution was dried with $MgSO_4$, the solvent was evaporated, and the semisolid residue was chromotographed on silica gel using $CHCl_3$/EtOAc (86:14) to obtain the pure product **5c** as a colorless oil (0.85 g, 73%): IR ($CHCl_3$) 1724 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.26 (br s, 16 H), 1.42−1.62 (br m, 4 H), 1.92 (s, 6 H), 2.46 (s, 3 H), 3.10−3.18 (m, 2 H), 3.40−3.46 (m, 4 H), 3.96−4.09 (m, 3 H), 4.85 (br m, 1 H), 7.37 (d, J = 8.3 Hz, 2 H), 7.81 (d, J = 8.3 Hz, 2 H); R_f ($CHCl_3$/EtOAc 86:14) 0.58; $[\alpha]^{25}_D$ −4.3 (*c* 1.00, $CHCl_3$/MeOH 4:1); FAB-MS $[M + H]^+$ calcd 632.1982, found 632.1994. Anal. Calcd for $C_{27}H_{44}NO_7Cl_3S$: C, 51.23; H, 7.01; N, 2.21. Found: C, 51.06; H, 6.99; N, 2.18.

Mosher Ester of 5a: 1-*O*-(5′-Carbomethoxypentyl)-2-(α-methoxy-α-trifluoromethylphenyl)acetyl-*sn*-glycero-3-tosylate (6a). To a stirred solution of **5a** (0.141 g, 0.38 mmol) and 4-(dimethylamino)pyridine (0.065 g, 0.53 mmol) in 5 mL of dry chloroform was added dropwise a solution of *S*-(+)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride (0.114 g, 0.45 mmol) in 1 mL of dry chloroform under nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight. The chloroform was removed under reduced pressure, and the residue was purified by preparative thin-layer chromatography (SiO_2, hexanes/EtOAc 2:1) to give the Mosher ester **6a** as a colorless oil (0.176 g, 79%): ^1H NMR (360 MHz, $CDCl_3$, δ) 1.21−1.25 (m, 2 H), 1.39−1.43 (m, 2 H), 1.51−1.58 (m, 2 H), 2.25 (t, J = 7.5 Hz, 2 H), 2.43 (s, 3 H), 3.26 (t, J = 6.5 Hz, 2 H), 3.43−3.52 (m, 2 H), 3.49 (s, 3 H), 3.64 (s, 3 H), 4.18 (dd, J = 11.0, 6.8 Hz, 1 H), 4.25 (dd, J = 11.0, 3.2 Hz, 1 H), 5.32−5.36 (m, 1 H), 7.31 (d, J = 8.2 Hz, 2 H), 7.36−7.71 (m, 5 H), 7.74 (d, J = 8.2 Hz, 2 H).

3-*O*-(5′Carbomethoxypentyl)-2-(α-methoxy-α-trifluoromethylphenyl)acetyl-*sn*-glycero-1-tosylate (7a). Compound **7a** was prepared under conditions similar to those described for **6a**, using the alcohol **5a′** obtained from (*S*)-glycidyl tosylate under the same reaction conditions as shown for the (*R*)-enantiomer: ^1H NMR (360 MHz, $CDCl_3$, δ) 1.23−1.32 (m, 2 H), 1.46−1.61 (m, 4 H), 2.27 (t, J = 7.5 Hz, 2 H), 2.42 (s, 3 H), 3.35−3.40 (m, 2 H), 3.47 (s, 3 H), 3.56−3.58 (m, 2 H), 3.64 (s, 3 H), 4.08 (dd, J = 10.9, 6.4 Hz, 1 H), 4.18 (dd, J = 10.9, 3.7 Hz, 1 H), 5.36−5.41 (m, 1 H), 7.29 (d, J = 8.2 Hz, 2 H), 7.36−7.51 (m, 5 H), 7.66 (d, J = 8.2 Hz, 2 H).

Mosher Ester of 5c: 1-*O*-[12′-(*N*-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)dodecyl]-2-(α-trifluoromethylphenyl)acetyl-*sn*-glycero-3-tosylate (6b). To a solution of **5c** (0.20 g, 0.316 mmol) in 12 mL of freshly distilled chloroform was added DMAP (0.046 g, 0.377 mmol), followed by MTPA chloride (0.096 g, 0.380 mmol). The solution was stirred at room temperature for 5 h, another portion of MTPA chloride (0.048 g, 0.190 mmol) was added, and the reaction mixture was kept overnight at room temperature. The solution was then washed with 5% $NaHCO_3$ (2 × 10 mL); the combined aqueous layers were extracted with 10 mL of $CHCl_3$, and the organic solution was dried with $MgSO_4$. The solvent was evaporated under reduced pressure to give the Mosher ester **6b** as a colorless oil. This residue was chromatographed on silica gel using $CHCl_3$/EtOAc (95:5) and then freeze-dried from benzene to obtain the pure product **6b** (0.240 g, 89%): ^1H NMR (500 MHz, $CDCl_3$, δ) 1.20−1.29 (br s, 16 H), 1.40 (br s, 2 H), 1.60 (br s, 2 H), 1.92 (s, 6 H), 2.44 (s, 3 H), 3.14 (m, 2 H), 3.27 (t, J = 6.6 Hz, 2 H), 3.46 (dd, J = 10.8, 5.3 Hz, 1 H), 3.51−3.54 (m, 1 H), 3.52 (s, 3 H), 4.20 (dd, J = 11.0, 6.9 Hz, 1 H), 4.28 (dd, J = 11.0, 3.2 Hz, 1 H), 5.35−5.40 (m, 1 H), 7.32 (d, J = 8.3 Hz, 2 H), 7.38−7.52 (m, 5 H), 7.75 (d, J = 8.3 Hz, 2 H); R_f ($CHCl_3$/EtOAc 95:5) 0.74.

3-*O*-[12′-(N-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)dodecyl-2-(α-methoxy-α-trifluoromethylphenyl)acetyl-*sn*-glycero-1-tosylate (7b). Compound **7b** was prepared under conditions similar to those described for **6b** using alcohol **5c′** obtained from (*S*)-glycidyl tosylate under the same reaction conditions as shown for the (*R*)-enantiomer: ^1H NMR (500 MHz, $CDCl_3$, δ) 1.24−1.29 (br s, 16 H), 1.51 (br s, 4 H), 1.92 (s, 6 H), 2.44 (s, 3 H), 3.12−3.16 (m, 2 H), 3.35−3.40 (m, 2 H), 3.50 (s, 3 H), 3.56−3.60 (m, 2 H), 4.10 (dd, J = 10.9, 6.4 Hz, 1 H), 4.20 (dd, J = 10.9, 3.7 Hz, 1 H), 5.39−5.43 (m, 1 H), 7.30 (d, J = 8.3 Hz, 2 H), 7.36−7.53 (m, 5 H), 7.68 (d, J = 8.3 Hz, 2 H).

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-glycero-3-tosylate (8a). To a solution of alcohol **5a** (5.3 g, 14.2 mmol) in 110 mL of dry dichloromethane were added pyridinium-*p*-toluenesulfonate (0.36 g, 1.42 mmol) and 3,4-dihydro-2*H*-pyran (1.78 g, 21.2 mmol), and the resulting mixture was stirred at room temperature for 4 h. The solution was then washed with saturated brine (2 × 40 mL), the organic phase was dried over $MgSO_4$, and the solvent was evaporated under reduced pressure with an evaporator. The residue was chromatographed on silica gel with hexane/EtOAc (2:1) to give 4.5 g (69%) of product **8a** as a colorless liquid: IR (Nujol) 1734 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.21−1.85 (m, 12 H), 2.31 (t, J = 7.5 Hz, 2 H), 2.45 (s, 3 H), 3.26−3.56 (m, 6 H), 3.67 (s, 3 H), 3.85−4.23 (m, 3 H), 4.64−4.74 (m, 1 H), 7.32 (d, J = 8.3 Hz, 2 H), 7.82 (d, J = 8.3 Hz, 2 H); R_f (hexane/EtOAc 2:1) 0.40. This compound was used for the next step without further treatment.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-glycero-3-acetate (9a). To a solution of tosylate **8a** (4.70 g, 10.25 mmol) in 80 mL of anhydrous acetonitrile were added tetraethylammonium acetate (8.04 g, 30.75 mmol) and activated molecular sieves (type 3A, 10 g), and the resulting mixture was stirred at room temperature for 36 h, at which time the TLC analysis showed that the conversion of the tosylate to the acetate was complete. The reaction mixture was then filtered through a pad of Celite, with suction, and the filtrate was evaporated under reduced pressure. The residue was chromatographed on silica gel with hexane/EtOAc (2:1) to give the product **9a** (3.2 g, 90%) as a colorless liquid: IR (Nujol), 1742 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.31−1.91 (m, 12 H), 2.07 (s, 3 H), 2.31 (t, J = 7.5 Hz, 2 H), 3.30−3.59 (m, 6 H), 3.75−4.35 (m, 3 H), 4.79 (br s, 1 H); R_f (hexanes−EtOAc 2:1) 0.42. Anal. Calcd for $C_{17}H_{30}O_6$: C, 58.94; H, 8.73. Found: C, 58.66; H, 8.49.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-glycerol (10a). To a solution of **9a** (3.20 g, 9.23 mmol) in 30 mL of dry methanol, cooled in an ice−water bath, was added anhydrous potassium carbonate (1.5 g). The resulting white suspension was stirred at 0 °C for 30 min, at which time the TLC analysis showed complete conversion of the acetate **9a** to alcohol **10a**. The solvent was evaporated under reduced pressure; the residue was dissolved in dichloromethane and passed through a short pad of silica gel, with suction. Further

elution with dichloromethane, followed by ethyl acetate, and subsequent evaporation of the solvent afforded the crude product **10a** which was chromatographed on silica gel using hexanes/ethyl acetate (1:1) to give pure **10a** (2.1 g), 75%) as a colorless oil: IR (Nujol) 3436, 1736 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.08−2.00 (m, 12 H), 2.32 (t, J = 7.5 Hz, 2 H), 3.25−4.12 (m, 9 H), 3.68 (s, 3 H), 4.52−4.90 (m, 1 H); R_f (hexanes/EtOAc 1:2) 0.46. Anal. Calcd for $C_{15}H_{28}O_6$: C, 59.19; H, 9.27. Found: C, 59.27; H, 9.24.

1-*O*-[6′-(*N*-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)hexyl]-2-tetrahydropyranyl-*sn*-glycero-3-tosylate (8b). To a solution of **5b** (2.35 g, 4.28 mmol) in 20 mL of dry dichloromethane were added pyridinium-*p*-toluenesulfonate (0.11 g, 0.429 mmol) and 3,4-dihydro-2*H*-pyran (0.60 mL, 6.58 mmol). The reaction mixture was stirred at room temperature for 20 h. After completion of the reaction, as shown by TLC, 30 mL of dichloromethane was added, and the resulting solution was washed with 2 × 50 mL of brine. The organic layer was dried over sodium sulfate, and the solvent was removed by a rotary evaporator to give a viscous oil that was purified by silica gel chromatography using dichloromethane/methanol (95:5) as eluant. The analytically pure product **8b** was obtained as a viscous oil (2.58 g, 95%): IR (neat) 1725 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.32 (br s, 6 H), 1.60 (br s, 8 H), 1.92 (s, 6 H), 2.45 (s, 3 H), 3.16 (m, 2 H), 3.42 (m, 5 H), 4.65 (br s, 0.5 H), 4.75 (br s, 0.5 H), 4.85 (br m, 1 H), 7.36 (d, J = 8.3 Hz, 2 H), 7.80 (d, J = 8.3 Hz, 2 H); R_f (CH_2Cl_2/CH_3OH) 0.67; FAB-MS $[M + H]^+$ calcd 632.1618, found 632.1617. Anal. Calcd for $C_{26}H_{40}NO_8SCl_3$: C, 49.33; H, 6.37; N, 2.21. Found: C, 48.98; H, 6.32; N, 2.09.

1-*O*-[12′-(*N*-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)dodecyl]-2-tethydropyranyl-*sn*-glycero-3-tosylate (8c). Compound **8c** was prepared according to the method described for compound **8b** and was afforded in 90% yield: IR (neat) 1723 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.26 (s, 16 H), 1.46 (br m, 10 H), 1.92 (s, 6 H), 2.43 (s, 3 H), 3.15 (m, 2 H), 3.32−3.54 (m, 5 H), 3.96−4.13 (m, 4 H), 4.64 (br s, 0.5 H), 4.74 (br s, 0.5 H), 4.8 (br m, 1 H), 7.32 (d, J = 8.3 Hz, 2 H), 7.80 (d, J = 8.3 Hz, 2 H); R_f (hexanes/EtOAc) 0.69; FAB-MS $[M + Na]^+$ calcd 738.2376, found 738.2408. Anal. Calcd for $C_{32}H_{52}Cl_3NO_8S$: C, 53.59; H, 7.31; N, 1.95. Found: C, 53.40; H, 7.30; N, 1.89.

1-*O*-[6′-(*N*-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)hexyl]-2-tetrahydropyranyl-*sn*-glyceryl-3-acetate (9b). To a solution of **8b** (2.56 g, 4.04 mmol) in 2 mL of dry acetonitrile was added 48 mL of 0.25 M tetraethylammonium acetate (12.13 mmol) in anhydrous acetonitrile. The reaction mixture was stirred at room temperature for 5 h. The solvent was then removed under reduced pressure; the resulting oil was dissolved in 50 mL of dichloromethane, and the resulting solution was washed with 50 mL of water. The organic layer was dried over sodium sulfate, the solvent was flash evaporated, and the crude product was chromatographed on silica gel with dichloromethane/methanol (95:5) to give a viscous oil, **9b** (1.64 g, 78%): IR (neat) 1731, 1210 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.26 (br s, 6 H), 1.50 (br s, 8 H), 1.92 (s, 6 H), 2.08 (s, 3 H), 3.10−3.17 (m, 2 H), 3.36−3.62 (m, 6 H), 3.87−4.20 (m, 3 H), 4.75 (br m, 1 H)), 4.9 (br m, 1 H); R_f (CH_2Cl_2/MeOH 95:5) 0.56; FAB-MS $[M + Na]^+$ calcd 542.1427, found 542.1455. Anal. Calcd for $C_{21}H_{36}Cl_3NO_7$: C, 48.42; H, 6.97; N, 2.69. Found: C, 48,17; H, 6.72; N, 2.77.

1-*O*-[12′-(*N*-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)dodecyl]-2-tetrahydropyranyl-*sn*-glyceryl acetate (9c). Compound **9c** was prepared according to the method described for compound **9b** and was afforded in 88% yield: IR (neat) 1731 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.26 (s, 18 H), 1.54 (s, 8 H), 1.92 (s, 6 H), 2.07 (s, 3 H), 3.10−3.16 (m, 2 H), 3.40−3.62 (m, 4 H), 3.82−4.24 (m, 5 H), 4.75 (br m, 1 H), 4.9 (br m, 1 H); R_f (hexanes/EtOAc 3:2) 0.71; FAB-MS $[M + Na]^+$ calcd 626.2394, found 626.2406. Anal. Calcd for $C_{27}H_{48}Cl_3NO_7$: C, 53.60; H, 8.00; N, 2.32. Found: C, 53.69; H, 7.78; N, 2.10.

1-*O*-[6′-(*N*-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)hexyl-2-tetrahydropyranyl-*sn*-glycerol (10b). To a solution of **9b** (1.28 g, 2.46 mmol) in 20 mL of dry methanol was added anhydrous potassium carbonate (0.68 g, 4.92 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 3 h. After complete conversion, as shown by TLC, 30 mL of water was added, and the resulting solution was extracted with 3 × 50 mL of dichloromethane. The combined organic layer was dried over sodium sulfate, and the solvent was removed by rotoevaporation. The oily residue was chromatographed on silica gel using CH_2Cl_2/MeOH (95:5) as eluant. The resulting oil was dissolved in 40 mL of benzene, and the solvent azeotrope solvent was removed by rotoevaporation. The product **10b** was further dried in benzene solution over molecular sieves (type 3 Å) for 24 h. The molecular sieves were removed by vacuum filtration, and the solvent was removed under reduced pressure. The resulting oil **10b** (0.89 g, 75%) was used for the next step without further treatment: IR (neat) 1726 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.26 (br s, 6 H), 1.46 (br s, 8 H), 1.92 (s, 6 H), 3.10−3.18 (m, 2 H), 3.45−4.10 (m, 9 H), 4.60 (br s, 0.5 H), 4.70 (br s, 0.5 H), 4.95 (br s, 1 H); R_f (CH_2Cl_2/MeOH 95:5) 0.41. Anal. Calcd for $C_{19}H_{34}NO_6Cl_3 \cdot 0.5H_2O$: C, 46.77; H, 7.02; N, 2.86. Found: C, 46.68; H, 6.84; N, 2.92.

1-*O*-[12′-(*N*-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)dodecyl-2-tetrahydropyranyl-*sn*-glycerol (10c). Compound **10c** was prepared according to the method described for compound **10b** and was afforded in 87% yield: IR (neat) 1723 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.27 (s, 18 H), 1.52 (br s, 8 H), 1.92 (s, 6 H), 3.10−3.18 (m, 2 H), 3.40−4.05 (m, 9 H), 4.60 (br s, 0.5 H), 4.76 (br s, 0.5 H), 4.95 (br s, 1 H); R_f (CH_2Cl_2/MeOH 95:5) 0.46; FAB-MS $[M + Na]^+$ calcd 584.2288, found 584.2295. Anal. Calcd for $C_{25}H_{46}Cl_3NO_6$: C, 53.33; H, 8.24; N, 2.49. Found: C, 53.05; H, 8.40; N, 2.48.

2-Methyl-5-*p*-toluenesulfonylmethyloxazoline (11). Boron trifluoride etherate (1.89 mL, 15.35 mmol) was added dropwise to a stirred solution of (*R*)-(−)-glycidyl tosylate (3 g, 13.14 mmol) in dry acetonitrile (120 mL) at 0 °C under nitrogen. The resulting solution was stirred at 0 °C for 15 min and then quenched with excess saturated $NaHCO_3$ solution (150 mL). The resulting mixture was stirred at room temperature for 2 h and then exhaustively extracted with ether (3 × 150 mL). The ethereal solution was washed with saturated brine (2 × 100 mL) and dried over anhydrous $MgSO_4$. Removal of the solvent gave a white solid which was crystallized from chloroform−hexane to give the desired oxazoline **11** as a white semicrystalline solid, 3.2 g (90%):[25] mp 109−112 °C; $[\alpha]^{25}_D$ −43 (*c* 2.1, $CHCl_3$); ^1H NMR ($CDCl_3$, δ) 1.87 (t, J = 1 Hz, 3 H), 2.45 (s, 3 H), 3.46 (ddq, J = 15.0, 7.1, 1.0 Hz, 1 H), 3.85 (ddq, J = 15.0, 10.2, 1.0 Hz, 1 H), 4.05 (m, 2 H), 4.62−4.73 (m, 1 H), 7.34 (d, J = 8.3 Hz, 2 H), 7.80 (d, J = 8.3 Hz, 2 H); R_f ($CHCl_3$/EtOAc 1:3) 0.32.

3-*p*-Toluenesulfonyl-2-acetoxypropyl(triphenylmethyl)amine (12). To a stirred solution of the oxazoline **11** (2.9 g, 10.77 mmol) in tetrahydrofuran (33 mL) was slowly added 0.5 N HCl (33 mL, 16.5 mmol) at 0 °C. The cooling bath was removed, and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with water (120 mL) and washed with ether (3 × 100 mL). The aqueous layer was freeze-dried to give 3.0 g of white amorphous solid. The crude material was suspended in dry chloroform (55 mL), and to it was added triethylamine (2.34 g, 23.15 mmol) with stirring at 0 °C. After the mixture was stirred for 5 min, triphenylmethyl chloride (2.57 g, 9.26 mmol) was added. The cooling bath was removed, and the resulting solution was stirred at room temperature for 3 h. The reaction mixture was washed with water (3 × 35 mL), and the organic layer was dried over anhydrous $MgSO_4$ solvent and then was evaporated. The crude oily residue was chromatographed (SiO_2, hexanes/EtOAc, 3:1) to yield the desired trityl derivative **12** as a white sticky solid (3.8 g, 67% overall): $[\alpha]^{25}_D$ −22.3 (*c* 1.00, $CHCl_3$); IR ($CHCl_3$) 1742, 1599 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 2.0 (s, 3 H), 2.30 (m, 2 H), 2.45 (s, 3 H), 4.25 (d, J = 5 Hz, 2 H), 5.00−5.10 (m, 1 H), 7.10−7.45 (m, 17 H), 7.75 (d, J = 8.3 Hz, 2 H); R_f (hexanes/EtOAc 2:1) 0.42; FAB-MS $[M + H]^+$ calcd 530.2001, found 530.2011. Anal. Calcd for $C_{31}H_{31}NO_5S \cdot 0.25H_2O$: C, 69.72; H, 5.95; N, 2.62; S, 5.99. Found: C, 69.76; H, 6.02; N, 2.48; S, 5.71.

3-Iodo-2-acetoxypropyl(triphenylmethyl)amine (13). To a stirred solution of the tosylate **12** (4.3 g, 8.12 mmol) in dry acetone (100 mL) was added excess sodium iodide (12 g,

81.2 mmol), and the resulting mixture was refluxed under nitrogen for 6 h. The acetone was evaporated under reduced pressure, and the residue was taken up in ether (150 mL) and washed with 10% sodium thiosulfate (2 × 50 mL) and water (2 × 50 mL). The organic solution was dried over anhydrous $MgSO_4$, the solvent was evaporated, and the crude residue was chromatographed (SiO_2, hexanes/EtOAc, 8:1) to furnish the desired iodide **13** as a semipure white solid (2.6 g, 66%). This was used in the next step without further purification: IR ($CHCl_3$) 3328, 2858, 1738, 1597 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 2.10 (s, 3 H), 2.35−2.55 (m, 2 H), 3.32−3.54 (m, 2 H), 4.84−4.96 (m, 1 H), 7.10−7.55 (m, 15 H); R_f (hexanes/EtOAc, 5:1) 0.48.

3-Cyano-2-acetoxypropyl(triphenylmethyl)amine (14). Sodium cyanide (0.66 g, 13.59 mmol) was added to a stirred solution of the iodide **13** (2.2 g, 4.53 mmol) in dry DMSO (45 mL). The resulting mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with water (400 mL) and extracted with ether (4 × 120 mL). The organic extract was washed with saturated brine (2 × 90 mL) and dried over anhydrous $MgSO_4$. Evaporation of the solvent was followed by chromatographic purification (SiO_2, hexanes/EtOAc, 3:1) and gave the nitrile **14** as a white gummy solid (0.87 g, 50%): IR ($CHCl_3$) 2248, 1742, 1597 cm^{-1}; $[\alpha]^{25}_D$ −5.1 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, δ) 2.10 (s 3 H), 2.40−2.50 (m, 2 H), 2.76−2.84 (m, 2 H), 5.04−5.12 (m, 1 H), 7.15−7.50 (m, 15 H); R_f (hexanes/EtOAc 3:1) 0.36; FAB-MS calcd for $C_{25}H_{24}N_2O_2$ (M^+) 384.1838, found 384.1863. Anal. Calcd for $C_{25}H_{24}N_2O_2$: C, 78.10; H, 6.29; N, 7.29. Found: C, 77.89; H, 6.36; N, 7.21.

3-Cyano-2-hydroxypropyl(triphenylmethyl)amine (15). Anhydrous potassium carbonate (0.5 g, 3.62 mmol) was added to a stirred solution of the acetate **14** (0.6 g, 1.56 mmol) in dry methanol (10 mL) at 0 °C. The cooling bath was removed, and the suspension was stirred at room temperature for 30 min. The methanol was removed under reduced pressure, and the residue was taken up in water (20 mL) and extracted with ether (3 × 25 mL). The ethereal extract was washed with water (20 mL) and saturated brine (20 mL) and was then dried over anhydrous $MgSO_4$. The solvent was evaporated, and the crude product was chromatographed (SiO_2, hexanes/EtOAc, 3:1) to give the cyanohydrin **15** as a white sticky solid (495 mg, 93%): IR ($CHCl_3$) 3472, 3328, 2249, 1599 cm^{-1}; $[\alpha]^{25}_D$ −3.0 (*c* 1.00, $CHCl_3$); ^1H NMR ($CDCl_3$, δ) 2.31 (d, J = 6.2 Hz, 2 H), 2.50 (d, J = 6.2 Hz, 2 H), 3.86−3.98 (m, 1 H), 7.10−7.50 (m, 15 H); R_f (hexanes/EtOAc 2:1) 0.37; FAB-MS calcd for $C_{23}H_{21}N_2O$ $[M + H]^+$ 341.1654, found 341.1660. Anal. Calcd for $C_{23}H_{22}N_2O$: C, 80.67; H, 6.48; N, 8.18. Found: C, 80.77; H, 6.43; N, 8.05.

(*S*)-γ-Amino-β-hydroxybutyric Acid (GABOB) (16). To a stirred solution of the cyanohydrin **15** (0.93 g, 2.72 mmol) in dry chloroform (15 mL) was added trifluoroacetic acid (465 mg, 4.08 mmol) at room temperature under nitrogen. The resulting solution was stirred for 30 min. The solution was then diluted with water (20 mL) and extracted with ether (2 × 20 mL). The aqueous layer was freeze-dried to give a pale brown viscous oil (500 mg) that was dissolved in concentrated H_2SO_4 (2.5 mL, 67.5 mmol) and heated on a steam bath for 15 min. Water (25 mL) was added and the solution was refluxed for 3 h. After cooling, the solution it was neutralized by the addition of solid lead carbonate. The solution was then heated on the steam bath for 1 h. The mixture was filtered under suction to remove the suspended solid, and the clear aqueous solution was freeze-dried to give 380 mg of pale brown amorphous solid. The solid was dissolved in a small amount of water (1 mL) and diluted with ethanol (50 mL), yielding a white solid precipitate. Filtration and drying under vacuum furnished the desired amino acid **16** as a white amorphous solid (311 mg, 96% overall: mp 209−212 °C (mp 214 °C); $[\alpha]^{23}_D$ +7.8 (*c* 1.7, H_2O) (lit[24] $[\alpha]^{25}_D$ −7.09 for the (*R*)-isomer); ^1H NMR (200 MHz, D_2O, δ) 2.33 (d, J = 6.6 Hz, 2 H), 2.80−3.12 (m, 2 H), 3.93−4.07 (m, 1 H). The NMR spectrum agrees exactly with that reported[24] and that of an authentic sample.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-glyceryl 2,2,2-Trichloro-*tert*-butyl 2-carbomethoxy-2-(*N*-triphenylmethylamino)ethyl Phosphite (17a). To a vigorously stirred solution of 2,2,2-trichloro-*tert*-butyl phosphodichloridite (4.0 g, 14.45 mmol) and diisopropylethylamine (4.67 g, 36.12 mmol) in 20 mL of dry THF was added dropwise a solution of *N*-tritylserine methyl ester (4.79 g, 12.04 mmol) in 20 mL of dry THF at −78 °C over 10 min, and then a solution of alcohol **10a** (4.4 g, 14.45 mmol) in 15 mL of dry THF was added slowly with vigorous stirring. The resulting mixture was stirred at −78 °C for 2 h. The reaction mixture was then slowly warmed to room temperature with continued stirring for an additional 1.5 h. The solvent was then removed under reduced pressure, and the residue was taken up in ethyl acetate and filtered through a pad of Celite, under suction, to remove the precipitated ammonium salt. The solvent was evaporated, and the oily residue was chromatographed on silica gel with hexanes/EtOAc (3:1) to give **17a** as a colorless viscous oil (7.5 g, 69%): IR (Nujol) 1743 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.10−1.73 (m, 12 H), 1.79 (br s, 6 H), 2.23 (t, J = 7.5 Hz, 2 H), 3.18 (s, 3 H), 3.51 (m, 6 H), 3.65 (s, 3 H), 3.96 (m, 6 H), 4.81 (m, 1 H), 7.19−7.55 (m, 15 H); R_f (hexanes/EtOAc 3:1) 0.58; FAB-MS $[M + H]^+$ $C_{42}H_{56}O_{10}NPCl_3$ calcd 870.2707, found 870.2715.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-glyceryl 2,2,2-Trichloro-*tert*-butyl 1-Carbomethoxy-1-*N*-triphenylmethylamino-1-carbomethoxy-2-propyl Phosphite (17b). To a vigorously stirred solution of 2,2,2-trichloro-*tert*-butylphosphodichloridite (767 mg, 3.30 mmol) and diisopropylethylamine (1.07 g, 8.25 mmol) in 5 mL of dry THF was added dropwise a solution of alcohol *N*-tritylthreonine methyl ester (1.4 g, 3.30 mmol) in 5 mL of dry THF at −78 °C under nitrogen. The solution was stirred at −78 °C for 15 min, and a solution of alcohol **10a** (0.84 g, 2.75 mmol) in 4 mL of dry THF was added slowly with vigorous stirring. The resulting mixture was stirred at −78 °C for 1.5 h. The reaction mixture was then slowly warmed to room temperature with continued stirring for an additional period of 1.5 h. The solvent was removed under reduced pressure, and the residue was taken up in ethyl acetate. The precipitated ammonium salt was removed by filtration through a pad of Celite, with suction. The solvent was evaporated, and the oily residue was chromatographed on silica gel using hexanes/EtOAc 3:1 as eluant to give **17b**, a colorless viscous oil (1.1 g, 45%): IR (Nujol) 1743 cm^{-1}; ^1H NMR ($CDCl_3$, 2 H) 1.10−1.71 (m, 15 H), 1.76 (bs, 6 H), 2.26 (t, J = 7.5 Hz, 2 H), 3.13 (s, 3 H), 3.24−3.57 (m, 6 H), 3.66 (s, 3 H), 3.74−4.17 (m, 5 H), 4.79 (m, 1 H), 7.20−7.45 (m, 15 H); R_f (hexanes/EtOAc 2:1) 0.59; FAB-MS calcd for $C_{43}H_{56}NO_{10}PCl_3$ $[M + H]^+$ 882.2707, found 882.2734.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-glyceryl 2,2,2-Trichloro-*tert*-butyl 1-*N*-Triphenylmethylamino-3-carbomethoxy-2-propyl Phosphite (17c). Compound **17c** was prepared in the same manner as that described for phosphite triester **17b** and was obtained in 42.5% yield: IR ($CHCl_3$) 3328, 2950, 2866, 1732, 1597 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.10−1.80 (m, 18 H), 2.25 (m, 4 H), 2.88 (m, 2 H), 3.40 (m, 6 H), 3.66 (superimposed singlets, 2 × 3 H), 3.86 (m, 4 H), 4.75 (m, 1 H), 7.10−7.50 (m, 15 H); R_f (hexanes/EtOAc 2:1) 0.50; FAB-MS calcd for $C_{43}H_{58}O_{10}NPCl_3$ $[M + H]^+$ 884.2864, found 884.2848.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-3-glyceroyl 2,2,2-Trichloro-*tert*-butyl 2-*N*-Tritylamino-2-carbomethoxyethyl Phosphate (18a). To a stirred solution of **17a** (7.59 g, 8.36 mmol) in 80 mL of dichloromethane was added 30% aqueous H_2O_2 (1.2 mL, 11.7 mmol). After being stirred for 2 h, the reaction mixture was washed with brine (2 × 30 mL). The organic layer was separated, dried over magnesium sulfate, and evaporated under reduced pressure. The oily residue was chromatographed on silica gel with hexanes/EtOAc (1:1) to give phosphotriester **18a** as a colorless viscous oil (7.4 g, 95%): IR (Nujol) 1737 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.11−1.77 (m, 12 H), 1.92 (s, 6 H), 2.28 (t, J = 7.5 Hz, 2 H), 3.20 (s, 3 H), 3.29−3.61 (m, 6 H), 3.65 (s, 3 H), 3.81−4.44 (m, 6 H), 4.78 (m, 1 H), 7.20−7.45 (m, 15 H); R_f (hexanes/EtOAc 1:1) 0.41; FAB-MS calcd for $C_{42}H_{56}O_{11}NPCl_3$ $[M + H]^+$ 886.2656, found 886.2612.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-3-glyceryl 2,2,2-Trichloro-*tert*-butyl 1-*N*-Triphenylmethylamino-1-carbomethoxy-2-propyl Phosphate (18b). Compound **18b** was prepared by employing the same experimental

conditions described for the corresponding phosphotriester **18a** and was obtained as a colorless viscous oil in 92% yield: IR ($CHCl_3$) 2951, 2867, 1735, 1591 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.09−1.78 (m, 15 H), 1.91 (bs, 6 H), 2.26 (t, $J = 7.5$ Hz, 2 H), 3.15 (s, 3 H), 3.22−3.60 (m, 6 H), 3.66 (s, 3 H), 3.88−4.28 (m, 5 H), 4.74 (m, 1 H), 7.20−7.45 (m, 15 H); R_f (hexanes/EtOAc 1:1) 0.50; FAB-MS calcd for $C_{43}H_{57}O_{11}NPCl_3$ $[M]^+$ 899.2734, found 899.2693.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-glyceryl 2,2,2-Trichloro-*tert*-butyl 1-*N*-Triphenylmethylamino-3-carbomethoxy-2-propyl Phosphate (18c). Compound **18c** was prepared under the same experimental conditions as those described for phosphotriester **18a** and was obtained as a viscous oil in 97% yield: IR ($CHCl_3$) 2951, 2867, 1735, 1597 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.20−1.70 (m, 12 H), 1.80 (m, 6 H), 2.25 (t, $J = 7.5$ Hz, 2 H), 2.45 (br s, 2 H), 2.95 (m, 2 H), 3.42 (m, 6 H), 3.65 (superimposed singlets, 2 × 3 H), 4.0 (m, 4 H), 4.75 (m, 1 H), 7.10−7.50 (m, 15 H); R_f (hexanes/EtOAc, 1:1) 0.45; FAB-MS calcd for $C_{43}H_{58}O_{11}NPCl_3$ $[M + H]^+$ 900.2813, found 900.2780.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-glycero-3-phospho-*N*-tritylserine Methyl Ester (19a). To a stirred solution of phosphotriester **18a** (1.5 g, 1.69 mmol) in 20 mL of dry acetonitrile were successively added triethylamine (3.3 g, 32.7 mmol), acetylacetone (2.42 g, 35.36 mmol), and zinc powder (1.5 g, 25.36 mmol). The resulting suspension was vigorously stirred at room temperature for 2 h. The reaction mixture was filtered with suction. The solvent was removed under reduced pressure, and the oily residue was chromatographed on silica gel with hexanes/EtOAc (1:1) and then with chloroform/methanol (4:1) to give the phosphodiester **19a** as a pale-yellow sticky solid (0.75 g, 61%). This was used in the next step without further treatment: IR ($CHCl_3$) 3329, 2948, 2864, 1733, 1599, 1443 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.02−1.90 (m, 12 H), 2.27 (t, $J = 7.4$ Hz, 2 H), 3.17 (br s, 3 H), 3.21−3.55 (m, 6 H), 3.64 (s, 3 H), 3.71−4.20 (m, 6 H), 4.76 (m, 1 H), 7.17−7.46 (m, 15 H); R_f (chloroform/methanol 4:1) 0.50.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-glycero-3-phospho-*N*-tritylthreonine Methyl Ester (19b). Compound **19b** was prepared by employing the same experimental conditions as those described for compound **19a** and was obtained as a pale-yellow sticky solid in 66% yield. This was used in the next step without further treatment: IR ($CHCl_3$) 3329, 2948, 2864, 1733, 1599, 1444 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.01−1.77 (m, 15 H), 2.12 (m, 2 H), 3.06 (br s, 3 H), 3.16−3.56 (m, 6 H), 3.64 (s, 3 H), 3.66−4.06 (m, 5 H), 4.78 (m, 1 H), 7.16−7.44 (m, 15 H); R_f (chloroform/methanol 4:1) 0.50.

1-*O*-(5′-Carbomethoxypentyl)-2-tetrahydropyranyl-*sn*-glycero-3-phospho-*γ*-*N*-triphenylmethylamino-*β*-hydroxybutyric Acid Methyl Ester (19c). Compound **19c** was prepared from compound **18c,** as described for **19a**, and it was obtained as a pale-yellow sticky solid in 76% yield. It was used for the next step without further treatment: IR ($CHCl_3$) 3330, 2948, 2865, 1733, 1599 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.10−1.80 (m, 12 H), 2.25 (m, 4 H), 2.70 (m, 2 H), 3.20−3.60 (m, 6 H), 3.64 (superimposed singlets, 2 × 3 H), 3.80 (m, 4 H), 4.76 (m, 1 H), 7.10−7.50 (m, 15 H); R_f ($CHCl_3$/MeOH 4:1) 0.49.

1-*O*-(5′-Carboxypentyl)-*sn*-3-glycero-3-phosphoserine (20a). Phosphodiester **19a** (300 mg, 0.41 mmol) was dissolved in tetrahydrofuran (6.2 mL). To this solution was added dropwise 0.2 N HCl (6.2 mL, 1.24 mmol) with stirring at room temperature. After the solution was stirred for 2 h, the reaction mixture was made basic by the dropwise addition of 2 N NaOH (1.2 mL, 2.46 mmol) and was further stirred for an additional 2 h. The reaction mixture was then neutralized by bubbling gaseous CO_2, diluted with water (20 mL), and washed with ether (2 × 35 mL). The aqueous layer was freeze-dried to give 480 mg of white solid which was dissolved in water (2 mL) and passed through a Sephadex G-10 column. The column was eluted with water, and the fractions containing the product were collected and freeze-dried to give 198 mg white solid. Chromatographic purification of this solid over a silica gel column (chloroform/methanol/water 3:6:1) gave the phospholipid **20a** (trisodium salt form) as a colorless hygroscopic solid (106 mg, 62% overall). A sample of the compound was converted to the free-acid form by passing it through a cation-exchange column (AG 50W-X8, hydrogen form) and eluting it with water: $[\alpha]^{25}_D$ −6.5 ± 0.3 (*c* 1.00, H_2O); ^1H NMR (D_2O, δ) 1.18−1.25 (m, 2 H), 1.42 (m, 4 H), 2.23 (t, $J = 7.4$ Hz, 2H), 3.38 (m, 4 H), 3.70 (m, 2 H), 3.83 (m, 1 H), 3.93 (m, 1 H), 4.10 (m, 2 H); R_f ($CHCl_3$/MeOH/H_2O 3:6:1) 0.42; FAB-MS calcd for $C_{12}H_{25}NO_{10}P$ $[M + H]^+$ 374.1216, found 374.1194.

1-*O*-(5′-Carboxypentyl)-*sn*-glycero-3-phosphothreonine (20b). Compound **20b** was prepared by employing the same experimental conditions as those described for compound **20a** and was obtained as a colorless hygroscopic solid in 59% overall yield: $[\alpha]^{25}_D$ −10 ± 0.5 (*c* 1.00, H_2O); ^1H NMR (360 MHz, D_2O, δ) 1.18−1.25 (m, 2 H), 1.31 (d, $J = 6.6$ Hz, 3 H), 1.41−1.51 (m, 4 H), 2.13 (t, $J = 7.4$ Hz, 2 H), 3.35−3.49 (m, 4 H), 3.56−3.57 (m, 1 H), 3.66−3.87 (m, 3 H), 4.40−4.59 (m, 1 H); R_f ($CHCl_3$/MeOH/H_2O 3:6:1) 0.44. Anal. Calcd for $C_{13}H_{24}NO_{10}PNa_2 \cdot 0.5H_2O$: C, 35.46; H, 5.72; N, 3.18. Found: C, 35.32; H, 6.11; N, 2.99.

1-*O*-(5′-Carboxypentyl)-*sn*-glycero-3-phospho-*γ*-amino-*β*-hydroxybutyric Acid (21). To a stirred solution of **19c** (0.897 g, 1.21 mmol) in 18 mL of dioxane was slowly added 1 N aqueous NaOH (6 mL, 6 mmol). After the reaction mixture was stirred at room temperature for 2 h, an additional 3 mL of 1 N NaOH was added. The solution was then stirred for another 2 h. The reaction mixture was then neutralized by the infusion of carbon dioxide bubbles, which resulted in the formation of a white precipitate. The suspension was centrifuged, and the supernatant was freeze-dried to give 1.6 g crude product. This was dissolved in 30 mL of chloroform and extracted with 10 mL of water. The chloroform layer was dried over magnesium sulfate, and evaporation of the solvent gave the trisodium salt as a white solid (0.79 g): IR ($CHCl_3$) 2945, 2863, 1712, 1606 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 1.10−1.70 (m, 12 H), 2.10 (br s, 2 H), 2.20−2.60 (m, 4 H), 3.30 (m, 6 H), 3.70 (m, 4 H), 4.61 (m, 1 H), 7.00−7.40 (m, 15 H). The crude trisodium salt was dissolved in 20 mL of dry chloroform, and to the solution was added trifluoroacetic acid (0.806 g, 7 mmol) with stirring, under nitrogen, at room temperature. The reaction mixture was kept at room temperature for 1 h, and the chloroform was then removed under reduced pressure. The oily residue was dissolved in 15 mL of tetrahydrofuran, and 0.2 N aqueous HCl (15 mL, 3 mmol) was added. After being stirred at room temperature for 2 h, the reaction mixture was neutralized with 10% $NaHCO_3$, diluted with water (25 mL), and extracted with ether (2 × 50 mL). The aqueous layer was freeze-dried, and the pale yellow solid that remained was dissolved in 2 mL of water and passed through a Sephadex G-10 column eluted with water. The ninhydrin positive fractions containing the product were combined, freeze-dried, and chromatographed on silica gel using $CHCl_3$/MeOH/H_2O (3:6:1) to give the pure phospholipid **21** as a colorless hygroscopic solid (0.285 g, 61% overall). The compound was analyzed as the sodium salt, obtained by passing a sample through a cation-exchange column (AG 50W-X8, sodium form), eluting with water, and freeze-drying the collected aqueous solution: $[\alpha]^{25}_D$ −1.6 (*c* 1.00, D_2O); ^1H NMR (360 MHz, D_2O, δ) 1.21 (m, 2 H), 1.40−1.48 (m, 4 H), 2.10 (t, $J = 7.5$ Hz, 2 H), 2.40 (dd, $J = 15.4$, 8.2 Hz, 1 H), 2.62 (dd, $J = 15.4$, 5.0 Hz, 1 H), 3.06 (dd, $J = 13.5$, 7.3 Hz, 1 H), 3.19 (dd, $J = 13.5$, 2.0 Hz, 1 H), 3.35−3.48 (m, 4 H), 3.68−3.88 (m, 3 H), 4.49−4.53 (m, 1 H); R_f ($CHCl_3$/MeOH/H_2O 3:6:1) 0.36. Anal. Calcd for $C_{13}H_{24}NO_{10}PNa_2 \cdot H_2O$: C, 33.40; H, 6.04; N, 2.99. Found: C, 33.52; H, 6.30; N, 2.89.

2-Oxo-2-{1′-*O*-[6′-(*N*-2‴,2‴,2‴-trichloro-*tert*-butoxycarbonylamino)hexyl]-2′−*O*-tetrahydropyranyl-*sn*-glyceryl}-1,3,2-dioxaphospholane (22a). To a solution of **10b** (0.89 g, 1.88 mmol) in 50 mL of freshly distilled benzene under nitrogen atmosphere were added triethylamine (0.52 mL, 3.76 mmol) and then 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.34 mL, 3.76 mmol). The reaction mixture was stirred at room temperature for 24 h. TLC, using dichloromethane/methanol (93:7), showed complete conversion to the phosphorylated product **22a**. The crystalline triethylamine hydrochloride that formed was filtered, and the solvent was evaporated under reduced pressure. The oily residue obtained, **22a** (1.1 g), was

used in the next step without further treatment: ^{1}H NMR ($CDCl_3$, δ) 1.26−1.75 (m, 14 H), 1.93 (s, 6 H), 3.15 (m, 2 H), 3.45−4.10 (m, 7 H), 4.20−4.50 (m, 6 H), 4.70−4.75 (br m, 1 H).

1-*O*-[6′-(N-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)hexyl-2-*O*-tetrahydropyranyl-*sn*-glycero-3-phosphocholine (23a). Compound **22a** (1.09 g, 1.87 mmol) was dissolved in 50 mL of anhydrous deaerated acetonitrile and was transferred to a pressure bottle. The solution was cooled in a dry ice/acetone bath (−78 °C). To the soluiton was added 1.5 mL of trimethylamine under nitrogen. The pressure bottle was sealed and kept in an oil bath at 65 °C with stirring for 48 h. The solution was then cooled to 0 °C, and the solid that precipitated was filtered and chromatographed on silica gel using chloroform/methanol/water (1:9:1) to obtain **23a** (1.03 g, 86% overall yield from **10b**). The compouned was isolated as a white powder after being freeze-dried from benzene: IR ($CHCl_3$) 1724 cm^{-1}; ^{1}H NMR ($CDCl_3$/CD_3OD 9.5:0.5, δ) 1.20−1.75 (m, 14 H), 1.92 (s, 6 H), 3.15 (m, 2 H), 3.30 (s, 9 H), 3.41−3.68 (br m, 6 H), 3.72−4.05 (br m, 5 H), 4.30 (br s, 2 H), 4.75−4.85 (br m, 1 H); R_f ($CHCl_3$/MeOH/H_2O 1:9:1) 0.45; FAB-MS $[M + H]^+$ calcd for $C_{24}H_{46}N_2O_9PCl_3$, 643.2085, found 643.2060.

1-*O*-(6′-Amino)hexyl-2-*O*-tetrahydropyranyl-*sn*-glycero-3-phosphocholine (24a). To a solution of **23a** (0.67 g, 1.04 mmol) in 7 mL of distilled dichloromethane was added glacial acetic acid (0.35 mL, to achieve concentration of 5% (v/v)) followed by zinc powder (1.36 g, 20.8 mmol, 7 μm, 97.5%, Aesar) under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 2 h, at which time TLC showed major conversion to the deprotected compound **23a**. The solvent was evaporated under reduced pressure. The residue was suspended in 20 mL of water, stirred for 5 min, and separated from the zinc by vacuum filtration. The filtrate was washed with 10 mL of chloroform. The organic layer was washed with 10 mL of water, and the combined aqueous layer was freeze-dried. The clear oily residue obtained was chromatographed first on Sephadex LH-20, with chloroform/methanol (1:1), and then on silica gel (12 g, using $CHCl_3$/MeOH/H_2O, 1:9:1) yielding **24a** as a pure product that was freeze-dried from a mixture of water and benzene (0.28 g, 61%): ^{1}H NMR ($CDCl_3$−CD_3OD 9.5:0.5, δ) 1.35 (br m, 4 H), 1.50 (br m, 8 H), 1.70 (br m, 2 H), 2.80 (m, 2 H), 3.30 (s, 9 H), 3.38−4.02 (m, 11 H), 4.24 (br m, 2 H), 4.74−4.84 (br m, 1 H); R_f ($CHCl_3$/MeOH/H_2O) 0.19. FAB-MS $[M + H]^+$ calcd for $C_{19}H_{42}N_2O_7P$ 441.2729, found 441.2730.

1-*O*-(6′-Amino)hexyl-*sn*-glycero-3-phosphocholine (25a). To a suspension of **24a** (0.22 g, 0.5 mmol) in 7.5 mL of tetrahydrofuran was added dropwise 0.2 N aqueous HCl (7.5 mL). The reaction mixture was stirred at room temperature for 2 h, at which time TLC showed the complete conversion to product **25a**. The solution was washed with 2 × 20 mL of ether, and the aqueous layer was freeze-dried. The yellow oily residue was dissolved in a minimum amount of water and then passed through a Sephadex G-10 column, eluting it with water. The pure fractions were combined, and the solvent was removed by freeze-drying to give pure **25a** (0.113 g, 58%): ^{1}H NMR (D_2O, δ) 1.28−1.36 (br m, 4 H), 1.48−1.68 (br m, 4 H), 2.90−2.96 (m, 2 H), 3.15 (s, 9 H), 3.42−3.58 (m, 4 H), 3.60−3.64 (m, 2 H), 3.72−3.88 (m, 2 H), 3.90−4.00 (m, 1 H), 4.25 (br m, 2 H); R_f ($CHCl_3$/MeOH/H_2O 1:9:1), 0.08; FAB-MS $[M + H]^+$ calcd for $C_{14}H_{34}N_2O_6P$ 357.2154, found 357.2158.

2-Oxo-{1′-*O*-[12″-(*N*-2‴,2‴,2‴-trichloro-*tert*-butoxycarbonylamino)dedecyl]-2′−*O*-tetrahydropyranyl-*sn*-glyceryl}-1,3,2-dioxaphospholane (22b). Compound **22b** was prepared by a method similar to that described for compound **22a**; however, the reaction conditions were somewhat different. To a solution of **10b** (1.13 g, 2.01 mmol) in 20 mL of freshly distilled benzene under nitrogen atmosphere were added triethylamine (0.42 mL, 3.02 mmol) and then 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.28 mL, 3.02 mmol) in an ice−water bath. The reaction mixture was stirred at room temperature for 3 h, whereupon TLC showed complete conversion to **22b**. The solid Et_3N·HCl that precipitated was suction filtered, and the filtrate was evaporated to give a colorless semisolid residue **22b** (1.34 g), which was dried in a vacuum over P_2O_5 for 20 min and used for the next step directly: ^{1}H NMR ($CDCl_3$, δ) 1.26−1.75 (m, 26 H), 1.93 (s, 6 H), 3.10−3.15 (m, 2 H), 3.45−4.10 (m, 7 H), 4.20−4.50 (m, 6 H), 4.70−4.76 (br m, 1 H); R_f ($CHCl_3$/MeOH 93:7), 0.65.

1-*O*-[12′-(*N*-2″,2″,2″-Trichloro-*tert*-butoxycarbonylamino)dodecyl]-2-*O*-tetrahydropyranyl-*sn*-glycero-3-phosphocholine (23b). Compound **23b** was prepared by a method similar to that described for compound **23a**, with some modification in the experimental conditions. Compound **22b** (1.34 g, 2.0 mmol) in 40 mL of anhydrous acetonitrile solution was transferred, under N_2, into a pressure bottle cooled in a dry ice/acetone bath (−78 °C). To this solution was added 2 mL of trimethylamine and, after the pressure bottle was sealed, the reaction mixture was kept in an oil bath at 65 °C overnight. The reaction mixture was then cooled to room temperature. TLC showed complete conversion to the product **23b**. The solvent was evaporated under reduced pressure and the oily residue was chromatographed twice on 12 g of silica gel using $CHCl_3$/MeOH/H_2O (1:9:1) as eluant. The product was isolated from the combined pure fractions and, following the evaporation of the solvent, freeze-dried from benzene to afford a white solid **23b** (1.08 g, 74% overall from alcohol **10b**): IR ($CHCl_3$) 1724 cm^{-1}; ^{1}H NMR ($CDCl_3$/CD_3OD 9.5:0.5, δ) 1.23 (s, 18 H), 1.38−1.80 (br m, 8 H), 1.90 (s, 6 H), 3.10−3.15 (m, 2 H), 3.37 (s, 9 H), 3.38−3.60 (br m, 7 H), 3.72−3.96 (m, 4 H), 4.28 (br s, 2 H), 4.78 (br m, 1 H); R_f ($CHCl_3$/MeOH/H_2O 1:9:1), 0.68. Anal. Calcd for $C_{30}H_{58}N_2O_9PCl_3$·H_2O: C, 48.30; H, 8.11; N, 3.75. Found: C, 48.29; 8.45; N, 3.60.

1-*O*-(12′-Amino)dodecyl-2-*O*-tetrahydropyranyl-*sn*-glycero-3-phosphocholine (24b). Compound **24b** was prepared by a method similar to that described for compound **24a,** with some modification of the experimental conditions. To a solution of **23b** (0.56 g, 0.77 mmol) in 6 mL of freshly distilled dichloromethane was added 0.3 mL of glacial acetic acid, followed by zinc powder (1.0 g, 20 equiv, 7 μm, 97.5% Aesar) under N_2 atmosphere. The reaction mixture was stirred at room temperature for 2 h. The solvent was then evaporated under reduced pressure, and the residue was suspended in 20 mL of water. To the resulting suspension was added 20 mL of chloroform/dichloromethane (1:1), and the solid was removed by suction filtration. The emulsion was treated with 5 mL of methanol, and after separation of the layers, the organic solvent was extracted with 15 mL of methanol/water (1:2). The aqueous solutions were combined, and the solvent was evaporated under reduced pressure. The white semisolid residue was chromatographed on 5 g of silica gel using chloroform/methanol/water as eluent. The pure fractions were combined, and the solvent was evaporated. The product was freeze-dried from an emulsion of benzene/water (1:1). The deprotected aminoalkyl product **24b** (0.307 g, 78%) was used for the next step without further treatment: ^{1}H NMR ($CDCl_3$/CD_3OD 9.5:0.5, δ) 1.24 (s, 18 H), 1.40−1.80 (br m, 8 H), 2.80 (m, 2 H), 3.32 (s, 9 H), 3.35−3.70 (m, 7 H), 3.72−4.04 (m, 4 H), 4.24 (br m, 2 H), 4.78 (br m, 1 H); R_f ($CHCl_3$/CH_3OH/H_2O 1:9:1), 0.24; FAB-MS $[M + H]^+$ calcd for $C_{25}H_{54}N_2O_7P$ 525.3668, found 525.3678.

1-*O*-(12′-Amino)dodecyl-*sn*-glycero-3-phosphocholine (25b). Compound **25b** was prepared following the same experimental conditions as those described for **25a** and afforded **25b** in 66% yield: ^{1}H NMR (D_2O, δ) 1.23 (br s, 16 H), 1.46−1.68 (br m, 4 H), 2.90−2.96 (m, 2 H), 3.15 (s, 9 H), 3.42−3.56 (m, 4 H), 3.58−3.62 (m, 2 H), 3.72−3.90 (m, 2 H), 3.92−3.98 (m, 1 H), 4.24 (br m, 2 H); R_f ($CHCl_3$/CH_3OH/H_2O 1:9:1), 0.12; FAB-MS $[M + H]^+$ calcd for $C_{20}H_{46}N_2O_6P$ 441.3093, found 441.3078.

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