Anal. Calcd for $C_{21}H_{24}O_4 \cdot 2H_2O$: C, 67.00; H, 7.49. Found: C, 67.33; H, 6.78.

20-Acetoxy-3,11-dioxo-1,4-*trans*-17(20)-pregnatrien-21-al (7e-*trans*): small prisms from ethanol; mp 207-210 °C; $[\alpha]_D$ +147°; λ_{max} 244 nm; ϵ 30 500; IR 1768 and 1200 (enol acetate), 1710 (11-ketone), 1690-1610 cm^{-1} (multiple peaks, conjugated carbonyls); CI-MS m/z 383 ($M^+ + 1$, 100), 341 ($M^+ + 1 - CH_3COH$, 13). NMR spectra were unsuccessful because compound decomposed in deuteriochloroform. Anal. Calcd for $C_{23}H_{26}O_5$: C, 72.23; H, 6.85. Found: C, 72.02; H, 7.11.

Acknowledgment. This work was supported by NIH Grant AM-31105. Mass spectral analyses were performed in The Rockefeller University Extended Range Mass Spectrometry Facility under the direction of Professor Frank Field and was supported by the Division of Research Resources of NIH. We thank Drs. F. Aladar Bencsath and Y. T. Ng for their assistance in performing the mass spectral scans. NMR spectra were obtained using the NMR facilities at the Rockefeller University purchased in part with funds from the National Science Foundation (PCM-7912083) and from the Camille and Henry Dreyfus Foundation. We would like to thank Pirjo Jänne for preparing the drawings and Richard Hale for typing the manuscript.

Registry No. **1a**, 50-23-7; **1b**, 53-06-5; **1c**, 152-58-9; **1d**, 50-24-8; **1e**, 53-03-2; *cis*-**3a**, 105562-13-8; *trans*-**3a**, 105562-12-7; *cis*-**3b**, 118916-30-6; *trans*-**3b**, 118864-84-9; *cis*-**3c**, 118864-85-0; *trans*-**3c**, 118864-86-1; *cis*-**3d**, 118864-87-2; *trans*-**3b**, 118864-88-3; *cis*-**3e**, 118724-35-9; *trans*-**3e**, 118724-36-0; *cis*-**7a**, 118864-89-4; *trans*-**7a**, 118864-90-7; *cis*-**7b**, 118864-91-8; *trans*-**7b**, 118864-92-9; *cis*-**7c**, 118864-93-0; *trans*-**7c**, 118864-94-1; *cis*-**7d**, 118866-09-4; *trans*-**7d**, 118864-95-2; *cis*-**7e**, 118724-37-1; *trans*-**7e**, 118724-38-2; *cis*-**8**, 118724-39-3; *trans*-**8**, 118724-40-6; **9a**, 95811-04-4; **9b**, 95909-27-6; **10a**, 118724-41-7; **10b**, 118724-42-8; **11a**, 98039-97-5; **11b**, 98040-02-9; **12a**, 118724-43-9; **12b**, 118724-44-0; **13a**, 118724-45-1; **13b**, 118724-46-2; **14**, 118724-47-3; methyl 11β,17,20α-trihydroxy-3-oxo-1,4-pregnadien-21-oate, 97232-42-3; methyl 11β,17,20β-trihydroxy-3-oxo-1,4-pregnadien-21-oate, 97274-84-5.

A General Method for the Synthesis of Glycerophospholipids and Their Analogues via H-Phosphonate Intermediates†

Ingvar Lindh and Jacek Stawiński*

Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Received September 27, 1988

A general chemical method for the synthesis of glycerophospholipids and their analogues via H-phosphonate intermediates has been developed. It was found that 1,2-dipalmitoylglycero-3-H-phosphonate, prepared by the reaction of 1,2-dipalmitoylglycerol with PCl_3/imidazole, reacts with various hydroxylic components (choline tosylate, *N*-(*tert*-butoxycarbonyl)ethanolamine, *N*-(*tert*-butoxycarbonyl)-L-serine) in the presence of condensing agents to produce in high yield the corresponding glycero-3-H-phosphonate diesters. These can be converted into natural phospholipids via oxidation with iodine or into thio or seleno analogues by using sulfur or selenium as oxidant, respectively.

The vital role played by phospholipids in many biological processes has in the last decade stimulated a numbers of studies concerning their chemistry, biochemistry, and physical properties.[1,2] Interactions of phospholipids with biopolymers such as peptides,[3] DNA,[4] and polysaccharides of cell structures[5,6] have been extensively investigated. Phospholipid analogues were found to be a valuable tool in studies concerning elucidation of the mechanism of some enzymatic reactions,[7] in probing biomembranes structures,[8] and in the preparation of liposomes with the desired properties.[9] Also therapeutical applications of phospholipids have been investigated that use these molecules as drug carriers[10] or as drugs per se.[9,11] Such studies caused high demand for phospholipids and their analogues of unequivocal structure and have resulted in an extensive expansion in the field of chemical synthesis of phospholipids.[12,13]

The most important stage in the chemical synthesis of phospholipids is phosphorylation, which leads to formation of a phosphodiester bond, the major structural element of these compounds. Due to considerable achievements during the past years, the synthetic chemistry of phospholipids has now at its disposal a variety of phosphorylation methods which make use of phosphodiester,[12-14] phosphotriester,[13,15] and phosphite[16] chemistries. Some other methods have also been developed.[16,17]

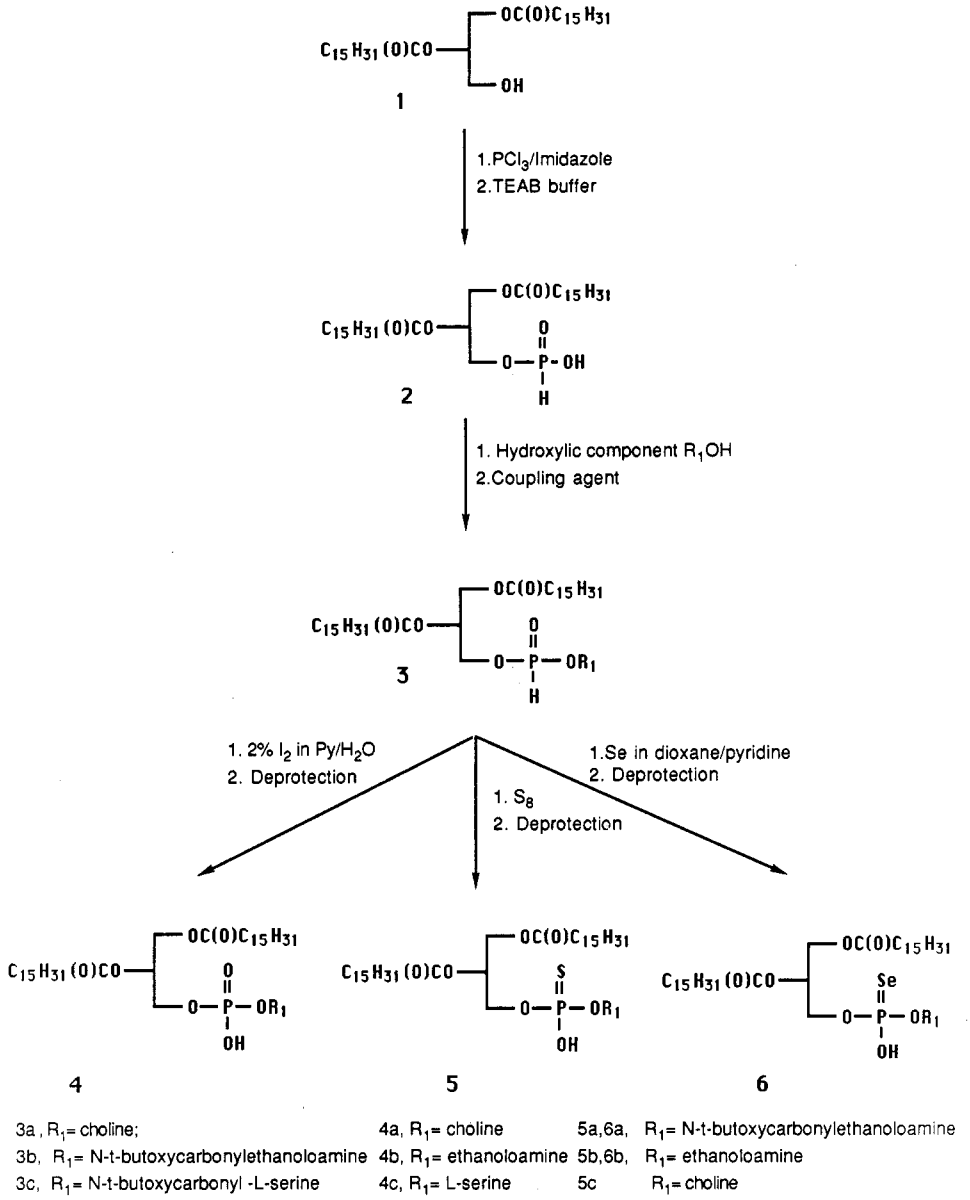
The most straightforward one, the phosphodiester method for phospholipids synthesis, consisting of condensation of glycerol phosphate with a suitable hydroxylic component, is inefficient in terms of both yield and la-

† The H is being used to emphasize that the phosphonic acid is unsubstituted.

(1) Eibel, H. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 257-271.
(2) Hanahan, D. J. *Ann. Rev. Biochem.* **1986**, *55*, 483-509.
(3) Tamm, L. K. *Biochemistry* **1986**, *25*, 7470-7476.
(4) Datema, K. P.; Spruijt, R. B.; Verduin, J. M.; Hemminga, M. A. *Biochemistry* **1987**, *26*, 6217-6223.
(5) Imoto, M.; Kusunoe, N.; Kusumoto, S.; Shibo, T. *Tetrahedron Lett.* **1988**, *29*, 2227-2230.
(6) Low, M. G.; Saltiel, A. R. *Science* **1988**, *239*, 268-275.
(7) Jiang, R.-T.; Shyy, Y.-J.; Tsai, M.-D. *Biochemistry* **1984**, *23*, 1661-1667.
(8) Vasilenko, I.; de Kruijff, B.; Verkleij, A. J. *Biochim. Biophys. Acta* **1982**, *685*, 144-152.
(9) Jett, M.; Alving, C. R. *Methods Enzymol.* **1987**, *141*, 459-466.
(10) Juliano, R. L. In *Liposomes: from Physical Structure to Therapeutical Application*; Knight, C. G., Ed.; Elsevier: Amsterdam, 1981; p 391.
(11) Satouchi, K.; Pinckard, R. N.; McMagnus, L. M.; Hanahan, D. J. *J. Biol. Chem.* **1981**, *256*, 4425-4432.
(12) Eibel, H. *Chem. Phys. Lipids* **1980**, *26*, 405-429.
(13) Stepanov, A. E.; Shvets, V. I. *Chem. Phys. Lipids* **1980**, *41*, 1-51.
(14) Aneja, R.; Chadha, J. S.; Davies, A. P. *Biochim. Biophys. Acta* **1970**, *218*, 102-111.
(15) Lammers, J. G.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1979**, *98*, 243-250.
(16) Nifant'ev, E. E.; Predvoditelev, D. A. *Bioorg. Khim.* **1981**, *7*, 1285-1309.
(17) Ramirez, F.; Ioannou, P. V.; Marecek, J. F.; Dodd, G. H.; Goldin, B. T. *Tetrahedron* **1977**, *33*, 599-608.

0022-3263/89/1954-1338$01.50/0

Scheme I. Synthesis of Phospholipids via H-Phosphonate Intermediates



3a, R_1 = choline; 4a, R_1 = choline 5a,6a, R_1 = N-t-butoxycarbonylethanoloamine
3b, R_1 = N-t-butoxycarbonylethanoloamine 4b, R_1 = ethanoloamine 5b,6b, R_1 = ethanoloamine
3c, R_1 = N-t-butoxycarbonyl -L-serine 4c, R_1 = L-serine 5c R_1 = choline

borious experimental procedure.[14] Phosphotriester chemistry, on the other hand, usually offers higher yield during the coupling step, but this is often offset by losses of a material during the removal of phosphate protecting groups.[15] The phosphoramidite approach, especially in the recent version reported by Stec et al.[18] affords phospholipids in good yield and opens possibilities for their modification at the phosphorus center as, e.g., isotope labeling or sulfurization. Unfortunately this method relies on phosphoramidites, which are rather reactive and difficult to handle for inexperienced people, and all starting materials have to be prepared in situ.[18]

Recently, the hydrogen phosphonate method, originally designed by us for the oligonucleotide synthesis,[19,20] attracted our attention as an alternative method for a chemical synthesis of phospholipids and their conjugates. Simple experimental procedures and high yield of phosphodiester formation on one hand and easy access to phospholipid analogues on the other, could make the H-phosphonate approach the method of choice for the phospholipids synthesis.

To demonstrate the utility of H-phosphonate intermediates in the synthesis of phospholipids, 1,2-dipalmitoyl-*sn*-glycero-3-H-phosphonate (**2**) was prepared by the reaction of 1,2-dipalmitoyl-*sn*-glycerol (**1**) with the PCl_3/imidazole reagent system[21] or with salicylchlorophosphite[22] as a phosphitylating reagent (Scheme I). After purification on a silica gel column and precipitation from a hexane–ether mixture, the glycero-3-H-phosphonate **2** was obtained as a solid in over 80% yield. 1,2-Dipalmitoyl-*rac*-glycero-3-H-phosphonate was also prepared in a similar way and it was used in most studies concerning optimization.

In a typical synthesis of phospholipid diesters, the glycero-H-phosphonate **2** was rendered anhydrous by repeated evaporation of added pyridine and then condensed

(18) Bruzik, K. S.; Salamonczyk, G.; Stec, W. J. *J. Org. Chem.* **1986**, *51*, 2368–2370.
(19) Garegg, P. J.; Regberg, T.; Stawinski, J.; Strömberg, R. *Chemica Scr.* **1985**, *25*, 280–282.
(20) Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R.; Henrichson, C. *Tetrahedron Lett.* **1986**, *27*, 4055–4058.
(21) Garegg, P. J.; Regberg, T.; Stawinski, J.; Strömberg, R. *Chemica Scr.* **1986**, *26*, 59–62.
(22) Marugg, J. E.; Tromp, M.; Kuyl-Yeheskiely, E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2661–2664.

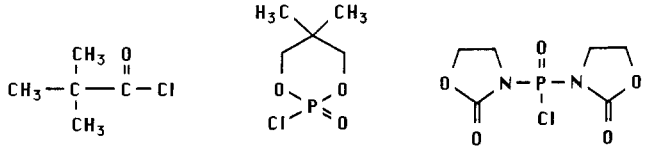
in the same solvent with a hydroxylic component, such as choline tosylate, *N*-(*tert*-butoxycarbonyl)ethanolamine, *N*-tritylethanolamine, or *N*-(*tert*-butoxycarbonyl)-L-serine in the presence of a coupling agent **7** (pivaloyl chloride, PV-Cl), **8** (5,5-dimethyl-2-oxo-2-chloro-1,3,2-dioxaphosphorinan, NPCl), or **9** (bis[2-oxo-3-oxazolidynyl]phosphinic chloride, OXP). When TLC analysis showed a complete conversion of **2** into the respective glycero-H-phosphonate diesters **3** (ca. 5 min when pivaloyl chloride was used as a condensing agent or 5–10 min in case of chlorophosphates **8** and **9**), the reaction mixture was simultaneously quenched and oxidized by addition of iodine in aqueous pyridine, followed by workup and purification on a silica gel column. The total yields from **2** to phospholipids **4** were ca. 80%.

Since TLC analysis at the various stages of synthesis showed virtually quantitative conversion of substrates into the products, we ascribe the lower than expected isolated yield of phospholipids **4** due to losses of the material during silica gel chromatography rather then to incomplete coupling, oxidation, or formation of side products.

For routine synthesis of phospholipids all synthetic steps can be carried out as a "one-pot" reaction. However, if it is desired, intermediates can be isolated, purified, and then used for the subsequent transformation.

Phosphitylation of compound **1** with PCl_3/imidazole or salicylchlorophosphite proceeds fast without acyl migration (TLC, ^{31}P NMR and ^{13}C NMR analyses) and without racemization (optical rotation). For practical purpose, chromatography of the glycero-3-H-phosphonate **2** can be omitted when PCl_3/imidazole is used as a phosphitylating reagent. In case of salicylchlorophosphite, a chromatographic purification seems to be necessary in order to removed decomposition products of the reagent.

Among the condensing reagents investigated, **7**–**9**, the most convenient one seems to be chlorophosphate **8**. It is a stable, crystalline compound, easy to prepare in large quantities,[23] and has good solubility in most organic solvents. This reagent ensures clean and reasonable fast coupling (5–10 min) without danger of side reactions even if the reaction mixture is left for a longer time. The other



7 **8** **9**

investigated chlorophosphate **9** also possesses similar properties, being an easily accessible,[24] stable, crystalline compound, and being slightly more reactive than chlorophosphate **8** in promoting condensations. The only inconvenience in using this reagent is its rather low solubility in organic solvents, which results in heterogeneous reaction mixtures, partly also because of the precipitation of decomposition products of the reagent. Pivaloyl chloride, which is commonly used in oligonucleotide synthesis,[25,26] seems to be unnecessarily reactive for the purpose of phospholipid synthesis. It may also lead to formation of side products if the reaction mixture is not quenched when the reaction is over.

To investigate the possibility of using a H-phosphonate diester of type **3** to produce phospholipids analogues having a modified phosphorus center, 1,2-dipalmitoyl-*sn*-glycero-3-H-phosphono-*N*-(*tert*-butoxycarbonyl)ethanolamine (**3b**) was obtained by using the procedure describe above and subjected to the reaction with elemental sulfur. The sulfurization was carried out under two reaction conditions, with sulfur in toluene/pyridine mixture, or by converting **3b** into the silyl ester with trimethylsilyl chloride followed by the addition of sulfur. TLC analysis showed that both methods are effective for the conversion of H-phosphonate diester **3b** into the thiophosphate derivative **5a** and that the reactions were almost complete after ca. 30 min. Removal of N-protecting groups under the standard acidic conditions[27] followed by silica gel chromatography afforded a major product in over 70% yield. However, the ^{31}P NMR spectrum of the isolated compound was not as one might expect to be for the compound **5b**, and instead of resonance(s) at ca. 60 ppm, two singlets at ca. 28 ppm were observed. The chemical shift value and the pattern of signals in the ^{31}P NMR spectrum indicated on a phosphorothioate triester containing a P(O)(SR) group and not on the desired phosphorothioate diester **5b** with a P(S)(OH) function. It was most likely that formation of a phosphorothioate triester occurred during the deprotection step. Strong acidic conditions used for the removal of the *tert*-butoxycarbonyl group from the ethanolamine moiety of **5a** may give rise to formation of *tert*-butyl carbocation, which in turn may react with the phosphorothioate diester to produce a phosphorothioate triester. To check this assumption, the ^{31}P NMR spectrum was recorded directly after sulfurization of **3b**. As expected the spectrum showed signal at ca. 60 ppm (phosphorothioate diester **5a**), and this was replaced by two singlets at ca. 28 ppm upon removal of the *tert*-butoxycarbonyl group. To eliminate this undesired reaction pathway, the deprotection was carried out in the presence of various carbocation scavengers, as e.g. anisole, thioanisole, or 1,2-ethanedithiol. As judged from TLC and from the ^{31}P NMR spectra, addition of anisole had a rather minor beneficial effect on the formation of phosphorothioate diester **5b** (two singlets at ca. 58 ppm), and thioanisole suppressed phosphorothioate triester formation by ca. 50%. The most efficient as a carbocation scavenger was found to be 1,2-ethanedithiol, which secured complete removal of *tert*-butoxycarbonyl group under acidic conditions from the ethanolamine moiety with a minimal (ca. 1–2%) formation of the phosphorothioate triesters. After such a modification of the deprotection procedure, we were able to synthesize the thio analogues **5b** and **5c** in a "one-pot" reaction in a total yield of ca. 90%.

A similar procedure was used for the preparation of seleno analogues of phospholipids. To this end, the H-phosphonate diester **3b** was produced in situ from **2**, and then a suspension of selenium in dioxane was added. The reaction was slower than that with sulfur, and the mixtures was left to stand overnight to ensure almost complete conversion to the seleno derivatives **6**. After workup and purification on a silica gel column, compounds **6a** was obtained in 98% yield. Removal of *tert*-butoxycarbonyl

(23) McConnell, R. L.; Coover, H. W. *J. Org. Chem.* **1959**, *24*, 630–635.
(24) Cabre-Castellvi, J.; Palomo-Coll, A.; Palomo-Coll, A. L. *Synthesis* **1981**, 616–620.
(25) Froehler, B. C.; Matteucci, M. D. *Tetrahedron Lett.* **1986**, *27*, 469–472.
(26) Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Strömberg, R.; Henrichson, C. *Tetrahedron Lett.* **1986**, *27*, 4051–4054.
(27) Eibel, H.; McIntyre, J. O.; Fleer, A. M.; Fleischer, S. *Methods Enzymol.* **1983**, *98*, 623–632.
(28) Chemical shift values in the ^{13}C and ^{31}P NMR spectra of phospholipids varied up to ±1 ppm from experiment to experiment, depending on sample concentration, slight changes in solvent composition, and way of preparation of samples. This is probably due to formation of micelles or other aggregation of phospholipid molecules.
(29) Orr, G. A.; Brewer, C. F.; Heney, G. *Biochemistry* **1982**, *21*, 3202–3206.

Table I. ^{13}C and ^{1}H NMR Data of Some Synthetic Phospholipids and Their Analogues[28]

compd	1-CH_2	2-CH	3-CH_2	α-CH_2O	β-CH_2	other	solvent	δ ^{31}P in ppm (solvent)
2	62.63	70.57 (7.8 Hz)	61.97 (3.7 Hz)			45.57 (CH_2-N), 8.58 (Me)	$CDCl_3$/MeOD (9:1, v/v)	4.60 (pyridine), $^{1}J_{PH}$ = 626 Hz, $^{3}J_{PH}$ = 6.8 Hz
3b	61.56	69.59 (6.4 Hz)	65.21	63.79	41.02	155.81 (C=O), 79.09 (t-Bu), 78.97 (t-Bu)	$CDCl_3$	8.65 and 8.43 (pyridine), $^{1}J_{PH}$ = 713 Hz, $^{3}J_{PH}$ = 9.3 and 9.1 Hz
	61.53	69.35 (5.5 Hz)						
4a	63.30	70.97 (8.3 Hz)	64.07 (4.6 Hz)	59.72 (4.6 Hz)	66.86	54.54 (NMe_3)	$CDCl_3$/MeOD/D_2O (50:50:15, v/v)	−0.45 (pyridine)
4b	62.76	70.61 (7.3 Hz)	69.13 (7.3 Hz)	61.96 (5.5 Hz)	40.79 (3.7 Hz)		$CDCl_3$/MeOD (4:1, v/v)	0.90 (pyridine)
4c	62.55	70.25	64.83			63.68 (CH_2, Ser), 54.11 (CH, Ser), 170.0 (C=O, Ser)	$CDCl_3$/MeOD (9:1, v/v)	0.00 (pyridine)
5b	69.13	70.74 (7.3 Hz)	64.44 (5.5 Hz) 64.35 (3.7 Hz)	62.23 (5.5 Hz)	40.59 (5.5 Hz)		$CDCl_3$/MeOD/D_2O (50:50:15, v/v)	58.44 and 58.42 (pyridine)
5c	62.90	70.24 (broad)	63.92 (5.5 Hz)	59.67	66.20 (broad)	54.76 (NMe_3)	$CDCl_3$	56.45[a,b] (pyridine)
6a	62.78	70.37 (11.0 Hz)	64.85 (7.4 Hz)	62.55 (5.5 Hz)	43.20 (3.7 Hz)	157.35 (C=O), 72.5 (t-Bu)	$CDCl_3$	52.43[a] (pyridine), $^{1}J_{PSe}$ = 809.8 Hz
6b	62.62	70.08 (9.1 Hz)	65.49	62.62	40.62		$CDCl_3$	55.38 and 55.23 (pyridine), $^{1}J_{PSe}$ = 807.4 Hz

[a] Signals from the two diastereoisomers were not resolved. [b] An enzymatic digestion with phospholipase A_2 and C[29] revealed presence of two diastereoisomers.

group from **6a** afforded **6b** in 56% yield.

All synthesized compound were characterized by TLC and spectral analysis and compared with commercial samples of natural phospholipids (**4a–c**). For the new compounds **2** and **6**, satisfactory elemental analysis data were obtained.

In conclusion, the hydrogen phosphonate approach was found to be an efficient and experimentally simple method for the phospholipid synthesis. The distinctive features of the method are (i) easy preparation of the key intermediate **2**, which can be stored for several month, (ii) coupling reactions are fast and clean, (iii) the possibility to isolate intermediates or to carry out the synthesis as a "one-pot" reaction, (iv) the lack of a protecting group at the phosphorus center simplifies the deprotection procedure, and (v) the possibility of synthesizing various phospholipid analogues, including isotope labeling, via changing the oxidation procedure.

Experimental Section

Materials and Methods. ^{13}C and ^{31}P NMR spectra were recorded on a JEOL FX-400 FT spectrometer. ^{13}C NMR spectra were referenced to the internal solvent signal, and for ^{31}P NMR spectra 1% H_3PO_4 in D_2O was used as an external standard (coaxial inner tube). TLC was carried out on Merck silica gel 60 F_{254} precoated plates with the following eluents: $CHCl_3$/MeOH/H_2O, 95:35:2 (v/v) (system A); $CHCl_3$/MeOH/H_2O, 66:33:4 (v/v) (system B); $CHCl_3$/MeOH, 1:1 (v/v) (system C); $CHCl_3$/MeOH, 5:1 (v/v) (system D); $CHCl_3$/MeOH, 6:1 (v/v) (system E); toluene/ethyl acetate, 1:1 (v/v) (system F). The spots were developed by iodine vapor or by charring after spraying with phosphomolybdic acid.

Pyridine, acetonitrile, and triethylamine were refluxed with CaH_2 overnight and then distilled and stored over molecular sieves (4 Å) or CaH_2. Tetrahydrofuran (THF) was distilled just before use from lithium aluminum hydride. Imidazole, 1,2-dipalmitoyl-*sn*-glycerol, choline chloride, and pivaloyl chloride were Aldrich commercial grade. Reference samples of **4a–c** were purchased from Sigma. *N*-(*tert*-Butoxycarbonyl)ethanolamine, *N*-(*tert*-butoxycarbonyl)-L-serine, and 1,2-dipalmitoyl-*rac*-glycerol were prepared according to the published procedures.[27] Choline tosylate was obtained from choline chloride via ion exchange.

1,2-Dipalmitoyl-*sn*-glycero-3-H-phosphonate Triethylammonium Salt (2). To a stirred solution of imidazole (1.7 g, 24.5 mmol, evaporated with toluene) in toluene (20 mL) at 0 °C was added dropwise PCl_3 (0.47 mL, 5.4 mmol) in toluene (5 mL) followed by triethylamine (1.95 mL, 14 mmol) in toluene (5 mL). Stirring was continued for 10 min, the temperature was lowered to −5 °C, and then 1,2-dipalmitoyl-*sn*-glycerol (1.0 g, 1.8 mmol, evaporated with toluene) in toluene (20 mL) was added dropwise during a period of 60 min. When TLC analysis showed a complete conversion of the starting material into a product with lower mobility, the reaction mixture was quenched by addition of water/pyridine (1:4, v/v, 100 mL). After 15 min, chloroform was added (300 mL), and the organic layer was washed with water (2 × 100 mL), dried with sodium sulfate, evaporated, and purified on a silica gel column with chloroform/methanol/water system (100:15:1, v/v). After concentration of appropriate fractions and precipitation from hexane/ethyl ether (1:1, v/v), a white powder was obtained. Yield: 1.1 g, 83%. $[\alpha]^{20}_{D}$ +16.8° (*c* 3.0, $CHCl_3$). Anal. Calcd for sodium salt of **2**, $C_{35}H_{68}O_7PNa$: C, 64.2; H, 10.5; P, 4.7. Found: C, 64.1; H, 10.5; P, 4.9. For the ^{13}C and ^{31}P NMR data, see Table I.

General Procedure for Synthesis of H-Phosphonate Intermediates of Type 3. Glycero-H-phosphonate **2** (0.1–0.2 mmol) and a hydroxylic component (choline tosylate, *N*-(*tert*-butoxycarbonyl)ethanolamine, or *N*-(*tert*-butoxycarbonyl)-L-serine) (1.5–2 equiv) were rendered anhydrous by evaporation of added pyridine and dissolved in the same solvent (1–2 mL), and then a condensing reagent (PV-Cl, NPCl or OXP) (2–3 equiv) was added. After the reaction was over (5–10 min, TLC), the mixture was directly subjected to further treatment (see below) in order to obtain **4**, **5**, or **6**. To isolate the intermediate **3**, the reaction mixture was quenched by addition of 0.1 M triethylammonium bicarbonate (TEAB), extracted with chloroform, and then chromatographed on a silica gel column.

General Procedure for Synthesis of Phospholipids 4. To the crude reaction mixture containing the H-phosphonate **3** was added iodine (2 equiv) in pyridine/water (98:2, v/v, 2 mL), and the mixture was stirred for 5 min. Then chloroform (30 mL) was added, the organic phase was washed with 5% aqueous sodium bisulfite, and the aqueous phase was washed back with chloroform (30 mL). The combined organic phase was concentrated to an oil under vacuum, and traces of pyridine were removed by evaporation of added toluene. If appropriate, phospholipids were subjected to deprotection by dissolving the crude reaction product in a solution containing dichloromethane (1 mL), trifluoroacetic acid (1 mL), and 70% perchloric acid (1 mL) and keeping it at 0 °C for 30 min. The reaction mixture was then diluted by addition of water (4 mL), chloroform (4 mL), and methanol (1 mL), the organic phase was washed with 0.5 M sodium carbonate (2 × 3 mL), and finally a combined aqueous phase was extracted

with chloroform (2 mL). Organic phases were combined and evaporated, and the residue was subjected to crystallization or purification on a silica gel column. Alternatively, the protected phospholipids can be purified on silica gel columns and then subjected to a deprotection step.

General Procedure for Synthesis of Phospholipid Analogues 5 and 6. To a crude reaction mixture containing the H-phosphonate **3** was added sulfur (2 equiv) in pyridine/toluene (1:1, v/v, 1 mL) or selenium (2 equiv) in dioxan (1 mL), and the suspension was stirred for 2 and 10 h, respectively. The reaction mixtures were diluted with chloroform (30 mL) and washed with water or with 0.1 TEAB. Further workup and deprotection were the same as described for **4** with the exception that the deprotection was carried out in the presence of 1,2-ethanedithiol (10 equiv).

1,2-Dipalmitoyl-*sn*-glycero-3-H-phosphono-*N*-(*tert*-butoxycarbonyl)ethanolamine (3b). Column chromatography: silica gel; eluent, toluene/ethyl acetate (1:1, v/v). Yield 98%. $[\alpha]^{20}_D$ +2.3° (*c* 2.6, CH_2CL_2). R_f 0.60 (system F). For the ^{13}C and ^{31}P NMR data, see Table I.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (4a). Column chromatography: silica gel; eluent, $CHCl_3/MeOH/H_2O$ (66:33:4, v/v). Yield 80%. $[\alpha]^{20}_D$ +7.7° (*c* 2.0, $CHCl_3$/MeOH, 1:1). R_f 0.45 (system B). For the ^{13}C and ^{31}P NMR data, see Table I.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (4b). Column chromatography: silica gel; eluent, $CHCl_3/MeOH/H_2O$ (100:15:1, v/v) and then $CHCl_3/MeOH/H_2O$ (95:35:2, v/v). Yield 86%. $[\alpha]^{20}_D$ +12.3° (*c* 2.2, $CHCl_3$/MeOH, 9:1). R_f 0.50 (system A). For the ^{13}C and ^{31}P NMR data, see Table I.

1,2-Dipalmitoyl-*sn*-glycero-3-phospho-L-serine (4c). Column chromatography: silica gel; eluent, $CHCl_3/MeOH/H_2O$ (65:25:4, v/v). Yield 81%. $[\alpha]^{20}_D$ +12.5° (*c* 1.5, $CHCl_3$/MeOH/H_2O, 65:25:4, v/v). R_f 0.40 (system B). For the ^{13}C and ^{31}P NMR data, see Table I.

1,2-Dipalmitoyl-*sn*-glycero-3-thiophosphoethanolamine (5b). Column chromatography: silica gel; eluent, $CHCl_3$/MeOH (5:1, v/v). Yield 93%. $[\alpha]^{20}_D$ +17.5° (*c* 1.3, $CHCl_3$/MeOH, 1:1, v/v). R_f 0.49 (system D). For the ^{13}C and ^{31}P NMR data, see Table I.

1,2-Dipalmitoyl-*sn*-glycero-3-thiophosphocholine (5c). Column chromatography: silica gel; eluent $CHCl_3$/MeOH (1:1, v/v). Yield 86%. $[\alpha]^{20}_D$ +16.0° (*c* 2.0, $CHCl_3$). R_f 0.60 (system C). For the ^{13}C and ^{31}P NMR data, see Table I.

1,2-Dipalmitoyl-*sn*-glycero-3-selenophospho-*N*-(*tert*-butylcarbonyl)ethanolamine (6a). Column chromatography: silica gel; eluent, $CHCl_3$/MeOH (6:1, v/v). Yield 98%. R_f 0.55 (system E). For the ^{13}C and ^{31}P NMR data, see Table I.

1,2-Dipalmitoyl-*sn*-glycero-3-selenophosphoethanolamine (6b). Column chromatography: silica gel; eluent, $CHCl_3$/MeOH (6:1, v/v). Yield 56%. $[\alpha]^{20}_D$ +17.6° (*c* 1.6, $CHCl_3$/MeOH, 2:1, v/v). R_f 0.37 (system E). For the ^{13}C and ^{31}P NMR data, see Table I. Anal. Calcd $C_{37}H_{74}O_7PNSe$: C, 58.8; H, 9.9; N, 1.9. Found: C, 58.5; H, 9.9; N, 1.7.

Acknowledgment. We are indebted to Prof. P. J. Garegg for his interest and to the Swedish National Board for Technical Development and the Swedish Natural Science Research Council for financial support.

Steady-State and Laser Flash Photolysis Studies of Norbornenobenzoquinones and Their Diels-Alder Adducts[1]

G. Mehta,*[,2a] S. Padma,[2a] and S. R. Karra[2a]

School of Chemistry, University of Hyderabad, Hyderabad 500134, India

K. R. Gopidas,[2b,c] D. R. Cyr,[2c,d] P. K. Das,*[,2c,e] and M. V. George*[,2b,c]

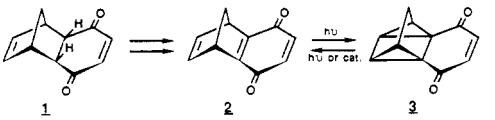
Department of Chemistry, Indian Institute of Technology, Kanpur 208016, India, and Radiation Laboratory and Department of Chemistry, University of Notre Dame, Notre Dame, Indiana 46556

Received July 28, 1988

Results of a photochemical study based on nanosecond laser flash photolysis and steady-state photolysis are reported for several norbornenobenzoquinones and their rigid Diels-Alder adducts. Products isolated from steady-state photolysis of a few representative cases suggest that the preferred mode is intramolecular [2 + 2] cycloaddition wherever feasible, and triplets have been implicated in this photoreaction. The 337.1-nm laser pulse excitation of the substrates in benzene gave rise to triplets (λ_{max}^T = 390-580 nm), characterized by short lifetimes (21 ns-1.05 μs) in fluid solutions at room temperature. The triplets were efficiently quenched by oxygen, ferrocene, *p*-methoxyphenol, HTEMPO, and azulene, but they exhibited reluctant quenching behavior toward DMHD and triethylamine. The lower limits of triplet yields ($\phi_{T,lim}$) for most of the substrates were measured by energy transfer to 9,10-diphenylanthracene (DPA). In some cases, the efficiency of energy transfer to DPA in benzene appeared to be small, probably owing to reversible charge transfer interaction competing favorably with energy transfer.

Introduction

The interesting tricyclic quinone **2** (2,3-norbornenobenzoquinone),[3a,b] readily available from the endo adduct **1** of 1,3-cyclopentadiene and *p*-benzoquinone, has not received much attention except for a few studies by Cookson and co-workers.[3c-e] Recently, some of us[4] have demonstrated the synthetic potential of **2** through Diels-Alder chemistry to prepare novel, strained polycyclic systems. In continuation, we became interested in utilizing the photochemistry of **2** and related quinones to gain access to highly strained quadricyclane derivatives **3**. In general,



1 2 3

(1) Document No. NDRL-3114 from the Notre Dame Radiation Laboratory.

(2) (a) University of Hyderabad. (b) Indian Institute of Technology, Kanpur. (c) University of Notre Dame. (d) Undergraduate research student from the University of Waterloo, Ontario, Canada. (e) Current address: 347A, Petroleum Laboratory, Research and Development, Philips Petroleum Company, Bartlesville, OK 74004.

(3) (a) Diels, O.; Alder, K. *Ber.* **1929**, *62*, 2337-2372. (b) Meinwald, J.; Wiley, G. A. *J. Am. Chem. Soc.* **1958**, *80*, 3667-3671. (c) Cookson, R. C.; Hill, R. R.; Hudec, J. *J. Chem. Soc.* **1964**, 3043-3062. (d) Cookson, R. C.; Hill, R. R. *J. Chem. Soc.* **1963**, 2023-2026. (e) Cookson, R. C.; Grundwell, E.; Hill, R. R.; Hudec, J. *J. Chem. Soc.* **1964**, 3062-3075.

(4) Mehta, G.; Padma, S. *J. Am. Chem. Soc.* **1987**, *109*, 7230-7232.

0022-3263/89/1954-1342$01.50/0