Synthesis of Chiral Diether and Tetraether Phospholipids: Regiospecific Ring Opening of Epoxy Alcohol Intermediates Derived from Asymmetric Epoxidation

David H. Thompson,*,† Chris B. Svendsen, Ciro Di Meglio, and Valerie C. Anderson‡

Biomolecular Materials Research Group, Department of Chemical and Biological Sciences, Oregon Graduate Institute of Science & Technology, 19600 N.W. von Neumann Drive, Beaverton, Oregon 97006-1999

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Diether and tetraether phospholipids have been synthesized using chiral epoxy alcohol starting materials (e.g. glycidol 3-nitrobenzenesulfonate esters or *tert*-butyldiphenylsilyl ethers). These chiral precursors provide control over the stereochemistry, substitution patterns, and steric properties of the phosphoglycerol backbone. Configuration at the *sn*-2 glycerol carbon was controlled by asymmetric epoxidation of allyl alcohol followed by acid-catalyzed, regioselective opening of the oxirane ring using excess aliphatic *n*-alcohols to give mono-*O*-alkylated glycerol intermediates in good yields. Nucleophilic attack at the less-hindered carbon of the oxirane ring was highly favored over attack at the sterically less accessible site, typically proceeding with regioselectivities of >10:1 and >95% ee's. Triflic acid, boron trifluoride etherate, toluenesulfonic acid, and tropylium tetrafluoroborate, all at 10 mol %, proved to be the most-effective catalysts compared with 10% cesium fluoride, 10% magnesium chloride, or 20% DDQ based on (i) comparison of initial rates of product formation, (ii) regioselectivity of attack on the glycidol nucleus, and (iii) isolated yields of 3-*O*-alkyl-*sn*-glycerol 1-(3′-nitrobenzenesulfonates). Ether linked phospholipids, produced by alkylation of *O*-alkylglycerol sulfonates with excess *n*-alkyl triflates in the presence of equimolar 1,8-bis(dimethylamino)-naphthalene, were isolated in 43–49% overall yields (from glycidol 3-nitrobenzenesulfonate) after tetrabutylammonium hydroxide deprotection and phosphorylation; treatment of the 3-*O*-alkylglycerol sulfonates with tetrabutylammonium hydroxide prior to alkylation at the *sn*-2 center led to internal displacement and oxirane ring reformation, rather than hydrolysis to 3-*O*-alkylglycerol as described in *J. Chromatogr.* **1990**, *506*, 611. 3,3′-*O*-Polymethylene diglycerol phospholipids (bolaamphiphiles) were also prepared by this route using glycidol 3-nitrobenzenesulfonate and bifunctional α,ω-diols as nucleophiles. Synthesis of sterically demanding ether lipids, via Sharpless epoxidation of cyclopentene-1-methanol, produced materials that exhibited larger molecular areas than the analogous 1,2-di-*O*-alkyl phosphatidic acids in monolayer experiments, confirming the restricted conformational flexibility of the cyclopentyl derivative at the air–water interface. Bolaamphiphiles adopted a U-shaped conformation at the air–water interface. Elution-mode HPLC of racemic 3-*O*-hexadecyl-2-*O*-[(3′,5′-dinitrophenyl)carbamoyl]glycerol 1-(3′-nitrobenzenesulfonate) on chiral naphthylalanine phases suggests that displacement-mode HPLC (Camacho-Torralba, P. L.; Vigh, G.; Thompson, D. H.; *J. Chromatogr.* **1993**, *641*, 31; **1993**, *646*, 259) may be used to obviate semipreparative chiral syntheses of alkyl glycerol ethers such as platelet activating factors, the antitumor agent ET-18-OCH_3, and other biologically active ether lipids.

Introduction

Alkyl glycerophospholipids, classically prepared from mannitol, serine, or isopropylidene glycerol derivatives,[1–4] are synthetic targets of great biological and pharmaceutical significance. These compounds are capable of exerting a host of different effects on biologic systems in both their chiral and racemic forms. Nonstereospecific effects include modification of cell membrane "bulk" properties relevant to tumor cytotoxicity,[5–11] cell growth inhibition via disruption of inositol phosphate signaling,[12–14] and stimulation of cellular differentiation.[15,16] Conversely, the action of platelet-activating factor (PAF; 1-*O*-alkyl-2-

† Current address: Department of Chemistry, Purdue University, 1393 Brown Building, West Lafayette, IN 47907-1393, Internet: davethom@chem.purdue.edu.

‡ Current address: Division of Neurosurgery, Oregon Health Sciences University, Portland, OR 97201.

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(1) Baumann, W. J. In *Ether Lipids: Chemistry and Biology*; Snyder, F., Ed.; Academic: New York, 1972; p 51.

(2) Paultauf, F. In *Ether Lipids: Biochemical and Biomedical Aspects*; Mangold, H. K., Paltauf, F., Eds.; Academic: New York, 1983; p 211.

(3) Eibl, H.; Woolley, P. *Chem. Phys. Lipids* **1988**, *47*, 47.

(4) Chandrakumar, N. S.; Hajdu, J. *J. Org. Chem.* **1983**, *48*, 1197.

(5) Berdel, W. E.; Munder, P. G. In *Platelet-Activating Factor and Related Lipid Mediators*; Snyder, F., Ed.; Plenum: New York, 1987; p 449.

(6) Berdel, W. E. *Onkologie* **1990**, *13*, 245.

(7) Kudo, I.; Nojima, S.; Chang, H. W.; Yanoshita, R.; Hayashi, H.; Kondo, E.; Nomura, H.; Inoue, K. *Lipids* **1987**, *22*, 862.

(8) Himmelmann, A. W.; Danhauser-Riedl, S.; Steinhauser, G.; Busch, R.; Modest, E. J.; Noseda, A.; Rastetter, J.; Vogler, W. R.; Berdel, W. E. *Cancer Chemother. Pharmacol.* **1990**, *26*, 437.

(9) Naito, M.; Kudo, I.; Mukai-Sato, Y.; Tsushima, S.; Nomura, H.; Nojima, S.; Inoue, K. *Cancer Immunol. Immunother.* **1987**, *24*, 158.

(10) Bazill, G. W.; Dexter, T. M. *Cancer Res.* **1990**, *50*, 7505.

(11) Noseda, A.; White, J. G.; Godwin, P. L.; Jerome, W. G.; Modest, E. J. *Exp. Mol. Pathol.* **1989**, *50*, 69.

(12) Seewald, M. J.; Olsen, R. A.; Sehgal, I.; Melder, D. C.; Modest, E. J.; Powis, G. *Cancer Res.* **1990**, *50*, 4458.

(13) Marasco, C. J.; Piantadosi, C.; Meyer, K. L.; Morris-Natschke, S.; Ishaq, K. S.; Small, G. W.; Daniel, L. W. *J. Med. Chem.* **1990**, *33*, 985.

(14) Workman, P.; Donaldson, J.; Lohmeyer, M. *Biochem. Pharmacol.* **1991**, *41*, 319.

(15) Honma, Y.; Kasukabe, T.; Okabe-Kado, J.; Hozumi, M.; Tsushima, S.; Nomura, H. *Cancer Chemother. Pharmacol.* **1983**, *11*, 73.

(16) Honma, Y.; Kasukabe, T.; Okabe-Kado, J.; Hozumi, M.; Tsushima, S.; Nomura, H. *Cancer Chemother. Pharmacol.* **1983**, *11*, 77.

0022-3263/94/1959-2945$04.50/0

O-acetyl-*sn*-glycerophosphocholine) derivatives occurs through stereospecific, receptor-mediated processes in platelet activation and inflammation.[17-21] Aside from their role as biological effectors, ether lipids also have promising technological applications in the areas of triggered release from liposomes[22-25] and the development of stabilized membrane films[26-29] for drug delivery, separations, and photochemical energy conversion. Development of facile, stereocontrolled synthetic methodologies for the preparation of PAF analogs[30-35] and other ether-linked phosphoglycerides, therefore, is of great biochemical and practical interest.

Approaches to phospholipid synthesis, utilizing chiral epoxy alcohol intermediates derived from Sharpless epoxidation,[36,37] have been reported by Burgos and Johnson,[38] Bittman and co-workers,[39-41] Henderson and Henderson,[42] and our laboratory.[43] These routes are based on acid-catalyzed, regioselective attack of fatty acid and fatty alcohol nucleophiles on chiral glycidol intermediates to generate diacyl, dialkyl, and alkyl acyl glycerol phospholipids. This paper describes the synthesis of chiral glycerol diethers, sterically constrained cyclopentyl diethers, bifunctional tetraethers containing two chiral glycerol segments coupled to α,ω-*O*,*O*′-polymethylene spacers (bolaform amphiphiles or bolalipids), and their characterization by transmission electron microscopy (TEM), Langmuir film balance, and Pirkle-phase HPLC.

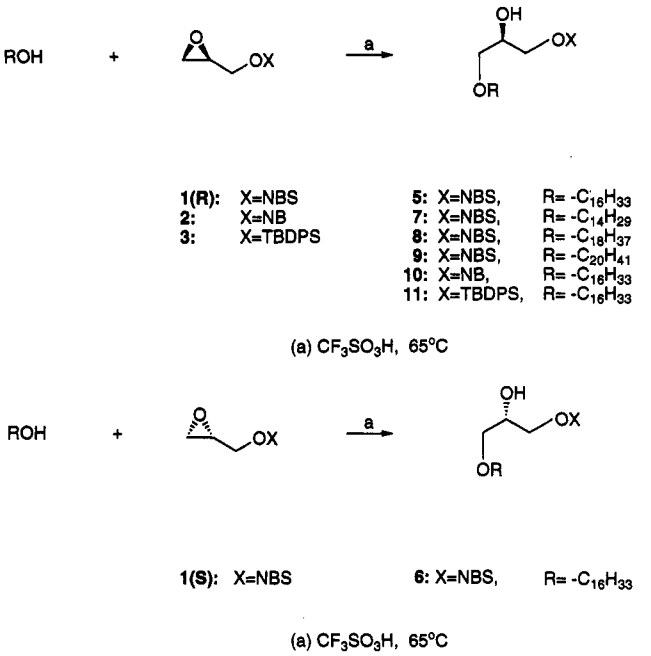


Figure 1. Alcohol C-3 addition to 2,3-epoxy alcohol derivatives.

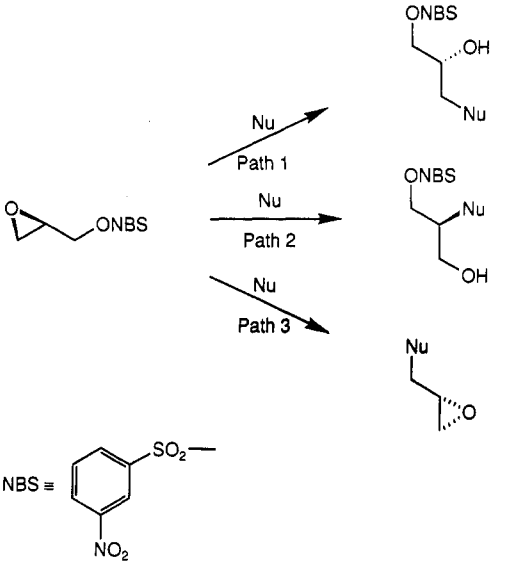
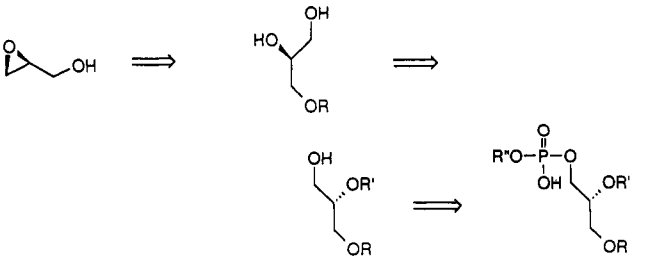


Figure 2. Three pathways for nucleophilic attack on glycidol esters.

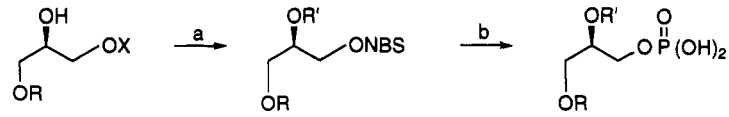
Scheme 1



Results and Discussion

Synthesis. Our approach to the synthesis of diether phospholipids (Scheme 1) is an extension of the method reported previously for the synthesis and chiral resolution of PAF intermediates by Pirkle-phase HPLC.[43] This route is based on Sharpless epoxidation of allylic alcohols to produce 2,3-epoxy alcohol precursors that are subject to

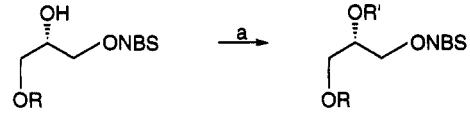
(17) Braquet, P.; Touqui, L.; Shen, T. Y.; Vargaftig, B. B. *Pharmacol. Rev.* **1987**, *39*, 97.
(18) Hanahan, D. J. *Ann. Rev. Biochem.* **1986**, *55*, 483.
(19) Houlihan, W. J. In *Platelet-Activating Factor in Endotoxin and Immune Diseases*; Handley, D. A., Saunders, R. N., Houlihan, W. J., Tomesch, J. C., Eds.; Marcel Dekker: New York, 1990; p 31.
(20) Morris-Natschke, S. L.; Gumus, F.; Marasco, C. J.; Meyer, K. L.; Marx, M.; Piantadosi, C.; Layne, M. D.; Modest, E. J. *J. Med. Chem.* **1993**, *36*, 2018, and refs therein.
(21) Kohler, C.; Carroll, M.; Tarrant, E.; Torley, L.; Wissner, A. *Inflammation* **1993**, *17*, 245.
(22) Jaeger, D. A.; Martin, C. A.; Golich, T. G. *J. Org. Chem.* **1984**, *49*, 4545.
(23) Anderson, V. C.; Thompson, D. H. In *Macromolecular Assemblies*; Stroeve, P., Balazs, A. C., Eds.; American Chemical Society: Washington, DC, 1992; p 154.
(24) Anderson, V. C.; Thompson, D. H. *Biochim. Biophys. Acta* **1992** *1109*, 33.
(25) Thompson, D. H.; Gerasimov, O.; Anderson, V. C. *Biochemistry*, submitted.
(26) Kim, J.-M.; Thompson, D. H. *Langmuir* **1992**, *8*, 637.
(27) Thompson, D. H.; Wong, K. F.; Humphry-Baker, R.; Wheeler, J.; Kim, J.-M.; Rananavare, S. B. *J. Am. Chem. Soc.* **1992**, *114*, 9035.
(28) Kim, J.-M.; Thompson, D. H. *MRS Symposium Ser.* **1992**, *277*, 93.
(29) Santaella, C.; Vierling, P.; Riess, J. G. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 567.
(30) Piwinski, J. J.; Kreutner, W.; Green, M. J. *Annu. Rep. Med. Chem.* **1987**, *22*, 73.
(31) Zeisig, R.; Arndt, D.; Brachwitz, H. *Die Pharmazie* **1990**, *45*, 809.
(32) Peters, U.; Bankova, W.; Welzel, P. *Tetrahedron* **1987**, *43*, 3803.
(33) Zeidler, J. M.; Zimmermann, W.; Roth, H. J. *Chem. Phys. Lipids* **1990**, *56*, 175.
(34) Morris-Natschke, S.; Surles, J. R.; Daniel, L. W.; Berens, M. E.; Modest, E. J.; Piantadosi, C. *J. Med. Chem.* **1986**, *29*, 2114.
(35) Joseph, J.; Shih, C. C.-Y.; Lai, C.-S. *Chem. Phys. Lipids* **1991**, *58*, 19.
(36) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.
(37) Hanson, R. M. *Chem. Rev.* **1991**, *91*, 437.
(38) Johnson, R. A.; Burgos, C. E.; Nidy, E. G. *Chem. Phys. Lipids* **1989**, *50*, 119.
(39) Guivisdalsky, P. N.; Bittman, R. *J. Am. Chem. Soc.* **1989**, *111*, 3077.
(40) Guivisdalsky, P. N.; Bittman, R. *J. Org. Chem.* **1989**, *54*, 4637.
(41) Guivisdalsky, P. N.; Bittman, R. *J. Org. Chem.* **1989**, *54*, 4643.
(42) Hendrickson, H. S.; Hendrickson, E. K. *Chem. Phys. Lipids* **1990**, *53*, 115.
(43) Camacho, P.; Geiger, E.; Vigh, G.; Webster, R.; Thompson, D. H. *J. Chromatogr.* **1990**, *506*, 611.



18: R = R' = -$C_{16}H_{33}$
20: R = R' = -$C_{14}H_{29}$
21: R = R' = -$C_{18}H_{37}$
22: R = R' = -$C_{20}H_{41}$

31: R = R' = -$C_{16}H_{33}$

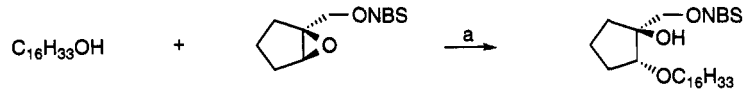
(a) R'OSO_2CF_3, PS, CH_2Cl_2, reflux; (b) Bu_4NOH, H_2O/THF, reflux; $POCl_3$, $CHCl_4$, 0°C; H_2O



19: R = R' = -$C_{16}H_{33}$
23: R = R' = -$C_{18}H_{37}$

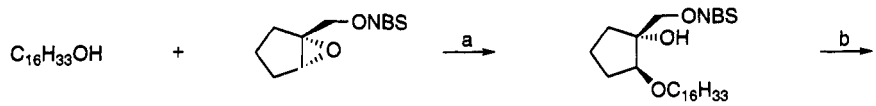
(a) R'OSO_2CF_3, PS, CH_2Cl_2, reflux; (b) Bu_4NOH, H_2O/THF; $POCl_3$, CCl_4; H_2O

Figure 3. Glycerol diether synthesis pathway.

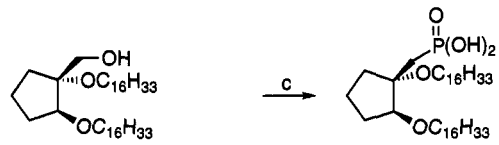


4(S) **12**

(a) CF_3SO_3H, $CHCl_3$, reflux



4(R) **13**



24 **32**

(a) CF_3SO_3H, $CHCl_3$, reflux; (b) $C_{16}H_{33}OSO_2CF_3$, PS, CH_2Cl_2, reflux; CsOAc, DMSO/DMF, 45 °C; LAH, THF; (c) $POCl_3$, CCl_4, 0 °C; H_2O

Figure 4. Cyclopentyl diether synthesis pathway.

alcoholysis in the presence of acid catalysts. Nucleophilic attack on the oxirane ring with high regioselectivity is the key step in the sequence (Figure 1). Since C-2 attack leads to inversion whereas C-3 addition produces retention at the *sn*-2 carbon, the overall efficiency of the reaction sequence may be suppressed by poor C-2:C-3 regioselectivity and/or displacement at C-1 to form glycidol alkyl ethers (Figure 2). Alkylation at the C-2 position with alkyl triflate followed by hydrolytic deprotection and phosphorylation at C-1 completes the glycerol ether pathway (Figure 3). Stepwise addition of alkyl chains, combined with the use of chiral glycerol synthons, therefore, provides a stereocontrolled route for the synthesis of mixed chain ether phospholipids. We have applied this approach to the preparation of di-*O*-alkyl phosphoglycerides, sterically demanding phosphatidic acids derived from cyclic allylic alcohols (Figure 4) and tetraether bolalipids capable of forming ultrathin, thermostable membranes (via double nucleophilic addition of α,ω-diols to glycidyl 3-nitrobenzenesulfonate, Figure 5).

Nucleophilic Oxirane Ring Opening at C_3 (Figure 1). Table 1 summarizes the results obtained with nucleophilic addition of aliphatic alcohols to 1–4 under a variety of reaction conditions. The reactions proceeded with high reigochemical yield, favoring attack at the glycidol ester C-3 vs C-2 carbon by a factor of 10 or more regardless of the conditions employed. Triflic acid-catalyzed reactions carried out in the absence of solvent gave the best reaction kinetics (Table 1) without compromising regioselectivity (typically in the range of 15:1 for C-3:C-2 addition) although the yields were slightly better using $CHCl_3$ as solvent.[44] High regioselectivities were also observed with the cyclopentyl epoxy alcohol 4 (~12:1 C-3:C-2 addition determined by NMR) in CH_2Cl_2

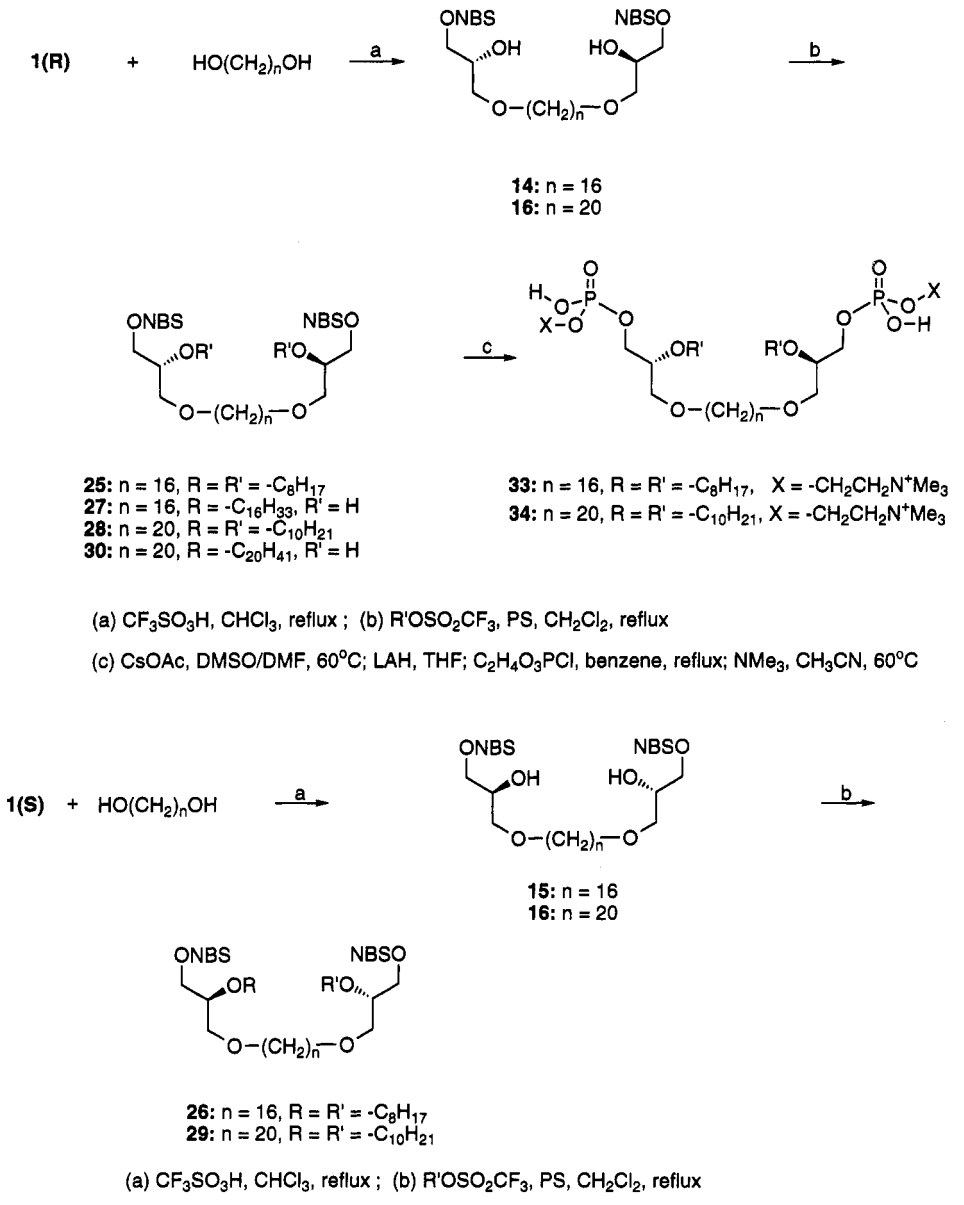


Figure 5. Diglycerol tetraether (bolaamphiphile) pathway.

using triflic acid; although BF_3·Et_2O catalysis was effective, it was slower (cf. Experimental Section for **12**) (Table 1).

The thioether derivative of **7** was obtained by addition of tetradecanethiol to **1** in 5% overall yield, significantly less than was reported by Hajdu and co-workers using glyceric acid (39%) or serine acid (36%) methyl esters as starting materials to prepare thioester and thioether phospholipids.[45] These methods, however, may be obviated by a very efficient method for preparing glycerol thioethers from *O*-protected glycidols using tetrabutylammonium fluoride as catalyst that has recently been reported.[46]

The catalytic activity of several protic and Lewis acids that reportedly accelerate nucleophilic addition of alcohols to epoxides were directly compared for 1-hexadecanol addition to **1**. No catalytic activity was observed using 10% (w/v) cesium fluoride, 10% (w/v) magnesium chloride, 20% (w/v) DDQ, or 1% (w/v) toluenesulfonic acid;

Table 1. Oxirane Ring Opening Reactions of Epoxy Alcohol Intermediates

reactants[a]	ratio	time (h)	products	yield (%)
1(R) + 1-hexadecanol	1:3	5	**5**	79
1(R) + 1-hexadecanol	1:5	24	**5**	56
1(R) + 1-hexadecanol	1:1	8.5	**5**	43
1(R) + 1-hexadecanol	1:1	21	**5**	36
1(R) + 1-hexadecanol[b]	1:3	4	**5**	71
1(R) + 1-hexadecanol[c]	1:1.2	24	**5**	82
1(S) + 1-hexadecanol	1:3	5	**6**	78
1(R) + 1-tetradecanol	1:3	5	**7**	83
1(R) + 1-octadecanol	1:3	5	**8**	75
1(R) + 1-eicosanol	1:3	5	**9**	68
2 + 1-hexadecanol	1:3	2	**10**	16
3 + 1-hexadecanol[d]	1:3	17	**11**	60
4(S) + 1-hexadecanol[e]	1:2.5	12	**12**	71
4(R) + 1-hexadecanol[e]	1:1.5	48	**13**	70

[a] All reactions were run neat (65 ± 5 °C) using 0.5–3 mol % triflic acid as catalyst unless otherwise noted. [b,c] These reactions were run in cyclohexane (b) and chloroform (c) solvent heated at 60–65 °C. [d] 7 mol % tropylium tetrafluoroborate catalyst. [e] Reactions run at 23 °C in CH_2Cl_2 using triflic acid (7.5 mol % v/v) as catalyst.

(44) If the starting materials were not intimately mixed in the neat reactions prior to triflic acid addition or the reaction was heated for longer than ~5 h, glycerol ether oligomers became the predominant reaction product.

(45) Bhatia, S. K.; Hajdu, J. *Tetrahedron Lett.* **1987**, *28*, 3767. Ibid. **1988**, *29*, 31.

(46) Albanese, D.; Landini, D.; Penso, M. *Synthesis* **1994**, 34.

Table 2. Catalyst Dependence on Initial Rate of 1-Tetradecanol Addition to 1 in $CDCl_3$ Solution

catalyst	$k_2^{25\,°C}$ ($M^{-1}\,s^{-1}$)[a]	$k_2^{65\,°C}$ ($M^{-1}\,s^{-1}$)[b]
triflic acid	5.2×10^{-4}	8×10^{-3}
boron trifluoride etherate	5.7×10^{-4}	1×10^{-2}
tropylium tetrafluoroborate	1.6×10^{-4}	8×10^{-3}
toluenesulfonic acid	1.8×10^{-4}	1×10^{-2}

[a] Rate constant precision is ±15%. [b] Rate constant precision is ±25%.

Table 3. Bolalipid Intermediates via Nucleophilic Addition to Glycidol Sulfonates

reactants	ratio	time (h)	product	yield (%)
1(R) + 1,16-hexadecanediol[a]	2:1	10	14	47
1(R) + 1,20-eicosanediol[a]	2:1	8	16	38
1(R) + 1,16-hexadecanediol[b]	2:1	48	14	68
1(S) + 1,16-hexadecanediol[b]	2:1	48	15	67
1(R) + 1,20-eicosanediol[b]	2:1	48	16	65
1(S) + 1,20-eicosanediol[b]	2:1	48	17	66

[a] Reactions were run neat at 82 ± 5 °C using 1% (v/v) triflic acid catalyst. [b] Reactions used 1% (v/v) triflic acid catalyst in chloroform heated at reflux.

reactions catalyzed with 5% (w/v) tropylium tetrafluoroborate were slower than those with 1% (v/v) boron trifluoride etherate or 1% (v/v) triflic acid. Further comparison at 25 °C in $CDCl_3$ using 10 mol % catalyst indicated that the initial rates for the triflic acid- and boron trifluoride·etherate-catalyzed reactions are similar, whereas tropylium- and toluenesulfonic acid-mediated reactions are slower by a factor of three, presumably due to poor catalyst solubility; these differences in initial rate become less pronounced at higher temperatures where the catalysts are more fully dispersed and the rates are comparable (Table 2). Since the catalytic activity of triflic acid and boron trifluoride etherate were essentially equivalent in reactions with 1, triflic acid was chosen for subsequent investigations because of its stability and more favorable reaction kinetics with hindered epoxy alcohol substrates.

Double nucleophilic additions of α,ω-polymethylene diols in the presence of excess 1 and 1% triflic acid (Figure 5) are summarized in Table 3. Unlike the monofunctional derivatives outlined in Table 1, the yields and regioselectivities of 3,3′-*O*-polymethylene diglycerols were greatly affected by reaction conditions. Facile conversions characterized the neat reactions of 1 with simple aliphatic monoalcohols; however, the higher reaction temperatures required to keep the diols in solution lead to higher degrees of glycerol oligomerization as a significant competitive reaction to the desired double nucleophilic additions. The best conditions for generating double C-3 oxirane ring opening product were found to be 1% triflic acid in $CHCl_3$ heated at reflux for ~48 h.

C_2 Alkylation (Figures 3–5). Alkylation of the C-2 position with alkyl triflates[47] occurred under a wide variety of reaction conditions (Table 4); however, it proceeded most efficiently in CH_2Cl_2 heated at reflux for 24–48 h using 1,8-bis(dimethylamino)naphthalene (PS) as base. This hindered, chelating proton acceptor produced fewer base-induced side reactions than others tested (see below) and enabled the rapid recovery of alkylated products upon acidification, extraction, and silica gel chromatography.

Deprotection and Phosphorylation. Simple diether intermediates were hydrolytically deprotected using a 10-fold excess of tetrabutylammonium hydroxide (40% wt/v, dissolved in 5 parts of THF) per equivalent of 3-nitrobenzenesulfonate ester; hindered diethers and tetraether bolaform lipid precursors were prepared via an acetate exchange/reductive deprotection sequence since the Bu_4NOH-mediated reaction led to elimination and oligomerization, respectively. The purified alcohols were then phosphorylated to produce the diether phosphatidic acid[48] and tetraether phosphatidylcholine[49,50] derivatives, respectively (Table 5). A notable exception to the reaction sequence described in our previous work[43] occurs during Bu_4NOH hydrolysis of the 3-nitrobenzenesulfonate protecting group from monoalkyl glycerol intermediates. The hydrolysis conditions reported result in reformation of the glycidol nucleus due to internal displacement of the C-1 sulfonate ester by the transiently formed C-2 alkoxide[51–53] rather than deprotection at the C-1 site. This side reaction was obviated by alkylation of the C-2 carbon prior to base-mediated deprotection of the glycerol intermediate at the C-1 site.

Table 4. Alkylation Reactions Using Triflate Esters of Aliphatic Alcohols

alcohol	triflate	base[a]	solvent	time (h)[b]	product	yield (%)
5	hexadecyl	Et_3N	Et_3N	12	18	22
5	hexadecyl	none	neat	12	18	38
5	hexadecyl	none	CH_2Cl_2	100	18	36
5	hexadecyl	DMAP	neat	13.5	18	48
5	hexadecyl	PS	CH_2Cl_2	48	18	89
6	hexadecyl	PS	CH_2Cl_2	50	19	92
7	tetradecyl	PS	CH_2Cl_2	48	20	89
8	octadecyl	PS	CH_2Cl_2	48	21	92
9	eicosyl	PS	CH_2Cl_2	46	22	91
6	octadecyl	PS	CH_2Cl_2	70	23	86
13	hexadecyl	MDTBP	CH_2Cl_2	120	24	53
14	octyl	PS	$CHCl_3$	48	25	82
15	octyl	PS	$CHCl_3$	48	26	83
14	hexadecyl	PS	$CHCl_3$	48	27	67
16	decyl	PS	$CHCl_3$	48	28	84
17	decyl	PS	$CHCl_3$	48	29	83
16	eicosyl	PS	$CHCl_3$	48	30	63

[a] DMAP = 4-(dimethylamino)pyridine. MDTBP = 4-methyl-2,6-di-*tert*-butylpyridine. PS = Proton Sponge (1,8-diaminonaphthalene). [b] Reactions in CH_2Cl_2 were heated at reflux; all others were carried out at 60 ± 10 °C.

Table 5. Ester Deprotection/Phosphorylation

reactant	phospholipid	yield (%)
18	31[a,b]	64
24	32[b,c]	32
25	33[c,d]	61
28	34[c,d]	61

[a] The deprotection reaction utilized 10 equiv of tetrabutylammonium hydroxide (in H_2O/THF) per equivalent of benzenesulfonate. [b] Phosphorylation was carried out in CCl_4 at 0 °C using stoichiometric $POCl_3/Et_3N$. [c] Deprotection was effected via acetate exchange, followed by LAH reduction (see Experimental Section). [d] Phosphorylation was performed as described in ref 71.

(47) Aoki, T.; Poulter, C. D. *J. Org. Chem.* **1985**, *50*, 5634.

(48) Wissner, A.; Schaub, R. E.; Sum, P.-E.; Kohler, C. A.; Goldstein, B. M. *J. Med. Chem.* **1985**, *28*, 1181.

(49) Yamauchi, K.; Sakamoto, Y.; Moriya, A.; Yamada, K.; Hosokawa, T.; Higuchi, T.; Kinoshita, M. *J. Am. Chem. Soc.* **1990**, *112*, 3188.

(50) Yamauchi, K.; Moriya, A.; Kinoshita, M. *Biochim. Biophys. Acta* **1989**, *1003*, 151.

(51) Klunder, J. M.; Ko, S. Y.; Sharpless, K. B. *J. Org. Chem.* **1986**, *51*, 3710.

(52) Cimetiere, B.; Jacob, L.; Julia, M. *Tetrahedron Lett.* **1986**, *27*, 6329.

(53) Pederson, R. L.; Liu, K. K.-C.; Rutan, J. F.; Chen, L.; Wong, C.-H. *J. Org. Chem.* **1990**, *55*, 4897.

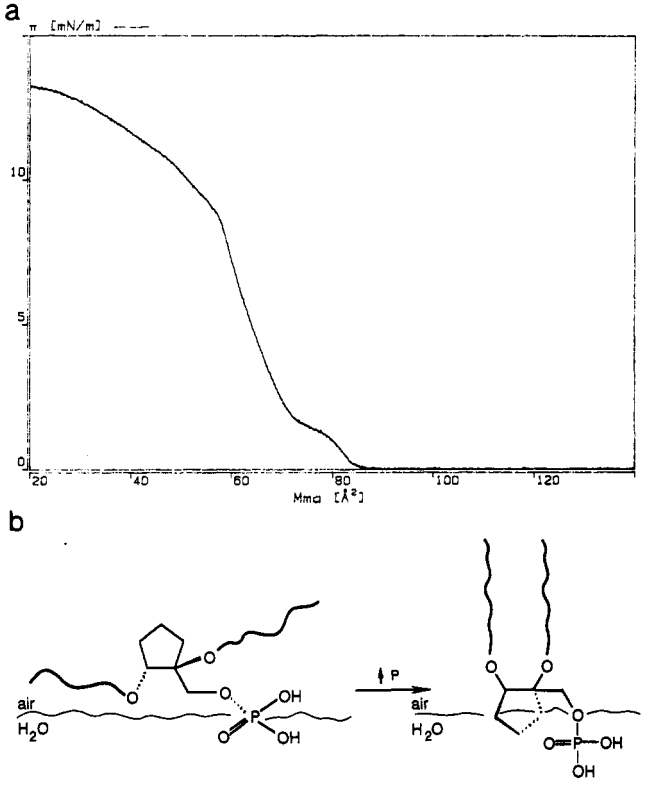


Figure 6. Monolayer behavior of **32**. A: π–A curve on pure water. B: Conceptual model of cyclopentyl di-*O*-hexadecyl ether monolayer packing in the air–water interface.

Monolayer Experiments. Conformationally restricted analogs of diacylglycerol have been shown to be competitive inhibitors of protein kinase C;[54] sterically demanding ester[55] and ether lipids[20,21,56–58] have also been used to assay phospholipase A_2 and PAF activity. We probed the increased steric demands of **32** resulting from conformationally restricted rotations about the C-2–C-3 bond of the cyclopentanoid headgroup relative to the glycerol diether of similar alkyl chain length by monolayer balance methods. Compression of **32** from the two-dimensional gas phase at the air–water interface showed a small increase in surface pressure at 85 Å^2/molecule prior to a large increase in surface pressure at 72 Å^2/molecule that occurs as the monolayer enters the liquid expanded phase (Figure 6A). We attribute the initial small rise in surface pressure at 85 Å^2/molecule to a torsional motion of the cyclopentyl headgroup about the C-2–C-3 bond (Figure 6B) that reduces alkyl chain interactions in the two-dimensional gas phase and ultimately provides better van der Waals interactions between the alkyl chains in the more condensed state below 70 Å^2/molecule; similar values have been reported by Paltauf and co-workers for 1,2-di-*O*-hexadecyl- and 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholines as they undergo two-dimensional condensation.[59] This increase in headgroup order is also reflected in the higher phase transition temperature and larger transition enthalpy observed for **32** relative to 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphate as well as for diastereomeric 1,3-dioxolane-based cationic surfactants[60] and cyclopentanoid analogs of dipalmitoyl phosphatidic acid[55] by differential scanning calorimetry.

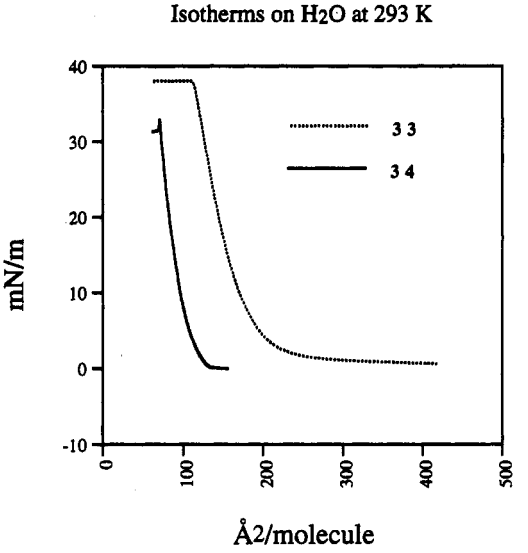


Figure 7. Monolayer behavior of 2,2′-di-*O*-decyl-3,3′-di-*O*-eicosamethylenebis(*sn*-glycero-1-phosphocholine) on pure water.

The pressure–area curves for **33** and **34** (Figure 7) are qualitatively similar to those reported by our laboratory for phosphatidic acid-type analogs;[26] a U-shaped conformation at the air–water interface is inferred since the very large molecular area observed is nearly twice that determined for 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine.[59]

Transmission Electron Microscopy. Electron micrographs (Figure 8) of diether lipid **31** and tetraether lipids **33** and **34** were obtained to determine whether the morphology of these lipids were closed membrane vesicles or multilamellar arrays characteristic of monopolar and bolaform[27] phospholipids, respectively. Large, unilamellar vesicles were formed when **31** was extruded through 0.1-μm Nucleopore filters,[61] whereas the bolaform lipids only formed stable vesicles in the presence of cholesterol (7:3 **34**:cholesterol); multilamellar arrays were observed below T_c and/or in the absence of cholesterol. The morphological behavior of **33** and **34** is consistent with that reported for phosphocholine,[49,50] phosphatidic acid,[26] and other bolaform amphiphiles.[62,63]

HPLC on Chiral Stationary Phases. Naphthylalanine-type Pirkle phases were used to develop displacement-mode HPLC separations of carbamate-derivatized ether lipids. The isocyanate intermediate formed by Curtius rearrangement of 3,5-dinitrobenzoyl azide[64] was trapped using racemic *O*-hexadecylglycerol 3′-nitrobenzenesulfonate, **5**, and **6** as nucleophiles and chromatographed on the chiral stationary phase with 7.5% (v/v) THF in *n*-hexane. Racemic carbamate gave baseline separations

(54) Teng, K.; Marquez, V. E.; Milne, G. W. A.; Barchi, J. J.; Kazanietz, M. G.; Lewin, N. E.; Blumberg, P. M.; Abushanab, E. *J. Am. Chem. Soc.* **1992**, *114*, 1059.
(55) Ahmad, T. Y.; Morrisett, J. D.; Pownall, H. J.; Gotto, A. M.; Brockman, H. L.; Sable, H. Z.; Lewis, E. O.; Hancock, A. J. *Chem. Phys. Lipids* **1990**, *55*, 231.
(56) Witzke, N. M.; Bittman, R. *J. Lipid Res.* **1985**, *26*, 623.
(57) Bittman, R.; Witzke, N. M. *Chem. Phys. Lipids* **1989**, *50*, 99.
(58) Wissner, A.; Schaub, R. E.; Sum, P.-E.; Kohler, C. A.; Goldstein, B. M. *J. Med. Chem.* **1985**, *28*, 1181.
(59) Paltauf, F.; Hauser, H.; Phillips, M. C. *Biochim. Biophys. Acta* **1971**, *249*, 539.
(60) Jaeger, D. A.; Subotkowski, W.; Mohebalian, J.; Sayed, Y. M.; Sanyal, B. J.; Heath, J.; Arnett, E. M. *Langmuir* **1991**, *7*, 1935.
(61) Hope, M. J.; Bally, M. B.; Webb, G.; Cullis, P. R. *Biochim. Biophys. Acta* **1985**, *812*, 55.
(62) Moss, R. A.; Li, J.-M. *J. Am. Chem. Soc.* **1992**, *114*, 9227.
(63) Fuhrhop, J. H.; Hungerbühler, H.; Siggel, V. *Langmuir* **1990**, *6*, 1295.
(64) Pirkle, W. H.; Pochapsky, T. C.; Mahler, G. S.; Corey, D. E.; Reno, D. S.; Alessi, D. M. *J. Org. Chem.* **1986**, *51*, 4991.

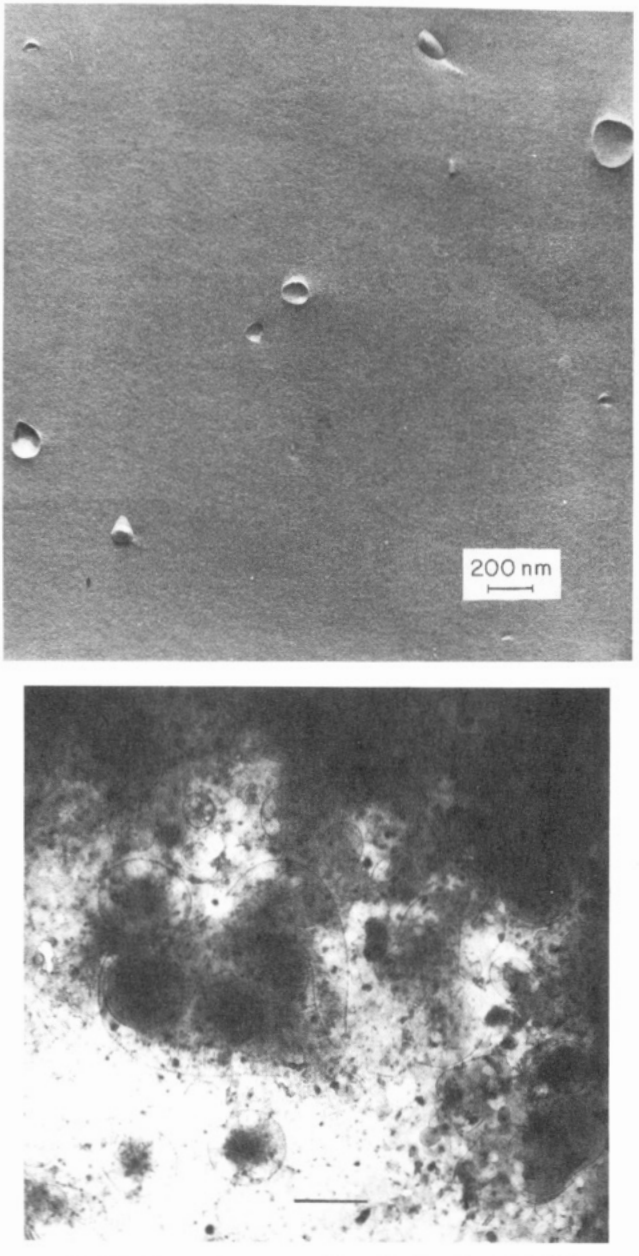


Figure 8. Electron microscopy of synthetic ether lipid dispersions. A, top: Freeze-fracture micrograph of **31**. B, bottom: Cryo-TEM micrograph of **34**. Scale bar = 2000 Å.

of *R*- and *S*-isomers, verified by subsequent injections of carbamates derived from **5** and **6** samples produced via Sharpless epoxidation. These conditions were then used to determine the enantiomeric excesses observed during nucleophilic ring opening of glycidol esters summarized above.

Stereochemical purities of **5** and **6** were maintained (i.e. less than 1% scrambling of the chiral center) during carbamate deprotection and rederivatization with 3,5-dinitrophenyl isocyanate (Figure 9). Displacement-mode separation of carbamate-derivatized racemic ether lipids into their chiral antipodes was accomplished on a semipreparative scale (27 mg) using two analytical naphthylalanine columns connected in series;[65,66] the *S*-enantiomer is eluted first at 1450 s followed by the *R*-enantiomer at 1520 s (the slight inflection in the slope at the displacer isotherm, ~1570 s, is due to a contaminant in the racemic carbamate sample). Resolution of the enantiomers under displacement conditions on naphthylalanine phases and the observed retention of stereochemistry upon reductive deprotection of the carbamate residue illustrates the utility of this approach in isolating semipreparative quantities of pure stereoisomers of glycerol ether intermediates for comparative biochemical and biophysical studies.

(65) Camacho-Torralba, P. L.; Vigh, G.; Thompson, D. H. *J. Chromatogr.* **1993**, *641*, 31.
(66) Camacho-Torralba, P. L.; Beeson, M. D.; Vigh, G.; Thompson, D. H. *J. Chromatogr.* **1993**, *646*, 259.

Conclusions

A facile stereocontrolled synthesis of ether lipids in moderate yields from allyl alcohol precursors has been developed. The six-step reaction pathway is applicable to a wide variety of enantiomerically pure, sterically constrained, and unsymmetrically substituted ether-linked phospholipids. Kinetic comparisons for acid-catalyzed alcoholysis of glycidol intermediates and an improved methodology for *sn*-2 glycerol alkylation using excess alkyl triflate and 1,8-bis(dimethylamino)naphthalene are also reported. Analytical HPLC on naphthylalanine Pirkle phases confirmed that stereochemical retention occurred during nucleophilic addition of aliphatic alcohols to glycidyl 3-nitrobenzenesulfonate and established the efficacy of 3,5-dinitro carbamate derivatization in the development of preparative-scale ether lipid separations. The ether-linked phospholipids synthesized by this route were characterized by electron microscopy and monolayer balance methods. Enzyme mechanism studies and the development of chemoselective thin films utilizing novel chiral bolaform amphiphiles prepared by these methods are currently in progress.

Experimental Section

All yields reported are for isolated products (>95% pure). Melting points were determined on a Mel-Temp II capillary melting point apparatus and are corrected. Boiling points are uncorrected. A 25-m fused silica column was used for GC/MS. HPLC was on 4.6 mm i.d. × 250 mm Rexchrom columns packed with 5 μm D-naphthylalanine-modified silica, using 150 mM *n*-heptyl 3,5-dinitrobenzoate as displacer, 7.5% (v/v) THF in *n*-hexane as eluent (15 °C), and a flow rate of 0.5 mL/min. Lipids were dispersed in buffer by extrusion.[61] Phase transition behavior of lipid suspensions were monitored with a Perkin Elmer DSC-7/Intracooler[26] while a home-built computer controlled monolayer balance with Wilhelmy plate was used to determine pressure–area isotherms. EM images were obtained as previously described.[27] NMR chemical shifts are reported in parts per million.

Materials. Solvents and reagents were purified by recrystallization or distillation from dessicant unless otherwise noted. Cumene hydroperoxide and *tert*-butyl hydroperoxide were dried over 4-Å molecular sieves for at least 2 h prior to use. Methylene chloride, chloroform, and triethylamine were distilled from P_2O_5 while titanium isopropoxide ($TiOiPr_4$) and the tartrate esters were distilled from dry, powdered 3-Å molecular sieves. (*R*)-Oxiranemethanol 3-nitrobenzenesulfonate (1(*R*)), (*S*)-oxiranemethanol 3-nitrobenzenesulfonate (1(*S*)), (*R*)-oxiranemethanol 4-nitrobenzoate (**2**), and (*R*)-oxiranemethanol *tert*-butyldiphenylsilyl ether (**3**) were prepared as described.[36] Boron trifluoride etherate, phosphorus oxychloride, *tert*-butyldiphenylsilyl chloride, and (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((*R*)-(+)-MTPA chloride) were distilled and stored under argon in reagent storage bottles with a glass stopcock and septum inlet. Triflic anhydride was prepared by P_2O_5 dehydration of triflic acid[67] and distilled twice from P_2O_5 before use. 1,8-Bis(dimethylamino)naphthalene (PS), 4-(dimethylamino)pyridine

(67) Burdon, J.; Farazmand, I.; Stacey, M.; Tatlow, J. C. *J. Chem. Soc.* **1957**, 2574.

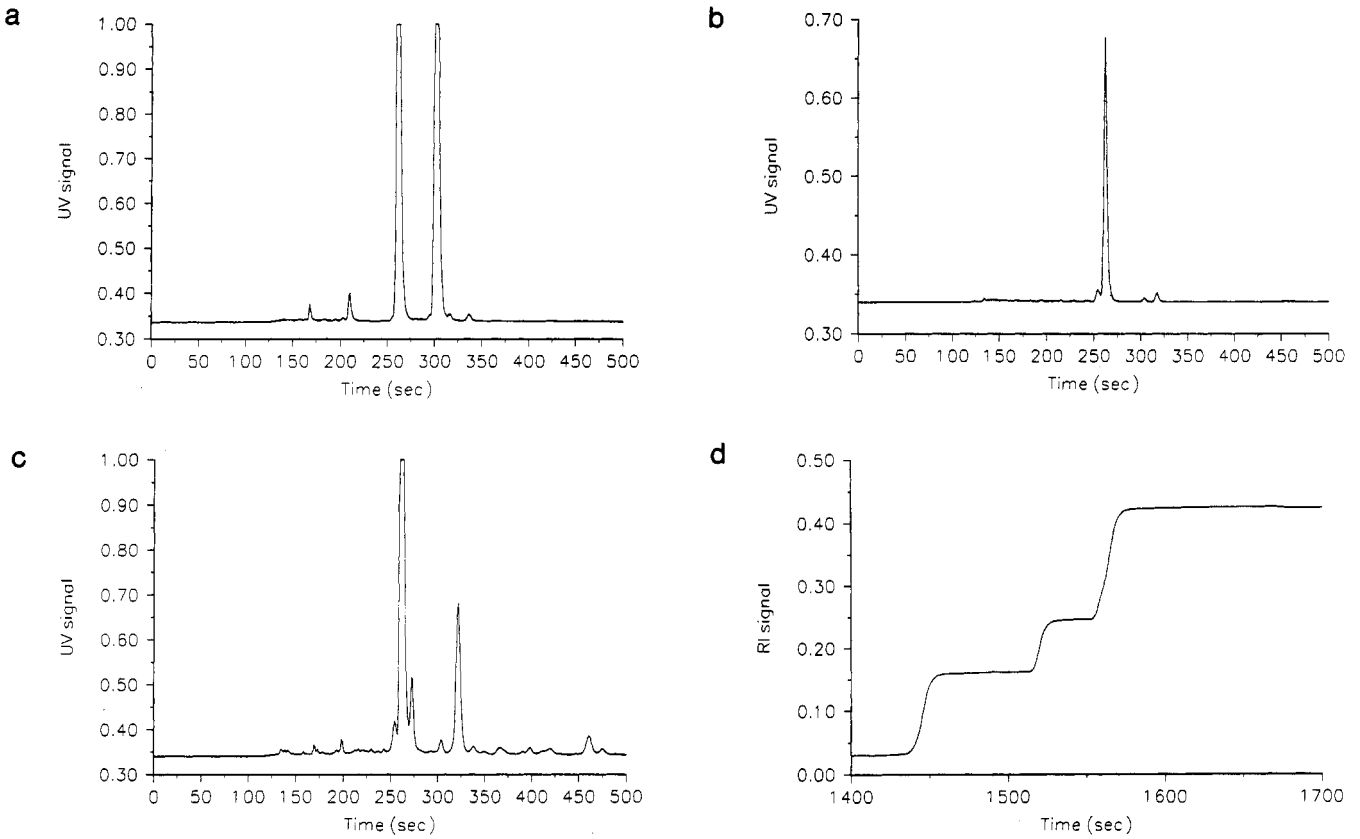


Figure 9. Pirkle-phase HPLC of 3,5-dinitrocarbamoyl derivative of **5**. A: Racemic *O*-hexadecylglycerol 3′-nitrobenzenesulfonate. B: **5**. C: **5**, after reductive cleavage of 3,5-dinitrocarbamoyl moiety and reformation by condensation with 3,5-dinitrophenyl isocyanate. D: Displacement mode chromatogram for **5** using 7.5% THF in hexane eluent (see refs 65 and 66 for details).

(DMAP), and 3-nitrobenzenesulfonyl chloride (NBSCl) were recrystallized from diethyl ether; triphenylmethyl chloride and *p*-toluenesulfonyl chloride were recrystallized from petroleum ether. All other reagents were used as received.

Kinetics Experiments. A stock solution was prepared by combining **1(R)** (0.375 g, 1.45 mmol), 1-tetradecanol (0.372 g, 1.74 mmol), and *p*-xylene as internal standard (0.108 mg, 1.02 mmol) into a 25-mL volumetric flask and diluting to volume with $CDCl_3$ (distilled from P_2O_5). Seven 5-mm NMR tubes were charged with the following acids: DDQ (8.5 mg, 0.037 mmol), triflic acid (32.5 μL, 0.367 mmol), boron trifluoride etherate (32.5 μL, 0.264 mmol), cesium fluoride (2.9 mg, 0.019 mmol), tropylium tetrafluoroborate (1.3 mg, 0.007 mmol), magnesium chloride (1.8 mg, 0.019 mmol), toluenesulfonic acid (0.4 mg, 0.002 mmol). An eighth tube contained no catalyst to serve as a control. Aliquots of the stock solution were transferred by syringe (3.25 mL) to each NMR tube; the tubes were then sealed, placed in a 60 °C oil bath, and periodically withdrawn for spectral analysis over a 38-h reaction time.

The data was analyzed by comparing the integrals of the resonances assigned to the sulfonyl ester α-methylenes of **1** and **5** (after normalizing to the *p*-xylene CH_3 integral) and calculating the product concentration at various time points. (As a first approximation, the rates of starting material disappearances and product appearance were assumed to be equal; this assumption is valid at early reaction times where the extent of 1,2-migration and oligomerization is negligible.) The observed rates of product formation were converted to second-order rate constants by dividing by the product of the initital concentrations of **1(R)** and tetradecanol.

A second set of experiments was carried out using the same quantities of **1(R)**, 1-tetradecanol, and *p*-xylene dissolved in 50 mL of $CDCl_3$ stock solution; $CDCl_3$ solutions of triflic acid, boron trifluoride etherate, tropylium tetrafluoroborate, and toluenesulfonic acid were added (25 μL) to 3.0-mL aliquots of the stock solution to give a final catalyst concentration of 10 mol %. Two series of reactions, catalyzed at 25 °C and 65 °C, were monitored periodically and the data analyzed as described above.

Determination of Chiral Purity. Enantiomeric excesses were determined by (1) NMR analysis of the Mosher ester, prepared from the glycidol adduct and (*R*)-(+)-MTPA chloride,[38] or (2) Pirkle-phase HPLC of carbamate derivatives, prepared from the glycerol ether intermediate and 3,5-dinitrobenzoyl azide,[64] on Rexchrom 250 mm × 4.6 mm i.d. columns using 7.5% (v/v) THF/*n*-hexane as eluent at a flow rate of 2 mL/min.[43] Carbamate deprotection was effected using lithium aluminum hydride (LAH) in THF on a 20-μmol scale; the resulting alcohol was then isolated, reprotected, and rechromatographed. Displacement mode HPLC was performed as described in refs 65 and 66.

Asymmetric Epoxidation Reactions. Allyl alcohol and 1-cyclopentenylmethanol were epoxidized as described by Sharpless and co-workers[36] and derivatized *in situ* by addition of 1.1 equiv each of NBSCl and triethylamine (Et_3N), and 1% DMAP as catalyst, in dichloromethane at −10 °C. *In situ* derivatization of (*R*)-oxiranemethanol with 4-nitrobenzoyl chloride (NBCl) or *tert*-butyldiphenylsilyl chloride (TBDPSCl) under similar conditions provided (*R*)-oxiranemethanol 4-nitrobenzoate (**2**) and (*R*)-oxiranemethanol *tert*-butyldiphenylsilyl ether (**3**). The general method utilized for asymmetric epoxidation is described for **4S**.

(*S*)-6-Oxabicyclo[3.1.0]hexane-1-methanol 3′-Nitrobenzenesulfonate (4(S)). Asymmetric epoxidation of 1-cyclopentene-1-methanol[68,69] (9.25 g, 94.3 mmol) at −24 °C (1 h) using titanium isopropoxide (1.41 mL, 4.74 mmol), (+)-diethyl tartrate (1.20 mL, 7 mmol), and *tert*-butyl hydroperoxide (63.5 mL) gave the epoxy alcohol in 89% yield (9.60 g, 84.2 mmol) after distillation[36] (51 °C @ 25 mmHg); derivatization with NBSCl, DMAP, and Et_3N gave **4(S)** in 71% yield after recrystallization from petroleum ether: mp 67–8 °C; ^1H NMR 8.89 (s, 1H), 8.66 (d, 1H), 8.37 (d, 1H), 7.92 (t, 1H), 4.47 (dd, 15 Hz, 2H), 3.47 (s, 1H), 2.0 (m, 2H), 1.78–1.32 (m, 4H); ^{13}C NMR 169.5, 148.5, 132.6, 130.0, 124.8, 124.2, 73.2, 63.3, 57.8, 29.2, 28.6, 21.1.

(68) Brown, J. B.; Henbest, H. B.; Jones, E. R. *J. Chem. Soc.* **1950**, 3635.
(69) Jorgensen, M. J. *Tetrahedron Lett.* **1962**, 559.

(*R*)-6-Oxabicyclo[3.1.0]hexane-1-methanol 3'-Nitrobenzenesulfonate (4(R)). This was prepared as described for 4(S) using 0.16 mL (0.54 mmol) of titanium isopropoxide, 7 mL of *tert*-butyl hydroperoxide, and 0.13 mL (0.76 mmol) of (-)-diethyl tartrate for Sharpless epoxidation to give 1.06 g (9.3 mmol) 4(R) in 86% yield from 1.06 g (10.8 mmol) 1-cyclopentene-1-methanol.

3-*O*-Hexadecyl-*sn*-glycerol 1-(3'-Nitrobenzenesulfonate) (5).[39] 1-Hexadecanol (14.03 g, 57.9 mmol) and 1(R) (5.0 g, 19.3 mmol) were combined in a 5-mL flask fitted with a $CaCl_2$ drying tube. The solids were heated to 65 ● 5 °C with vigorous stirring to emulsify the phase-separated melt prior to addition of triflic acid (100 μL).[44] After 5 h, the reaction mixture was cooled, dissolved in 30 mL of CH_2Cl_2, filtered through a 1-in. plug of silica gel, evaporated under reduced pressure, and isolated by flash column chromatography[70] using 3:1 hexane:ethyl acetate as eluent to give 7.65 g of 5 (79% yield; ≥99% ee by NMR of Mosher ester; 98.8% ee by HPLC): TLC R_f = 0.18, 3:1 hexane:ethyl acetate; mp 58 °C; ^{1}H NMR 8.78 (s, 1H), 8.53 (d, 1H), 8.28 (d,1H), 7.81 (t, 1H), 4.20 (dd, 2H), 4.01 (m, 1H), 3.47 (t, 2H), 3.42 (t, 2H), 2.19 (br s, 1H), 1.52 (m, 2H), 1.27 (br s, 26H), 0.89 (t, 3H); ^{13}C NMR 133.5, 131.5, 128.0, 123.4, 72.2, 72.0, 70.8, 68.2, 32.3, 29.8, 29.6, 29.4, 29.2, 26.3, 23.1, 14.2; Mosher ester ^{1}H NMR 8.57 (d, J = 8 Hz, 1H), 8.20 (d, J = 8 Hz, 1H), 7.88 (s, 1H), 7.77 (s, 1H), 7.73–7.31 (m, 5H), 5.42 (s, 1H), 4.42 (dd, J_{AC} = 2.9 Hz, J_{AB} = 11 Hz, 1H), 4.35 (dd, J_{AB} = 11 Hz, J_{BC} = 7.3 Hz, 1H), 3.25–3.87 (m, 7H), 1.44 (m, 2H), 1.28 (m, 28H), 0.90 (t, J = 7 Hz, 3H).

1-*O*-Hexadecyl-*sn*-glycerol 3-(3'-Nitrobenzenesulfonate) (6).[39] 1-Hexadecanol (2.51 g, 10.4 mmol) and 1(S) (1.057 g, 4.08 mmol) were treated as for 5 and isolated by Chromatotron using 3:1 hexane:ethyl acetate to give 6 in 78% yield (1.60 g, 3.19 mmol; ≥99% ee by NMR of Mosher ester; 98.3% ee by HPLC): Mosher ester ^{1}H NMR 8.48 (d, J = 8 Hz, 1H), 8.07 (d, J = 8 Hz, 1 H), 7.85 (br s, 1H), 7.73 (br s, 1H), 7.44 (m, 5H), 5.42 (s, 1H), 4.35 (dd, J_{AC} = 3 Hz, J_{AB} = 11 Hz, 1H), 4.25 (dd, J_{AB} = 11 Hz, J_{BC} = 7 Hz, 1H), 3.54 (m, 7H), 1.50 (m, 2H), 1.26 (m, 28H), 0.88 (t, J = 7 Hz).

3-*O*-Tetradecyl-*sn*-glycerol 1-(3'-Nitrobenzenesulfonate) (7). Reaction of 1(R) (1.36 g, 5.25 mmol) and 1-tetradecanol (3.38 g, 15.8 mmol) gave 7 in 83% yield (2.06 g, 4.35 mmol; ≥99% ee by NMR of Mosher ester) using the procedure described for 5: TLC R_f = 0.12, 3:1 hexane:ethyl acetate; mp 41 °C; Mosher ester ^{1}H NMR 8.46 (d, J = 7 Hz, 1H), 8.20 (d, J = 7 Hz, 1H), 7.95 (br s, 1H), 7.77 (t, J = 7 Hz, 1H), 7.43 (m, 5H), 5.41 (s, 1H), 4.42 (dd, J_{AC} = 3 Hz, J_{AB} = 11 Hz, 1H), 4.35 (dd, J_{BC} = 7 Hz, J_{AB} = 11 Hz, 1H), 4.35 (dd, J_{BC} = 7 Hz, J_{AB} = 11 Hz, 1H), 3.44 (m, 7H), 1.42 (s, 2H), 1.26 (m, 24H), 0.88 (t, J = 7 Hz, 3H).

3-*O*-Octadecyl-*sn*-glycerol 1-(3'-Nitrobenzenesulfonate) (8).[39] The octadecyl derivative 8 (1.88 g, 35.5 mmol) was obtained in 75% yield using a modification of the method described for 5 (≥99% ee by NMR of Mosher ester): TLC R_f = 0.16, 3:1 hexane:ethyl acetate: mp 65 °C; Mosher ester ^{1}H NMR 8.52 (br s, 1H), 8.19 (br s, 1H), 7.74 (br s, 1H), 7.43 (m, 5H), 5.42 (br s, 1H), 4.40 (br d, J = 11 Hz, 2H), 3.25–3.77 (m, 7H), 1.44 (br s, 2H), 1.27 (br s, 32H), 0.90 (br s, 3H).

3-*O*-Eicosyl-*sn*-glycerol 1-(3'-Nitrobenzenesulfonate) (9). Using the procedure described for 5, 1-eicosanol (3.45 g, 11.6 mmol) and 1(R) (1.00 g, 3.86 mmol) gave 9 (1.47 g, 2.63 mmol) in 68% yield (≥99% ee by NMR of Mosher ester): TLC R_f = 0.14, 3:1 hexane:ethyl acetate; mp 70 °C; Mosher ester ^{1}H NMR 8.51 (br s, 2H), 8.19 (br s, 1H), 7.77 (s, 1H), 7.73–7.31 (m, 5H), 5.41 (s, 1H), 4.39 (m, 1H), 4.35 (m, 1H), 3.22–3.87 (m, 7H), 1.44 (m, 2H), 1.28 (m, 36H), 0.89 (t, J = 7 Hz, 3H).

3-*O*-Hexadecyl-*sn*-glycerol 1-(4'-Nitrobenzoate) (10). The NB derivative 10 (0.67 g, 1.44 mmol) was obtained in 16% yield by heating 2 (2.0 g, 8.96 mmol) and 1-hexadecanol (6.52 g, 26.9 mmol) for 2 h as in 5: TLC R_f = 0.29, 3:1 hexane:ethyl acetate; ^{1}H NMR 8.25 (m, 4H), 4.46 (m, 2H), 4.04 (m, 1H), 3.54 (m, 4H), 1.60 (m, 2H), 1.26 (br s, 26H), 0.88 (t, 3H); mp 64 °C. The regioisomer of 10, alkylated at the *sn*-2 carbon, was also recovered in 2% yield (0.10 g, 0.22 mmol); the amount of this product increased at the expense of 10, with increasing reaction time: TLC R_f = 0.21, 3:1 hexane:ethyl acetate.

3-*O*-Hexadecyl-*sn*-glycerol 1-(*tert*-butyldiphenylsilyl Ether) (11).[40] Tropylium tetrafluoroborate (40 mg, 0.22 mmol), 1-hexadecanol (2.33 g, 9.6 mmol), and 3 (1.0 g, 3.2 mmol) were heated for 17 h at 70 °C in a 5-mL flask under a N_2 atmosphere and then cooled and dissolved in 110 mL of CH_2Cl_2. The solution was washed with $NaHCO_3$ (2 × 100 mL) and H_2O (100 mL), dried with $MgSO_4$, and evaporated, and 1.064 g of 11 (1.9 mmol, 59.9% yield) was isolated by flash chromatography using 3:1 hexane:ethyl acetate as eluent: TLC R_f = 0.60, 3:1 hexane:ethyl acetate; ^{1}H NMR 7.70 (m, 4H), 7.46 (m, 6H), 3.90 (m, 1H), 3.76 (d, 2H), 3.52 (d, 2H), 3.46 (t, 2H), 2.50 (d, 1H), 1.56 (m, 2H), 1.29 (br s, 26H), 1.08 (s, 9H), 0.88 (t, 3H).

(1*S*,2*R*)-2-(Hexadecyloxy)-1-[[(3'-nitrobenzenesulfonyl)-oxy]methyl]cyclopentan-1-ol (12). 1-Hexadecanol (9.084 g, 37.5 mmol) and 4(S) (4.502 g, 15 mmol) were dissolved in 25 mL of CH_2Cl_2 (under dry argon), heated briefly to dissolve the hexadecanol, and cooled to room temperature. Triflic acid (10 μL; 7.5 mol %) was added and the solution stirred for 12 h. The reaction mixture was washed twice with saturated $NaHCO_3$ and water and dried over $MgSO_4$. Silica gel chromatography (3:1 hexane:ether) followed by recrystallization from petroleum ether gave 12 in 71% yield (5.27 g, 10.7 mmol, ≥88.9% ee by NMR of Mosher ester): ^{1}H NMR 8.89 (s, 1H), 8.63 (d, 1H), 8.37 (d, 1H), 7.89 (t, 1H), 4.34 (dd, 2H), 3.63 (m, 1H), 3.42 (m, 1H), 3.24 (m, 1H), 1.89–1.58 (m, 8H), 1.26 (m, 26H), 0.89 (t, 3H); ^{13}C NMR 138.2, 133.3, 130.6, 128.2, 123.3, 123.2, 85.1, 82.1, 74.6, 69.2, 33.6, 31.9, 29.8, 29.7, 29.6, 29.4, 28.8, 26.1, 22.7, 20.6, 14.1.

BF_3·Et_2O-catalyzed (5 mol %) ring opening of 4(S) (0.50 g, 1.67 mmol) with 1-hexadecanol (0.67 g, 2.8 mmol) in CH_2Cl_2 at room temperature under Bittman's conditions[41] was substantially slower, producing a 70% yield of 12 over a 96-h reaction period: Mosher ester ^{1}H NMR 8.52 (d, J = 8 Hz, 1H), 8.27 (d, J = 8 Hz, 1H), 8.13 (t, J = 8 Hz, 1H), 7.8 (t, J = 8 Hz, 1H), 7.65 (m, 5H), 7.40 (m, 9H), 4.39 (d, J = 10 Hz, 1H), 4.21 (d, J = 10 Hz, 1H), 3.61 (s, 11H), 3.40 (m, 1H), 3.20 (m, 1H), 1.79 (m, 9H), 1.29 (m, 2H), 1.26 (m, 28H), 0.89 (t, 3H).

(1*R*,2*S*)-2-(Hexadecyloxy)-1-[[(3'-nitrobenzenesulfonyl)-oxy]methyl]cyclopentane-1-ol (13). This was prepared via the triflic acid-catalyzed route as described above using 4(R) and 1.5 equiv of 1-hexadecanol to give 13 in 70% isolated yield.

3,3'-Di-*O*-hexadecamethylenedi-*sn*-glycerol 1,1'-Bis(3'-nitrobenzenesulfonate) (14). Triflic acid (1% v/v) was added to a chloroform solution of 1(R) and 1,16-hexadecanediol via microliter syringe. The reaction mixture was heated at reflux for ~48 h before filtration through a 1-in. plug of silica gel. The filtrate was evaporated and chromatographed using 9:1 $CHCl_3$:acetone to give pure 14 in 68% yield: ^{1}H NMR 8.78 (s, 2H), 8.56 (d, 2H), 8.31 (d, 2H), 7.80 (t, 2H), 4.20 (m, 4H), 4.05 (m, 2H), 3.42 (m, 4H), 2.4 (d, 2H), 1.58 (m, 4H), 1.27 (br s, 24H); Mosher ester ^{1}H NMR 8.52 (br s, 2H), 8.22 (br s, 2H), 7.98 (br s, 2H), 7.80 (br s, 2H), 7.25–7.73 (m, 10H), 5.42 (br s, 2H), 4.43 (dd, J_{AC} = 3 Hz, J_{AB} = 11 Hz, 2H), 4.38 (dd, J_{AB} = 11 Hz, J_{BC} = 7 Hz, 2H), 3.90–3.15 (m, 14H), 1.45 (m, 4H), 1.27 (m, 24H).

1,1'-Di-*O*-hexadecamethylenedi-*sn*-glycerol-3,3'-Bis(3'-nitrobenzenesulfonate) (15). This was prepared as described for 14 in 67% yield, except 1(S) was used as substrate.

3,3'-Di-*O*-eicosamethylenedi-*sn*-glycerol 1,1'-Bis(3'-nitrobenzenesulfonate) (16). This was prepared as described for 14 in 65% yield, except 1,20-eicosanediol was used as substrate: ^{1}H NMR 8.75 (s, 2H), 8.54 (d, 2H), 8.29 (d, 2H), 7.78 (t, 2H), 4.18 (m, 4H), 3.99 (m, 2H), 3.39 (m, 4H), 2.42 (d, 2H), 1.58 (m, 4H), 1.27 (br s, 32H); Mosher ester ^{1}H NMR 8.52 (d, J = 7 Hz, 2H), 8.19 (d, 8 Hz, 2H), 7.98 (d, J = 7 Hz, 2H), 7.77 (d, J = 8 Hz, 2H), 7.49 (m, 10H), 5.42 (br s, 2H), 4.43 (dd, J_{AC} = 3 Hz, J_{AB} = 11 Hz, 2H), 4.35 (dd, J_{BC} = 7 Hz, J_{AB} = 11 Hz, 2H), 3.48 (m, 14H), 1.43 (m, 4H), 1.27 (m, 36H).

1,1'-Di-*O*-eicosamethylenedi-*sn*-glycerol 3,3'-Bis(3'-nitrobenzenesulfonate) (17). This was prepared as described for 14 in 66% yield, except 1(S) was used as substrate.

Alkyl Triflates. Triflate esters of 1-hexanol, 1-octanol, 1-tetradecanol, 1-hexadecanol, 1-octadecanol, and 1-eicosanol were prepared as described by Aoki and Poulter.[47] The triflate ester products were dissolved in pentane and purified by filtration through a 1-in. plug of silica gel. The purified products were stored as pentane solutions ($<C_{16}$) or as solids at 5 °C (after evaporation for $\geq C_{16}$) prior to use.

2,3-Di-*O*-hexadecyl-*sn*-glycerol 1-(3'-Nitrobenzenesulfonate) (18). A 10-mL round-bottom flask was charged with alcohol

(70) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

5 (0.617 gm, 1.23 mmol), PS (0.418 g, 1.95 mmol), hexadecyl triflate (0.731 g, 1.95 mmol), and 5 mL of CH_2Cl_2. The solution was heated at reflux for approximately 48 h. When no further reaction progress was detected by TLC, the orange suspension was evaporated to dryness (after addition of HCl to protonate the excess base) and triturated with ether, and the ether solution was filtered through a 1-in. layer of silica gel to remove low R_f impurities. The eluent was evaporated and the product isolated by Chromatotron using 2:1 hexane:ethyl acetate to give 18 (0.797 g, 1.10 mmol, 89% yield): TLC R_f = 0.70, 2:1 hexane:ethyl acetate; ^{1}H NMR 8.80 (s, 1H), 8.53 (d, 1H), 8.25 (d, 1H), 7.80 (t, 1H), 4.30–4.15 (m, 2H), 3.65 (m, 1H), 3.5–3.3 (m, 6H), 1.45 (m, 4H), 1.25 (m, 52H), 0.85 (t, 6H); ^{13}C NMR 148.3, 138.3, 133.8, 130.9, 128.1, 123.5, 76.6, 72.1, 71.6, 70.8, 68.7, 32.3, 30.1, 29.8, 29.6, 29.4, 26.6, 26.5, 22.8, 14.6. Pirkle phase HPLC indicated that no scrambling of the *sn*-2 chirality occurred during alkylation. When the same reaction was run using chloroform (heated at reflux) as solvent, the reaction time was reduced to 36 h, giving 18 in 84% yield.

1,2-Di-*O*-hexadecyl-*sn*-glycerol 3-(3′-Nitrobenzenesulfonate) (19).[41] The diether 19 was prepared as described for 18 (CH_2Cl_2), except that 6 (1.556 g, 3.10 mmol) was heated for ~2 d with hexadecyl triflate (2.32 g, 6.20 mmol) to give 2.072 g 19 (92% yield).

2,3-Di-*O*-tetradecyl-*sn*-glycerol 1-(3′-Nitrobenzenesulfonate) (20). The monoether 7 (0.198 g, 0.42 mmol), PS (0.223 g, 1.04 mmol), and tetradecyl triflate (0.463 g, 1.33 mmol) were heated for 48 h in CH_2Cl_2 and isolated as in 18 to give 20 (0.249 g, 3.72 mmol, 89% yield): ^{1}H NMR 8.80 (s, 1H), 8.52 (d, 1H), 8.28 (d, 1H), 7.80 (t, 1H), 4.38–4.15 (m, 2H), 3.67 (m, 1H), 3.6–3.35 (m, 6H), 1.30 (m, 48H), 0.90 (t, 6H).

2,3-Di-*O*-octadecyl-*sn*-glycerol 1-(3′-Nitrobenzenesulfonate) (21). The diether 21 was prepared in 92% yield as described for 18 (CH_2Cl_2): TLC R_f = 0.65, 2:1 hexane:ethyl acetate.

2,3-Di-*O*-eicosyl-*sn*-glycerol 1-(3′-Nitrobenzenesulfonate) (22). The monoether 9 (1.114 g, 2.00 mmol) and eicosyl triflate (1.72 g, 3.99 mmol) were heated with PS (0.857 g, 4.00 mmol) in CH_2Cl_2 for 46 h to give 22 (1.517 g, 1.81 mmol, 91% yield).

1-*O*-Hexadecyl-2-*O*-octadecyl-*sn*-glycerol 3-(3′-Nitrobenzenesulfonate) (23). The diether 23 was prepared in 86% yield as described for 18 (CH_2Cl_2) except that 1.02 mmol of 6 and 1.58 mmol of octadecyl triflate were heated for 70 h: TLC R_f = 0.67 in 2:1 hexane:ethyl acetate; ^{1}H NMR 8.79 (s, 1H), 8.53 (d, 1H), 8.26 (d, 1H), 7.81 (t, 1H), 4.31 (m, 2H), 3.64 (m, 1H), 3.56–3.32 (m, 6H), 1.49 (m, 4H), 1.27 (br s, 56H), 0.89 (t, 6H).

(1*R*,2*S*)-1,2-Bis(hexadecyloxy)-1-(hydroxymethyl)cyclopentane (24). 3′-Nitrobenzenesulfonate Ester. The sterically constrained monoether 13 (0.24 g, 0.45 mmol) was dissolved with hexadecyl triflate (1.66 g, 4.43 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (0.93 g, 4.6 mmol) in CH_2Cl_2 and heated at reflux under N_2 for 5 d. The solution was washed with HCl, water, and saturated $NaHCO_3$ and dried over $MgSO_4$. The organic phase was concentrated to give a brown oil; this was filtered through a 1-in. column of silica gel (eluting with ethyl acetate), chromatographed using 15:1 hexane:ether as eluent, and evaporated to give 24 (0.25 g, 53% yield). The tosyl analog of 24 was prepared in a similar manner, except using a 23 h reaction time, to give the alkylated product in 50% yield. ^{1}H NMR: 7.84 (d, 2H), 7.36 (d, 2H), 4.22 (dd, 2H), 3.64 (m, 1H), 3.38 (m, 1H), 3.25 (t, 2H), 2.46 (s, 3H), 1.98–1.35 (m, 10H), 1.28 (br s, 52H), 0.91 (t, 6H).

2,2′-Di-*O*-dioctyl-3,3′-di-*O*-hexadecamethylenedi-*sn*-glycerol 1,1′-Bis(3′-nitrobenzenesulfonate) (25). Octyl triflate, PS, and 14 in $CHCl_3$ were heated at reflux for ~48 h. The reaction mixture was then filtered and chromatographed as for 18 to give 25 in 62% yield: ^{1}H NMR 8.89 (s, 2H), 8.63 (d, 2H), 8.37 (d, 2H), 7.89 (t, 2H), 4.47–4.16 (m, 4H), 3.67–3.60 (m, 2H), 3.53–3.33 (m, 12H), 1.47 (m, 8H), 1.29 (s, 44H), 0.89 (t, 6H).

2,2′-Di-*O*-octyl-1,1′-di-*O*-hexadecamethylenedi-*sn*-glycerol 3,3′-Bis(3′-nitrobenzenesulfonate) (26). This was prepared as for 25 above, except 15 was used in the alkylation reaction: ^{1}H NMR 8.82 (s, 2H), 8.59 (d, 2H), 8.30 (d, 2H), 7.85 (t, 2H), 4.38–4.20 (m, 4H), 3.64 (m, 2H), 3.54–3.35 (m, 12H), 1.60 (m, 2H), 1.50 (m, 6H), 1.32 (s, 44H), 0.92 (t, 6H).

2-*O*-Hexadecyl-3,3′-di-*O*-hexadecamethylenedi-*sn*-glycerol 1,1′-Bis(3′-nitrobenzenesulfonate) (27). This material was prepared as described for 25 using 14 and limiting hexadecyl triflate: ^{1}H NMR 8.89 (s, 2H), 8.64 (d, 2H), 8.37 (d, 2H), 7.89 (t, 2H), 4.35–4.12 (m, 4H), 3.68–3.60 (m, 2H), 3.53–3.32 (m, 7.3H), 1.47 (m, 4H), 1.29 (s, 64H), 0.89 (t, 6H).

2,2′-Di-*O*-decyl-3,3′-di-*O*-eicosamethylenedi-*sn*-glycerol 1,1′-Bis(3′-Nitrobenzenesulfonate) (28). The procedure was similar to that described for 25, except 16 was used as substrate to give 28 in 61% yield: ^{1}H NMR 8.89 (s, 2H), 8.61 (d, 2H), 8.34 (d, 2H), 7.87 (t, 2H), 4.39–4.13 (m, 4H), 3.68 (m, 2H), 3.55–3.34 (m, 10H), 1.47 (m, 4H), 1.26 (s, 60H), 0.87 (t, 6H).

2,2′-Di-*O*-decyl-1,1′-di-*O*-eicosamethylenedi-*sn*-glycerol 3,3′-Bis(3′-nitrobenzenesulfonate) (29). Alkylation of 17 was carried out as described for 25.

2-*O*-Eicosyl-3,3′-di-*O*-eicosamethylenedi-*sn*-glycerol 1,1′-Bis(3′-nitrobenzenesulfonate) (30). The procedure described for 25 was used to alkylate 16: ^{1}H NMR 8.76 (t, 1H), 8.48 (m, 1H), 8.23 (m, 1H), 7.84 (m, 1H), 7.47 (m, 2H), 7.32 (m, 2H), 4.35–3.90 (m, 6H), 3.68 (t, 2H), 3.49–3.26 (m, 8H), 1.74 (d, 1H), 1.59–1.42 (m, 6H), 1.27 (s, 66H), 0.87 (t, 3H).

2,3-Di-*O*-hexadecyl-*sn*-glycerol 1-Phosphate (31). Tetrabutylammonium hydroxide (1 mL of 40% aqueous solution) and 18 (0.750 g, 1.03 mmol) were heated at reflux in 5 mL THF and the product alcohol isolated by neutralization of the excess base with 2 N HCl, evaporation, trituration of the residue with CH_2Cl_2, and filtration of the CH_2Cl_2 solution through a 1-in. plug of silica gel. The deprotected alcohol was purified by Chromatotron using 2:1 hexane:diethyl ether (0.374 g, 0.69 mmol, 67% yield, R_f = 0.37): ^{1}H NMR 3.76–3.38 (m, 9H), 2.23 (br s, 1H), 1.50 (m, 4H), 1.31 (br s, 52H), 0.88 (t, 6H). Phosphorylation of thealcohol[58] was carried out in 6.4 mL of CCl_4 using $POCl_3$ (0.186 g, 1.21 mmol) and triethylamine (0.122 g, 1.21 mmol) to give 32 (0.406 g, 0.66 mmol, 95% yield): ^{1}H NMR 4.30–3.35 (br m, 11H), 1.57 (m, 4H), 1.40–1.05 (m, 52H), 0.89 (t, 6H). ^{13}C NMR: 71.9, 71.0, 69.3, 66.0, 57.8, 31.9, 29.8, 29.2, 25.8, 22.6, 14.1.

1,2-Di-*O*-(hexadecyloxy)cyclopentane-1-methanephosphonic Acid (32). The tosyl analog of 24 (0.187 g) was dissolved with cesium acetate (0.16 g, 0.83 mmol) in 4:1 DMSO:DMF and heated at 45 °C for 3 d. The acetate intermediate was isolated[41] and reduced with LAH to give 0.050 g (46% yield) of the dialkylglycerol after purification by silica gel chromatography (4:1 hexane:ethyl acetate). The alcohol was phosphorylated as described for 31 to give 32 in 70% yield: ^{1}H NMR 4.24 (dd, 2H), 3.61 (m, 1H), 3.38 (dt, 1H), 3.28 (t, 2H), 3.22 (dt, 1H), 1.97–1.38 (m, 10H), 1.21 (br s, 52H), 0.84 (t, 6H).

2,2′-Di-*O*-octyl-3,3′-di-*O*-hexadecamethylenebis(*sn*-glycero-1-phosphocholine) (33). Tetraether 25 (250 mg, 0.226 mmol) was dissolved in 1 mL of 4:1 DMSO:DMF with cesium acetate (659 mg, 3.43 mmol), heated at 60 °C for ~16 h, and isolated[41] to give the tetraether diacetate in 82% yield: R_f = 0.71 in 5:2 ether:hexane.

The tetraether diacetate from the previous reaction (150 mg, 0.179 mmol) was reduced with LAH (15 mg, 0.394 mmol) in THF (~16 h at 67 °C) and purified by Chromatotron using 3:2 diethyl ether:hexane as eluent (60% yield): R_f = 0.16; ^{1}H NMR 3.76–3.40 (m, 18H), 1.65 (m, 10H), 1.40–1.25 (s, 60H), 0.89 (t, 6H). The tetraether diol above (73 mg, 0.10 mmol) was lyophilized and dissolved in 5 mL of benzene containing Et_3N (103 mg, 1.02 mmol) and 2-chloro-2-oxo-1,3,2-dioxaphospholane (93 mg, 1.02 mmol) on an ice bath. The coolant was removed and the mixture stirred for 36 h at room temperature. After filtering to remove Et_3N·HCl, the filtrate was evaporated *in vacuo* and the residue dissolved in CH_3CN (5 mL). The CH_3CN solution was transferred to a 20-mL pressure vessel into which dry Me_3N (1 mL) was condensed, using a dry ice–acetone bath. The bath was removed and the reaction heated at 65 °C with stirring for 48 h; a white solid precipitated during the course of the reaction. The reaction was then cooled to room temperature and the volatile components removed under vacuum. The residue was dissolved in 2:1 chloroform:methanol (25 mL) and washed with water. The organic layer was concentrated and purified by column chromatography using 8:6:4:1 (v/v) chloroform:acetone:methanol: concentrated NH_4OH initially and then increasing the ammonium hydroxide content to 8:6:4:3 (v/v) chloroform:acetone:methanol: NH_4OH to give a colorless solid (90 mg, 82% yield): R_f 0.37,

8:6:4:3 (v/v) chloroform:acetone:methanol:concd NH_4OH:[71] ^1H NMR 4.47–4.16 (m, 8H), 3.97 (m, 4H), 3.67–3.60 (m, 2H), 3.53–3.33 (m, 12H), 3.25 (s, 18H), 1.47 (m, 8H), 1.29 (s, 44H), 0.89 (t, 6H).

2,2′-Di-*O*-decyl-3,3′-di-*O*-eicosamethylenebis(*sn*-glycero-1-phosphocholine) (34).[50] Deprotection of **28** with subsequent phosphorylation followed the procedure described for **33**. **34**: ^1H NMR 4.39–4.13 (m, 8H), 3.97 (m, 4H), 3.68 (m, 2H), 3.55–3.34 (m, 10H), 3.25 (s, 18H), 1.47 (m, 4H), 1.26 (s, 60H), 0.87 (t, 6H).

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Supplementary Material Available: ^1H NMR data for all new compounds and for the kinetics experiments utilizing various acid catalysts are available (63 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(71) Hajdu, J.; Bhatia, S. K. *Synthesis* **1989**, 16.