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Synthesis of polymerizable and deuterated phosphatidylcholines for use in membrane studies

Xu, Zhenchun, Ph.D.

City University of New York, 1992

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SYNTHESIS OF POLYMERIZABLE AND DEUTERATED PHOSPHATIDYLCHOLINES FOR USE IN MEMBRANE STUDIES

by

ZHENCHUN XU

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1992

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

January 30, 1992
Date

Robert Bittman
Chair of Examining Committee

1/30/92
Date

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Ruth E. Stark

Supervisory Committee

The City University of New York

Abstract

SYNTHESIS OF POLYMERIZABLE AND DEUTERATED PHOSPHATIDYLCHOLINES FOR USE IN MEMBRANE STUDIES

by

Zhenchun Xu

Adviser: Professor Robert Bittman

In this Dissertation, (*Z*)-1-methoxybut-1-en-3-yne has served as a useful synthon for the synthesis of the positional isomers and homologues of conjugated diacetylenic alcohols and acids. This new and efficient way is an alternative to the use of the Cadiot-Chodkiewicz heterocoupling reaction for the preparation of diacetylenic carboxylic acids. The butadiyne-synthon approach to conjugated diacetylenic alcohols has been extended to the preparation of the identical-acid, mixed-acid, mixed-ether, and ether/ester phosphatidylcholines (PC) containing conjugated diacetylene chains by using previous methods for PC synthesis. The ether-containing PCs are prepared by using diacetylenic alcohols as nucleophiles in the ring-opening reaction of various chiral glycidyl derivatives. The chiral purity of each stereoisomer of the ring-opened products was established by examination of the diastereomeric mixture of the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid ester by chiral high-pressure liquid chromatography.

Liposomes prepared from four diacetylenic PCs containing eighteen-carbon chains have been examined in terms of lamellar structure,

polymerizability, and permeability properties. The structures of bilayers of these lipids were determined at low resolution by low angle *X*-ray diffraction. Among these PC's, only 1,2-di-(4',6'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine polymerized effectively upon UV irradiation at room temperature, as estimated by Dr. David G. Rhodes. The results of permeation experiments of PC liposomes indicate that unpolymerized and polymerized liposomes of 1,2-di-(4',6'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine underwent osmotic swelling with acetamide, glycerol, and urea more rapidly than did liposomes of stearoyl-oleoyl PC, but the initial rates of swelling of polymerized liposomes of 1,2-di-(4',6'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine were 3-10 times lower than those of unpolymerized liposomes of 1,2-di-(4',6'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine. The molecular packing of monolayers of three diacetylenic PCs at the air/water interface was studied by pressure/area measurements, and their domain structure was analyzed using 1% phosphatidylethanolamine-rhodamine labeled monolayers by fluorescence microscopy by Hui et al.

Three partially deuterated PCs were prepared by acetylenic coupling reaction, catalytic reduction with deuterium, and conversion of the deuterium-labeled fatty acids into PC. One of the PC-d_8 isomers was used recently in the direct determination of conformational disorder in bilayer membranes by FT-infrared spectroscopy [Mendelsohn, R.; Davies, M. A.; Schuster, H. F.; Xu, Z.; Bittman, R. *Biochemistry* **1991**, *30*, 8558-8563]. The quantitative detection of a specific, position-dependent kink (gtg') and isolated gauche (gtt) rotamer has been performed using CD_2 rocking modes.

Acknowledgments

I would like to express my appreciation to my thesis advisor, Dr. Robert Bittman, for his guidance in the phospholipid synthesis and his advice and instruction during the entire period of my graduate study at The City University of New York.

I would like to thank my committee members, Dr. William Berkowitz, Dr. Robert Engel, and Dr. Ruth E. Stark, who guided and helped me in finishing my thesis work with their experience. I would also like to thank Dr. David C. Locke for his assistance in HPLC and GC-MS.

I extend my thanks to all the colleagues who not only helped me in the research but provided such a nice atmosphere in the laboratory. Thanks to all the other members of the department who offered their assistance.

Very special thanks to my wife and best friend Xinxin Sun for her patience, understanding, and encouragement during these years.

Finally, I would like to acknowledge the financial support from the NIH.

Dedicated to those who made this thesis possible:

My parents, my wife, and my wonderful daughter Ying Xu.

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Abbreviations

DCC	*N, N'*-dicyclohexylcarbodiimide
DDPC	didienoyl phosphatidylcholine
DIPT	diisopropyl tartrate
DMAP	4-(dimethylamino)pyridine
DMF	*N,N*-dimethylformamide
DMPC	dimyristoylphosphatidylcholine
GPC	glycerophosphocholine
HMPA	hexamethylphosphoramide
HPLC	high-pressure liquid chromatography
MDPC	monodienoyl phosphatidylcholine
MDPE	monodienoyl phosphatidylethanolamine
MOM	methoxymethyl
MTPA	α-methoxy-α-(trifluoromethyl)-phenylacetic acid
PC	phosphatidylcholine
PDC	pyridinium dichromate
SOPC	stearoyl-oleoyl phosphatidylcholine
TBDPS	*tert*-butyldiphenylsilyl
THF	tetrahydrofuran
TLC	thin-layer chromatography
UV	ultraviolet

CHAPTER 1. SYNTHESIS OF POLYMERIZABLE PHOSPHATIDYLCHOLINES FOR USE IN MEMBRANE STUDIES

INTRODUCTION

The cell membrane plays an important role in many essential biological phenomena. The various cell membranes are built from a matrix of polar lipids. Phosphatidylcholine (PC) is one of the most important and abundant components of lipids. The chemical synthesis of 1,2-distearoyl-*sn*-glycero-3-phosphocholine made available a phospholipid for the first time in pure form for use in biochemical and biophysical studies [1]. The various approaches used to prepare PCs can be found in previous review articles [2].

Previous syntheses of polymerizable lipids

Liposomes or vesicles have been used as biological membrane models and have become a subject of increasingly active research since the early 1960s. These closed spherical supramolecular structures are composed of lipid bilayers that enclose an aqueous volume [3]. Unfortunately, because liposomes or vesicles are thermodynamically unstable, their short lifetime limits some long-term applications in research and medicine. Their intrinsic unstability is one likely reason for the relatively slow application of liposomes in the pharmaceutical industry, besides their nonselective targeting [4]. In the last few years, numerous methods to stablize model membranes have been developed, mainly by using polymeric systems. If liposomes or vesicles could be prepared in polymerized forms, they should not only retain their many important properties, but also should be more stable.

The synthesis of polymerizable amphiphiles and their incorporation

into liposomes have been reported since 1980. Many lipids containing polymerizable groups have been made. For example, lipids containing diacetylenic, butadienic, acryloylic, methacryloylic, and sulfhydryl groups within hydrophobic chains have been prepared and used in polymerized membranes [5]. A cationic ammonium salt with a methacrylate at the end of one hydrocarbon chain was made by Regen et al., and methacryloyl lipids have been used in studies of the permeability characteristics of polymeric bilayer membranes [6]. The synthesis of polymerizable diacetylenic lipids by using acetylenic-coupling reactions has been developed [7]. Diacetylenic lipids can be used as new stable biomembranes and cell models and as carriers for biologically active substances. Their physical characterisation, polymerization characteristics, and photospectra have been examined. A polymerizable mixed-acid PC analogue containing a double bond and an ester group has been prepared, and shows reduced permeability and enhanced stability after its polymerization [8]. Regen et al. prepared some interesting thiol-disulfide-based PCs that can be oxidatively polymerized, and reversibly and reductively depolymerized [9].

The possible relevance of such polymerizable-depolymerizable PCs to drug delivery system and membrane modeling has been investigated. O'Brien et al. incorporated polymerizable units into an amino acid backbone to make a new diacetylenic lipid, which can make free standing polydiacetylene films [10]. A way of synthesizing polymerizable monodienoyl-PC (MDPC) and didienoyl-PC (DDPC) and monodienoyl-phosphatidylethanolamine (MDPE) containing butadiene units has been developed [11]. Their physical characterization has been examined, and their

possible use to stabilize model biomembranes has been studied.

Polymerizable phospholipids constitute an important new class of biomaterials with many potential applications such as models for biological membranes [12], carriers of drugs [13], devices for solar energy conversion [14], and new types of cosmetic formulation [15].

Diacetylenic PC and its polymerization

Diacetylenic PC is one of the most important and potentially available components of polymerizable lipids. Diacetylenic PC liposomes and multilayers undergo extensive photopolymerization below their transition temperature [7a,16]. The polymerization of a diacetylenic monomer under UV irradiation forms a polyconjugated polymer and proceeds as a 1,4-addition reaction [17]. During the polymerization, the color changes to red, blue or purple [5a]. The intensity of the color produced is related to the amount of conversion of monomeric PC to polymer within the liposome and depends on experimental factors. *X*-ray crystallography [18] and Raman spectroscopy [19] have shown that the resulting polymer consists of conjugated double and triple bonds (Figure 1). Conjugated diacetylenic chains have been incorporated into various polymerizable phospholipids for use in model membranes; UV irradiation induces crosslinking, resulting in a phospholipid polymer in the membrane bilayer. This kind of polymerizable phospholipid has the ability to transform bilayers into more stable microstructures [20]. Therefore, diacetylenic PCs stabilized by photopolymerization have been the subject of extensive studies and have a wide range of potential applications, such as the possible use of liposomes

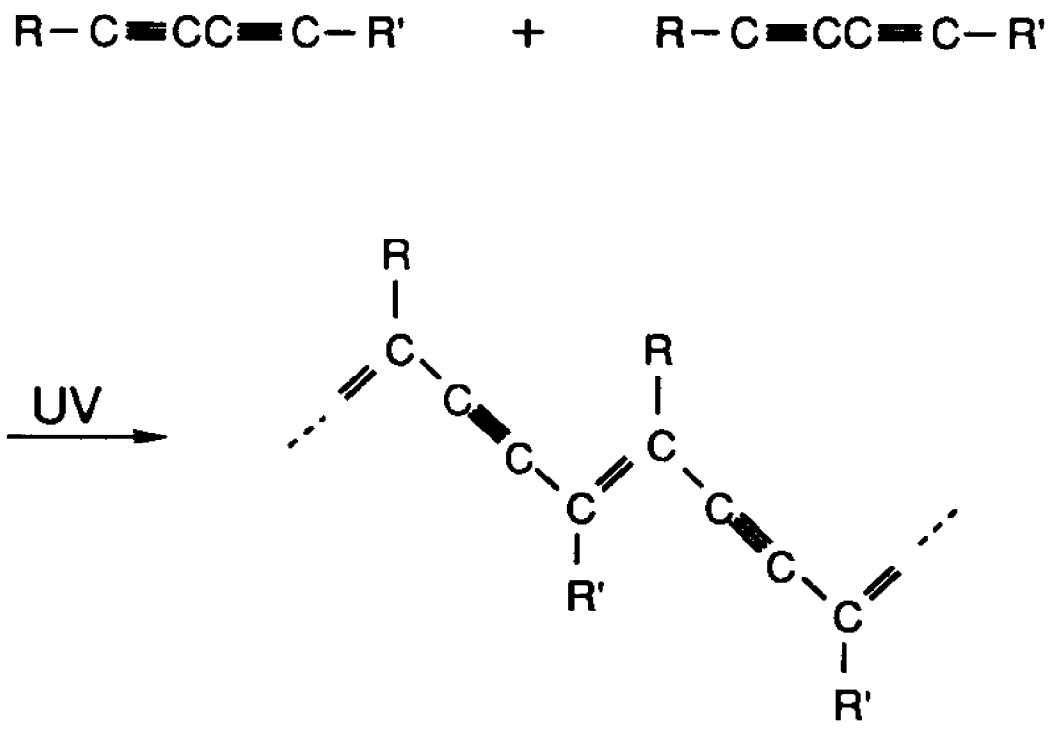
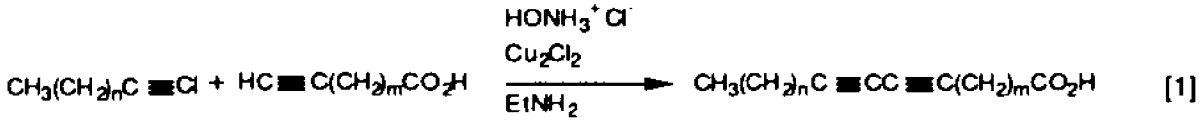


Fig. 1. Formation of the polyconjugated PC polymer from photolysis of diacetylene-PC via 1,4-addition reactions.

as drug carriers.

Conjugated diacetylenic alcohols and acids

Because of the current interest in the behavior of poly(diacetylene) chains in long-chain phospholipids as stabilizing units in membranes [7,10], an efficient preparation of long-chain conjugated diacetylenic alcohols and acids in PCs was developed in this thesis. In general, unsymmetrical diynoic acids have been prepared by coupling of a 1-haloalkyne (usually the iodoalkyne) with a metal alkynoic acid (the Cadiot-Chodkiewicz reaction, eq. 1) [21]. Diynoic acids have also been prepared by the Cu_2Cl_2-catalyzed oxidative coupling of ω-alkynoic acids with terminal alkynes [22]. This acetylenic-coupling reaction has also been used to prepare long-chain conjugated (enyne [23] and diyne [24]) alcohols. Disadvantages of this method for preparation of conjugated diacetylenic alcohols and acids are (a) higher ω-alkynoic acids are obtained in low yields (generally <50%) [21], (b) preparation of 1-haloalkynes is required in the Cadiot-Chodkiewicz reaction, and (c) unsymmetrical ω-diynoic acids obtained are frequently contaminated by symmetrical diynoic acids [22].



[1]

Zweifel and Rajagopalan reported that the nucleophilic butadiyne synthons 1,4-bis(trimethylsilyl)-1,3-butadiyne (**2**) and 4-lithio-1-(trimethylsilyl)butadiyne (**3**) can be prepared from (*Z*)-1-methoxybut-1-en-3-

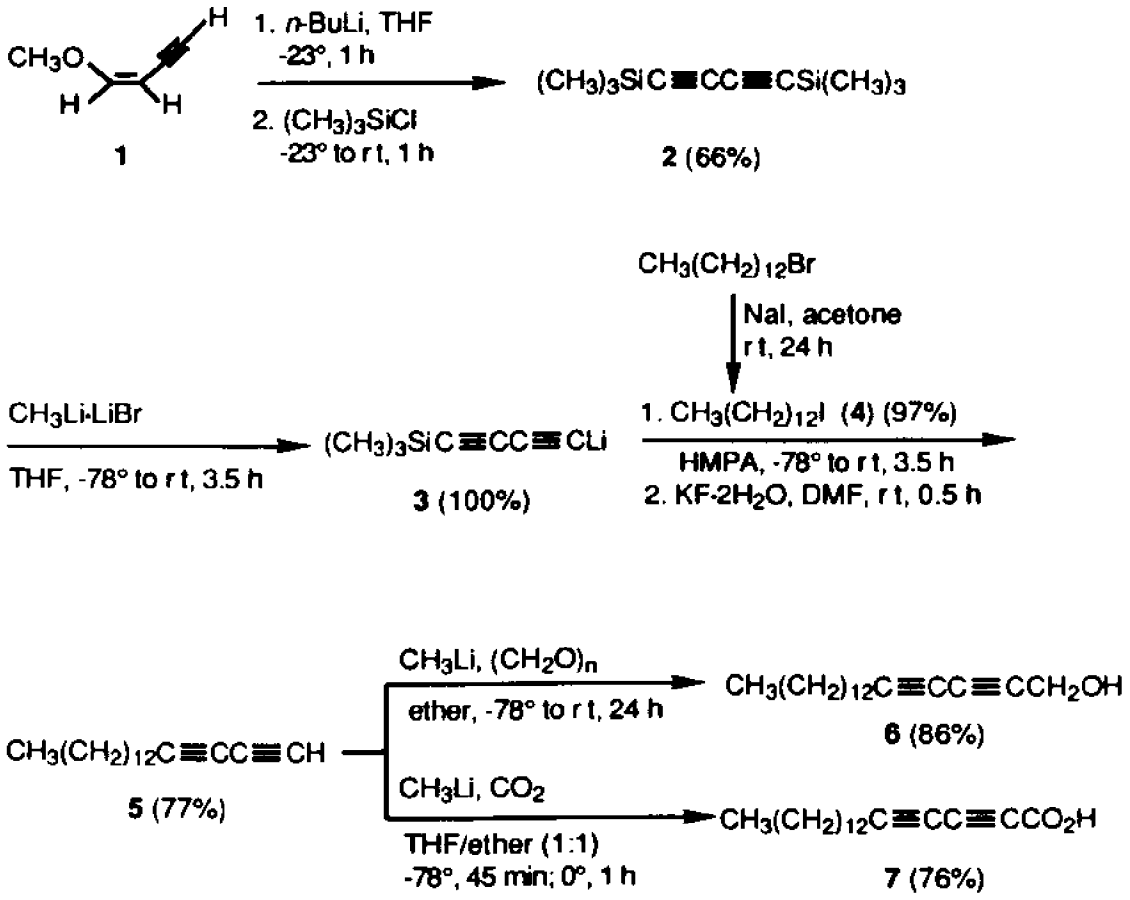
yne (**1**) via a series of metalation-elimination-metalation reactions [25]. This report suggested to us that **1** would be useful for the preparation of diacetylenic acids and alcohols. (*Z*)-1-Methoxybut-1-en-3-yne is commercially available as a 50% solution in methanol-water, and should be stored at -20 °C after purification [26]. Therefore, the efficient preparation of conjugated diacetylenic alcohols (**6**, **11**) and acids (**7**, **12**) outlined in Schemes 1 and 2 was developed [27]. The butadiyne units are located next to the hydroxyl and carboxyl groups in compounds **6** and **7**, respectively, as shown in Scheme 1, whereas there are some methylene groups between them in compounds **11** and **12**, as shown in Scheme 2. The various positional isomers of the conjugated diacetylene along the hydrocarbon chain have been prepared. The availability of such isomers makes possible a study of the structural requirements for efficient polymerization of lipid diacetylenes in multilayer films (refer to results in this thesis, pp. 37-39).

Conjugated diacetylenic phospholipids

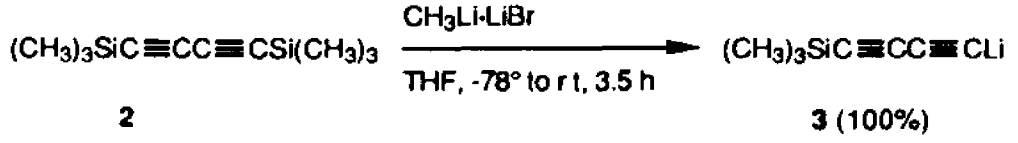
The conjugated diacetylenic alcohols and acids were used to prepare conjugated diacetylenic PCs. Identical-acid (**13**) and mixed-acid (**14**) PCs have been prepared by using previously published methods for PC synthesis involving diacylation of *sn*-glycerophosphocholine·$CdCl_2$ complex, followed by phospholipase A_2 treatment and acylation of the resulting lyso-PC (Scheme 3). Both acyl chains in PCs **13a**, **13b**, and **14a** carry the diacetylene, whereas only one acyl chain carries the diacetylene in PCs **14b** and **14c**.

Mixed-ether and ether/ester PCs are important groups of biologically active molecules in membrane research [28]. Many glycerol derivatives have

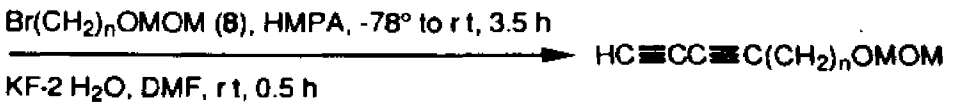
Scheme 1. Synthesis of Diacetylenic Alcohol 6 and Acid 7



Scheme 2. Synthesis of Diacetylenic Alcohols 11a-f and Acids 12a-f

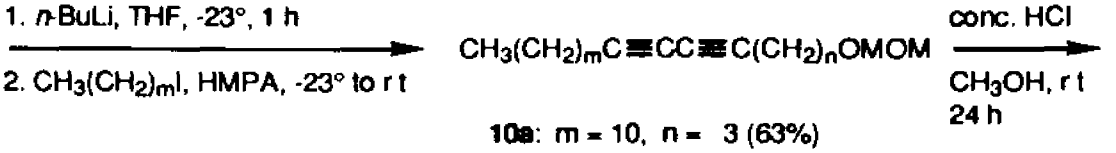


2 **3** (100%)

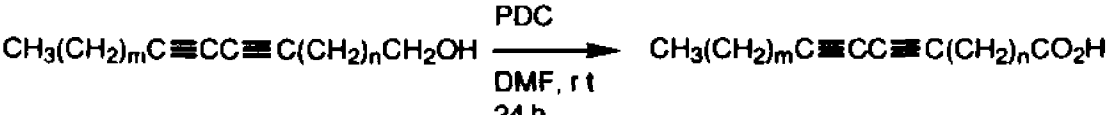


8a: n = 3 (91%)
8b: n = 4 (86%)
8c: n = 7 (88%)
8d: n = 11 (95%)

9a: n = 3 (84%)
9b: n = 4 (78%)
9c: n = 7 (91%)
9d: n = 11 (89%)



10a: m = 10, n = 3 (63%)
10b: m = 9, n = 4 (71%)
10c: m = 6, n = 7 (78%)
10d: m = 2, n = 11 (73%)
10e: m = 9, n = 11 (77%)
10 f: m = 11, n = 11 (79%)



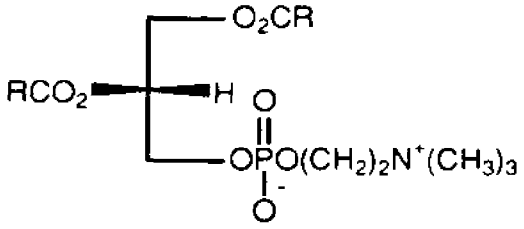
11a: m = 10, n = 2 (92%)
11b: m = 9, n = 3 (93%)
11c: m = 6, n = 6 (92%)
11d: m = 2, n = 10 (93%)
11e: m = 9, n = 10 (93%)
11 f: m = 11, n = 10 (91%)

12a: m = 10, n = 2 (72%)
12b: m = 9, n = 3 (60%)
12c: m = 6, n = 6 (75%)
12d: m = 2, n = 10 (75%)
12e: m = 9, n = 10 (85%)
12 f: m = 11, n = 10 (86%)

Scheme 3. Synthesis of Identical-Acid Phosphatidylcholines 13a-c and Mixed-Acid Phosphatidylcholines 14a-c

$CH_3(CH_2)_m(C\equiv C)_k(CH_2)_nCO_2H$ —(GPC·$CdCl_2$, DMAP, DCC; $CHCl_3$, r t, 60 h)→

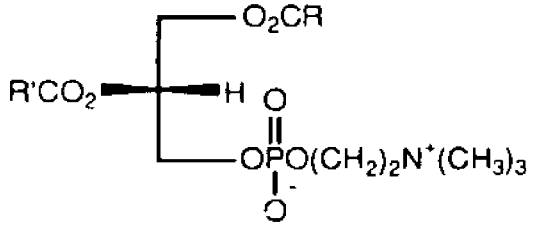
12a: k = 2, m = 10, n = 2
12g: k = 2, m = 4, n = 8
12h: k = 0, m = 8, n = 0



—(1. phospholipase A_2; 2. R'CO_2H, DMAP, DCC, $CHCl_3$, r t, 48 h)→

R = $CH_3(CH_2)_m(C\equiv C)_k(CH_2)_n$

13a: k = 2, m = 10, n = 2 (81%)
13b: k = 2, m = 4, n = 8 (52%)
13c: k = 0, m = 8, n = 0 (63%)



R = $CH_3(CH_2)_m(C\equiv C)_k(CH_2)_n$

R' = $CH_3(CH_2)_{m'}(C\equiv C)_{k'}(CH_2)_{n'}$

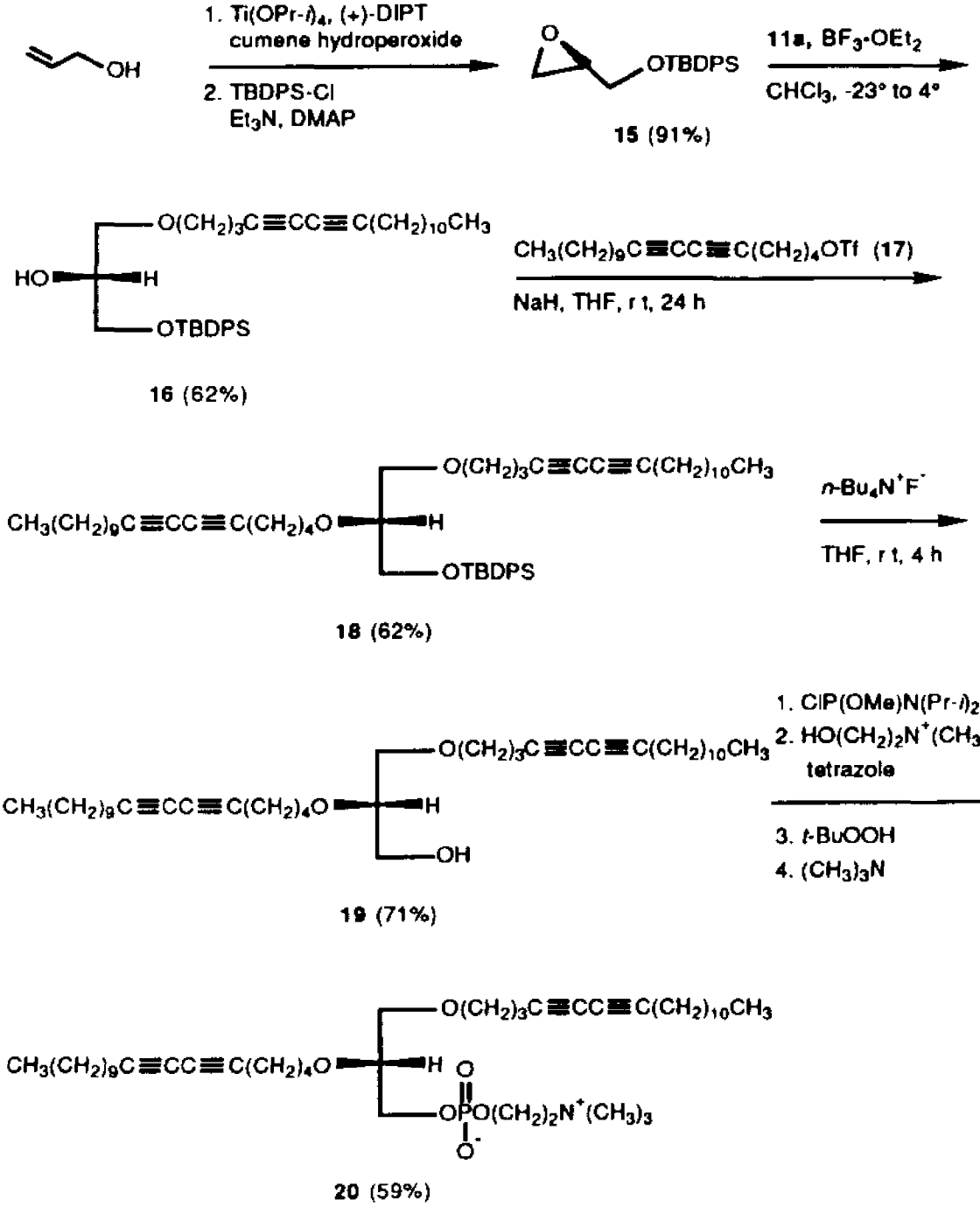
14a: k = 2, m = 10, n = 2, k' = 2, m' = 9, n' = 3 (54%)
14b: k = 0, m = 8, n = 0, k' = 2, m' = 4, n' = 8 (60%)
14c: k = 0, m = 16, n = 0, k' = 2, m' = 10, n' = 2 (59%)

been used as starting materials in chemical syntheses of diether and ether/ester PCs, such as 2,3-*O*-isopropylidene-*sn*-glycerol [29], 1-*O*-benzyl-3-*O*-trityl-*rac*-glycerol [30], and 1,3-benzylideneglycerol [31]. Epoxides have also used as important lipid precursors due to their many advantages. They are available in optically active form, have high chemical reactivity, and are easily prepared. For example, optically active acylglycerols are available from (*S*)-glycidol [32], *rac*-mono- and diacylglycerols [33] and phospholipids are available from *rac*-glycidol [34], and *rac*-1,3-diacylglycerols are available from *rac*-glycidyl esters [35]. In our laboratory, Bittman et al. demonstrated the use of the *p*-toluenesulfonate, *m*-nitrobenzenesulfonate, and *tert*-butyldiphenylsilyl (TBDPS) ether derivatives of (*R*)- and (*S*)-glycidol as precursors in the syntheses of diacyl [36], diether [37], and ether/ester [38] glycerophospholipids. To obtain isomerically and enantiomerically pure 1(3)-*O*-alkyl-2-*O*-methyl-glycerophosphocholines and ether/ester PCs, they developed a new route of asymmetric synthesis from glycidyl derivatives, which are prepared by asymmetric epoxidation of allyl alcohol followed by in situ sulfonation or silylation. The key step is the BF_3-etherate-catalyzed regio- and stereospecific nucleophilic opening of the glycidyl derivatives at C_3 with various alcohols in high yield. Their methodology is flexible and can, therefore, be applied to prepare diacetylenic mixed-ether and ether/ester PCs with some modification. For example, because hydrogenolysis (which is used to deprotect the benzyl group) would destroy the polymerizable butadiyne unit, it was necessary to find another suitable protecting group in the preparation of ether/ester PCs.

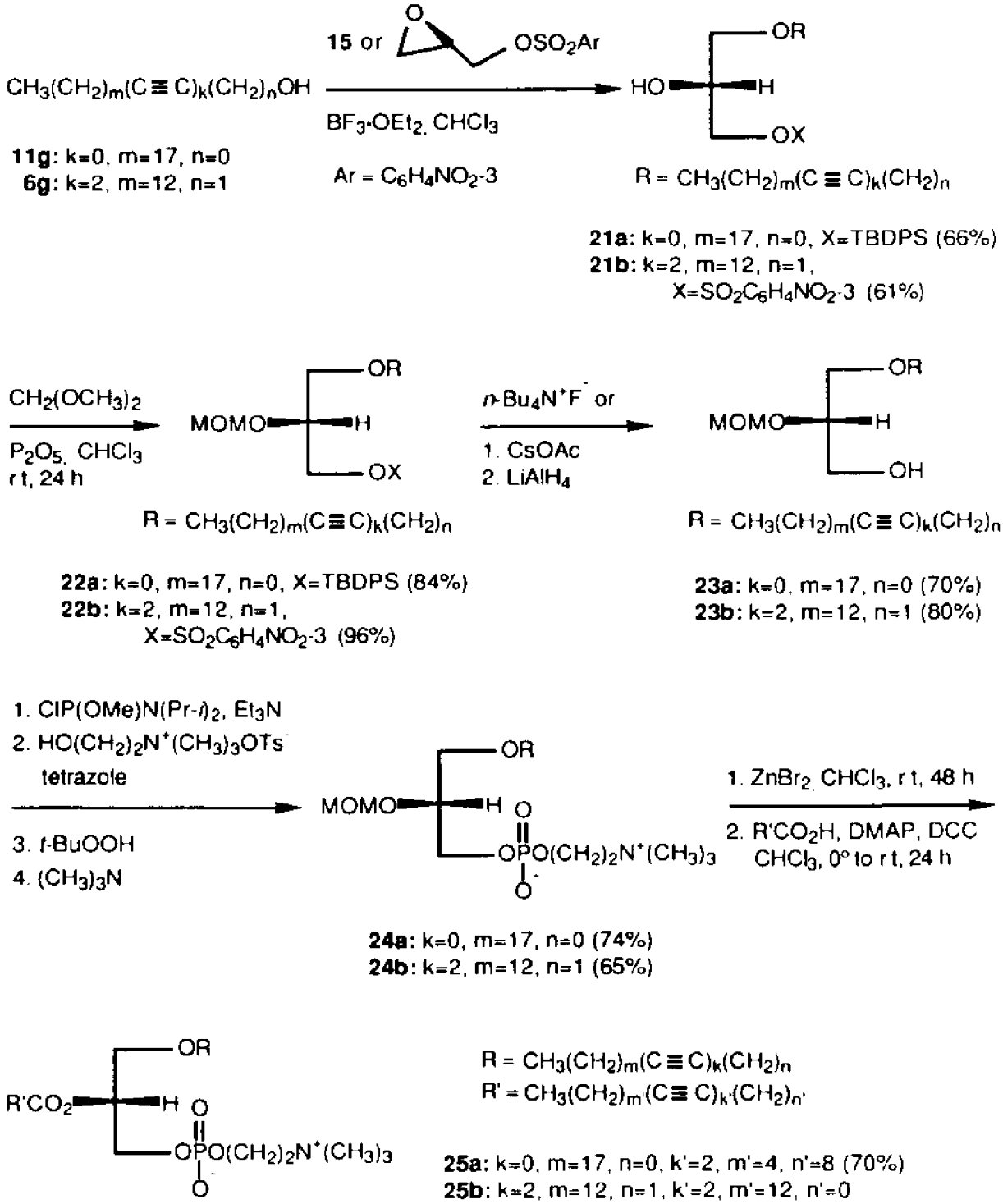
In this thesis, conjugated diacetylenic mixed-ether and ether/ester PCs

are prepared by using diacetylenic alcohols as nucleophiles in the ring-opening reaction of chiral glycidyl derivatives. The TBDPS ether derivative of (*R*)-glycidol has been used to prepare a mixed-ether PC containing butadiyne units (**20**) as shown in Scheme 4. Scheme 5 shows that both the TBDPS and *m*-nitrobenzenesulfonate ether derivatives of (*R*)-glycidol can be used in the preparation of ether/ester PCs containing butadiyne units (**25**). Moreover, the optical purity of derivatives of ring-opening products **21b** and **21b'** has been examined by chiral HPLC. The bilayer structure, polymerizability, and permeability properties of some conjugated diacetylenic PCs have been also examined. The molecular packing of monolayers of three diacetylenic PCs at the air/water interface was studied by pressure/area measurements, and their domain structure was analyzed using 1% phosphatidylethanolamine-rhodamine labeled monolayers by fluorescence microscopy by Drs. Sek Wen Hui and Hao Yu.

Scheme 4. Synthesis of Mixed-Ether Phosphatidylcholine 20



Scheme 5. Synthesis of Ether/Ester Phosphatidylcholines 25a,b

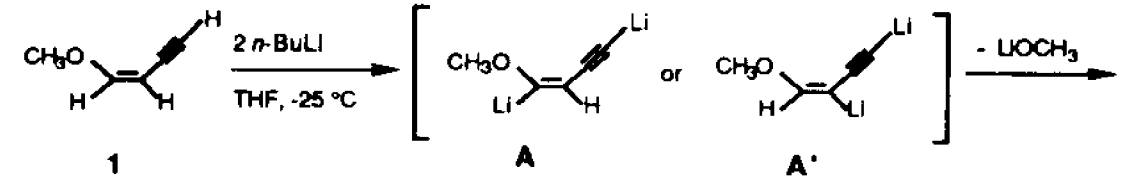


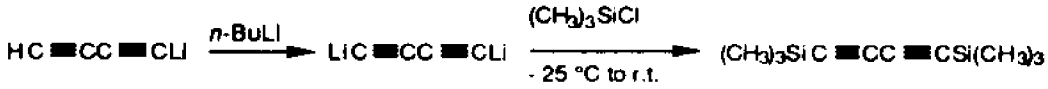
RESULTS

Mechanism

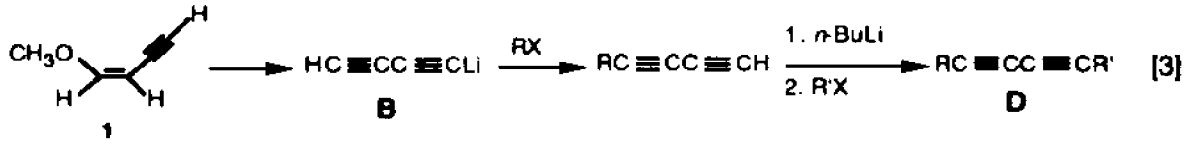
Because butadiyne is a chemically active but very unstable synthon, it may be used as a basic skeleton in a protected form and then coupled with two different 1-haloalkynes at its two sides to prepare the desired unsymmetrical diacetylenic alcohols and acids. The trialkylsilyl group was shown to be a protecting group for terminal acetylenes [39]. Zweifel and Rajagopalan have used **1** to prepare 1,4-bis(trimethylsilyl)-1,3-butadiyne (**2**), a stable crystalline precursor of butadiyne [25]. They considered that the mechanism of the reaction proceeds via a sequence of metalation-elimination-metalation reactions to furnish the dilithio diyne **C** through intermediates **A** and **B**, which then reacts with trimethylsilyl chloride to produce **2** (eq. 2). The initial approach to unsymmetrical diyne **D** was via treatment of **1** with 2 equivalents of *n*-butyllithium to give lithiated butadiyne **B**, which would react with an alkyl halide to give a terminal diyne. Treatment of the latter with another equivalent of *n*-butyllithium and coupling with another alkyl halide would give unsymmetrical conjugated diynes **D** (eq. 3). Several alkyl halides and different reaction conditions were tried, but they failed. In the first coupling reaction, the major product was **E**. Two minor products, **F** and **G**, were also obtained. Scheme 6 outlines a possible mechanism that may explain the formation of a mixture of compounds **E**, **F**, and **G**. Lithiated butadiyne **B** can be further reacted with an alkyl halide by three possible ways: (a) coupling with an alkyl halide to get compound **F**; (b) disproportionation and then coupling with an alkyl halide to give compound **E**; (c) reaction with intermediates **A** and **A'**, and then coupling with an alkyl

halide to obtain compounds **E** and **G**.





[2]



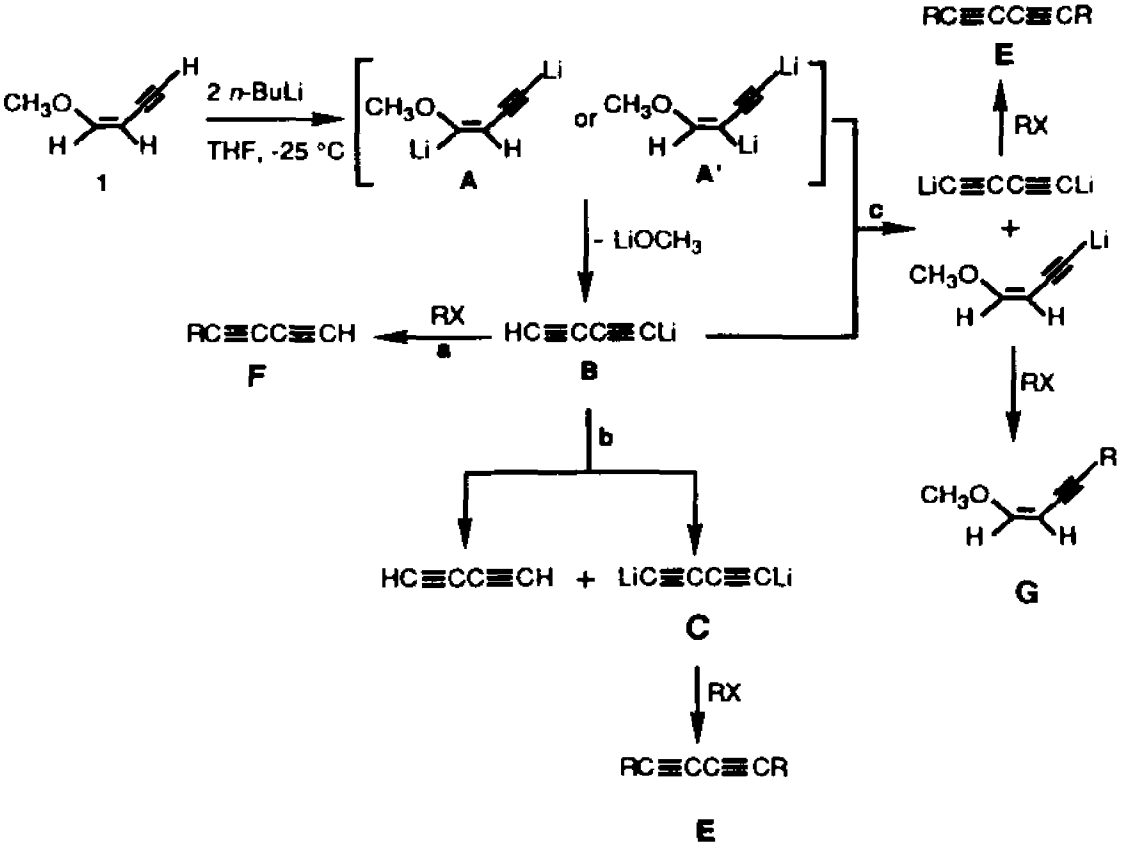
[3]

Synthesis of conjugated diacetylenic alcohols and acids (see Schemes 1 and 2, pp. 7 and 8)

The cleavage of **2** with methyllithium-lithium bromide complex in tetrahydrofuran (THF) occurs quantitatively to yield lithium trimethylsilylbutadiyne (**3**), which is then alkylated by using primary alkyl halides in THF-hexamethylphosphoramide (HMPA) (1:1), giving 4-alkyl-1-trimethylsilylbuta-1,3-diynes [40]. Schemes 1 and 2 show the application of the butadiyne synthon methodology to the synthesis of conjugated long-chain diacetylenic alcohols (**6**, **11**) and acids (**7**, **12**).

Unsymmetrical diacetylenes **6** and **7** were prepared from (*Z*)-1-

Scheme 6. A Possible Mechanism for the Resulting Reaction Mixture



methoxybut-1-en-3-yne (**1**) as outlined in Scheme 1. The unstable starting material **1** was converted to **2** according the procedure of Zweifel and Rajagopalan [21]. Lithium trimethylsilylbutadiyne (**3**) was obtained from **2** by monodesilylation with methyllithium-lithium bromide complex in THF, as described previously [40], and then was coupled with 1-iodotridecane (**4**) to give 1,3-heptadecadiyne (**5**) in 77% yield. Terminal diynes are unstable, and a polymerization reaction takes place during redistillation [41]. Thus the generated terminal diynes should be stored at -70 or -20 °C for a very limited period (e.g. about 2 weeks) to avoid extensive polymerization. Compound **5** reacted with methyllithium and then with dry paraformaldehyde in ether or with dry ice in THF/ether, giving 2,4-octadecadiyn-1-ol (**6**) in 86% yield or 2,4-octadecadiynoic acid (**7**) in 76% yield, respectively, by using previous procedures with some modification, such as the use of methyllithium instead of butyllithium [41].

When other positional isomers of diacetylenic alcohols and acids were prepared in which $n \neq 0$ in **11** and **12**, the first reaction in an attempted synthesis is to couple **3** with an alkyl halide to obtain an alkyl terminal diyne, which is then coupled with a hydroxy-protected bromide. However, there are several problems in this attempted synthetic approach. First, the yields of most of the first coupling reactions are low (about 50%). Second, it is difficult to separate the resulting alkyl terminal diyne from the unreacted alkyl halide, since they have similar R_f values. Third, when the generated alkyl terminal diyne is reacted with 4-bromobutan-1-ol tetrahydropyranyl ether or with methoxymethyl (MOM)-protected 4-bromobutan-1-ol, the resulting mixture was always complicated and contained by-products. Therefore, the order of

successive coupling reactions was changed. The revised and successful procedure involves coupling first with a protected ω-bromoalkan-1-ol, and then coupling with an alkyl halide.

Scheme 2 shows that lithium trimethylsilylbutadiyne (**3**) was coupled with the MOM-protected ω-bromoalkan-1-ols **8**, giving ω-(methoxymethoxy)-1,3-alkadiynes **9**. The MOM-protected ω-bromoalkan-1-ols were prepared in 86-95% yields from ω-bromoalkan-1-ols and dimethoxymethane by using phosphorus pentoxide in methylene chloride as outlined previously [42]. It was expected that the product of the first coupling reaction would be 4-alkyl-1-trimethylsilylbuta-1,3-diyne [40], which then can be converted to a terminal diyne by using potassium fluoride dihydrate [43]. However, in this thesis these two reaction steps were combined in situ, directly giving **9** in 78-91% yields. Coupling of the lithium salt of **9** with an alkyl iodide gave unsymmetrical diynes **10** in good yields. *n*-Butyllithium was used instead of methyllithium because it is easier to handle. The use of HMPA as co-solvent to promote alkylation has been reported previously [44]. It was also found that addition of HMPA improved the yields of the alkylation reaction. As expected, alkyl iodides gave higher yields than the corresponding bromides. Conjugated diacetylenic alcohols **11** can be easily prepared in 91-93% yields by deprotection of MOM-protected alcohols **10** using concentrated HCl in methanol at room temperature. Conjugated diacetylenic acids **12** were obtained in 60-86% yields by oxidation of the alcohols **11** by using pyridinium dichromate (PDC) in dimethylformamide [45].

Synthesis of identical-acid and mixed-acid PCs (see Scheme

3, p. 9)

Scheme 3 shows the preparation of identical-acid (**13a-c**) and mixed-acid (**14a-c**) PCs from *sn*-glycerophosphocholine (GPC)-cadmium chloride complex. This approach is a standard procedure for preparing symmetric-chain diacyl PCs. Identical-acid PCs (**13a-c**) were obtained by acylation of GPC·$CdCl_2$ with diacetylenic or saturated fatty acids (**12**) using 4-(dimethylamino)pyridine (DMAP) and *N,N'*-dicyclohexylcarbodiimide (DCC) in chloroform at room temperature. Identical-acid PC was easily converted to lyso-PC using phospholipase A_2. The latter was coupled with another diacetylenic acid using DMAP and DCC, giving the desired mixed-acid PCs (**14a-c**).

Synthesis of mixed-ether PC (see Scheme 4, p. 12)

Scheme 4 shows the preparation of mixed-ether PC (**20**) using a chiral glycidyl derivative as the starting material. Since alkoxide ion attack on glycidyl arenesulfonate is known to give direct displacement of the arenesulfonate group through ring opening followed by epoxidation [46], the TBDPS ether of (*R*)-glycidol was selected instead of (*R*)-glycidyl arenesulfonate.

The asymmetric epoxidation of allylic alcohol by the use of catalytic amounts of $Ti(O\text{-}i\text{-}Pr)_4$ and tartrate ester in the presence of molecular sieves, followed by trapping glycidol with TBDPS chloride at -20 °C, gave (*R*)-(+)-oxiranemethanol TBDPS ether (**15**) in 91% yield [47]. The BF_3 etherate-catalyzed opening of **15** with diacetylenic alcohol **11a** in chloroform took place, affording the opening product **16** in 62% yield. Etherification with 5,7-octadecadiynyl triflate, which was prepared from 5,7-octadecadiyn-1-ol (**11b**)

and trifluoromethanesulfonic anhydride using sodium hydride in THF, gave compcund **18** in 62% yield. Desilylation of **18** in the presence of tetra-*n*-butylammonium fluoride [48] provided 1,2-di-*O*-alkyl-*sn*-glycerol **19** in 71% yield.

Numerous procedures for phosphorylation of glycerol derivatives have been reported. Several methods of phosphorylation, such as the coupling of glycerol derivative with (2-bromoethyl)phosphodichloridate followed by displacement of the bromide with trimethylamine [49], the 2-chloro-2-oxo-1,3,2-dioxaphospholane-trimethylamine sequence [50], and the nucleophilic opening of cyclic phosphates with tertiary amines by using trimethylsilyl triflate [51], have been tried here. Since the yields were not high, we used phosphitylation with P(III) amide chloride followed by oxidation instead of the phosphorylation approach. The treatment of **19** with *N,N*-diisopropylmethylphosphonamidic chloride gave an amido diester. After TLC monitoring indicated that no starting alcohol remained, excess 1*H*-tetrazole and choline tosylate were added to form the triester. Oxidation of the phosphite ester with *tert*-butyl hydroperoxide, followed by demethylation with anhydrous trimethylamine generated the desired mixed-ether diacetylenic PC (**20**) [52]. The above four successive steps were performed in situ without isolation in 59% overall yield.

Synthesis of ether/ester PCs (see Scheme 5, p. 13)

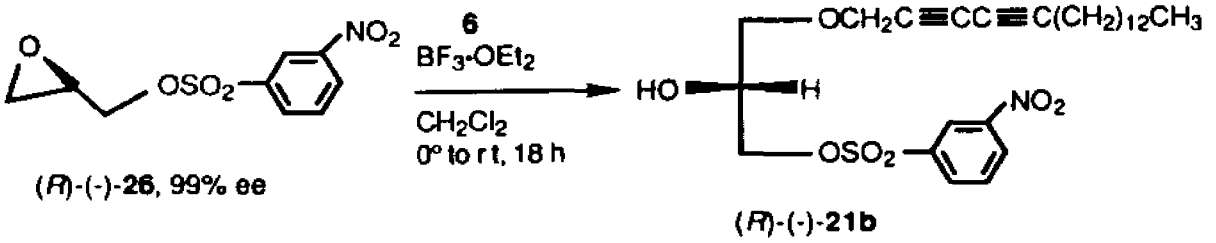
Scheme 5 shows the preparation of ether/ester PCs (**25a,b**) using chiral glycidyl derivatives as starting materials. In the preparation of ether/ester PC, the first step is the ring opening of glycidyl derivatives with alcohol using BF_3 etherate as catalyst. Methoxymethyl was used as a

protecting group in this synthetic scheme. Desilylation of **22a** with tetra-*n*-butylammonium fluoride followed by phosphorylation gave diether PC **24a**. Attempts to remove the MOM group by using $TiCl_4$ and concentrated HCl failed. The use of zinc bromide for the deprotection of methoxymethyl group in chloroform was successful, however, giving lyso-PC. The subsequent conversion of lyso-PC to ether/ester PC **25a** was accomplished in situ without isolation using the standard procedure [53] in 70% yield.

Glycidyl arenesulfonate can be used instead of the TBDPS ether **15** in the preparation of ether/ester PC. The use of glycidyl 3-nitrobenzenesulfonate as starting material is better than the corresponding glycidyl tosylate because acetate displacement proceeds much more rapidly with the former than the latter [38a]. Furthermore, glycidyl 3-nitrobenzenesulfonate can be obtained in very high enantiomeric excess (ee) (99% ee) after two recrystallizations from ethanol [54] and gave the ring-opening product in higher ee than did the glycidyl tosylate [38a].

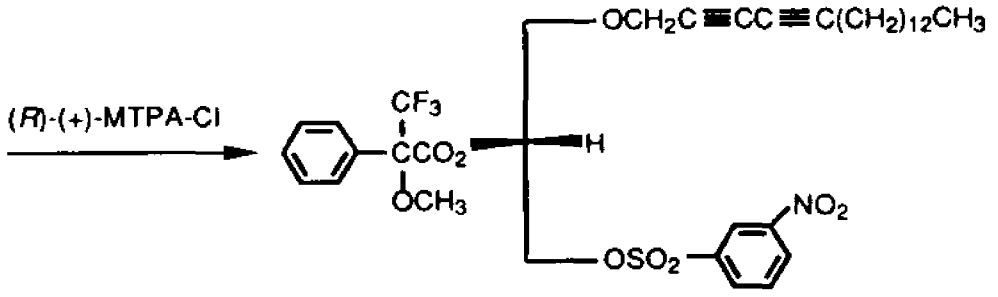
Scheme 7 shows the preparation of (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid ((*R*)-(+)-MTPA) [55] esters (**27**, **27'**) of (*R*)-(-)- and (*S*)-(+)-1(3)-*O*-(2',4'-octadecadiynyl)-*sn*-glycero-3(1)-*O*-*m*-nitrobenzenesulfonate (**21b**, **21b'**) by ring opening of (*R*)-(-)- and (*S*)-(+)-glycidyl 3-nitrobenzenesulfonate followed by acylation with (*R*)-(+)-MTPA chloride. Here, glycidyl 3-nitrobenzenesulfonate (**26**) has been selected as the starting material instead of the TBDPS ether **15**, and its high stereoselectivity in the ring-opening reaction has been examined by chiral HPLC analysis (Fig. 2). The diastereomeric mixture of **27** and **27'** was

Scheme 7. Synthesis of the Mosher Ester of 21b and 21b'

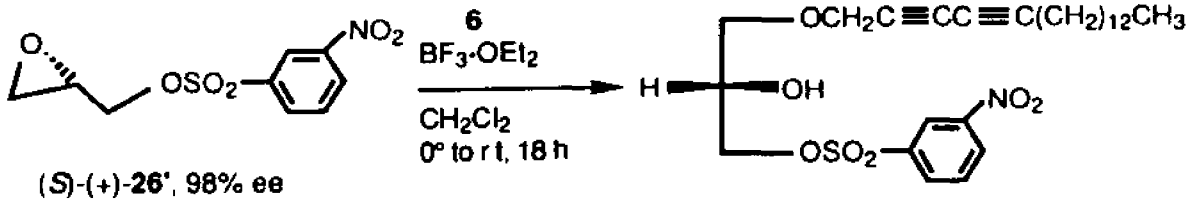


(*R*)-(-)-**26**, 99% ee

(*R*)-(-)-**21b**

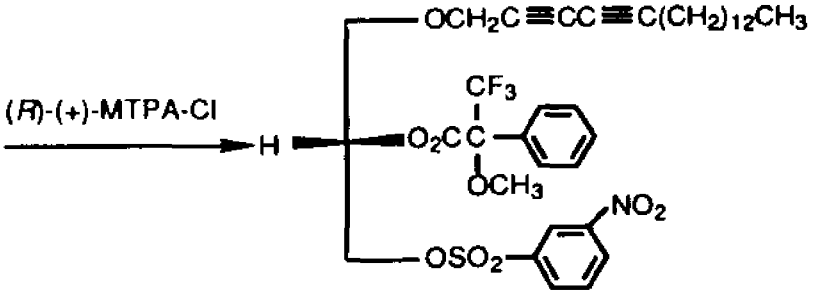


(*R*)-**27**, 95.6% ee



(*S*)-(+)-**26'**, 98% ee

(*S*)-(+)-**21b'**



(*S*)-**27'**, 96.4% ee

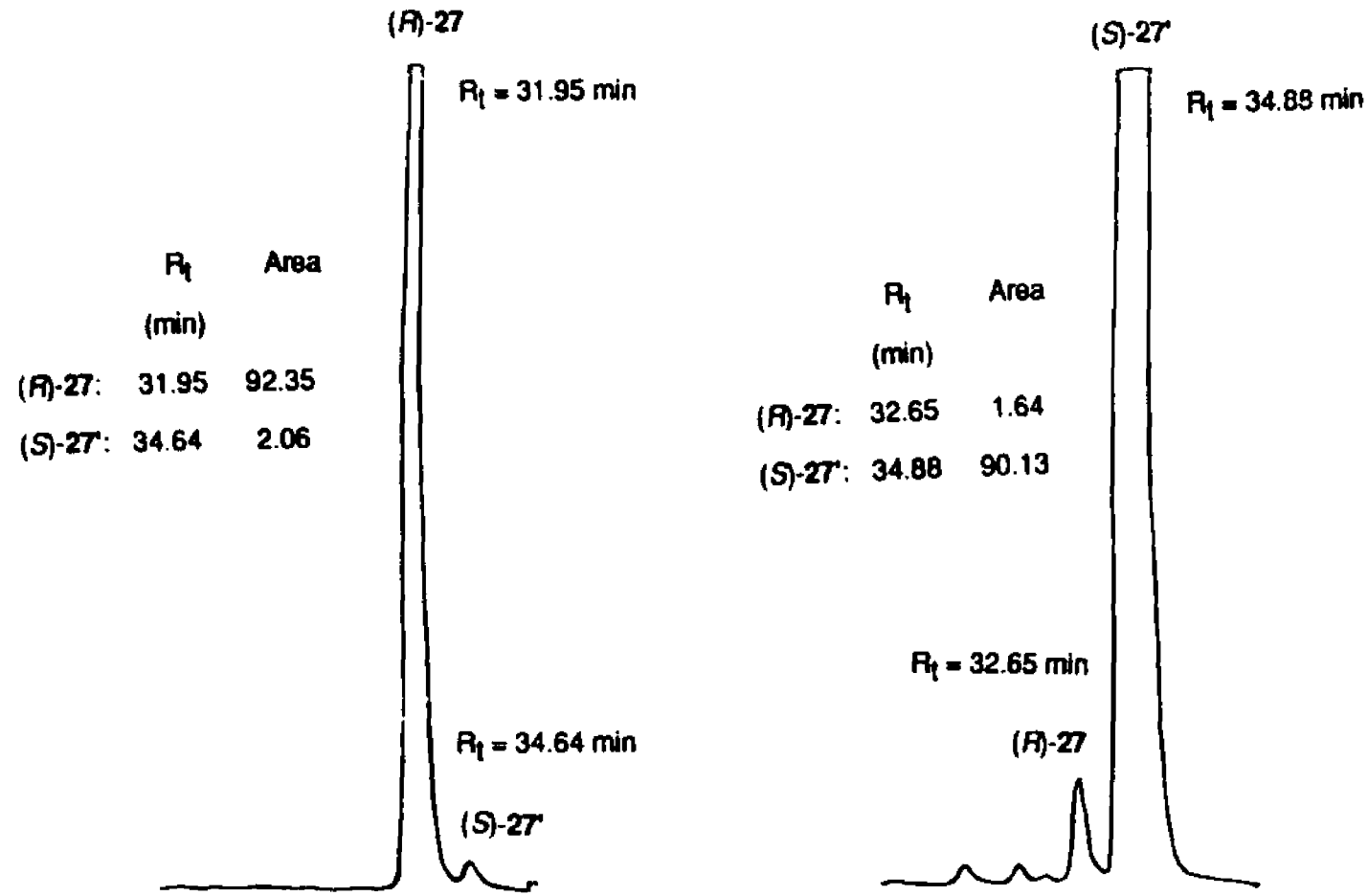


Fig. 2. Analysis of the diastereomeric ratio of the mixture of **27** and **27'** obtained from (*R*)-(-)-**26** (left) and (*S*)-(+)-**26** (right) ring-opening reactions by chiral HPLC (UV, 245 nm; eluent, hexane/2-propanol, 87.5:12.5; flow, 0.45 mL/min).

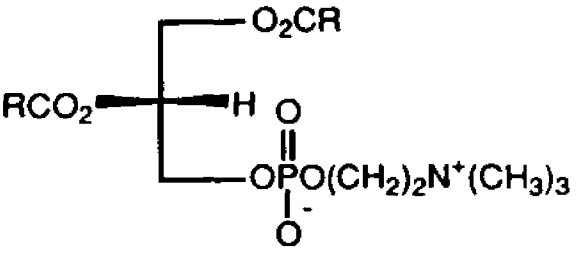
analyzed by HPLC to determine the ee of the ring-opened products **21b** and **21b'**. A chiral stationary-phase column (Pirkle type IA, 4.6 x 250 mm, J. T. Baker) and a flow rate of 0.45 mL/min (elution with hexane/2-propanol 87.5:12.5) were used. Under the above conditions, base-line separation of the diastereomeric (*R*)-(+)-MTPA esters of (*R*)-(-)-**21b** and (*S*)-(+)-**21b'** was achieved (R_t of **27**, 31.95 and 32.65 min; R_t of **27'**, 34.64 and 34.88 min). The percent ee values of the (*R*)-(+)-MTPA esters of (*R*)-(-)-**21b** and (*S*)-(+)-**21b'** are 95.6 and 96.4, respectively. The results from Figure 2 indicated that the BF_3-catalyzed ring opening of **26** and **26'** by diacetylenic alcohol **6** proceeded with very high stereoselectivity and without any significant loss of chiral purity. This is consistent with results obtained previously using long-chain saturated alcohols [37c]. There is one principal difference between the uses of glycidyl TBDPS ether (**15**) and glycidyl 3-nitrobenzenesulfonate (**26**) as starting materials in the preparation of ether/ester PC. Instead of the use of tetra-*n*-butylammonium fluoride for the desilylation in the former, displacement using cesium acetate followed by reduction with lithium aluminum hydride as catalyst was used to remove the 3-nitrobenzenesulfonate group in the latter.

Bilayer structure, polymerizability, and permeability properties of liposomes from diacetylenic PCs

Direct structural investigation for polymerizable diacetylenic phospholipid bilayers is useful in order to obtain a better understanding of the relation of monomer structure to assembly morphology and properties. Four diacetylenic PCs (C_{18} PC derivatives **13a**, **13b**, **14a**, and **20**) have been examined in terms of their lamellar structure, polymerizability, and

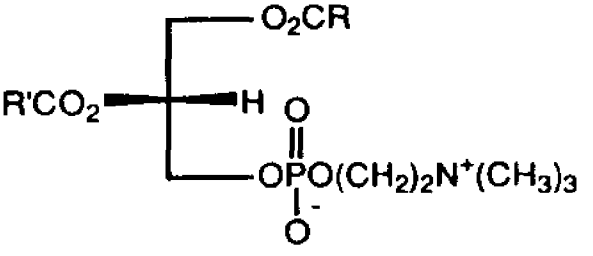
permeability properties (Fig. 3). The structures of bilayers of these lipids have been determined at low resolution by low angle *X*-ray diffraction by Rhodes et al. [56]. These PCs all have 18-carbon chains but differ with respect to the ether/ester linkage at *sn*-1 and *sn*-2 positions and the relative position of the diacetylene moiety. It was found that only **13a** exhibited the 'typical' bilayer profile, while **13b**, **14a**, and **20** showed evidence of interdigitation and/or significant disorder. Figure 4 shows a 'typical' bilayer profile for PC **13a**, whereas Figure 5 shows a 'typical' interdigitation profile for PC **13b**. The intensity distribution of the lamellar reflections for PC **13a** indicated a more conventional bilayer structure and increased ordering in the multilayer. The electron density profile of PC **13a** is characteristic of a well-ordered lipid bilayer, with an electron density minimum in the bilayer center and extended shoulders on the main headgroup electron density peaks corresponding to the glycerol backbone and diacetylene as shown in Figure 6.

The microstructure of the monolayers is very important with regard to their uniformity and stability, but very little is known about the microstructure of monolayers formed by these polymerizable lipids. Therefore, microdomain structures in polymerizable (**13a**) and nonpolymerizable diacetylenic PC (**13b** and **14a**) monolayers have been determined by pressure/area measurements and by fluorescence microscopy [57]. During first-time compression, an overshot or "bump" was detected in the π/A isotherms of monolayers of PC **13a** only, indicating a lack of nucleation for the growth of domains. When monolayers of PC **13a** are exposed to UV while compressed to less than 40 Å^2/molecule, polymerization occurs as indicated by the inelastic behavior of the monolayer. PC **13b** and **14a** do not produce



$R = CH_3(CH_2)_m(C{\equiv}C)_k(CH_2)_n$

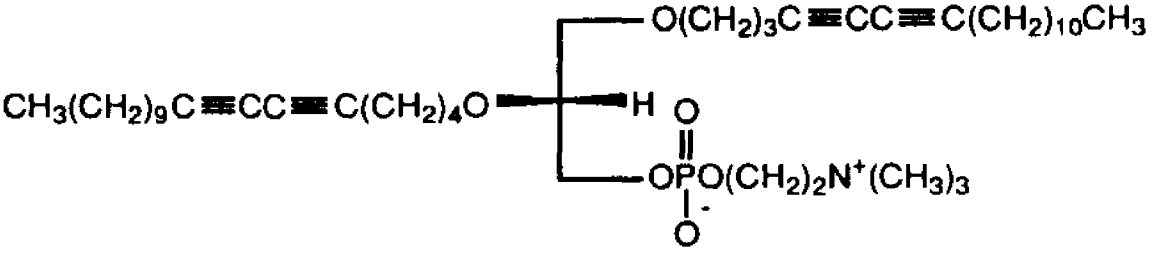
13a: k = 2, m = 10, n = 2
13b: k = 2, m = 4, n = 8



$R = CH_3(CH_2)_m(C{\equiv}C)_k(CH_2)_n$

$R' = CH_3(CH_2)_{m'}(C{\equiv}C)_{k'}(CH_2)_{n'}$

14a: k = 2, m = 10, n = 2, k' = 2, m' = 9, n' = 3



20

Fig. 3. Four diacetylenic phosphatidylcholines **13a**, **13b**, **14a**, and **20**, which have been examined in terms of lamellar structure, polymerizability, and permeability properties.

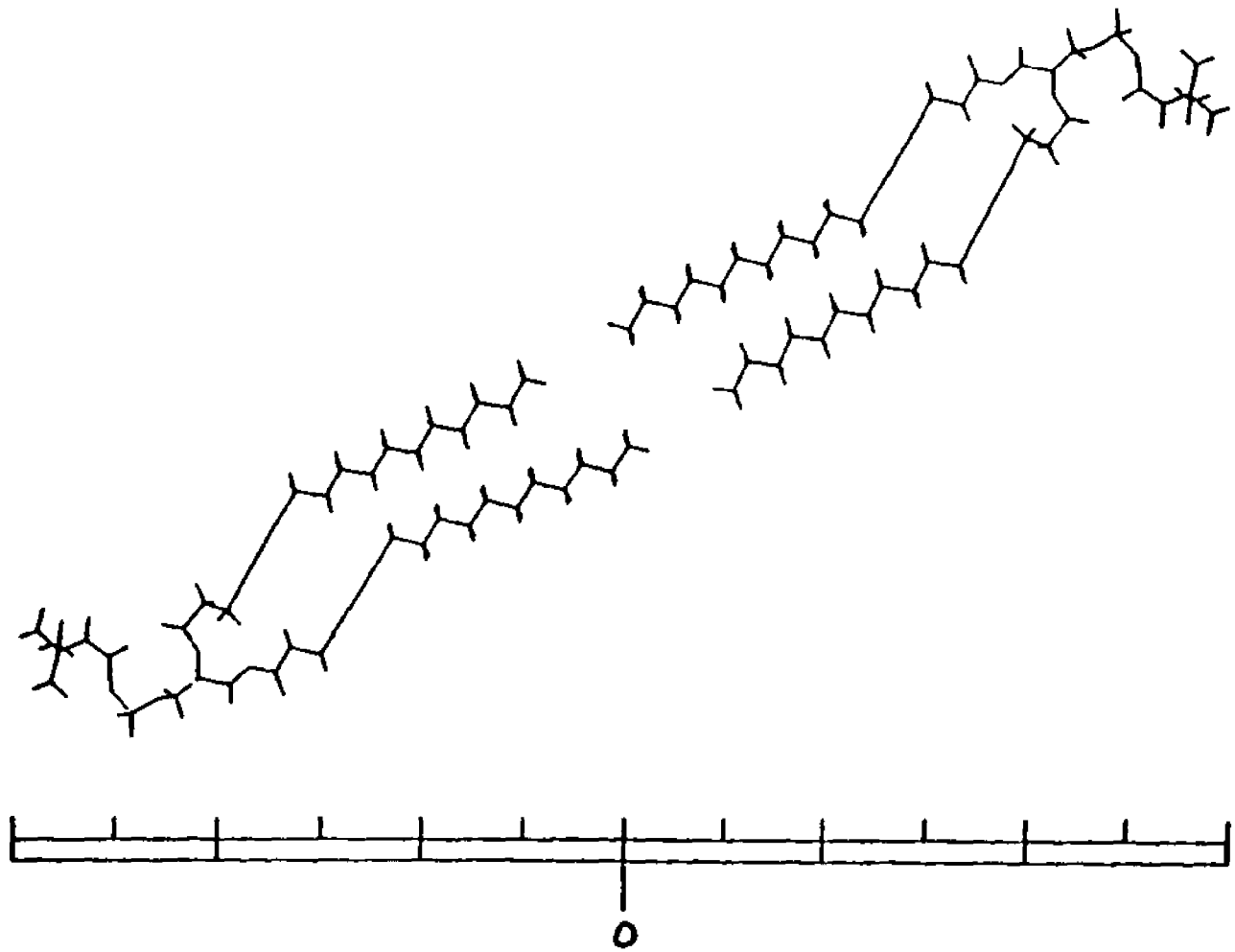


Fig. 4. A typical bilayer profile for PC **13a** obtained by X-ray diffraction by D. G. Rhodes (University of Connecticut Health Center, Farmington, CT).

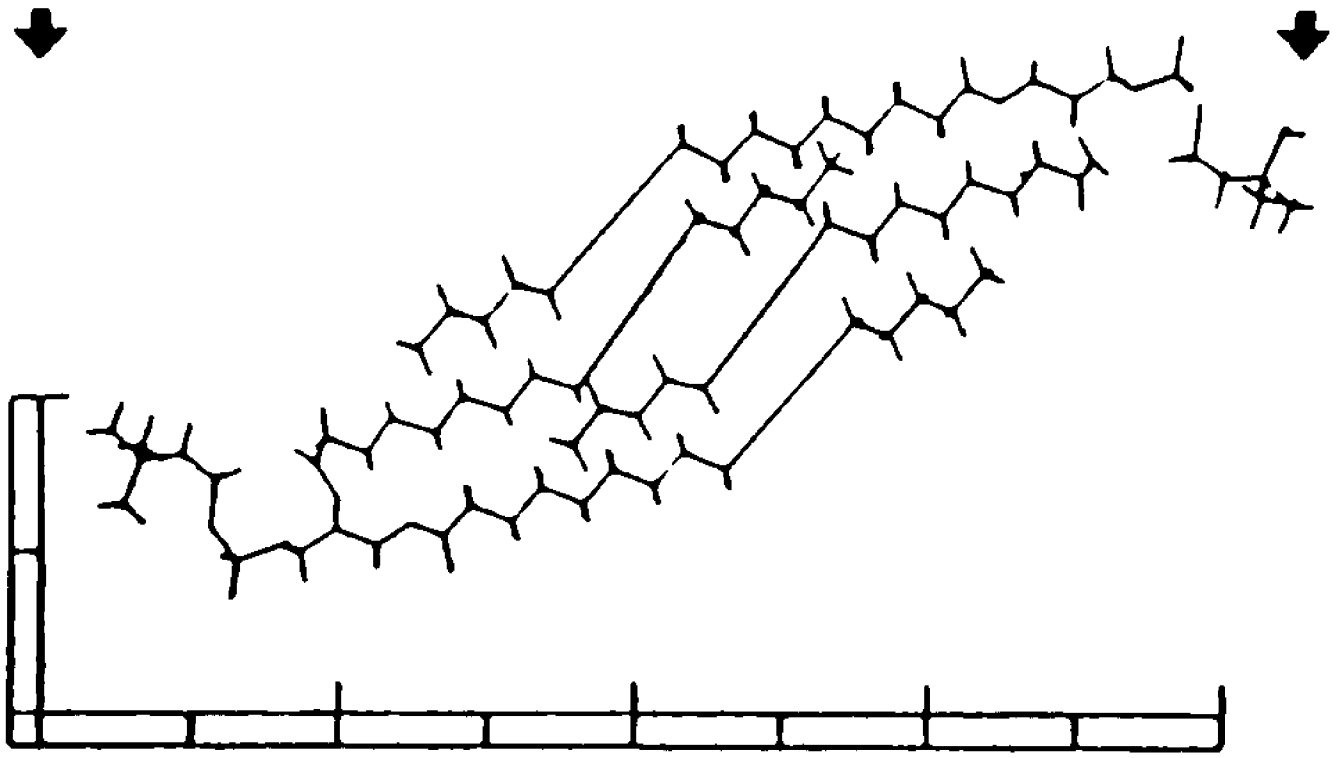


Fig. 5. A typical interdigitation profile for PC **13b** obtained by X-ray diffration by D. G. Rhodes.

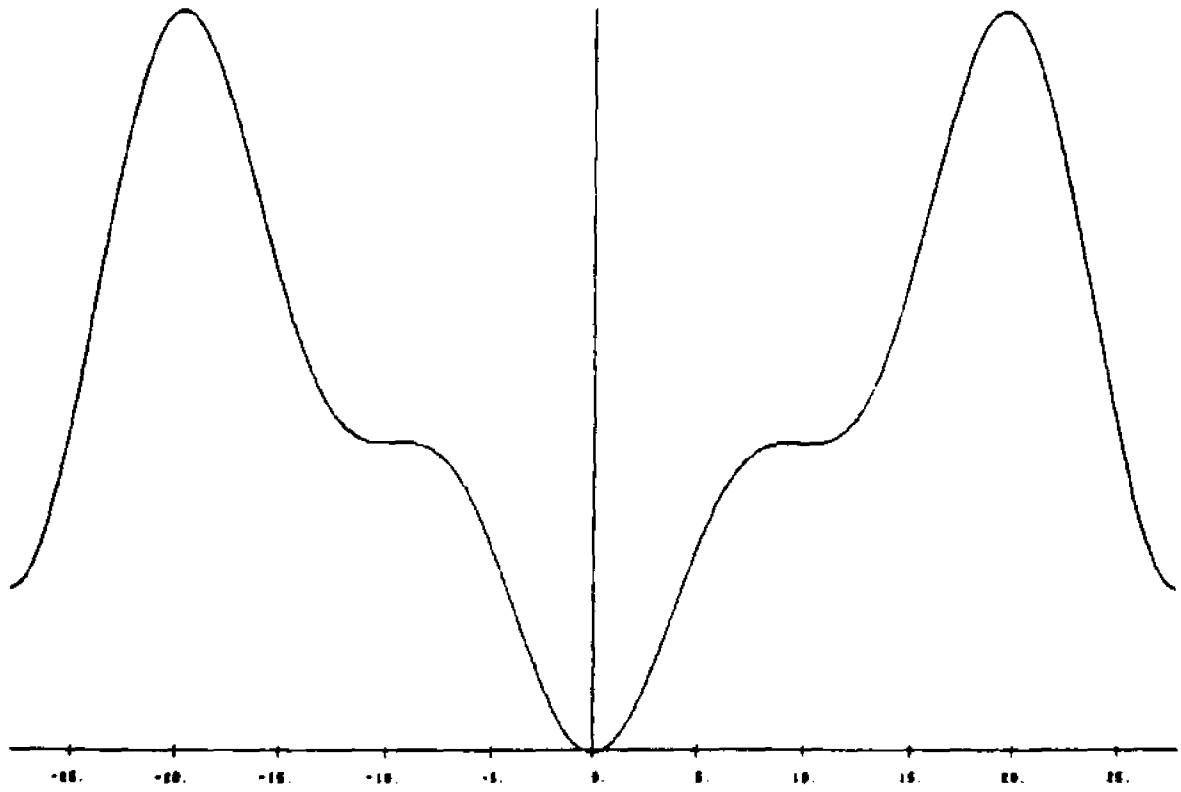


Fig. 6. The electron density profile of PC **13 a.**

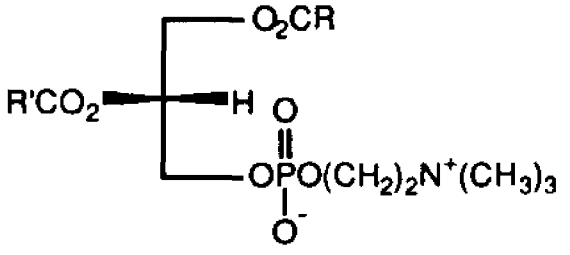
visible solid domains, and do not become polymerized under the same experimental conditions.

It was found that only PC **13a** polymerized effectively upon UV irradiation (254 nm) at room temperature. This derivative polymerized in seconds to a deep blue color. When the temperature exceeds 35-40 °C, however, there is an irreversible transition to orange.

Previous studies of permeation in lipid bilayers indicate that: (1) bilayer membranes are permeable to water and small, neutral, nonpolar molecules; (2) the diffusion of solutes across bilayers depends on the structure and dynamics of the lipid molecules comprising the bilayer; (3) the permeability of solute across liposomal bilayers can be monitored spectrophotometrically; (4) osmotically induced shrinking of liposomes is accompanied by an increase in absorbance, and osmotically induced swelling by a decrease in absorbance; (5) the initial rate of liposome swelling, dV/dt, arising from nonelectrolyte entry is determined from the initial rate of the reciprocal of the absorbance change, d(1/A)/dt, where absorbance arises because of light scattering [58].

It has been shown that liposomes of 1,2-di-(10',12'-tricosadiynoyl)-*sn*-glycero-3-phosphocholine (**H**) and 1-octadecanoyl-2-(10',12'-heptacosadiynoyl)-*sn*-glycero-3-phosphocholine (**I**) are more permeable to glycerol than dimyristoyl-PC (DMPC) liposomes above T_m before and after polymerization, although polymerization reduced the permeability [59]. Polymerization markedly enhances the stability of liposomes. We examined the permeability behavior of liposomes of stearoyl-oleoyl-PC (SOPC), and of unpolymerized and polymerized 1,2-di-(4',6'-octadecadiynoyl)-*sn*-glycero-3-

phosphocholine (**13a**). Table 1 shows the effect of polymerization on the initial swelling rates of these liposomes exposed to hyperosmolar solutions of acetamide, glycerol, and urea. Representative absorbance versus time plots of acetamide permeability are shown in Figures 7-9. The results indicate that unpolymerized and polymerized liposomes of **13a** underwent osmotic swelling with acetamide, glycerol, and urea more rapidly than did liposomes of SOPC, but the initial rates of swelling of polymerized liposomes of **13a** were 3-10 times lower than those of unpolymerized liposomes of **13a**.



SOPC: R = $CH_3(CH_2)_{16}$

R' = $CH_3(CH_2)_7CH{=}CH(CH_2)_7$

13a: R, R' = $CH_3(CH_2)_{10}C{\equiv}CC{\equiv}C(CH_2)_2$

Table 1. Effect of Polymerization on the Initial Swelling Rates of PC 13a Liposomes Exposed to Hyperosmolar Solutions of Nonelectrolytes*

PC	Δ(1/A) per min		
	Acetamide	Glycerol	Urea
SOPC	4.20 ± 0.30	2.59 ± 0.23	0.91 ± 0.09
Unpolymerized PC **13a**	24.05 ± 0.83	18.32 ± 0.61	9.86 ± 0.45
Polymerized PC **13a**	2.80 ± 0.18	4.36 ± 0.46	2.40 ± 0.14

* Permeabilities were measured as described under Experimental Section, and values are the average of five determinations at 23-24 °C.

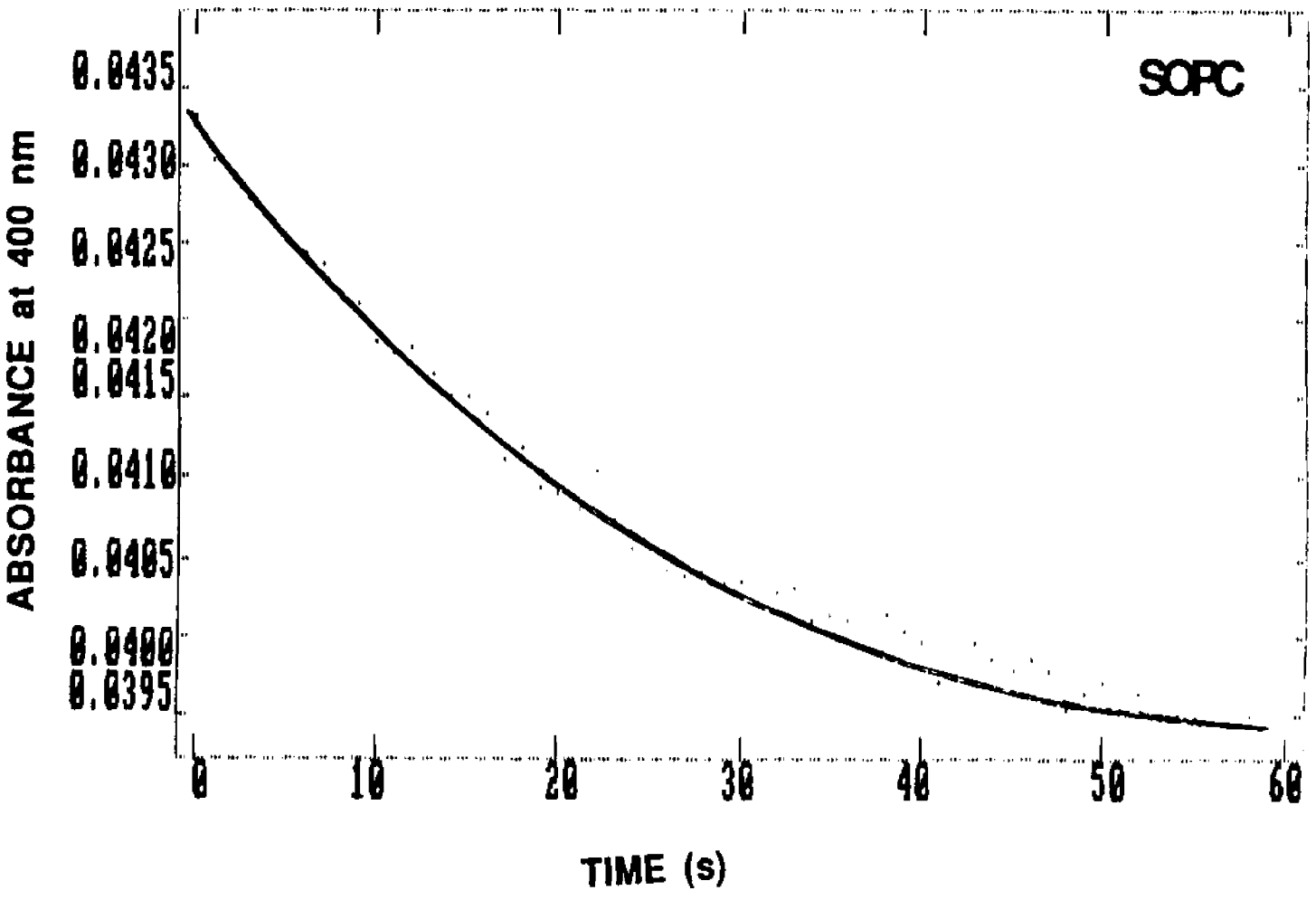


Fig. 7. Absorbance versus time plot of acetamide permeability of liposomes of SOPC.

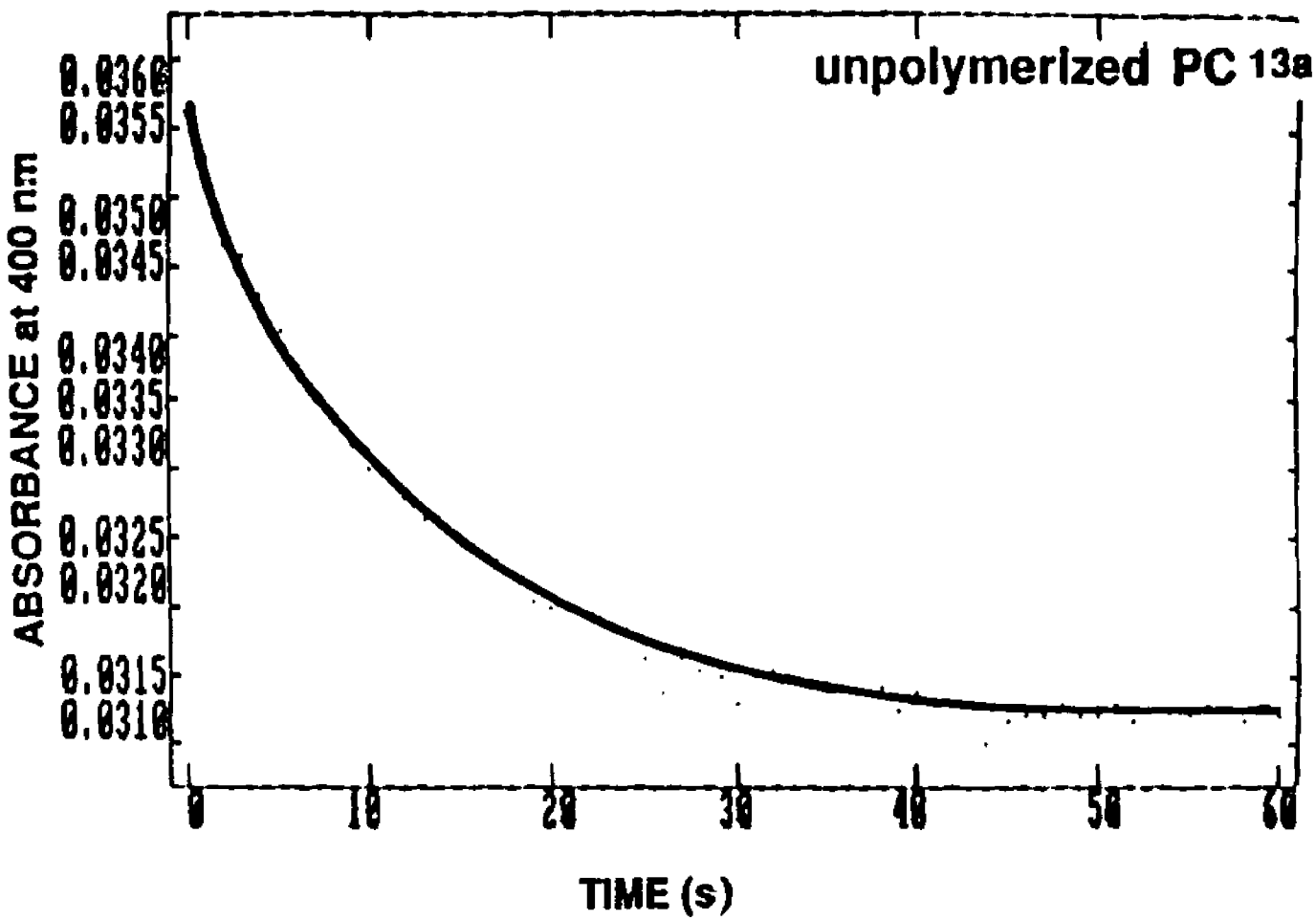


Fig. 8. Absorbance versus time plot of acetamide permeability of liposomes of unpolymerized PC **13a**.

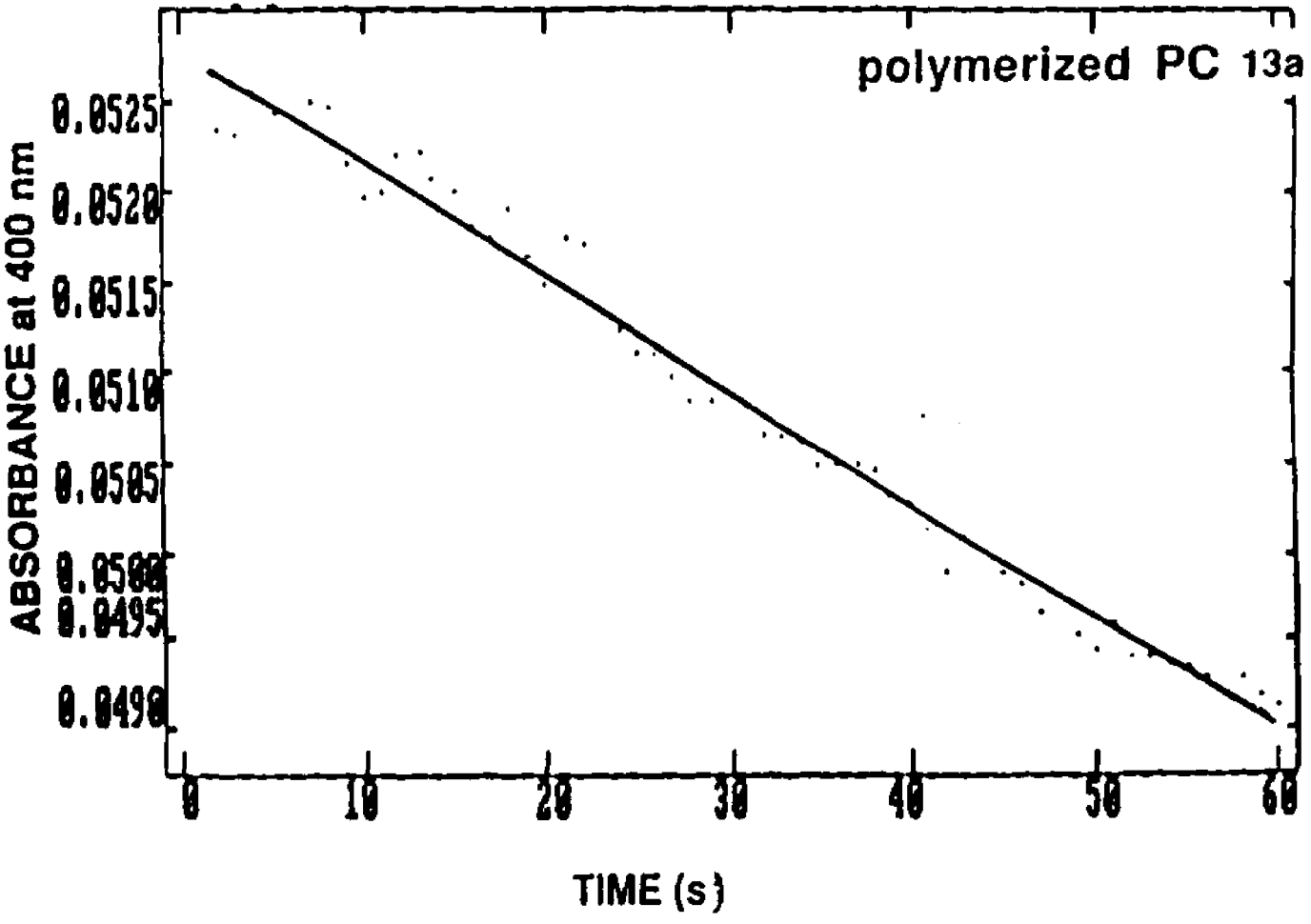


Fig. 9. Absorbance versus time plot of acetamide permeability of liposomes of polymerized PC **13a**.

DISCUSSION

Syntheses of conjugated diacetylenic compounds

In the present thesis (*Z*)-1-methoxybut-1-en-3-yne (**1**) has served as a useful synthon, via butadiyne derivatives **2** and **3**, for the synthesis of the positional isomers and homologues of conjugated diacetylenic alcohols and acids. This new and efficient way is an alternative of the Cadiot-Chodkiewicz heterocoupling reaction and provides a more direct access to conjugated diacetylenic compounds than does the latter. The above conjugated diacetylenic alcohols and acids have been used in the preparation of the identical-acid and mixed-acid, mixed-ether, and ether/ester PCs containing a conjugated diacetylenic unit.

It is also found that the TBDPS ether and *m*-nitrobenzenesulfonate derivatives of (*R*)-glycidol are useful chiral building blocks for the synthesis of mixed-ether and ether/ester diacetylenic PCs. Moreover, BF_3 etherate serves as an excellent catalyst in the opening reactions of *m*-nitrobenzenesulfonate derivative of (*R*)-glycidol, giving regioselectivity and optical purity in high yield. The chiral purity of each stereoisomer of **21b** was established by examination of the diastereomeric mixture of the (*R*)-(+)-MTPA ester by chiral HPLC.

The synthesis of conjugated diacetylenic alcohols, acids, and PCs proceeds from commercially available starting materials. This methodology is flexible and can be applied to prepare related analogues. All of these conjugated diacetylenic alcohols, acids, and PCs except 8,10-octadecadiynoic, 12,14-pentacosadiynoic, and 12,14-heptacosadiynoic acids are new compounds and have not been reported previously. They will be

useful for the study of effect of polymerization on phospholipid properties.

Morphology and polymerizability of conjugated diacetylenic PCs

The existing conjugated diacetylenic PCs are very long-chain identical-acid PCs in which both conjugated diyne units are located farther away from the glycerol backbone (at least 9 carbons) [5a]. *X*-ray crystallography has shown that the *sn*-2 chain has a bend near the lipid-water interface of the PC bilayer. If the two acyl chains are identical, the *sn*-2 chain is shorter than the *sn*-1 chain by about 1.5 C-C bond length difference. In order to optimize the intra- and intermolecular cross-linking, it may be preferred that the relative positions of the conjugated diyne units in the *sn*-1 and *sn*-2 chains should be approximately the same in the PC bilayer. Therefore, the insertion of an additional methylene group into the *sn*-2 chain may bring the diacetylenes into register in the *sn*-1 and *sn*-2 chains.

Among the above prepared PCs, **13a-c** are identical-acid PCs. PCs **13a** and **13b** are conjugated diacetylenic positional isomers. The former constitutes a typical bilayer and polymerizes easily, whereas the latter forms a typical interdigitation structure and does not polymerize under the same conditions. PCs **14a-c** all are mixed-acid PCs. To my knowledge, conjugated diacetylenic mixed-ether and ether/ester PCs have not yet been reported. PC **20** is a mixed-ether PC, whereas PCs **25a,b** are ether/ester PCs. Compared with **13a**, there is one carbon position apart of diyne units between the *sn*-1 and *sn*-2 chains in PC **14a**. Only one hydrophobic chain contains a conjugated diacetylenic unit in PCs **14b,c** and **25a**, so only intermolecular cross-linking can take place during polymerization. In PC **25b**, the

conjugated diyne units in both of the *sn*-1 and *sn*-2 chains are located close to the glycerol backbone.

The polymerizability of diacetylenic PCs depends on a number of factors related to the position and orientation of the diacetylenic bonds in adjacent molecules relative to each other and their assembly morphogenesis. In order for polymer formation to occur, a PC must be a well-ordered solid [56]. The polymerization of diacetylenic PC bilayer is facilitated if the hydrophobic chains of the PC are in a crystallizable lattice, and the polymerization is inhibited if the chains are in a disordered lamellar phase [7c]. Moreover, a shift in the intermonomer distance occurs during polymerization, and the lattice may have some internal flexibility to accommodate the conformational change induced by polymer formation [56].

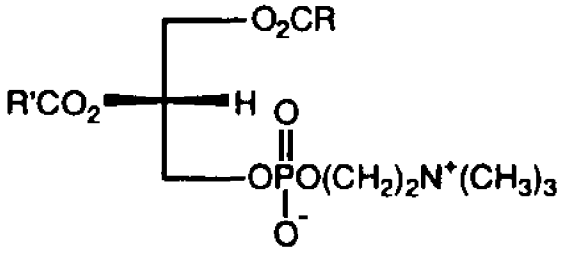
All the above prepared PCs were tested for polymerizability in aqueous dispersion. Only PC **13a** polymerized effectively upon UV irradiation, turning deep blue in seconds, indicating the formation of long, well-ordered polymer. The exact juxtaposition of diacetylenic bonds in adjacent chains in PC **13a** is suitable for its special molecular alignment and polymerization [57]. That PC **13a** can polymerize at room temperature is probably due to the proximity of the diacetylene bonds to the headgroup [56]. Other PCs did not polymerize under the same conditions. This result is consistent with the evidence of interdigitation and/or significant disorder in PCs **13b**, **14a**, and **20** by low angle *X*-ray diffraction. One structural difference in PC **14a** and PC **20** from PC **13a** is that the diacetylenic bonds in the two adjacent chains are one carbon position apart, instead of being at the same position in PC **13a**. It is expected that the diacetylenic bonds in the

two adjacent chains are at same depth in the bilayers of PC **14a** and PC **20**, so their polymerization might be improved. Because PC **14a** and PC **20** have very short proximal hydrophobic chain segments, the glycerol backbone conformation and packing may strongly influence the packing (alignment) of the diacetylenic bonds. Moreover, because PC **14a** and PC **20** are not long enough to establish extended all-*trans* linear domains, there are some disorders by constraints of the adjacent regions of the molecule [56]. These modifications could result in diminished polymerizability. Although the diacetylenic bonds in the adjacent chains in PC **13b** are at the same position, they are located further toward the terminal methyl group. Their thermal motion could reduce the chance to become polymerized or even to become aligned with respect to each other. It is expected that PC **13b** does not polymerize due to its very low critical temperature (<11 °C) [57].

Effect of polymerization on the permeability of PC liposomes

It is expected that polymer formation in PC liposomes will alter the permeability characteristics of the liposomes. Experimental data indicated that the rate of decrease in absorbance immediately following the absorbance maximum depends on the permeability of the liposomes to added nonelectrolyte [60]. A reduced permeability of [^{14}C]sucrose in methacryloyl-substituted PCs after photopolymerization compared with before the polymerization has been reported [8]. Polymeric methacryloyl and butadiene liposomes also show decreased membrane permeability of [^{3}H]glucose after polymerization [6b,61]. It has been reported that UV irradiation had no effect on the permeability of DMPC liposomes to glycerol,

whereas UV radiation markedly reduced the permeability of the diacetylene-containing lipids [59]. Table 2 shows the glycerol permeability data of Chapman et al. [59] for aqueous dispersions of 1,2-di-(10',12'-tricosadiynoyl)-*sn*-glycero-3-phosphocholine (**H**), 1-octadecanoyl-2-(10',12'-heptacosadiynoyl)-*sn*-glycero-3-phosphocholine (**I**), and DMPC before and after polymerization. In this thesis the effect of polymerization on the permeabilities of liposomes of SOPC, unpolymerized and polymerized **13a** to nonelectrolytes has been examined. As compared with SOPC liposomes, the higher permeability to nonelectrolytes of PC **13a** liposomes before polymerization may be the result of increased polarity in the bilayer center owing to the presence of triple bonds in the hydrophobic chains of PC. After polymerization this enhanced permeability is reduced, corresponding to the cross-linked polymer networks by virtue of the reactive triple bonds in PC **13a** liposomes. Similar results in which a reduced permeability to nonelectrolytes was found after polymerization were described previously [6b,8,59-61].



H: R, R' = $CH_3(CH_2)_9(C{\equiv}C)_2(CH_2)_8$

I: R = $CH_3(CH_2)_{16}$

R' = $CH_3(CH_2)_{13}(C{\equiv}C)_2(CH_2)_8$

Table 2. Glycerol Permeability of Aqueous Dispersions of PC H and I and DMPC Before and After Polymerization**

[Data from ref. 59]

Lipid	Δ(1/A) per min Before	After
Same-Chain PC **H**	1.43	0.64
Mixed-Chain PC **I**	> 10	0.43
DMPC	0.20	0.20

** Dispersions were polymerized by placing them 5 cm from a UV lamp (Mineralight R-52, UV Products, Inc.). The initial absorbance was adjusted to 0.6-0.8 by varying the measured wavelength between 400 and 800 nm. Values are the average of two determinations at 50 °C.

EXPERIMENTAL SECTION

General Procedures

The solvents used were dried as follows: THF was refluxed over sodium benzophenone ketyl for several hours immediately prior to use. Acetone was stored over calcium sulfate for at least one week. Alcohol-free chloroform, dichloromethane, HMPA, and DMF were distilled from calcium hydride and stored over type 3A molecular sieves. Triethylamine was dried and stored over calcium hydride. DCC was melted in an oven (110 °C) and the supernatant was used. DMAP was recrystallized from $CHCl_3/Et_2O$ (1:1) before use. Choline tosylate and 1*H*-tetrazole were dried for 5 h over phosphorus pentoxide under vacuum at 78 °C. Commercially available BF_3-OEt_2 from Aldrich (8.1 M) was distilled and then diluted with 9 volumes of dichloromethane. Alcohol-free chloroform and paraformaldehyde were obtained from J. T. Baker (Phillipsburg, NJ). ω-Bromoalkan-1-ol, 1-octadecanol, 1-bromotridecane, 1-iodopropane, 1-iodoheptane, 1-iodoundecane, (*Z*)-1-methoxybut-1-en-3-yne, *n*-butyllithium, methyllithium-lithium bromide complex, decanoic acid, allyl alcohol, TBDPS chloride, methyllithium, phosphorus pentoxide, DMAP, DCC, anhydrous zinc bromide, trifluoromethanesulfonic anhydride, tetra-*n*-butylammonium fluoride, sodium hydride, triethylamine, 1*H*-tetrazole, dimethoxymethane, *tert*-butyl hydroperoxide, (*R*)-(-)- and (*S*)-(+)-glycidyl-3-nitrobenzenesulfonate, *N,N*-diisopropylmethylphosphonamidic chloride, and chlorotrimethylsilane were purchased from Aldrich. Potassium fluoride dihydrate was from Mallinckrodt Chemical Works. Phospholipase A_2 (from porcine pancreas) and *sn*-

glycerophosphocholine-cadmium chloride complex were from Sigma Chemical Company. 1-Stearoyllyso-PC was from Avanti Polar Lipids, Inc. Anhydrous trimethylamine was from Fluka Chemical Corp. 10,12-Octadecadiynoic acid was from Farchan Laboratories, Inc. Sodium iodide was from MCB, Inc. Choline tosylate was prepared as described previously [62]. PDC was prepared according to a procedure of Corey and Schmidt [63]. (*R*)-(+)-MTPA-Cl was obtained from Aldrich and Fluka Chemical Corp. 1-Iododecane and 1-iododododecane were prepared by the reaction of the corresponding *n*-alkyl bromides with sodium iodide in acetone at room temperature for 24 h, followed by vacuum distillation. Metricel filters (0.45 μm) of Gelman Scientific were obtained from Baxter Healthcare Corp.

Melting points are uncorrected. Silica gel GF TLC plates of 0.25-mm thickness (Analtech, Newark, DE) were used to monitor reactions, with 10% sulfuric acid in ethanol and/or short-wavelength ultraviolet light to visualize the spots or with molybdate spray for phospholipids as described previously [62]. Flash chromatography was carried out with silica gel 60 (230-400 ASTM mesh) of E. Merck, purchased from Aldrich. ^{1}H NMR spectra were recorded on an IBM-Bruker WP 200-MHz spectrometer or on a GE QE 300-MHz spectrometer, and chemical shifts are given in parts per million from tetramethylsilane as internal standard. Infrared spectra were recorded on a Perkin-Elmer FT-IR 1600 series spectrometer. HPLC was carried out on a Perkin-Elmer Model 410 system equipped with a LC-235 diode array detector and LCI-100 recorder-integrator. Rotation values were recorded on a JASCO DIP-140 Digital Polarimeter. Liposome swelling was recorded on an Aviv 14DS UV-VIS-IR spectrophotometer. Elemental analyses were performed by

Desert Analyties, Tucson, AZ, and by Oneida Research Services, Inc., Whitesboro, NY.

Syntheses

1,4-Bis(trimethylsilyl)-1,3-butadiyne (2). This compound was prepared in 66% yield as described previously [25].

1-Iodotridecane (4). To a solution of 15 g (100 mmol) of sodium iodide in 100 mL of acetone was added 13.2 g (50 mmol) of 1-bromotridecane. The reaction mixture was stirred for 3 days at room temperature. The white precipitate (NaBr) was filtered and acetone was removed. Hexane and water were added. The organic layer was washed with 10% aqueous sodium thiosulfate solution, water, and dried over magnesium sulfate. After removal of the solvents, 15.0 g (48.4 mmol, 97%) of 1-iodotridecane (**4**) was obtained as a colorless liquid by vacuum distillation; bp 138-140 °C/3 mm Hg ; ^{1}H NMR (200 MHz, $CDCl_3$), δ 3.19 (t, J = 6.97 Hz, 2 H, CH_2I), 1.82 (m, 2 H, $C\underline{H}_2CH_2I$), 1.26-1.42 (m, 20 H, $(CH_2)_{10}$), 0.88 (t, J = 6.22 Hz, 3 H, ω-CH_3).

1,3-Heptadecadiyne (5). To a solution of 3.89 g (20 mmol) of 1,4-bis(trimethylsilyl)-1,3-butadiyne (**2**) in 40 mL of THF was added dropwise 13.36 mL (20 mmol) of methyllithium-lithium bromide complex (a 1.5 M solution in ether) at -78 °C. After the reaction mixture was stirred for 3.5 h at room temperature under nitrogen, a solution of 6.21 g (20 mmol) of 1-iodotridecane (**4**) in 30 mL of HMPA was added dropwise at -78 °C, and

stirring was continued for 3.5 h at room temperature under nitrogen. The pH was adjusted to 7.0 by addition of 3 N HCl at 0 °C. The organic layer was separated and the aqueous layer was extracted three times with hexane. The combined organic layer was dried over magnesium sulfate and evaporated. A slurry of 3.76 g (40 mmol) of potassium fluoride dihydrate in 40 mL of DMF was added and the mixture was stirred for 30 min at room temperature. The pH was adjusted to 2.0 by addition of 3 N HCl. The aqueous layer was extracted three times with hexane. The combined organic layer was washed with saturated sodium bicarbonate solution, 10% sodium thiosulfate solution, brine, and dried over magnesium sulfate. Removal of the solvents gave a residue that was purified by flash chromatography (elution with hexane) to yield 3.60 g (15.5 mmol, 77%) of 1,3-heptadecadiyne (**5**) as a colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.26 (t, J = 6.55 Hz, 2 H, $CH_2C{\equiv}C$), 1.95 (s, 1 H, $C{\equiv}CH$), 1.26-1.54(m, 22 H, $(CH_2)_{11}$), 0.88 (t, J = 6.23 Hz, 3 H, ω-CH_3).

2,4-Octadecadiyn-1-ol (**6**). To a solution of 94 mg (0.40 mmol) of 1,3-heptadecadiyne (**5**) in 2 mL of ethyl ether was added dropwise 267 μL (0.40 mmol) of methyllithium (a 1.5 M solution in ether). After the reaction mixture was stirred for 0.5 h at -78 °C under nitrogen, 100 mg (3.30 mmol) of paraformaldehyde (dried for 5 h over phosphorus pentoxide under vacuum at 78 °C) in 3 mL of THF were added at -78 °C. The mixture was warmed slowly to room temperature and stirred overnight. It was poured into 20 mL of ice water and shaken vigorously. The organic layer was separated and the aqueous layer was extracted three times with 15 mL of ether. The combined ethereal layer was washed with 15 mL of saturated ammonium chloride

solution, then dried over magnesium sulfate. Removal of the solvents gave a yellow solid that was purified by flash chromatography (elution with 9:1 hexane/EtOAc) to yield 91.2 mg (0.35 mmol, 86%) of 2,4-octadecadiyn-1-ol (**6**) as a white solid, mp 68-69 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.30 (d, J = 5.77 Hz, 2H, CH_2OH), 2.28 (t, J = 6.84 Hz, 2 H, $CH_2C{\equiv}C$), 1.81 (s, 1 H, OH), 1.26-1.57 (m, 22 H, $(CH_2)_{11}$), 0.88 (t, J = 6.38 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{30}O$ (262.43): C, 82.38; H, 11.52. Found: C, 82.86; H, 11.69.

2,4-Octadecadiynoic acid (**7**). To a solution of 81.2 mg (0.35 mmol) of 1,3-heptadecadiyne (**5**) in 2 mL of THF/ether (1:1, v/v) was added dropwise 260 μL (0.36 mmol) of methyllithium (a 1.4 M solution in ether). The reaction mixture was stirred for 45 min at -78 °C. Dry ice was placed in a flask, and carbon dioxide was allowed to pass through 10 mL of concentrated sulfuric acid and then into the reaction mixture over a period of 1 h at 0-5 °C. The pH was adjusted to 2.0 by addition of 2 N HCl. Water (15 mL) and 15 mL of ether were added, and the organic layer was separated. The aqueous layer was extracted three times with 15 mL of ether. The combined organic layer was washed with saturated sodium bicarbonate solution, brine, and dried over magnesium sulfate. Removal of the solvents gave 73.2 mg (0.27 mmol, 76%) of 2,4-octadecadiynoic acid (**7**) as a white solid; IR ($CHCl_3$) 2245 ($C{\equiv}CC{\equiv}C$), 1688 cm^{-1} (C=O); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.35 (t, 2 H, $CH_2C{\equiv}C$), 1.26-1.56 (m, 22 H, $(CH_2)_{11}$), 0.88 (t, J = 6.40 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{28}O_2$ (276.42): C, 78.21; H, 10.21. Found: C, 77.40; H, 10.33.

General Procedure for the Preparation of 1-Bromo-ω-(methoxymethoxy)alkane (8). To a stirred suspension of 5.0 g (35.2 mmol) of phosphorus pentoxide in 70 mL (60.2 g, 791 mmol) of dimethoxymethane and 40 mL of methylene chloride was added dropwise a solution of 10.0 mmol of ω-bromoalkan-1-ol in 10 mL of methylene chloride. After the reaction mixture was stirred overnight at room temperature, the solution was decanted to obtain a residue which was dissolved in 40 mL of water and extracted three times with methylene chloride. The combined organic layer was washed with saturated sodium bicarbonate solution and dried over sodium sulfate. The solvents were removed to give a residue that was purified by flash chromatography (elution with 15:1 hexane/EtOAc) to yield 1-bromo-ω-(methoxymethoxy)alkane (8) as a colorless liquid.

1-Bromo-3-(methoxymethoxy)propane (8a): 91% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.63 (s, 2 H, OCH_2O), 3.66 (t, J = 5.86 Hz, 2 H, CH_2O), 3.53 (t, J = 6.50 Hz, 2 H, CH_2Br), 3.37 (s, 3 H, OCH_3), 2.12 (m, 2 H, $CH_2C\underline{H}_2CH_2$). Anal. Calcd for $C_5H_{11}O_2Br$: C, 32.81; H, 6.06. Found: C, 33.15; H, 5.94.

1-Bromo-4-(methoxymethoxy)butane (8b): 86% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.61 (s, 2 H, OCH_2O), 3.56 (t, J = 6.13 Hz, 2 H, CH_2O), 3.45 (t, J = 6.62 Hz, 2 H, CH_2Br), 3.36 (s, 3 H, OCH_3), 1.68-2.10 (m, 4 H, $(CH_2)_2$). Anal. Calcd for $C_6H_{13}O_2Br$: C, 36.57; H, 6.65. Found: C, 36.21; H, 6.37.

1-Bromo-7-(methoxymethoxy)heptane (8c): 88% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.62 (s, 2 H, OCH_2O), 3.52 (t, J = 6.45 Hz, 2 H, CH_2O), 3.41 (t, J = 6.80 Hz, 2 H, CH_2Br), 3.36 (s, 3 H, OCH_3), 1.38-1.90 (m, 10 H,

$(CH_2)_5$). Anal. Calcd for $C_9H_{19}O_2Br$: C, 45.20; H, 8.01. Found: C, 44.96; H, 7.84.

1-Bromo-11-(methoxymethoxy)undecane (8d): 95% yield; ^1H NMR (200 MHz, $CDCl_3$) δ 4.62 (s, 2 H, OCH_2O), 3.51 (t, J = 6.48 Hz, 2 H, CH_2O), 3.40 (t, J = 6.99 Hz, 2 H, CH_2Br), 3.36 (s, 3 H, OCH_3), 1.29-1.89 (m, 18 H, $(CH_2)_9$). Anal. Calcd for $C_{13}H_{27}O_2Br$: C, 52.88; H, 9.22. Found: C, 52.38; H, 9.06.

General Procedure for the Preparation of ω-(Methoxymethoxy)-1,3-alkadiyne (9). To a solution of 10.0 mmol of 1,4-bis(trimethylsilyl)-1,3-butadiyne (**2**) in 20 mL of THF was added dropwise 6.7 mL (10.0 mmol) of methyllithium-lithium bromide complex (a 1.5 M solution in ether) at -78 °C. After the reaction mixture was warmed to room temperature and stirred for 3.5 h under nitrogen, a solution of 12.0 mmol of 1-bromo-ω-(methoxymethoxy)alkane (**8**) in 20 mL of HMPA was added dropwise at -78 °C. Then the mixture was stirred for 30 min at room temperature. The pH was adjusted to 7.0 by addition of 3 N HCl at 0 °C. After the layers were separated, the aqueous layer was extracted three times with 20 mL of hexane. Removal of the solvents from the combined organic layer gave a residue. A slurry of 1.88 g (20 mmol) of potassium fluoride dihydrate in 20 mL of DMF was added, and the mixture was stirred for 30 min at room temperature. The reaction mixture was poured into 15 mL of 3 N HCl solution with stirring at 0 °C. The layers were separated and the aqueous layer was extracted three times with hexane. The combined organic layer was washed with 3 N HCl, saturated sodium bicarbonate solution, brine, and dried over

sodium sulfate. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 20:1 hexane/EtOAc) to yield ω-(methoxymethoxy)-1,3-alkadiyne (**9**) as a light-yellow liquid.

7-(Methoxymethoxy)-1,3-heptadiyne (9a): 84% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.58 (s, 2 H, OCH_2O), 3.57 (t, J = 6.05 Hz, 2 H, CH_2O), 3.32 (s, 3 H, OCH_3), 2.36 (t, J = 6.99 Hz, 2 H, $CH_2C{\equiv}C$), 1.95 (s, 1 H, $C{\equiv}CH$), 1.75-1.82 (m, 2 H, $CH_2C\underline{H}_2CH_2$).

8-(Methoxymethoxy)-1,3-octadiyne (9b): 78% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.60 (s, 2 H, OCH_2O), 3.54 (t, J = 5.91 Hz, 2 H, CH_2O), 3.35 (s, 3 H, OCH_3), 2.32 (t, J = 6.20 Hz, 2 H, $CH_2C{\equiv}C$), 2.01 (s, 1 H, $C{\equiv}CH$), 1.65 (m, 4 H, $(CH_2)_2$).

11-(Methoxymethoxy)-1,3-undecadiyne (9c): 91% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.61 (s, 2 H, OCH_2O), 3.52 (t, J = 6.49 Hz, 2 H, CH_2O), 3.36 (s, 3 H, OCH_3), 2.26 (t, J = 6.81 Hz, 2 H, $CH_2C{\equiv}C$), 1.98 (s, 1 H, $C{\equiv}CH$), 1.35-1.62 (m, 10 H, $(CH_2)_5$). Anal. Calcd for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68. Found: C, 73.95; H, 9.41.

15-(Methoxymethoxy)-1,3-pentadecadiyne (9d): 89% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.62 (s, 2 H, OCH_2O), 3.52 (t, J = 6.52 Hz, 2 H, CH_2O), 3.36 (s, 3 H, OCH_3), 2.26 (t, J = 6.68 Hz, 2 H, $CH_2C{\equiv}C$), 1.97 (s, 1 H, $C{\equiv}CH$), 1.28-1.58 (m, 18 H, $(CH_2)_9$). Anal. Calcd for $C_{17}H_{28}O_2$: C, 77.22; H, 10.67. Found: C, 77.01; H, 10.60.

General Procedure for the Preparation of 1-(Methoxymethoxy)alkadiyne (10). To a solution of 2.0 mmol of ω-(methoxymethoxy)-1,3-alkadiyne (**9**) in 8 mL of THF was added dropwise 1.5

mL (2.4 mmol) of *n*-butyllithium (a 1.6 M solution in hexane) at -23 °C. After the reaction mixture was stirred for 1 h at -23 °C under nitrogen, a solution of 2.4 mmol of 1-iodoalkane in 8 mL of HMPA was added dropwise. The reaction mixture was stirred for 30 min at -23 °C and then for 1.5 h at room temperature. The pH was adjusted to 6.0 with 3 N HCl. After the layers were separated, the aqueous layer was extracted three times with hexane. The combined organic layer was washed with saturated sodium bicarbonate solution, brine, and dried over sodium sulfate. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 25:1 hexane/EtOAc) to yield 1-(methoxymethoxy)alkadiyne (**10**) as a colorless liquid.

1-(Methoxymethoxy)-4,6-octadecadiyne (10a): 63% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.62 (s, 2 H, OCH_2O), 3.61 (t, J = 6.14 Hz, 2 H, CH_2O), 3.36 (s, 3 H, OCH_3), 2.38 (t, J = 7.01 Hz, 2 H, $C{\equiv}CCH_2(CH_2)_2O$), 2.24 (t, J = 6.83 Hz, 2 H, $CH_2C{\equiv}C$), 1.80 (m, 2 H, CH_2CH_2O), 1.26-1.55 (m, 18 H, $(CH_2)_9$), 0.88 (t, J = 6.40 Hz, 3 H, ω-CH_3).

1-(Methoxymethoxy)-5,7-octadecadiyne (10b): 71% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.59 (s, 2 H, OCH_2O), 3.51 (t, J = 6.05 Hz, 2 H, CH_2O), 3.33 (s, 3 H, OCH_3), 2.18-2.31 (m, 4 H, $(CH_2C{\equiv}C)_2$), 1.23-1.68 (m, 20 H, $(CH_2)_{10}$), 0.85 (t, J = 6.37 Hz, 3 H, ω-CH_3).

1-(Methoxymethoxy)-8,10-octadecadiyne (10c): 78% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.61 (s, 2 H, OCH_2O), 3.51 (t, J = 6.47 Hz, 2 H, CH_2O), 3.36 (s, 3 H, OCH_3), 2.24 (t, J = 6.64 Hz, 4 H, $(CH_2C{\equiv}C)_2$), 1.27-1.62 (m, 20 H, $(CH_2)_{10}$), 0.88 (t, J = 6.43 Hz, 3 H, ω-CH_3).

1-(Methoxymethoxy)-12,14-octadecadiyne (10d): 73% yield;

^{1}H NMR (200 MHz, $CDCl_3$) δ 4.62 (s, 2 H, OCH_2O), 3.52 (t, J = 6.51 Hz, 2 H, CH_2O), 3.36 (s, 3 H, OCH_3), 2.19-2.26 (m, 4 H, $(CH_2C{\equiv}C)_2$), 1.26-1.69 (m, 20 H, $(CH_2)_{10}$), 0.98 (t, J = 7.33 Hz, 3 H, ω-CH_3).

1-(Methoxymethoxy)-12,14-pentacosadiyne (10e): 77% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.62 (s, 2 H, OCH_2O), 3.52 (t, J = 6.55 Hz, 2 H, CH_2O), 3.36 (s, 3 H, OCH_3), 2.24 (t, J = 6.77 Hz, 4 H, $(CH_2C{\equiv}C)_2$), 1.26-1.62 (m, 34 H, $(CH_2)_{17}$), 0.88 (t, J = 6.39 Hz, 3 H, ω-CH_3).

1-(Methoxymethoxy)-12,14-heptacosadiyne (10f): 79% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.62 (s, 2 H, OCH_2O), 3.52 (t, J = 6.56 Hz, 2 H, CH_2O), 3.36 (s, 3 H, OCH_3), 2.24 (t, J = 6.79 Hz, 4 H, $(CH_2C{\equiv}C)_2$), 1.26-1.63 (m, 38 H, $(CH_2)_{19}$), 0.88 (t, J = 6.41 Hz, 3 H, ω-CH_3).

General Procedure for the Preparation of Alkadiyn-1-ol (11). To a solution of 1.0 mmol of 1-(methoxymethoxy)alkadiyne (**10**) in 25 mL of methanol was added 3 mL of 37% hydrochloric acid. The reaction mixture was stirred for 24 h at room temperature. After the reaction mixture was evaporated to remove methanol, 25 mL of water and 25 mL of chloroform were added and the mixture was stirred for 5 min. The layers were separated and the aqueous layer was extracted three times with chloroform. The combined organic layer was washed with saturated sodium bicarbonate solution, brine, and dried over sodium sulfate. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 6:1 hexane/EtOAc) to yield alkadiyn-1-ol (**11**) as a light-yellow liquid or white solid.

4,6-Octadecadiyn-1-ol (11a): 92% yield; mp 37-39 °C; ^{1}H NMR

(200 MHz, $CDCl_3$) δ 3.74 (t, *J* = 6.16 Hz, 2 H, $C\underline{H}_2OH$), 2.38 (t, *J* = 6.95 Hz, 2 H, $C{\equiv}CC\underline{H}_2(CH_2)_2O$), 2.24 (t, *J* = 6.85 Hz, 2 H, $CH_2C{\equiv}C$), 1.70-1.83 (m, 3 H, $C\underline{H}_2CH_2O$ and $O\underline{H}$), 1.23-1.55 (m, 18 H, $(CH_2)_9$), 0.88 (t, *J* = 6.39 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{30}O$: C, 82.38; H, 11.52. Found: C, 82.00; H, 11.56.

5,7-Octadecadiyn-1-ol (11b): 93% yield; light-yellow liquid; ^1H NMR (200 MHz, $CDCl_3$) δ 3.67 (t, *J* = 5.84 Hz, 2 H, $C\underline{H}_2OH$), 2.21-2.34 (m, 4 H, $(CH_2C{\equiv}C)_2$), 1.26-1.66 (m, 21 H, $(CH_2)_{10}$ and OH), 0.88 (t, *J* = 5.83 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{30}O$: C, 82.38; H, 11.52. Found: C, 82.36; H, 11.58.

8,10-Octadecadiyn-1-ol (11c): 92% yield; mp 23-24 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 3.63 (t, *J* = 6.48 Hz, 2 H, $C\underline{H}_2OH$), 2.25 (t, *J* = 6.59 Hz, 4 H, $(CH_2C{\equiv}C)_2$), 1.27-1.56 (m, 21 H, $(CH_2)_{10}$ and OH), 0.88 (t, *J* = 6.36 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{30}O$: C, 82.38; H, 11.52. Found: C, 82.56; H, 11.76.

12,14-Octadecadiyn-1-ol (11d): 93% yield; mp 33-34 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 3.63 (t, *J* = 6.55 Hz, 2 H, $C\underline{H}_2OH$), 2.19-2.26 (m, 4 H, $(CH_2C{\equiv}C)_2$), 1.27-1.64 (m, 20 H, $(CH_2)_{10}$), 0.98 (t, *J* = 7.33 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{30}O$: C, 82.38; H, 11.52. Found: C, 81.45; H, 11.39.

12,14-Pentacosadiyn-1-ol (11e): 93% yield; mp 58-59 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 3.64 (t, *J* = 6.34 Hz, 2 H, $C\underline{H}_2OH$), 2.24 (t, *J* = 6.61 Hz, 4 H, $(CH_2C{\equiv}C)_2$), 1.26-1.51 (m, 35 H, $(CH_2)_{17}$ and OH), 0.88 (t, *J* = 6.01 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{25}H_{44}O$: C, 83.27; H, 12.30. Found: C, 83.22; H, 12.16.

12,14-Heptacosadiyn-1-ol (11f): 91% yield; mp 58-59 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 3.64 (t, *J* = 6.34 Hz, 2 H, $C\underline{H}_2OH$), 2.24 (t, *J* = 6.73 Hz, 4 H, $(CH_2C{\equiv}C)_2$), 1.26-1.55 (m, 39 H, $(CH_2)_{19}$ and OH), 0.88 (t, *J* = 6.06

Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{27}H_{48}O$: C, 83.44; H, 12.45. Found: C, 83.22; H, 12.64.

General Procedure for the Preparation of Alkadiynoic acid (12). To 0.5 mmol of alkadiyn-1-ol (**11**) in 2 mL of DMF was added a solution of 1.5 g (4.0 mmol) PDC in 3 mL of DMF. The reaction mixture was stirred for 24 h at room temperature. The reaction mixture was poured into 10 volumes of water, acidified with 3 N HCl until the pH reached 4.0, and extracted three times with ether. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 500:100:5 hexane/EtOAc/ 85% formic acid) to yield alkadiynoic acid (**12**) as a white solid.

4,6-Octadecadiynoic acid (12a): 72% yield; mp 74-75 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.20 (br s, 1 H, CO_2H), 2.59 (br s, 4 H, $C{\equiv}CCH_2CH_2CO_2$), 2.24 (t, J = 6.82 Hz, 2 H, $CH_2C{\equiv}C$), 1.26-1.55 (m, 18 H, $(CH_2)_9$), 0.89 (t, J = 6.38 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{28}O_2$: C, 78.21; H, 10.21. Found: C, 78.17; H, 10.34.

5,7-Octadecadiynoic acid (12b): 60% yield; mp 47-49 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.21 (br s, 1 H, CO_2H), 2.51 (t, J = 7.35 Hz, 2 H, CH_2CO_2), 2.36 (t, J = 6.79 Hz, 2 H, $C{\equiv}CC\underline{H}_2(CH_2)_2CO_2$), 2.24 (t, J = 6.83 Hz, 2 H, $CH_2C{\equiv}C$), 1.85 (m, 2 H, $C\underline{H}_2CH_2CO_2$), 1.26-1.56 (m, 16 H, $(CH_2)_8$), 0.88 (t, J = 6.40 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{28}O_2$: C, 78.21; H, 10.21. Found: C,77.91; H, 10.31.

8,10-Octadecadiynoic acid (12c): 75% yield; mp 42-43 °C (lit. [22] mp 41.5-42 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 11.23 (br s, 1 H, CO_2H), 2.36 (t, J = 7.38 Hz, 2 H, CH_2CO_2), 2.25 (t, J = 6.55 Hz, 4 H, $(CH_2C{\equiv}C)_2$),

1.27-1.71 (m, 18 H, $(CH_2)_9$), 0.88 (t, J = 6.34 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{28}O_2$: C, 78.21; H, 10.21. Found: C, 78.36; H, 10.26.

12,14-Octadecadiynoic acid (12d): 75% yield; mp 58-59 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.62 (br s, 1 H, CO_2H), 2.35 (t, J = 7.44 Hz, 2 H, CH_2CO_2), 2.23 (m, 4 H, $(CH_2C{\equiv}C)_2$), 1.28-1.67 (m, 18 H, $(CH_2)_9$), 0.99 (t, J = 7.33 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{28}O_2$: C, 78.21; H, 10.21. Found: C, 77.34; H, 10.36.

12,14-Pentacosadiynoic acid (12e): 85% yield; mp 57-59 °C (lit. [21c] mp 52-54 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.72 (br s, 1 H, CO_2H), 2.35 (t, J = 7.43 Hz, 2 H, CH_2CO_2), 2.24 (t, J = 6.77 Hz, 4 H, $(CH_2C{\equiv}C)_2$), 1.26-1.67 (m, 32 H, $(CH_2)_{16}$), 0.88 (t, J = 6.40 Hz, 3 H, ω-CH_3).

12,14-Heptacosadiynoic acid (12f): 86% yield; mp 62-63 °C (lit. [21c] mp 38-40 °C); ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.81 (br s, 1 H, CO_2H), 2.35 (t, J = 7.45 Hz, 2 H, CH_2CO_2), 2.24 (t, J = 6.77 Hz, 4 H, $(CH_2C{=}C)_2$), 1.26-1.67 (m, 36 H, $(CH_2)_{18}$), 0.88 (t, J = 6.36 Hz, 3 H, ω-CH_3).

1,2-Di-(4',6'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (13a). To a suspension of 247 mg (0.5 mmol) of *sn*-glycerophosphocholine-cadmium chloride complex (dried for 5 h over phosphorus pentoxide under vacuum at 78 °C), 122 mg (1.0 mmol) of DMAP, and 586 mg (2.12 mmol) of 4,6-octadecadiynoic acid (**12a**) in 6 mL of freshly distilled alcohol-free chloroform was added 437 mg (2.12 mmol) of DCC. The reaction mixture was stirred for 60 h at room temperature under nitrogen in the dark. Chloroform (15 mL) was added and the mixture was filtered through a Celite 545 pad, which was washed with 15 mL of chloroform. Removal of

the solvents gave a residue that was purified by flash chromatography (elution first with 100% $CHCl_3$, then with 90:10, 60:40, 20:80 $CHCl_3/CH_3OH$) to yield a white solid. The suspended silica gel was removed by filtering a chloroform solution of the solid through a 0.45-μm Metricel filter three times. The product was lyophilized with benzene, affording 352 mg (0.45 mmol, 81%) of 1,2-di-(4',6'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (**13a**) as a white solid; $[\alpha]^{25}_D$ +4.41° (*c* 1.125, $CHCl_3/CH_3OH$ 1:1); ^1H NMR (300 MHz, $CDCl_3$) δ 5.21 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.74-4.42 (m, 14 H, $POC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2CHC\underline{H}_2$, $(\underline{H}_2O)_3$), 3.33 (s, 9 H, $N(CH_3)_3$), 2.56 (m, 8 H, $(OCCH_2CH_2C{\equiv}C)_2$), 2.24 (t, 4 H, $(CH_2C{\equiv}C)_2$), 1.19-1.55 (m, 36 H, $(CH_2)_{18}$), 0.87 (t, 6 H, $(\omega\text{-}CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.42 ($CH_2O\underline{C}O$), 171.12 ($CH_2O\underline{C}O$), 78.42, 78.34, 77.42, 77.00, 76.58, 74.99, 70.99, 70.91, 66.24, 66.17, 66.05, 64.99, 63.24, 59.32, 59.28, 54.26, 32.95, 32.77, 31.87, 29.60, 29.49, 29.32, 29.10, 28.92, 28.33, 22.65, 19.15, 14.97 ($\omega\text{-}CH_3$), 14.08 ($\omega\text{-}CH_3$). Anal. Calcd for $C_{44}H_{72}O_8NP{\cdot}3H_2O$: C, 63.82; H, 9.49; N, 1.69. Found: C, 63.38; H, 9.10; N, 1.72.

1,2-Di-(10',12'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (**13b**). This compound was prepared from 10,12-octadecadiynoic acid (**12g**) in 52% yield by using the procedure described for the preparation of 1,2-di-(4',6'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (**13a**); $[\alpha]^{25}_D$ -3.10° (*c* 1.55, $CHCl_3/CH_3OH$ 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 5.17 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.67-4.42 (m, 12 H, $POC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2CHC\underline{H}_2$, $(\underline{H}_2O)_2$), 3.35 (s, 9 H, $N(CH_3)_3$), 2.14-2.35 (m, 12 H, $(CH_2CO_2)_2$, $(CH_2C{\equiv}C)_4$), 1.30-1.56 (m, 36 H, $(CH_2)_{18}$), 0.90 (t, J = 6.97 Hz, 6

H, (ω-CH_3)$_2$). Anal. Calcd for $C_{44}H_{72}O_8NP{\cdot}2H_2O$: C, 65.24; H, 9.46; N, 1.73; P, 3.82. Found: C, 64.69; H, 9.04; N, 1.39; P, 3.15.

1,2-Didecanoyl-*sn*-glycero-3-phosphocholine (13c). This compound was prepared from decanoic acid (**12h**) in 63% yield by using the procedure described for the preparation of 1,2-di-(4',6'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (**13a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.20 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.66-4.38 (m, 8 H, $POC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2CHC\underline{H}_2$), 3.40 (s, 9 H, $N(CH_3)_3$), 2.24-2.33 (m, 4 H, $(CH_2CO_2)_2$), 1.58 (br s, 4 H, $(C\underline{H}_2CH_2CO_2)_2$), 1.27 (m, 24 H, $(CH_2)_{12}$), 0.88 (t, J = 6.39 Hz, 6 H, (ω-CH_3)$_2$).

1-(4',6'-Octadecadiynoyl)-2-(5'',7''-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (14a). To a solution of 70 mg (0.090 mmol) of 1,2-di-(4',6'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (**13a**) in 10 mL of 49:1 ether/CH_3OH solution were added 2 mL of 100 mM sodium borate buffer (pH 7.4) containing 4 mg/mL of calcium acetate and 300 μL (150 units) of phospholipase A_2 solution. The reaction mixture was stirred for 96 h at room temperature in the dark. TLC analysis (elution with 95:35:6 $CHCl_3/CH_3OH/H_2O$) indicated complete conversion of diacyl-PC **13a** (R_f = 0.48) into the desired lyso-PC (R_f = 0.27). Water (10 mL) was added and the mixture was extracted three times with 25 mL of ether. The lyso-PC remained in the aqueous layer, whereas the liberated fatty acid was in the ether layer. The aqueous layer was extracted three times with 25 mL of 2:1 $CHCl_3/CH_3OH$ solution, and then the combined organic layer was dried over sodium sulfate. After removal of the solvents, lyso-PC was obtained as a

white solid. A solution of 24 mg (0.2 mmol) of DMAP and 55 mg (0.2 mmol) of 5,7-octadecadiynoic acid (**12b**) in 2 mL of freshly distilled alcohol-free chloroform was added at 0 °C, followed by 83 mg (0.4 mmol) of DCC. The reaction mixture was stirred for 48 h at room temperature (water bath) under nitrogen in the dark. Removal of the solvents gave a residue that was purified by flash chromatography (elution first with 100% $CHCl_3$, then with 90:10, 60:40, 20:80 $CHCl_3/CH_3OH$) to yield a light yellow residue. The suspended silica gel was removed by filtering a chloroform solution of the residue through a 0.45-μm Metricel filter three times. The product was lyophilized with benzene, affording 38 mg (0.049 mmol, 54%) of 1-(4',6'-octadecadiynoyl)-2-(5",7"-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (**14a**) as a white solid; $[\alpha]^{25}_D$ +7.15° (*c* 1.02, $CHCl_3/CH_3OH$ 1:1); ^1H NMR (300 MHz, $CDCl_3$) δ 5.20 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.59-4.42 (m, 14 H, $POC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2CHC\underline{H}_2$, $(\underline{H}_2O)_3$), 3.36 (s, 9 H, $N(CH_3)_3$), 2.21-2.85 (m, 12 H, $(CH_2CO_2)_2$, $(CH_2C{\equiv}CCH_2)_2$), 1.26-1.83 (m, 36 H, $(CH_2)_{18}$), 0.88 (t, J = 6.34 Hz, 6 H, $(\omega\text{-}CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.34 ($CH_2O\underline{C}O$), 171.36 ($CH_2O\underline{C}O$), 104.87, 78.38, 78.13, 77.42, 77.26, 77.21, 77.00, 76.57, 75.96, 74.92, 70.65, 66.36, 66.21, 66.04, 65.05, 64.98, 64.89, 63.44, 63.35, 63.29, 54.46, 34.75, 32.93, 32.76, 31.86, 29.58, 29.47, 29.30, 29.19, 29.08, 28.89, 28.52, 28.31, 26.97, 25.84, 23.54, 22.65, 19.15, 18.53, 14.95 ($\omega\text{-}CH_3$), 14.09 ($\omega\text{-}CH_3$). Anal. Calcd for $C_{44}H_{72}O_8NP{\cdot}4H_2O$: C, 62.46; H, 9.53; N, 1.66. Found: C, 61.82; H, 9.15; N, 1.54.

1-Decanoyl-2-(10',12'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (14b). This compound was prepared by phospholipase

A_2 treatment of 1,2-didecanoyl-*sn*-glycero-3-phosphocholine (**13c**) followed by acylation of the lyso-PC with 10,12-octadecadiynoic acid (**12g**). The same procedure described for the preparation of 1-(4',6'-octadecadiynoyl)-2-(5",7"-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (**14a**) was used. The product was obtained as a white solid in 60% overall yield; $[\alpha]^{25}_D$ +2.95° (*c* 0.84, $CHCl_3/CH_3OH$ 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 5.20 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.70-4.42 (m, 16 H, $POC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2CHC\underline{H}_2$, $(\underline{H}_2O)_4$), 3.33 (s, 9 H, $N(CH_3)_3$), 2.21-2.34 (m, 8 H, $(CH_2CO_2)_2$, $(CH_2C{\equiv}C)_2$), 1.27-1.56 (m, 32 H, $(CH_2)_{16}$), 0.86-0.93 (m, 6 H, $(\omega\text{-}CH_3)_2$). Anal. Calcd for $C_{36}H_{64}O_8NP{\cdot}2H_2O$: C, 61.25; H, 9.71; N, 1.98; P, 4.39. Found: C, 61.08; H, 9.79; N, 2.20; P, 4.65.

1-Octadecanoyl-2-(4',6'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (14c). To a suspension of 105 mg (0.2 mmol) of 1-octadecanoyl-*sn*-glycero-3-phosphocholine in 2 mL of freshly distilled alcohol-free chloroform were added 55.3 mg (0.2 mmol) of 4,6-octadecadiynoic acid (**12a**) and 24 mg (0.2 mmol) of DMAP in 2 mL of freshly distilled alcohol-free chloroform, followed by 83 mg (0.4 mmol) of DCC at 0 °C. The reaction mixture was stirred for 0.5 h at 0 °C and then for 48 h at room temperature (water bath) under nitrogen in the dark. Removal of the solvents gave a residue that was purified by flash chromatography (elution first with 100% $CHCl_3$, then with 90:10, 60:40, 20:80 $CHCl_3/CH_3OH$) to yield a light yellow solid. The suspended silica gel was removed by filtering a chloroform solution of the solid through a 0.45-μm Metricel filter three times. The product was lyophilized with benzene, affording 92.4 mg (0.12 mmol, 59%) of 1-octadecanoyl-2-(4",6"-octadecadiynoyl)-*sn*-glycero-3-

phosphocholine (**14c**) as a light-yellow solid; $[\alpha]^{25}_D$ -2.92° (*c* 1.27, $CHCl_3/CH_3OH$ 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 5.21 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.67-4.62 (m, 8 H, $POC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2CHC\underline{H}_2$), 3.36 (s, 9 H, $N(CH_3)_3$), 2.20-2.56 (m, 8 H, $(CH_2CO_2)_2$, $(CH_2C{\equiv}C)_2$), 1.25-1.61 (m, 50 H, $(CH_2)_{25}$), 0.88 (t, *J* = 6.01 Hz, 6 H, $(\omega\text{-}CH_3)_2$). Anal. Calcd for $C_{44}H_{80}O_8NP{\cdot}H_2O$: C, 66.05; H, 10.33; N, 1.75; P, 3.87. Found: C, 65.87; H, 10.40; N, 2.01; P, 3.99.

(*R*)-(+)-Oxiranemethanol TBDPS Ether (**15**). Epoxide **15** was prepared in 91% yield by asymmetric epoxidation of allyl alcohol using L-(+)-diisopropyl tartrate (DIPT) followed by trapping of the resulting glycidol with TBDPS chloride using the procedure of Gao et al. [47] with minor modification; $[\alpha]^{25}_D$ +2.41° (*c* 3.20, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.37-7.67 (m, 10 H, phenyl), 3.66-3.90 (m, 2 H, $OC\underline{H}_2CH$), 3.14 (br s, 1 H, $OC\underline{H}CH_2$), 2.58-2.77 (m, 2 H, CH_2OSi), 1.06 (s, 9 H, $SiC(CH_3)_3$).

1-*O*-(4',6'-Octadecadiynyl)-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**16**). To a mixture of 1.31 g (5.0 mmol) of 4,6-octadecadiyn-1-ol (**11a**) and 2.30 g (6.5 mmol) of (*R*)-(+)-oxiranemethanol TBDPS ether (**15**) in 40 mL of alcohol-free chloroform was added 50 drops of a solution of 10% $BF_3{\cdot}OEt_2$ in CH_2Cl_2 at -23 °C under nitrogen. The reaction mixture was stirred for 30 min at -23 °C and then for 48 h at 4 °C. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 9:1 hexane/EtOAc) to yield 1.78 g (3.09 mmol, 62%) of 1-*O*-(4',6'-octadecadiynyl)-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**16**) as a colorless liquid; $[\alpha]^{25}_D$ +1.27° (*c* 1.95, $CHCl_3$); $[\alpha]^{25}_D$ +1.43° (*c* 2.24, hexane); ^1H NMR (200 MHz,

$CDCl_3$) δ 7.37-7.69 (m, 10 H, phenyl), 3.87 (br s, 1 H, $CH_2C\underline{H}CH_2$), 3.48-3.71 (m, 6 H, $C\underline{H}_2OC\underline{H}_2CHC\underline{H}_2$), 2.47 (br s, 1 H, OH), 2.27 (m, 4 H, $(CH_2C{\equiv}C)_2$), 1.75 (m, 2 H, $OCH_2C\underline{H}_2CH_2C{\equiv}C$), 1.26-1.58 (m, 18 H, $(CH_2)_9$), 1.07 (s, 9 H, $SiC(CH_3)_3$), 0.88 (t, J = 6.36 Hz, 3 H, ω-CH_3). ^{13}C NMR (75 MHz, $CDCl_3$), δ 135.85, 135.69, 135.49, 133.08, 129.75, 129.66, 127.71, 104.84, 77.72, 77.42, 77.00, 76.58, 76.46, 71.51, 70.76, 70.63, 69.67, 65.62, 65.11, 64.65, 64.56, 31.87, 29.57, 29.44, 29.30, 29.01, 28.81, 28.36, 28.28, 26.80, 22.65, 19.20, 19.15, 15.97, 14.11.

1-*O*-(4',6'-Octadecadiynyl)-2-*O*-(5'',7''-octadecadiynyl)-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**18**). To a solution of 236 μL (395 mg, 1.4 mmol) of trifluoromethanesulfonic anhydride and 111 mg (1.4 mmol) of pyridine in 4 mL of CH_2Cl_2 was slowly added a solution of 280 mg (1.07 mmol) of 5,7-octadecadiyn-1-ol (**11b**) in 2.5 mL of CH_2Cl_2. The reaction mixture was stirred for 2 h at room temperature. Water (15 mL) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with brine, and dried over a mixture of anhydrous sodium carbonate and magnesium sulfate (1:1, v/v). Removal of the solvents under vacuum gave 338 mg of crude 5,7-octadecadiynyl triflate (**17**) as a light brown viscous oil. To a suspension of 310 mg (0.57 mmol) of 1-*O*-(4',6'-octadecadiynyl)-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**16**) and 17 mg (0.71 mmol) of sodium hydride in 8 mL of THF was added a solution of 280 mg (0.71 mmol) of crude 5,7-octadecadiynyl triflate (**17**) in 6 mL of THF. After the reaction mixture was stirred overnight at room temperature, 15 mL of water was added. The resulting mixture was

extracted with ether and dried over magnesium sulfate. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 20:1 hexane/EtOAc) to yield 274 mg (0.33 mmol, 62%) of 1-*O*-(4',6'-octadecadiynyl)-2-*O*-(5'',7''-octadecadiynyl)-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**18**) as a colorless oil; $[\alpha]^{25}_D$ +2.14° (*c* 1.535, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.26-7.70 (m, 10 H, phenyl), 3.36-3.70 (m, 9 H, CH_2CHCH_2, $(OCH_2)_2$), 2.20-2.34 (m, 8 H, $(CH_2C{\equiv}C)_4$), 1.26-1.78 (m, 40 H, $(CH_2)_{20}$), 1.05 (s, 9 H, $SiC(CH_3)_3$), 0.88 (t, *J* = 6.37 Hz, 6 H, $(\omega\text{-}CH_3)_2$). Anal. Calcd for $C_{55}H_{82}O_3Si$: C, 80.63; H, 10.09. Found: C, 79.41; H, 10.08.

1-*O*-(4',6'-Octadecadiynyl)-2-*O*-(5'',7''-octadecadiynyl)-*sn*-glycerol (19). To a solution of 106 mg (0.13 mmol) of 1-*O*-(4',6'-octadecadiynyl)-2-*O*-(5'',7''-octadecadiynyl)-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**18**) in 3 mL of THF was added 300 μL (0.3 mmol) of 1 M solution of tetra-*n*-butylammonium fluoride in THF. The reaction mixture was stirred for 4 h at room temperature under nitrogen. After removal of the solvents, 10 mL of water was added. The reaction mixture was extracted three times with 15 mL of ether and then dried over sodium sulfate. Removal of the solvents under vacuum gave a residue that was purified by flash chromatography (elution with 4:1 hexane/EtOAc) to yield 53 mg (0.09 mmol, 71%) of 1-*O*-(4',6'-octadecadiynyl)-2-*O*-(5'',7''-octadecadiynyl)-*sn*-glycerol (**19**) as a colorless liquid; $[\alpha]^{25}_D$ -8.04° (*c* 2.165, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 3.51-3.74 (m, 9 H, CH_2CHCH_2, $(OCH_2)_2$), 2.20-2.33 (m, 8 H, $(CH_2C{\equiv}C)_4$), 2.02 (s, 1 H, OH), 1.26-1.84 (m, 40 H, $(CH_2)_{20}$), 0.88 (t, *J* = 5.98 Hz, 6 H, $(\omega\text{-}CH_3)_2$). Anal. Calcd for $C_{39}H_{64}O_3$: C, 80.63; H, 11.10. Found: C, 80.58; H, 11.42.

1-*O*-(4',6'-Octadecadiynyl)-2-*O*-(5'',7''-octadecadiynyl)-*sn*-glycero-3-phosphocholine (20). To a solution of 52 mg (0.09 mmol) of 1-*O*-(4',6'-octadecadiynyl)-2-*O*-(5'',7''-octadecadiynyl)-*sn*-glycerol (**19**) and 75 μL (54.5 mg, 0.54 mmol) of triethylamine in 2 mL of CH_2Cl_2 was added with a syringe 22.5 μL (23 mg, 95% pure, 0.11 mmol, about 20% molar excess) of *N,N*-diisopropylmethylphosphonamidic chloride. The reaction mixture was stirred for 5 min at -5 °C and then concentrated to dryness under vacuum. 1*H*-Tetrazole (25 mg, 0.36 mmol) and 74 mg (0.27 mmol) of choline tosylate (both were dried overnight over phosphorus pentoxide under vacuum at 78 °C) were placed in the reaction flask, and the mixture was dissolved in 5 mL of 1:1 acetonitrile/THF solution. The reaction mixture was stirred for 3 h at room temperature and then evaporated to dryness under vacuum. After the residue was mostly dissolved in 3 mL of THF, 33 μL (0.1 mmol, 10% excess) of a 3 M solution of anhydrous *tert*-butyl hydroperoxide in 2,2,4-trimethylpentane was added. The mixture was stirred for 3 h at room temperature. After addition of 5 mL of ethyl acetate, the resulting solution was washed with triethylammonium hydrogen carbonate buffer (1 M, pH 7.5) to remove the excess of 1*H*-tetrazole and choline tosylate. The aqueous layer was extracted three times with chloroform. The combined organic layer was concentrated to dryness and the residue was rendered anhydrous by repeated evaporation with dry 2-propanol. Finally, 4 mL of toluene was added and the mixture was transferred to a pressure bottle. Anhydrous trimethylamine (1 mL) was added. The reaction mixture was stirred for 18 h at room temperature. Removal of the excess trimethylamine gave a residue that was purified by flash chromatography (elution with 95:35:6

$CHCl_3/CH_3OH/H_2O$) to yield a light yellow residue. The suspended silica gel was removed by filtering a chloroform solution of the residue through a 0.45-μm Metricel filter three times. The product was lyophilized with benzene, affording 39.6 mg (0.053 mmol, 59%) of 1-*O*-(4',6'-octadecadiynyl)-2-*O*-(5'',7''-octadecadiynyl)-*sn*-glycero-3-phosphocholine (**20**) as a light yellow solid; $[\alpha]^{23}_D$ -2.27° (*c* 0.90, $CHCl_3/CH_3OH$ 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 4.41 (m, 2 H, $CHCH_2OP$), 3.86 (m, 4 H, $POCH_2CH_2N$), 3.53 (m, 7 H, $CH_2OCH_2CHCH_2$), 3.31 (s, 9 H, $N(CH_3)_3$), 2.20-2.34 (m, 8 H, $(CH_2C{\equiv}C)_4$), 1.26-1.91 (m, 40 H, $(CH_2)_{20}$), 0.88 (t, J = 6.22 Hz, 6 H, $(\omega\text{-}CH_3)_2$). Anal. Calcd for $C_{44}H_{76}O_6NP{\cdot}2H_2O$: C, 67.57; H, 10.31; N, 1.79; P, 3.96. Found: C, 67.81; H, 10.32; N, 1.80; P, 3.99.

1-*O*-Octadecyl-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (21a). This compound was prepared from 1-octadecanol in 66% yield by using the procedure described for the preparation of 1-*O*-(4',6'-octadecadiynyl)-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**16**) with minor modification; $[\alpha]^{25}_D$ +2.36° (*c* 1.975, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.25-7.69 (m, 10 H, phenyl), 3.33-3.92 (m, 8 H, $CH_2OCH_2CH(OH)CH_2$), 1.25-1.55 (m, 32 H, $(CH_2)_{16}$), 1.06 (s, 9 H, $SiC(CH_3)_3$), 0.88 (t, J = 6.34 Hz, 3 H, $\omega\text{-}CH_3$). Anal. Calcd for $C_{37}H_{62}O_3Si$: C, 76.23; H, 10.72. Found: C, 75.85; H, 10.39.

1-*O*-(2',4'-Octadecadiynyl)-*sn*-glycero-3-*O*-*m*-nitrobenzenesulfonate (21b). To a mixture of 1.05 g (4.01 mmol) of 2,4-octadecadiyn-1-ol (**6**) and 866 mg (3.34 mmol) of (*R*)-(-)-glycidyl 3-

nitrobenzenesulfonate* in 30 mL of dry methylene chloride was added 30 drops of a 10% solution of BF_3-OEt_2 in methylene chloride at 0 °C under nitrogen. The reaction mixture was stirred for 18 h at room temperature. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 3:1 hexane/EtOAc) to yield 1.24 g (2.38 mmol, 71%) of 1-*O*-(2',4'-octadecadiynyl)-*sn*-glycerol-3-*O*-*m*-nitrobenzenesulfonate (**21b**) as a white solid, mp 37-39 °C; $[\alpha]^{25}_D$ -14.3° (*c* 2.0, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.27-8.80 (m, 4 H, phenyl), 4.04-4.23 (m, 5 H, $C\underline{H}_2OSO_2$, $OC\underline{H}_2C{\equiv}C$, $CH_2C\underline{H}CH_2$), 3.58 (d, 2 H, $C\underline{H}_2OCH_2C{\equiv}C$), 2.28 (t, *J* = 6.87 Hz, 2 H, $CH_2C{\equiv}C$), 1.14-1.61 (m, 22 H, $(CH_2)_{11}$), 0.88 (t, *J* = 6.29 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{27}H_{39}O_7NS$: C, 62.17; H, 7.54; N, 2.68. Found: C, 62.25; H, 7.66; N, 2.68.

* (*S*)-(+)-Glycidyl 3-nitrobenzenesulfonate of 99% ee is reported to have $[\alpha]^{25}_D$ +23.0° (*c* 2.14, $CHCl_3$) [54]. For our lot of (*R*)-(-)-glycidyl 3-nitrobenzenesulfonate, we obtained $[\alpha]^{25}_D$ -21.72°. After recrystallization twice from ethanol, we obtained $[\alpha]^{25}_D$ -23.36° (*c* 2.14, $CHCl_3$).

1-*O*-Octadecyl-2-*O*-methoxymethyl-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (22a). To a solution of 1.95 g (3.3 mmol) of 1-*O*-octadecyl-3-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**21a**) in 60 mL of alcohol-free chloroform and 60 mL of dimethoxymethane was added 18 g (127 mmol) of phosphorus pentoxide. After the reaction mixture was stirred overnight at room temperature under nitrogen, 100 mL of 10% aqueous sodium carbonate solution was added to remove excess of phosphorus pentoxide. The layers were separated and the aqueous layer was extracted

three times with chloroform. The combined organic layer was washed with saturated sodium bicarbonate solution, brine, and dried over sodium sulfate. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 20:1 hexane/EtOAc) to yield 1.80 g (2.87 mmol, 86%) of 1-*O*-octadecyl-2-*O*-methoxymethyl-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**22a**) as a colorless liquid; $[\alpha]^{25}_D$ +6.51° (*c* 2.97, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.40-7.70 (m, 10 H, phenyl), 4.71 (s, 2 H, OCH_2O), 3.36-3.88 (m, 7 H, $CH_2OCH_2CHCH_2$), 3.34 (s, 3 H, OCH_3), 1.26-1.64 (m, 32 H, $(CH_2)_{16}$), 1.05 (s, 9 H, $SiC(CH_3)_3$), 0.90 (t, *J* = 6.34 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{39}H_{66}O_4Si$: C, 74.71; H, 10.61. Found: C, 74.46; H, 10.55.

1-*O*-(2',4'-Octadecadiynyl)-2-*O*-methoxymethyl-*sn*-glycero-3-*O*-*m*-nitrobenzenesulfonate (22b). This compound was prepared from 1-*O*-(2',4'-octadecadiynyl)-*sn*-glycero-3-*O*-*m*-nitrobenzenesulfonate (**21b**) in 96% yield by using the procedure described for the preparation of 1-*O*-octadecyl-2-*O*-methoxymethyl-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**22a**) with minor modification; $[\alpha]^{25}_D$ -10.83° (*c* 1.33, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 7.27-8.77 (m, 4 H, phenyl), 4.64 (s, 2 H, OCH_2O), 4.23-4.29 (m, 2 H, CH_2OSO_2), 4.14 (s, 2 H, $OCH_2C{\equiv}C$), 3.95 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.59 (d, 2 H, $C\underline{H}_2OCH_2C{\equiv}C$), 3.34 (s, 3 H, OCH_3), 2.28 (t, *J* = 6.92 Hz, 2 H, $CH_2C{\equiv}C$), 1.25-1.63 (m, 22 H, $(CH_2)_{11}$), 0.87 (t, *J* = 6.77 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{29}H_{43}O_8NS$: C, 61.57; H, 7.66; N, 2.48. Found: C, 61.75; H, 7.70; N, 2.57.

1-*O*-Octadecyl-2-*O*-methoxymethyl-*sn*-glycerol (23a). This compound was prepared from 1-*O*-octadecyl-2-*O*-methoxymethyl-3-*O*-(*tert*-

butyldiphenylsilyl)-*sn*-glycerol (**22a**) in 70% yield by using the procedure described for the preparation of 1-*O*-(4',6'-octadecadiynyl)-2-*O*-(5'',7''-octadecadiynyl)-*sn*-glycerol (**19**) with minor modification; mp 37-39 °C; $[\alpha]^{25}_D$ -17.9° (*c* 2.205, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 4.76 (s, 2 H, OCH_2O), 3.37-3.83 (m, 7 H, $CH_2OCH_2CHCH_2$), 3.42 (s, 3 H, OCH_3), 2.78 (br s, 1 H, OH), 1.25-1.60 (m, 32 H, $(CH_2)_{16}$), 0.88 (t, J = 6.34 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{23}H_{48}O_4$: C, 71.08; H,12.45. Found: C, 71.05; H, 12.47.

1-*O*-(2',4'-Octadecadiynyl)-2-*O*-methoxymethyl-*sn*-glycerol (23b). To a solution of 671 mg (1.19 mmol) of 1-*O*-(2',4'-octadecadiynyl)-2-*O*-methoxymethyl-*sn*-glycero-3-*O*-*m*-nitrobenzenesulfonate (**22b**) in 25 mL of DMF/DMSO (4:1) was added 911 mg (4.75 mmol) of cesium acetate. The reaction mixture was stirred overnight at room temperature under nitrogen. Diethyl ether (500 mL) was added and the reaction mixture was washed with water twice. The organic layer was separated and dried over anhydrous sodium sulfate. The solvents were concentrated under vacuum to about 30 mL, and the solution was then cooled to 0 °C. Lithium aluminium hydride (200 mg, 5.27 mmol) was added. The reaction mixture was stirred for 0.5 h at 0 °C and for 1 h at room temperature. Water (40 mL) was added, and the mixture was filtered and extracted with chloroform. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 2:1 hexane/EtOAc) to yield 359 mg (0.94 mmol, 80%) of 1-*O*-(2',4'-octadecadiynyl)-2-*O*-methoxymethyl-*sn*-glycerol (**23b**) as a liquid; $[\alpha]^{25}_D$ -26.92° (*c* 1.27, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 4.76 (s, 2 H, OCH_2O), 4.24 (s, 2 H, $OCH_2C{\equiv}C$), 3.63-3.80 (m, 5 H, CH_2CHCH_2), 3.43 (s, 3 H, OCH_3),

2.67 (br s, 1 H, OH), 2.28 (t, J = 6.77 Hz, 2 H, $CH_2C{\equiv}C$), 1.26-1.53 (m, 22 H, $(CH_2)_{11}$), 0.88 (t, J = 6.29 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{23}H_{40}O_4$: C, 72.98; H, 10.12. Found: C, 71.71; H, 10.00.

1-*O*-Octadecyl-2-*O*-methoxymethyl-*sn*-glycero-3-phosphocholine (24a). This compound was prepared from 1-*O*-octadecyl-2-*O*-methoxymethyl-*sn*-glycerol (**23a**) in 74% yield by using the procedure described for the preparation of 1-*O*-(4',6'-octadecadiynyl)-2-*O*-(5",7"-octadecadiynyl)-*sn*-glycero-3-phosphocholine (**20**); mp 194-197 °C; $[\alpha]^{25}_D$ +3.21° (*c* 2.25, $CHCl_3/CH_3OH$ 1:1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.62-4.78 (m, 2 H, OCH_2O), 4.29 (br s, 2 H, $CHC\underline{H}_2OP$), 3.40-3.90 (m, 9 H, $POCH_2CH_2N$, CH_2OCH_2CH), 3.36 (s, 3 H, OCH_3), 3.34 (s, 9 H, $N(CH_3)_3$), 1.13-1.53 (m, 32 H, $(CH_2)_{16}$), 0.88 (t, J = 6.36 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{28}H_{60}O_7NP{\cdot}H_2O$: C, 58.82; H, 10.93; N, 2.45. Found: C, 58.76; H, 10.71; N, 2.31.

1-*O*-(2',4'-Octadecadiynyl)-2-*O*-methoxymethyl-*sn*-glycero-3-phosphocholine (24b). This compound was prepared from 1-*O*-(2',4'-octadecadiynyl)-2-*O*-methoxymethyl-*sn*-glycerol (**23b**) in 65% yield by using the procedure described for the preparation of 1-*O*-(4',6'-octadecadiynyl)-2-*O*-(5",7"-octadecadiynyl)-*sn*-glycero-3-phosphocholine (**20**); $[\alpha]^{25}_D$ -2.48° (*c* 1.08, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.68-4.79 (m, 2 H, OCH_2O), 3.56-4.47 (m, 19 H, $POCH_2CH_2N$, $CH_2OCH_2CHCH_2$, $(H_2O)_4$), 3.38 (s, 3 H, OCH_3), 3.26 (s, 9 H, $N(CH_3)_3$), 2.27 (t, J = 6.78 Hz, 2 H, $CH_2C{\equiv}C$), 1.26-1.53 (m, 22 H, $(CH_2)_{11}$), 0.88 (t, J = 6.31 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{28}H_{52}O_7NP{\cdot}H_2O$: C, 59.66; H, 9.66; N, 2.49; P, 5.49. Found: C, 59.60; H, 9.49; N, 2.32; P, 5.28.

1-*O*-Octadecyl-2-(10',12'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (25a). To a solution of 114.3 mg (0.21 mmol) of 1-*O*-octadecyl-2-*O*-methoxymethyl-*sn*-glycero-3-phosphocholine (**24a**) in 4 mL of alcohol-free chloroform was added 232 mg (1.03 mmol) of anhydrous zinc bromide. The reaction mixture was stirred 2 days at room temperature. TLC analysis (elution with 60:30:3:2 $CHCl_3/CH_3OH/NH_4OH/H_2O$) showed complete conversion of **24a** (R_f= 0.35) into the desired lyso-PC (R_f= 0.20). A solution of 114 mg (0.41 mmol) of 10,12-octadecadiynoic acid (**12g**) and 50 mg (0.41 mmol) of DMAP in 2 mL of freshly distilled alcohol-free chloroform was added, followed by 170 mg (0.82 mmol) of DCC at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and then for 2 days at room temperature under nitrogen in the dark. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 40:10:1 $CHCl_3/CH_3OH/NH_4OH$) to yield a white residue. The suspended silica gel was removed by filtering a chloroform solution of the residue through a 0.45-μm Metricel filter three times. The product was lyophilized with benzene, affording 111.2 mg (0.15 mmol, 70%) of 1-*O*-octadecyl-2-(10',12'-octadecadiynoyl)-*sn*-glycero-3-phosphocholine (**25a**) as a white solid; $[\alpha]^{25}_D$ -11.4° (c 0.83, C_6H_6); ^1H NMR (200 MHz, $CDCl_3$) δ 5.13 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.42-4.30 (m, 10 H, $POC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2CHC\underline{H}_2OC\underline{H}_2$), 3.37 (s, 9H, $N(CH_3)_3$), 2.24 (m, 6H, $(CH_2C{\equiv}C)_2$, CH_2CO_2), 1.26-1.54 (m, 50 H, $(CH_2)_{25}$), 0.90 (m, 6 H, $(\omega\text{-}CH_3)_2$). Anal. Calcd for $C_{44}H_{82}O_7NP{\cdot}2H_2O$: C, 65.72; H, 10.78; N, 1.74. Found: C, 65.41; H, 10.65; N, 1.76.

3-*O*-(2',4'-Octadecadiynyl)-*sn*-glycero-1-*O*-*m*-

nitrobenzenesulfonate (21b'). This compound was prepared from 2,4-octadecadiyn-1-ol (**6**) and (*S*)-(+)-glycidyl 3-nitrobenzenesulfonate* in 60% yield by using the procedure described for the preparation of 1-*O*-(2',4'-octadecadiynyl)-*sn*-glycerol-3-*O*-*m*-nitrobenzenesulfonate (**21b**) as a white solid, mp 37-39 °C.

* (*S*)-(+)-Glycidyl 3-nitrobenzenesulfonate of 99% ee is reported to have $[\alpha]^{25}_D$ +23.0° (*c* 2.14, $CHCl_3$) [54]. For our lot of (*S*)-(+)-glycidyl 3-nitrobenzenesulfonate, we obtained $[\alpha]^{25}_D$ +21.08°. After recrystallization twice from ethanol, we obtained $[\alpha]^{25}_D$ +22.62° (*c* 2.14, $CHCl_3$).

(*R*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid ester (27) of 1-*O*-(2',4'-octadecadiynyl)-*sn*-glycero-3-*O*-*m*-nitrobenzenesulfonate (21b). To a mixture of 6 mg (0.05 mmol) of DMAP and 30 µL of triethylamine in 0.4 mL of dry methylene chloride was added 15 mg (0.029 mmol) of 1-*O*-(2',4'-octadecadiynyl)-*sn*-glycero-3-*O*-*m*-nitrobenzenesulfonate (**21b**), followed by 10 µL of neat (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride. The solution became warm and turned orange. It is important to monitor the reaction by TLC to ensure complete reaction (about 15 min). The reaction was quenched by addition of 20 µL of 3-(dimethylamino)propylamine and concentrated. The residue was applied to a short plug of silica gel in order to remove polar impurities. Elution with 4:1 hexane/EtOAc yielded 14.7 mg (0.02 mmol, 69%) of (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid ester (**27**) of 1-*O*-(2',4'-octadecadiynyl)-*sn*-glycero-3-*O*-*m*-nitrobenzenesulfonate (**21b**) as a colorless liquid. HPLC (UV 254 nm, flow 0.45 mL/min; elution: hexane/2-propanol, 87.5:12.5, v/v)

indicated 95.6% ee (see Fig. 2, p. 23).

(*R*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid ester (27') of 3-*O*-(2',4'-octadecadiynyl)-*sn*-glycero-1-*O*-*m*-nitrobenzenesulfonate (21b'). This compound was obtained as a colorless liquid from 3-*O*-(2',4'-octadecadiynyl)-*sn*-glycero-1-*O*-*m*-nitrobenzenesulfonate (**21b'**) and (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride in 58% yield by using the procedure described for the preparation of (*R*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid ester (**27**) of 1-*O*-(2',4'-octadecadiynyl)-*sn*-glycero-3-*O*-*m*-nitrobenzenesulfonate (**21b**). HPLC (UV 254 nm, flow 0.45 mL/min; elution: hexane/2-propanol, 87.5:12.5, v/v) indicated 96.4% ee (see Fig. 2, p. 23).

Spectrophotometric Determination of Permeability Properties of Liposomes

Preparation of aqueous dispersions. Stock solutions of polymerizable PC **13a** (2 mM) and SOPC (0.5 mM) were prepared in chloroform. Liposomes were prepared by pipetting the desired amounts from the stock solution into 20-mL vials, and removing the chloroform under nitrogen. The lipids were evaporated to dryness under vacuum, and the resulting thin lipid film was suspended in a solution of 60 mM KCl by agitation on a Vortex mixer for 3 min. One glass bead (3 mm diameter) was added per 2 mL of suspending KCl solution prior to mixing. The concentration of lipid in liposomes was 0.5 mM. All liposomes contained 4 mol % of dicetyl phosphoric acid, which was added to confer a net negative charge on the

bilayers. Stock solutions of acetamide, glycerol, and urea (0.40 M) were prepared in deionized water.

Polymerization of aqueous dispersions. Dispersions were polymerized by placing them 3 cm from a 254-nm ultraviolet lamp (UVGL-58, UVP, San Gabriel, CA) at room temperature. Prior to and during irradiation, PC **13a** dispersions were purged throughly with nitrogen. Dispersions of PC **13a** polymerized effectively, as evidenced by the color change to purple within 30 min.

Assay of nonelectrolyte permeability. Absorbance versus time plots of nonelectrolytes (acetamide, glycerol, and urea) permeability of liposomes of SOPC, unpolymerized and polymerized PC **13a** were recorded on an Aviv 14DS UV-VIS-IR spectrophotometer (see Fig. 7-9, pp. 33-35). In each experiment, a 0.1-mL aliquot of liposomes suspended in 60 mM KCl solution was rapidly injected into a cuvette containing 0.7 mL of 0.40 M nonelectrolyte solution. The solutes used were acetamide, glycerol, and urea. Time-dependent changes in absorbance occurring after addition of liposomes were recorded continuously. The initial rate of swelling was measured from the tangent to the initial slope in terms of $\Delta(1/A)$ per min, where A is the absorbance at 400 nm at 23-24 °C. The absorbance was measured after a short time period, e.g., 20-60 s after injecting, and was then converted to $\Delta(1/A)$ per min (see Table 2, p. 41).

CHAPTER 2. SYNTHESIS OF DEUTERATED PHOSPHATIDYLCHOLINES FOR USE IN MEMBRANE STUDIES

INTRODUCTION

Specifically deuterated fatty acids and derived lipids have been employed as nonradioactive tracers in investigations of biological processes, such as the studies of membrane structure and properties using ^1H and ^2H NMR and FT-IR techniques. They can be used to attenuate the background signals of phospholipids, especially to selectively eliminate some undesirable signals in an NMR spectrum. Mendelsohn et al. proposed and demonstrated a new method "for the direct determination of conformational disorder (trans-gauche isomerization) as a function of acyl-chain position in bilayer membranes by using CD_2 rocking modes in phospholipids" by FT-IR [64]. Recently, a method based on the conformation-dependent coupling between CD_2 rocking frequencies of two successive CD_2 groups for the quantitative detection of specific, position-dependent kink (gtg') and isolated gauche (gtt) conformers has also been developed [65].

The various approaches used to prepare deuterated fatty acids and derived lipids can be found in previous review articles [66]. Basically there are only two methods of introducing deuterium into organic molecules: exchange and reduction. Some partially deuterated fatty acids can be obtained by using reduction of acetylenic, diacetylenic, or polyacetylenic acids. Because scatter of label can be a problem in synthetic work, Wilkinson's catalyst, tris(triphenylphosphine)chlororhodium, has been developed and studied extensively [67]. $[(C_6H_5)_3P]_3RhCl$ catalyzes *cis*

addition of deuterium to multiple bonds without deuterium scattering or bond isomerization at room temperature and pressure. In this thesis PCs partially deuterated at two consecutive carbons (CD_2CD_2) (**34a-c**) have been prepared from commercially available starting materials. Deuterated PC **34c** has been used recently in the direct determination of conformational disorder in bilayer membrane by FT-IR by Dr. Richard Mendelsohn. In addition, three partially deuterated alkanes (**37a-c**) have been prepared via reaction of an 1-alkyne with 1-iodoalkane, followed by catalytic reduction with deuterium. These compounds will be used by Dr. Mendelsohn for calibration of the conformations of the acyl chains of dipalmitoyl-PC-d_8 bilayers.

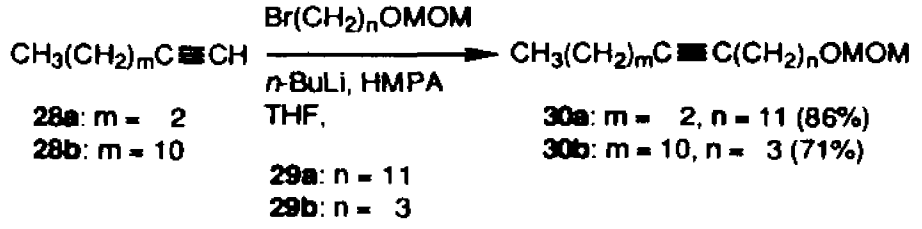
RESULTS AND DISCUSSION

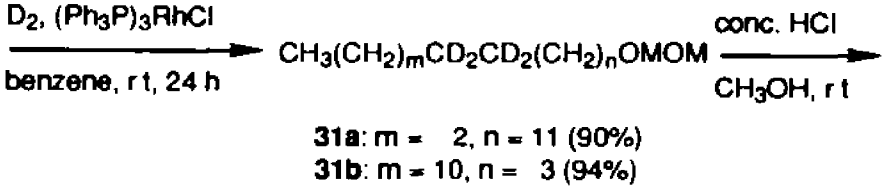
Two PCs (**34a-b**) containing CD_2CD_2 units at sites either close to the ω-methyl group or close to the glycerol backbone have been prepared from terminal alkynes as shown in Scheme 8. The acetylenic coupling reaction has not been used very much with deuterated fragments. In most reactions, the deuterium is introduced by catalytic reduction with deuterium after the acetylenic coupling reaction. Terminal alkyne **28** was coupled with the MOM-protected ω-bromoalkan-1-ol **29** in THF/HMPA (1:1.2) by using *n*-butyllithium, giving ω-(methoxymethoxy)alkyne **30**. Because the catalytic reduction using Pd results in extensive multiple bond migration, the locations of the deuterium atoms cannot be controlled. Therefore, Wilkinson's catalyst, tris-(triphenylphosphine)chlororhodium, was selected as the catalyst in the reduction with deuterium in benzene, and did not cause deuterium scatter. The resulting ω-(methoxymethoxy)alkanes **31** are deprotected easily in high yield using concentrated HCl in methanol at room temperature. Partially deuterated fatty acids **33** were obtained by oxidation of the alcohols **32** by using chromium trioxide in glacial acetic acid. Partially deuterated PCs **34** were prepared by acylation of GPC·$CdCl_2$ complex with the acids **33** using DMAP and DCC in chloroform at room temperature. However, hexadecan-1-ol-1,7,7,8,8-d_5 (**32c**) was prepared directly from the catalytic reduction of the commercially available 7-hexadecyn-1-ol with deuterium using tris(triphenylphosphine)chlororhodium.

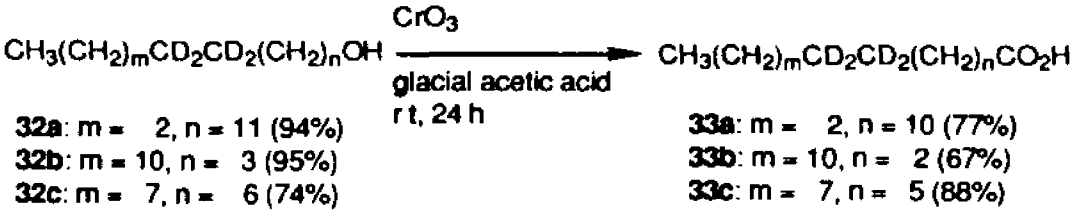
Partially deuterated alkanes can be used for calibration of the conformations of the acyl chains in deuterated PC bilayers. Scheme 9 shows

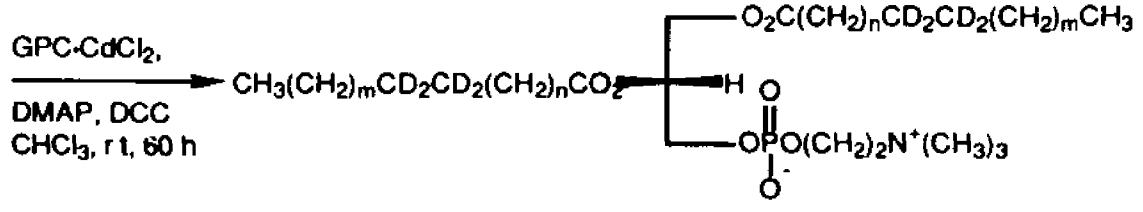
the preparation of partially deuterated alkanes **37a-c** using the acetylenic coupling reaction of 1-alkyne with 1-iodoalkane, followed by catalytic reduction.

Scheme 8. Synthesis of Deuterated Phosphatidylcholines 34a-c

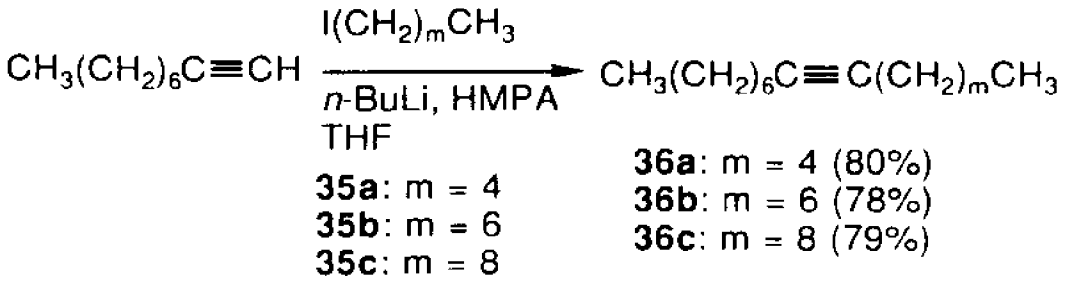


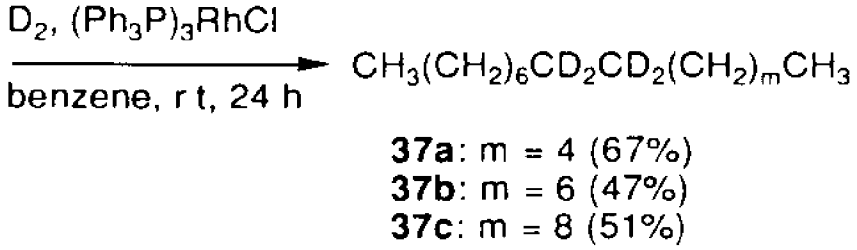






Scheme 9. Synthesis of Deuterated Alkanes 37a-c





EXPERIMENTAL SECTION

General Procedures

The procedures for drying the solvents were as described in pp. 42-44. 1-Pentyne, 1-tridecyne, 1-nonyne, and 7-hexadecyn-1-ol were purchased from Farchan Laboratories, Inc. 1-Iodopentane, 1-iodoheptane, 1-iodononane, D_2O (99.8 atom % D) and 10% silver nitrate silica gel were from Aldrich. Triphenylphosphine was from Fluka Chemical Corp. Rhodium (III) chloride hydrate was from MCB. Chromium trioxide was from Mallinckrodt Chemical Works. Degassing is performed by evacuating and flushing four times with deuterium gas contained in a balloon.

Syntheses

1-(Methoxymethoxy)-12-hexadecyne (30a). To a solution of 681 mg (10 mmmol) of 1-pentyne (**28a**) in 10 mL of THF was added dropwise 7.5 mL (12 mmol) of *n*-butyllithium (a 1.6 M solution in hexane) at -23 °C. After the reaction mixture was stirred for 0.5 h at -23 °C, a solution of 1.60 g (5.4 mmol) of 1-bromo-11-(methoxymethoxy)undecane (**29a**) in 12 mL of HMPA was added dropwise at -23 °C. The mixture was stirred for 2 h at room temperature. Water (40 mL) and 40 mL of ether were added. After the mixture was stirred and separated, the aqueous layer was extracted three times with ether. The combined organic layer was washed with saturated sodium bicarbonate solution, brine, and dried over sodium sulfate. Removal of the solvents gave a light-yellow liquid that was purified by flash chromatography (elution with 20:1 hexane/EtOAc) to yield 1.31 g (4.64 mmol,

86%) of 1-(methoxymethoxy)-12-hexadecyne (**30a**) as a colorless liquid; ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.62 (s, 2 H, OCH_2O), 3.52 (t, J = 6.54 Hz, 2 H, $CH_2C\underline{H}_2O$), 3.36 (s, 3 H, OCH_3), 2.08-2.16 (m, 4 H, $(CH_2C{\equiv}C)_2$), 1.28-1.59 (m, 20 H, $(CH_2)_{10}$), 0.97 (t, J = 7.30 Hz, 3 H ω-CH_3). Anal. Calcd for $C_{18}H_{34}O_2$ (282.47): C, 76.54; H, 12.13. Found: C, 76.18; H, 12.23.

1-(Methoxymethoxy)-4-hexadecyne (30b). This compound was prepared from 1-tridecyne (**28b**) and 1-bromo-3-(methoxymethoxy)propane (**29b**) in 71% yield by using the procedure described for the preparation of 1-(methoxymethoxy)-12-hexadecyne (**30a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.61 (s, 2 H, OCH_2O), 3.61 (t, J = 6.24 Hz, 2 H, $CH_2C\underline{H}_2O$), 3.36 (s, 3 H, OCH_3), 2.10-2.30 (m, 4 H, $(CH_2C{\equiv}C)_2$), 1.69-1.84 (m, 2 H, $C{\equiv}CCH_2C\underline{H}_2CH_2O$), 1.26-1.50 (m, 18 H, $(CH_2)_9$), 0.88 (t, J = 6.29 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{18}H_{34}O_2$ (282.47): C, 76.54; H, 12.13. Found: C, 76.31; H, 11.82.

1-(Methoxymethoxy)hexadecane-12,12,13,13-d_4 (31a). Flasks and other glassware were washed with D_2O four times and stored in an oven (110 °C) prior to use. The catalyst tris(triphenylphosphine)-chlororhodium* (200 mg, 0.22 mmol) was suspended in 25 mL of dry, degassed benzene. The suspension was evacuated and flushed four times with deuterium gas, which was generated from the reaction of sodium (washed with dry ether and benzene) and a mixture of methanol-d_4 and D_2O. 1-(Methoxymethoxy)-12-hexadecyne (**30a**) (490 mg, 1.73 mmol) in 5 mL of dry, degassed benzene was added in one portion. The reaction mixture was

degassed and flushed three times with D_2 gas and then stirred for 24 h at room temperature under an atmosphere of deuterium using a balloon. After the catalyst was removed by filtration through Celite 545, the solution was evaporated on a rotary evaporator. The residue was purified by flash chromatography (elution with 20:1 hexane/EtOAc) to yield 453 mg (90%) of 1-(methoxymethoxy)hexadecane-12,12,13,13-d_4 (**31a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.62 (s, 2 H, OCH_2O), 3.51 (t, J = 6.54 Hz, 2 H, $CH_2C\underline{H}_2O$), 3.36 (s, 3 H, OCH_3), 1.52-1.62 (m, 2 H, $C\underline{H}_2CH_2O$), 1.25-1.38 (m, 22 H, $(CH_2)_{11}$), 0.88 (t, J = 6.71 Hz, 3 H, ω-CH_3); MS, m/z 289 (M^+-1). Anal. Calcd for $C_{18}H_{34}D_4O_2$ (290.53): C, 74.42; H, 13.18. Found: C, 74.17; H, 13.22.

* Tris(triphenylphosphine)chlororhodium was prepared from triphenylphosphine and rhodium (III) chloride hydrate in 93% yield according to the procedure of Young et al. [68] and stored under vacuum. However, best results were obtained when freshly prepared catalyst was used.

1-(Methoxymethoxy)hexadecane-4,4,5,5-d_4 (31b). This compound was prepared from 1-(methoxymethoxy)-4-hexadecyne (**30b**) in 94% yield by using the procedure described for the preparation of 1-(methoxymethoxy)hexadecane-12,12,13,13-d_4 (**31a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.62 (s, 2 H, OCH_2O), 3.52 (t, J = 6.59 Hz, 2 H, $CH_2C\underline{H}_2O$), 3.36 (s, 3 H, OCH_3), 1.52-1.67 (m, 2 H, $C\underline{H}_2CH_2O$), 1.26-1.37 (m, 22 H, $(CH_2)_{11}$), 0.88 (t, J = 6.41 Hz, 3 H, ω-CH_3); MS, m/z (relative intensities) 290 (M^+, 19), 289 (M^+-1, 100). Anal. Calcd for $C_{18}H_{34}D_4O_2$ (290.53): C, 74.42; H, 13.18. Found: C,

74.26; H, 13.42.

Hexadecan-1-ol-12,12,13,13-d_4 (32a). To a solution of 419 mg (1.44 mmol) of 1-(methoxymethoxy)hexadecane-12,12,13,13-d_4 (**31a**) in 40 mL of methanol was added 6 mL of 37% hydrochloric acid. The reaction mixture was stirred overnight at room temperature. After removal of methanol, water and chloroform were added, and the organic layer was separated. The aqueous layer was extracted three times with chloroform. The combined organic layer was washed with saturated sodium bicarbonate solution, brine, and dried over sodium sulfate. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 6:1 hexane/EtOAc) to yield 334 mg (1.36 mmol, 94%) of hexadecan-1-ol-12,12,13,13-d_4 (**32a**) as a white solid, mp 45-46 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.63 (t, J = 6.52 Hz, 2 H, $C\underline{H}_2OH$), 1.25-1.60 (m, 25 H, $(CH_2)_{12}$ and OH), 0.88 (t, J = 6.71 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{16}H_{30}D_4O$ (246.48): C, 77.97; H, 13.90. Found: C, 77.69; H, 13.73.

Hexadecan-1-ol-4,4,5,5-d_4 (32b). This compound was prepared from 1-(methoxymethoxy)hexadecane-4,4,5,5-d_4 (**31b**) in 95% yield by using the procedure described for the preparation of hexadecan-1-ol-12,12,13,13-d_4 (**32a**); mp 48-49 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.64 (t, J = 6.56 Hz, 2 H, $C\underline{H}_2OH$), 1.25-1.60 (m, 25 H, $(CH_2)_{12}$ and OH), 0.88 (t, J = 6.41 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{16}H_{30}D_4O$ (246.48): C, 77.97; H, 13.90. Found: C, 78.06; H, 13.67.

Hexadecan-1-ol-1,7,7,8,8-d_5 (32c). 7-Hexadecyn-1-ol (850 mg, 3.57 mmol) was dissolved in 5 mL of chloroform, and the hydroxyl proton was exchanged by treatment four times with 5 mL of D_2O (99.8 atom % deuterium). The organic layer was separated and dried over sodium sulfate. Removal of the solvents gave 7-hexadecyn-1-ol-1-d, which was dried. tris(Triphenylphosphine)chlororhodium (60 mg, 0.066 mmol) was suspended in 15 mL of dry, degassed benzene. The suspension was evacuated and flushed four times with deuterium gas, which was generated from the reaction of sodium (washed with dry ether and benzene) and a mixture of methanol-d_4 and D_2O. 7-Hexadecyn-1-ol-1-d (734 mg, 3.07 mmol) in 3 mL of dry, degassed benzene was added. The reaction mixture was degassed and flushed three times with D_2 gas and then stirred for 24 h at room temperature under an atmosphere of deuterium using a balloon. After the catalyst was removed by filtration through Celite 545, the solution was evaporated on a rotary evaporator. The residue was purified by flash chromatography (elution with 7:1 hexane/EtOAc) to yield 654 mg (74%) of hexadecan-1-ol-1,7,7,8,8-d_5 (**32c**) as a white solid; mp 47-48 °C; IR ($CHCl_3$) 3416 (OH), 2182, 2080 cm^{-1} (CD_2CD_2); ^1H NMR (200 MHz, $CDCl_3$) δ 3.62 (t, J = 6.55 Hz, 2 H, CH_2OD), 1.49-1.59 (m, 2 H, CH_2CH_2OD), 1.15-1.43 (m, 22 H, $(CH_2)_{11}$), 0.88 (t, J = 6.40 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{16}H_{29}D_5O$ (247.48): C, 77.65; H, 13.85. Found: C, 77.93; H, 13.61.

Hexadecanoic-12,12,13,13-d_4 acid (33a). To 295 mg (1.2 mmol) of hexadecan-1-ol-12,12,13,13-d_4 (**32a**) in 60 mL of glacial acetic acid

was added dropwise 0.7 g (7.0 mmol) of chromium trioxide in 10 mL of 90% acetic acid. The reaction mixture was stirred for 24 h at room temperature. Water was added, and the product was extracted with ether. The ether layer was washed with water and dried over sodium sulfate. Removal of the solvents gave a residue that was purified by flash chromatography (elution with 200:50:1 hexane/EtOAc/85% formic acid) to yield 242 mg (0.93 mmol, 77%) of hexadecanoic-12,12,13,13-d_4 (**33a**) as a white solid; mp 56-57 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.35 (t, J = 7.45 Hz, 2 H, CH_2CO_2), 1.60-1.67 (m, 2 H, $C\underline{H}_2CH_2CO_2$), 1.25-1.28 (m, 20 H, $(CH_2)_{10}$), 0.88 (t, J = 6.68 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{16}H_{28}D_4O_2$ (260.46): C, 73.78; H, 12.38. Found: C, 73.51; H, 12.17.

Hexadecanoic-4,4,5,5-d_4 acid (33b). This compound was prepared from hexadecan-1-ol-4,4,5,5-d_4 (**32b**) in 67% yield by using the procedure described for the preparation of hexadecanoic-12,12,13,13-d_4 acid (**33a**); mp 61-62 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.34 (t, J = 7.52 Hz, 2 H, CH_2CO_2), 1.61 (t, J = 7.47 Hz, 2 H, $C\underline{H}_2CH_2CO_2$), 1.25 (m, 20 H, $(CH_2)_{10}$), 0.88 (t, J = 6.18 Hz, 3 H, ω-CH_3). Anal. Calcd for $C_{16}H_{28}D_4O_2$ (260.46): C, 73.78; H, 12.38. Found: C, 73.88; H, 12.19.

The acid was esterified as described below and characterized as the methyl ester. To a solution of 0.6 mL of 50% aqueous potassium hydroxide in 2 mL of ether was added 206 mg (2 mmol) of *N*-methyl-*N*-nitrosourea with shaking. The reaction mixture was stirred for 0.5 h at 0 °C. The etheral supernatant was transferred to 26 mg (0.1 mmol) of acid **33b**. The mixture

was stirred for 0.5 h at room temperature. After removal of the solvents, the methyl ester of hexadecanoic-4,4,5,5-d_4 acid was obtained. MS of the methyl ester, m/z (relative intensities) 275 (M^++1, 18), 274 (M^+, 100).

Hexadecanoic-7,7,8,8-d_4 acid (33c). This compound was prepared from hexadecan-1-ol-7,7,8,8-d_4 (**32c**) in 88% yield by using the procedure described for the preparation of hexadecanoic-12,12,13,13-d_4 acid (**33a**); mp 54-55 °C; IR ($CHCl_3$) 2180, 2078 (CD_2CD_2), 1706 cm^{-1} (C=O); ^1H NMR (200 MHz, $CDCl_3$) δ 2.35 (t, J = 7.42 Hz, 2 H, CH_2CO_2), 1.25-1.63 (m, 22 H, $(CH_2)_{11}$), 0.88 (t, J = 6.12 Hz, 3 H, ω-CH_3); MS of the methyl ester, m/z 274 (M^+). Anal. Calcd for $C_{16}H_{28}D_4O_2$ (260.46): C, 73.78; H, 12.38. Found: C, 73.62; H, 12.21.

1,2-Di-(hexadecanoyl-12,12,13,13,12',12',13',13'-d_8)-*sn*-glycero-3-phosphocholine (34a). To a suspension of 110 mg (0.25 mmol) of *sn*-glycerophosphocholine, cadmium chloride complex (dried for 5 h over phosphorus pentoxide under vacuum at 78 °C), 61 mg (0.50 mmol) of DMAP, and 130 mg (0.50 mmol) of hexadecanoic-12,12,13,13-d_4 acid (**33a**) in 5 mL of freshly distilled alcohol-free chloroform was added 206 mg (1.0 mmol) of DCC at 0 °C. The reaction mixture was stirred for 0.5 h at 0 °C and then for 60 h at room temperature under nitrogen. Chloroform (10 mL) was added and the mixture was filtered through a Celite 545 pad, which was washed with 10 mL of chloroform. Removal of the solvents gave a residue that was purified by flash chromatography (elution first with 100% $CHCl_3$, then

with 90:10, 60:40, 20:80 $CHCl_3/CH_3OH$) to yield a white solid. The suspended silica gel was removed by filtering a chloroform solution of the solid through a 0.45-μm Metricel filter three times. The product, 1,2-di-(hexadecanoyl-12,12,13,13,12',12',13',13'-d_8)-*sn*-glycero-3-phosphocholine (**34a**), was lyophilized with benzene, affording 119 mg (64%) as a white solid; $[\alpha]^{25}_D$ -5.58° (*c* 1.25, $CHCl_3/CH_3OH$ 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 5.21 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.67-4.14 (m, 12 H, $POC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2CHC\underline{H}_2$, $(H_2O)_2$), 3.36 (s, 9 H, $N(CH_3)_3$), 2.30 (t, J = 7.53 Hz, 4 H, $(CH_2CO_2)_2$), 1.52-1.63 (m, 4 H, $(C\underline{H}_2CH_2CO_2)_2$), 1.07-1.34 (m, 40 H, $(CH_2)_{20}$), 0.88 (t, J = 6.41 Hz, 6 H, $(\omega\text{-}CH_3)_2$). Anal. Calcd for $C_{40}H_{72}D_8O_8NP{\cdot}2H_2O$: C, 61.75; H, 10.88; P, 3.98. Found: C, 61.60; H, 10.81; P, 3.82.

1,2-Di-(hexadecanoyl-4,4,5,5,4',4',5',5'-d_8)-*sn*-glycero-3-phosphocholine (34b). This compound was prepared from hexadecanoic-4,4,5,5-d_4 acid (**33b**) in 61% yield by using the procedure described for the preparation of 1,2-di-(hexadecanoyl-12,12,13,13,12',12',13',13'-d_8)-*sn*-glycero-3-phosphocholine (**34a**); $[\alpha]^{25}_D$ -6.66° (*c* 0.70, $CHCl_3/CH_3OH$ 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 5.21 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.67-4.37 (m, 12 H, $POC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2CHC\underline{H}_2$, $(H_2O)_2$), 3.36 (s, 9 H, $N(CH_3)_3$), 2.23-2.34 (m, 4 H, $(CH_2CO_2)_2$), 1.52-1.63 (m, 4 H, $(C\underline{H}_2CH_2CO_2)_2$), 1.07-1.34 (m, 40 H, $(CH_2)_{20}$), 0.88 (t, J = 6.40 Hz, 6 H, $(\omega\text{-}CH_3)_2$). Anal. Calcd for $C_{40}H_{72}D_8O_8NP{\cdot}2H_2O$: C, 61.75; H, 10.88; P, 3.98. Found: C, 61.57; H, 10.75; P, 3.79.

1,2-Di-(hexadecanoyl-7,7,8,8,7',7',8',8'-d_8)-*sn*-glycero-3-phosphocholine (34c). This compound was prepared from hexadecanoic-7,7,8,8-d_4 acid (**33c**) in 72% yield by using the procedure described for the preparation of 1,2-di-(hexadecanoyl-12,12,13,13,12',12',13',13'-d_8)-*sn*-glycero-3-phosphocholine (**34a**); $[\alpha]^{25}_D$ +5.31° (*c* 1.27, $CHCl_3/CH_3OH$ 1:1); IR ($CHCl_3$) 2179, 2077 (CD_2CD_2), 1735 cm^{-1} (C=O); ^1H NMR (300 MHz, $CDCl_3$) δ 5.24 (m, 1 H, $CH_2C\underline{H}CH_2$), 3.48-4.43 (m, 8 H, $POC\underline{H}_2C\underline{H}_2N$, $C\underline{H}_2CHC\underline{H}_2$), 3.43 (s, 9 H, $N(CH_3)_3$), 2.29-2.37 (m, 4 H, $(CH_2CO_2)_2$), 1.61-1.71 (m, 4 H, $(C\underline{H}_2CH_2CO_2)_2$), 1.20-1.45 (m, 40 H, $(CH_2)_{20}$), 0.92 (t, J = 6.56 Hz, 6 H, $(\omega\text{-}CH_3)_2$). Anal. Calcd for $C_{40}H_{72}D_8O_8NP{\cdot}2H_2O$: C, 61.75; H, 10.88; P, 3.98. Found: C, 61.63; H, 10.85; P, 3.84.

6-Tetradecyne (36a). To a solution of 1.24 g (10 mmmol) of 1-nonyne in 10 mL of THF was added dropwise 7.5 mL (12 mmol) of *n*-butyllithium (a 1.6 M solution in hexane) at -23 °C. After the reaction mixture was stirred for 0.5 h at -23 °C, a solution of 1.98 g (10 mmol) of 1-iodopentane (**35a**) in 12 mL of HMPA was added dropwise at -23 °C. After the mixture was stirred for 2 h at room temperature, 40 mL of water and 40 mL of ether were added. The aqueous layer was extracted three times with ether (40 mL). The combined organic layer was washed with saturated sodium bicarbonate solution, brine, and dried over sodium sulfate. Removal of the solvents gave a residue that was purified by flash chromatography (elution with hexane) to yield 1.55 g (8.0 mmol, 80%) of 6-tetradecyne (**36a**) as a colorless liquid; ^1H

NMR (200 MHz, $CDCl_3$) δ 2.14 (m, 4 H, $(CH_2C{\equiv}C)_2$), 1.28-1.48 (m, 16 H, $(CH_2)_8$), 0.90 (t, J = 7.04 Hz, 3 H, $C{\equiv}C(CH_2)_4C\underline{H}_3$), 0.88 (t, J = 6.32 Hz, 3 H, $C\underline{H}_3(CH_2)_6C{=}C$).

8-Hexadecyne (36b). This compound was prepared from 1-nonyne and 1-iodoheptane (**35b**) in 78% yield by using the procedure described for the preparation of 6-tetradecyne (**36a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.14 (t, J = 6.71 Hz, 4 H, $(CH_2C{\equiv}C)_2$), 1.28-1.57 (m, 20 H, $(CH_2)_{10}$), 0.88 (t, J = 6.28 Hz, 6 H, $(\omega\text{-}CH_3)_2$).

10-Octadecyne (36c). This compound was prepared from 1-nonyne and 1-iodononane (**35c**) in 79% yield by using the procedure described for the preparation of 6-tetradecyne (**36a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.13 (t, J = 6.75 Hz, 4 H, $(CH_2C{\equiv}C)_2$), 1.27-1.47 (m, 24 H, $(CH_2)_{12}$), 0.88 (t, J = 6.37 Hz, 6 H, $(\omega\text{-}CH_3)_2$).

Tetradecane-6,6,7,7-d_4 (37a). The catalyst tris(triphenylphosphine)chlororhodium (300 mg, 0.33 mmol) was suspended in 30 mL of dry, degassed benzene. The suspension was evacuated and flushed four times with deuterium gas, which was generated from the reaction of sodium (washed with dry ether and benzene) and a mixture of methanol-d_4 and D_2O. 6-Tetradecyne (**36a**) (480 mg, 2.47 mmol) in 6 mL of dry, degassed benzene was added. The reaction mixture was degassed and flushed three

times with D_2 gas and then stirred for 48 h at room temperature under an atmosphere of deuterium using a balloon. After the catalyst was removed by filtration through Celite 545, the solvent was evaporated on a rotary evaporator. The residue was purified by flash chromatography with 10% silver nitrate silica gel (elution with hexane) under red light to yield 246 mg (49%) of tetradecane-6,6,7,7-d_4 (**37a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25-1.34 (m, 20 H, $(CH_2)_{10}$), 0.88 (t, J = 6.47 Hz, 6 H, $(\omega\text{-}CH_3)_2$); MS, m/z (relative intensities) 203 (M^++1, 15), 202 (M^+, 100), 201 (M^+-1, 10).

Hexadecane-8,8,9,9-d_4 (37b). This compound was prepared from 8-hexadecyne (**36b**) in 47% yield by using the procedure described for the preparation of tetradecane-6,6,7,7-d_4 (**37a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25-1.53 (m, 24 H, $(CH_2)_{12}$), 0.88 (t, J = 6.38 Hz, 6 H, $(\omega\text{-}CH_3)_2$); MS, m/z (relative intensities) 231.5 (M^++1, 16), 230.5 (M^+, 100), 229.5 (M^+-1, 2).

Octadecane-10,10,11,11-d_4 (37c). This compound was prepared from 10-octadecyne (**36c**) in 51% yield by using the procedure described for the preparation of tetradecane-6,6,7,7-d_4 (**37a**); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.25-1.34 (m, 28 H, $(CH_2)_{14}$), 0.88 (t, J = 6.44 Hz, 6 H, $(\omega\text{-}CH_3)_2$); MS, m/z (relative intensities) 259 (M^++1, 21), 258 (M^+, 100), 257 (M^+-1, 24).

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