°C for 1 h. The solvent was removed, and the residue was chromatographed on silica gel. Elution with chloroform–hexane afforded a mixture of 7 and 8 (1.14 g, 80%, 78:12 by gas chromatography) as a colorless oil: R_f 0.70 ($CHCl_3$); IR 1710 (C=O), 1280 and 1130 cm^{-1} (C–F). Anal. Calcd for $C_{10}H_{11}OF_3$: C, 58.82; H, 5.43; F, 27.91. Found: C, 58.23; H, 5.30; F, 27.77. The isomeric mixture was separated by gas chromatography. 7: ^1H NMR δ 6.30 (m, 1 H), 6.15 (m, 1 H), 3.30 (m, 1 H), 3.10 (m, 2 H), 2.50 (m, 1 H), 2.30 (s, 3 H), 1.50 (m, 2 H); MS, *m/e* 204 (M^+). 8: ^1H NMR δ 6.25 (m, 1 H), 6.00 (m, 1 H), 3.26 (m, 1 H), 3.06 (m, 2 H), 2.58 (m, 1 H), 2.15 (s, 3 H), 1.78 (m, 1 H), 1.56 (m, 1 H); MS, *m/e* 204 (M^+).

3-Acetyl-4-(trifluoromethyl)-2-pyrazoline (9). To an ether solution (5 mL) of 1 (1.38 g, 10 mmol) was added a solution of CH_2N_2 (10 mmol) in ether (20 mL). The solvent was removed, and the residue was chromatographed on silica gel. Elution with dichloromethane afforded crude **9**, which was recrystallized from CH_2Cl_2–hexane to give yellow needles (1.64 g, 91%): mp 75–76 °C; R_f 0.29 (CH_2Cl_2); ^1H NMR δ 6.73 (br, 1 H), 4.00 (m, 3 H), 2.43 (s, 3 H); IR 1625 (C=O), 1520 (C=N), 1260, 1160, and 1130 cm^{-1} (C–F); MS, *m/e* 180 (M^+). Anal. Calcd for $C_6H_7ON_2F_3$: C, 40.00; H, 3.92; N, 15.55; F, 31.64. Found: C, 39.49; H, 3.84; N, 15.54; F, 31.62.

2-(1-(Trifluoromethyl)-3-oxobutyl)pyrrole (10). A solution of 1 (1.34 g, 9.7 mmol) and pyrrole (0.65 g, 9.7 mmol) in dichloromethane (10 mL) was refluxed for 7 h. The solvent was removed, and the residue was chromatographed on silica gel. Elution with dichloromethane afforded crude **10**, which was recrystallized from $CHCl_3$–hexane to give white needles (1.30 g, 65%): mp 59 °C; R_f 0.33 (CH_2Cl_2); ^1H NMR δ 8.42 (br, 1 H), 6.72 (q, 1 H), 6.33 (t, 2 H), 4.05 (m, 1 H), 3.03 (d, 2 H), 2.18 (s, 3 H); IR 3390 (N–H), 1715 (C=O), 1295, 1150, and 1095 cm^{-1} (C–F); MS, *m/e* 205 (M^+). Anal. Calcd for $C_9H_{10}NF_3$: C, 52.69; H, 4.91; N, 6.83; F, 27.99. Found: C, 52.61; H, 4.75; N, 6.73; F, 27.78.

Reaction of 1 with Furan. A solution of 1 (1.59 g, 12 mmol) and furan (1.65 g, 24 mmol) in dichloromethane (5 mL) was stirred at −5 °C for 1 h. The solvent was removed, and the residue was chromatographed on silica gel. Elution with dichloromethane afforded 2-(1-(trifluoromethyl)-3-oxobutyl)furan (ca. 40 mg) as an oil: R_f 0.55 (CH_2Cl_2); ^1H NMR δ 7.35 (q, 1 H), 6.35 (t, 2 H), 4.14 (m, 1 H), 3.05 (d, 2 H), 2.21 (s, 3 H). The same reaction was also carried out at 40 °C for 1 h. Chromatography followed by gel filtration with Sephadex LH-20 with methanol as eluant gave the Diels–Alder adduct, 5-acetyl-6-(trifluoromethyl)-7-oxanorborn-2-ene (0.42 g, 17%): R_f 0.75 (CH_2Cl_2); ^1H NMR δ 6.52 (m, 1 H), 6.31 (m, 1 H), 5.30 (m, 1 H), 5.13 (m, 1 H), 3.30 (m, 1 H), 2.72 (m, 1 H), 2.23 (s, 3 H); IR 1710 (C=O), 1275, and 1115 cm^{-1} (C–F); MS, *m/e* 206 (M^+).

Registry No. (*E*)-1, 101395-81-7; (*Z*)-1, 101518-49-4; 2, 101518-39-2; 3, 101518-40-5; 4, 101518-41-6; 5, 101518-42-7; 6, 101518-43-8; 7, 101518-44-9; 8, 101628-00-6; 9, 101518-45-0; 10, 101518-46-1; (acetylmethylene)triphenylphosphorane, 1439-36-7; trifluoroacetyl ethyl hemiacetal, 433-27-2; trifluoroacetaldehyde, 75-90-1; ethyl magnesium iodide, 10467-10-4; cyclopentadiene, 542-92-7; pyrrole, 109-97-7; furan, 110-00-9; 2-[(1-trifluoromethyl)-3-oxobutyl]furan, 101518-47-2; 5-acetyl-6-(trifluoromethyl)-7-oxonorborn-2-ene, 101518-48-3.

A General Method for the Synthesis of Glycerophospholipids

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The synthesis of phospholipids has been a subject of interest in various laboratories over many years, and numerous synthetic procedures have been reported to date.[1-4] The progress in this area has often relied upon the development of new methods originally designed for the synthesis of nucleic acid fragments.[5] Recently, the synthesis of phosphoric acid diesters has been improved with a procedure for the selective introduction of alkoxy functions at the phosphorus atom using phosphoroamidites.[6,7] Although this method is now being widely used for the synthesis of oligodeoxyribonucleotides on solid-phase support,[8] it has not yet been sufficiently exploited to prove its suitability for the synthesis of other groups of phosphodiesters, such as phospholipids and their analogues. Only a few syntheses of phospholipids have been published using the amidophosphite reagents.[9,10]

Lately, phosphorothioate analogues of glycerophospholipids have been shown to be useful in studies of the mechanisms of phospholipases[11-14] as well as nonperturbing probes of phospholipid bilayer organization.[15,16] The procedures described for the syntheses of these analogues involve repeated substitution of the chlorine atoms at the phosphorothioyl center such as in method of Vasilenko et al.,[15] or can be used for the preparation of phosphatidylcholines.[17] Both methods in practice are not reproducible with respect to yield and purity of the products.[18] Phosphorothioyl analogues of phospholipids are not easily accessible by the general routes described for the preparation of natural phospholipids such as the diester method of Aneja and Davies[5] or the alkylation procedures of Eibl[2] and Toccane et al.[19] The phosphoramidite method of phosphodiester synthesis involving trivalent phosphorus intermediates offers the advantage of a simple introduction of sulfur at the phosphorus atom, thus avoiding nucleophilic displacement steps at the phosphorothioyl center.[20] The phosphoramidite method is also applicable for the synthesis of phospholipids bearing an isotope label in their phosphate function due to the oxidation of P (III) intermediates with an oxygen-labeled water/iodine reagent.[21]

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Scheme I

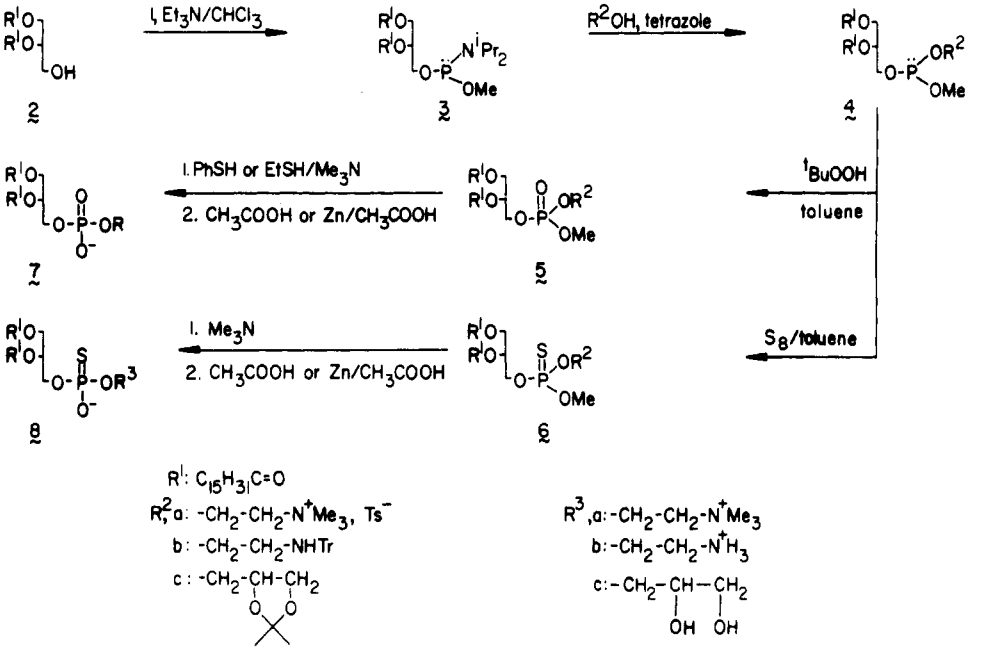

Table I. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphoesters 7 and 3-Thiophosphoesters 8 Prepared

R^3	product	yield,[a] %	$\delta_{^{31}P}$[b]	$\delta_{^{13}C}$[b,j]	$[\alpha]^{20}_D$, deg (c, solv)
$CH_2CH_2N^+Me_3$	**7a**	60	−1.5[c]	55.0 (NMe), 60.1 (α-CH_2), 63.65 (1-*sn*-CH_2), 64.4 (3-*sn*-CH_2), 67.3 (β-CH_2), 71.3 (2-*sn*-CH)	+6.34[g] (2.0, 1:1 $CHCl_3$-MeOH)
	8a	75	55.3[d]	54.9 (NMe), 60.1 (α-CH_2), 63.6 (1-*sn*-CH_2), 64.5 (3-*sn*-CH_2), 67.1 (β-CH_2), 71.2 (2-*sn*-CH)	+10.3[h] (2.7, $CHCl_3$)
$CH_2CH_2N^+H_3$	**7b**	61	−0.5[c]	41.0 (β-CH_2), 62.3 (α-CH_2), 63.4 (1-*sn*-CH_2), 64.2 (3-*sn*-CH_2), 71.5 (2-*sn*-CH)	+6.93[i] (2.1, 2:1 $CHCl_3$-MeOH)
	8b	60	57.94,[e,k] 58.06	41.0 (β-CH_2), 62.3 (α-CH_2), 63.3 (1-*sn*-CH_2), 64.6 (3-*sn*-CH_2), 71.3 (2-*sn*-CH)	+10.6 (2.3, 1:1 $CHCl_3$-MeOH)
$CH_2CHOHCH_2OH$	**7c**	71	0.7[c]	63.0, 63.1 [1(1′)-*sn*-CH_2], 64.0 (3-*sn*-CH_2), 66.9 (3′-*sn*-CH_2), 71.0, 71.6 [2(2′)-*sn*-CH]	+6.93 (2.1 2:1 $CHCl_3$-MeOH)
	8c	59	59.86,[d,k] 60.15	63.0, 63.1 [1(1′)-*sn*-CH_2], 64.4 (3-*sn*-CH_2), 67.1 (3′-*sn*-CH_2), 70.9, 71.4 [2(2′)-*sn*-CH]	+6.84 (2.2, $CHCl_3$)

[a] Yields refer to chromatographically pure compounds and are not optimized. [b] ^{31}P and ^{13}C NMR spectra were run at 24.3 and 22.63 MHz, respectively. [c] 50 mg in 2 mL of chloroform–methanol (1:1 v/v). [d] 50 mg in 2 mL of chloroform. [e] 50 mg in 2 mL of chloroform + 3 equiv of Et_3N. [f] In chloroform–methanol–water (50:50:15 v/v). [g] Lit.[22] $[\alpha]_D$ +6.6°. [h] Lit.[11] $[\alpha]^{20}_D$ +10.4°. [i] Lit.[23] $[\alpha]^{22}_D$ +6.03°. [j] Only chemical shift of carbon atoms of glycerol backbone and head groups are given; all other resonances present in the spectra were characteristic of fatty acid residues. [k] Signals arising from two diastereomers, relative ratio 1:1.

We now report a convenient procedure for the synthesis of glycerophospholipids and their phosphorothioyl analogues bearing various polar head group. Chloro-(*N*,*N*-diisopropylamino)methoxyphosphine (**1**) was used as the phosphitylating reagent in the present study. The general synthetic procedure is outlined in Scheme I.

All phospholipids synthesized were 1,2-dipalmitoyl-*sn*-glycero-3-phosphoesters.

1,2-Dipalmitoyl-*sn*-glycerol (**2**) was first condensed with chloro-(*N*,*N*-diisopropylamino)methoxyphosphine (**1**) in chloroform in the presence of an excess of triethylamine at ambient temperature. The resulting amido diester **3** was reacted with the second hydroxyl component such as choline tosylate, *N*-tritylethanolamine, or 1,2-isopropylidene-*sn*-glycerol in the presence of 3–4 equiv of tetrazole in THF–acetonitrile, giving triesters **4**.

Each of the intermediates **4** was treated with *tert*-butyl hydroperoxide or elemental sulfur in toluene affording phosphoryl or phosphorothioyl derivatives **5** and **6**, respectively, which were next subjected to deprotection steps. The progress of all reactions was followed by TLC and/or ^{31}P NMR. It is important to note that in all cases the yields of phosphorotriesters **5** and **6** were essentially quantitative as judged by the ^{31}p NMR spectra of the respective reaction mixtures.

In case of phosphorothioyl derivatives **6**, their treatment with anhydrous trimethylamine in toluene was sufficient to remove the methyl group from the phosphorothioate function.

Phosphoryl compounds **5** were generally more resistant and required application of stronger nucleophiles or higher temperatures. Thus, **5d** was converted to the corresponding diester **7d** by demethylation with an ethanethiol/trimethylamine mixture. Deprotection of phosphoethanolamine derivative **5b** with trimethylamine required higher temperatures and longer reaction times than those for phosphocholine **5a**.

N-Trityl blocking groups of **5b** and **6b** were removed by the action of Zn/acetic acid. The removal of the isopropylidene group from **5c** and **6c** was achieved with 70% aqueous acetic acid at 30–40 °C.

All crude phospholipid preparations were extensively purified by means of silica gel chromatography using the solvent systems listed below. The yields of final products and their analytical data are given in Table I. ^{13}C, ^{31}P, and ^1H NMR spectra further identified all of the synth-

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sized compounds. Compounds **7a–c** were also identified by the TLC which was compared to respective standards of natural phospholipids.

Experimental Section

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (7a): Typical Procedure. a. Synthesis of Triester 5a. (–)-1,2-Dipalmitoyl-*sn*-glycerol [2, 1 mmol, $[\alpha]_D$ –2.28° (*c* 3.3, $CHCl_3$)] was carefully dried at room temperature in vacuo in a round-bottom, two-neck flask equipped with Teflon stopcock and magnetic stirrer and capped with a rubber septum.

While the mixture was stirred at room temperature, dry chloroform (5 mL), triethylamine (2 mmol), and chloro-(*N,N*-diisopropylamino)methoxyphosphine (1.2 mmol) were successively added into the flask by using a syringe. The progress of the condensation was monitored by following the disappearance of starting (–)-dipalmitin (**2**) (TLC on Merck silica gel plates, 3:2 ether–hexane. After the reaction was complete (5 min), the solvent and excess triethylamine were evaporated under vacuum, and solid tetrazole (4 mmol) and choline tosylate (2–3 mmol) were added, the mixture was kept under vacuum for 1 h. All reactants were solubilized by the addition of acetonitrile–THF (1:1 v/v) mixture (5–10 mL). After 30 min the solvents were removed by evaporation and replaced with anhydrous toluene (10 mL). The resulting heterogeneous mixture was treated with *tert*-butyl hydroperoxide (2 mmol). The suspension thus obtained was stirred at ambient temperature for 10 h. At this time the reaction mixture was washed with triethylammonium bicarbonate buffer (1.5 M, pH 7.0). The organic phase was concentrated, and the residue was rendered anhydrous by repeated evaporation with anhydrous benzene.

b. Deprotection of Triester 5a. The anhydrous semisolid from the above preparation was suspended in toluene (3 mL) and treated with anhydrous trimethylamine (3 mL, stored in a stoppered ampule over NaH).

The resulting solution was kept at room temperature for 10 h. After the deprotection reaction was judged complete (TLC), trimethylamine and solvent were evaporated, and the crude product was purified to homogeneity by chromatography on silica gel (2.5 × 30 cm column, Merck 230–400 mesh silica gel) using chloroform–methanol–water (66:33:4) as the eluting solvent.

1,2-Dipalmitoyl-*sn*-glycero-3-thiophosphocholine (8a). This compound was prepared by closely following the above procedure except that elemental sulfur instead of *tert*-butyl hydroperoxide was used to obtain trialkyl phosphorothioate **6a** from 4. Product purification was carried out with chloroform–methanol (1:1) for the chromatography.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (7b). The corresponding triester **5b** was prepared in the same manner as described for **5a** using *N*-tritylethanolamine (2 mmol) as the second hydroxyl component. The deprotection procedure involved demethylation of **5b** with Me_3N/toluene (1:1 v/v, 5 mL) at 50 °C for 20 h and detritylation with Zn/CH_3COOH (200 mg of Zn in 10 mL of CH_3COOH) at 45 °C for 9 h. The product was purified with CH_2Cl_2–MeOH–H_2O (80:20:3) as the eluting solvent for chromatography.

1,2-Dipalmitoyl-*sn*-glycero-3-thiophosphoethanolamine (8b). The intermediate trialkyl phosphorothioate **6b** was obtained similarly as described above. Deprotection of triester **6b** was performed with Me_3N/toluene (6 h at 50 °C) and with Zn/CH_3COOH (1.5 h at 40 °C). The product was purified with $CHCl_3$–MeOH (5:1) as solvent for chromatography.

1,2-Dipalmitoyl-*sn*-glycero-3′-phospho-*sn*-glycerol (7c). The respective triester **5c** was obtained in a similar manner by using 1,2-isopropylidene-*sn*-glycerol $[\alpha]^{19}_D$ + 15.17° (neat) as the second hydroxyl component. Deprotection of the resulting triester **5c** was achieved by using a solution of ethanethiol–trimethylamine in toluene (200 mg EtSH/100 mg Me_3N in 5 mL toluene) at 45 °C for 3 h. After removal of this reagent, the isopropylidene group was removed with 70% aqueous CH_3COOH (3 h at 35–40° C). Purification of **7c** was carried out with CH_2Cl–MeOH–H_2O (80:20:3) as solvent system for chromatography. Attempts at conversion of **7c** into the ammonium salt by aqueous workup of its chloroform solution with a solution of ammonium chloride failed. Elemental analysis of the final product indicated a 56% content of ammonium salt and a 46% content of the free acid.

1,2-Dipalmitoyl-*sn*-glycero-3-thiophospho-3′-*sn*-glycerol (8c). This product was prepared similarly to **7c** except trimethylamine in toluene was used to demethylate triester **5c**.

Aqueous acetic acid (70%) at room temperature was applied to remove the 1,2-isopropylidene protecting group. The product was chromatographed with acetone–CH_2Cl_2–MeOH (100:10:4) as solvent. Elemental analysis of the product showed a 24% content of ammonium salt $C_{38}H_{78}NO_9PS$ and 76% of free acid $C_{38}H_{75}O_9PS$ present in the sample, %C ± 0.4, %H ± 0.1, %N ± 0.0, %P ± 0.4.

Registry No. (–)-**2**, 30334-71-5; **5a**, 102152-55-6; **5b**, 102152-58-9; **5c**, 102210-89-9; **6a**, 102152-57-8; **6b**, 102152-59-0; (+)-**7a**, 63-89-8; (+)-**7b**, 923-61-5; (+)-**7c**, 74313-95-4; (+)-**7c**·NH_3, 102152-60-3; (+)-**8a**, 82916-29-8; (+)-**8b**, 102281-30-1; (+)-**8c**, 102152-61-4; (+)-**8c**·NH_3, 102282-26-8; chloro-(*N,N*-diisopropylamine)methoxyphosphine, 86030-43-5; choline tosylate, 55357-38-5; *N*-tritylethanolamine, 24070-16-4; (+)-1,2-isopropylidene-*sn*-glycerol, 22323-82-6.

On the Use of the *O*-Methylmandelate Ester for Establishment of Absolute Configuration of Secondary Alcohols

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With the current emphasis on obtaining enantiomerically pure compounds, methods for resolution, determination of enantiomeric purities, and determination of absolute configurations become important.[1] Reagents that function in all three regards are most desirable. Raban and Mislow[2] pointed out the utility of *O*-methylmandelate derivatives for determination of enantiomeric purity. Dale and Mosher[3] examined this approach in more detail–especially with respect to the mandelate esters and α-(trifluoromethyl)-*O*-methylmandelate esters. For these two, conformational models were developed to rationalize the sense of nonequivalence. The model chose a conformation for the former esters in which the OH group eclipses the carbonyl group of the ester due to hydrogen bonding and, for the latter esters, one in which the trifluoromethyl group eclipses the carbonyl group. No model was proposed for the *O*-methylmandelate although the trends in chemical shift differences between the diastereomeric pairs followed the mandelate esters. Little use of this empirical method for assignment of absolute configuration has been made. Furthermore, the *O*-methylmandelates have been less used because of the problems of racemization during esterification and the lack of a model for assignment of absolute configuration. Since *O*-methylmandelates appeared to represent a facile approach to all three goals for resolving

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