Synthesis of Glycerophosphonolipids Containing Aminoalkylphosphonic Acids

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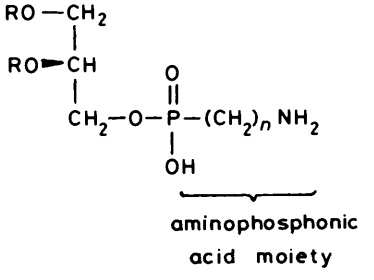
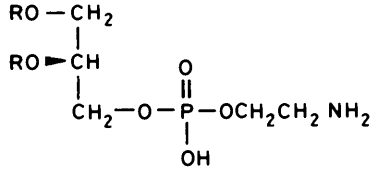
1,2-Di-*O*-octadecyl- and 1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-(2-aminoethyl)phosphonate (**21**) and (**22**) and the (3-aminopropyl)phosphonate analogues (**23**) and (**24**) were prepared with the aim of obtaining enzyme (lipase)-stable liposomes. New reactions were devised and classic methodologies were modified in order to transform D-mannitol (**6**) into optically pure 1,2-di-*O*-substituted *sn*-glycerols (**12**) and (**13**) (R = $C_{18}H_{37}$ and $C_{15}H_{31}CO$). Benzyloxycarbonylaminoalkylphosphonic acids (**5a**) and (**5b**) were then coupled with the glycerols by means of condensing reagents such as 2,4,6-triisopropylbenzenesulphonyl chloride. The resulting *N*-protected lipids were catalytically hydrogenated to furnish compounds (**21**)—(**24**) in overall yields of 10—20% from compound (**6**).

(2-Aminoethyl)phosphonic acid (**4a**) has been discovered in a variety of aquatic and terrestrial animals as well as in microorganisms.[1] The lipid-fractions were the most abundant source of (**4a**), and have been shown to contain glycerophosphonolipids (**1**)[2] and other types of lipid.[3] Although the physiological roles of the abnormal lipids remain obscure, (**4a**) has been reported to be both chemically[4,5] and biologically[4,6] stable and, unlike biomembranes made from common glycerophospholipids (**3**), those containing the phosphonate (**1**) have been found to be resistant to enzymatic hydrolysis,[7] thus, possibly, stabilizing the biological cell structure against a potentially harmful environment.[8]



(**1**) *n* = 2
(**2**) *n* = 3

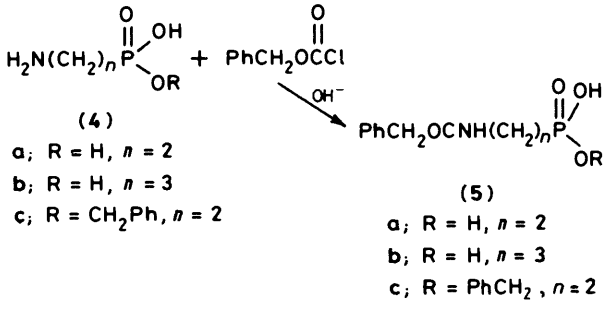


(**3**)

R = long-chain alkyl or acyl group

Scheme 1.

These aspects suggest that artificial liposomes made from the phosphonolipid (**1**) would bear the abnormal membrane properties aforementioned and may have wide use in the fields of chemistry and biochemistry. We therefore undertook the synthesis of 1,2-di-*O*-octadecyl-*sn*-glycerol 3-(2-aminoethyl)-phosphonate (**21**) and 1,2-di-*O*-hexadecanoyl-*sn*-glycerol 3-(2-aminoethyl)phosphonate (**22**) which are representative natural phosphonolipids. Such lipids were not found in nature three decades ago when synthetic studies were carried out by Baer and co-workers according to the procedures cited below.[9-12] We investigated the synthesis using new reactions and modifying the classic methodologies in order to obtain the phosphonolipids using simple procedures and in high yields. Prepared also were unknown lipids (**2**), (**23**) and (**24**) bearing the (3-aminopropyl)phosphonic acid moiety.



(**4**)
a; R = H, *n* = 2
b; R = H, *n* = 3
c; R = CH_2Ph, *n* = 2

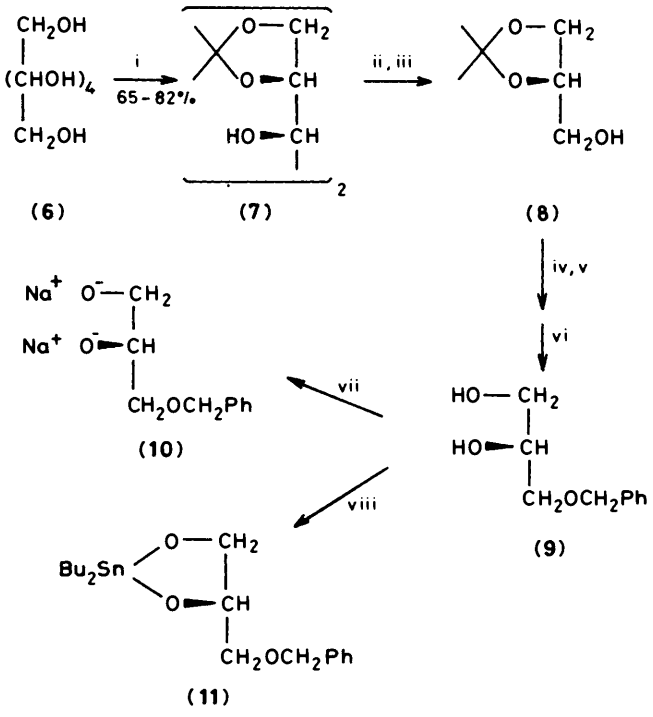
(**5**)
a; R = H, *n* = 2
b; R = H, *n* = 3
c; R = $PhCH_2$, *n* = 2

Scheme 2.

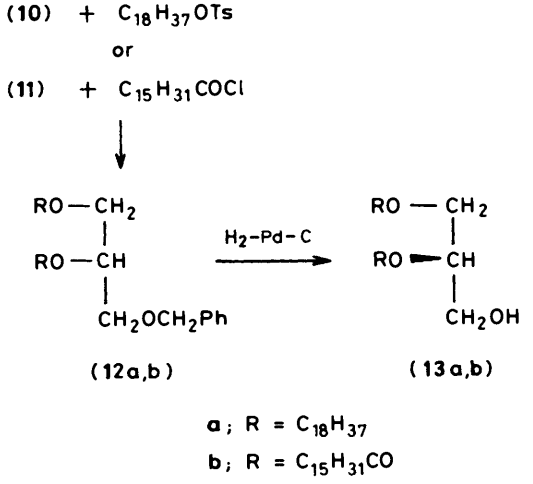
Results and Discussion

It was planned to prepare the phosphonolipids (**1**) and (**2**) by condensing an *N*-protected aminophosphonic acid with a 1,2-di-*O*-acyl (or alkyl)-*sn*-glycerol. The benzyloxycarbonyl group (Cbz) was chosen as the *N*-protecting group because it was easily removable by catalytic hydrogenolysis. Thus, compound (**4**) was treated with benzyloxycarbonyl chloride (Cbz-Cl) in the presence of alkali to furnish benzyloxycarbonylaminoalkylphosphonic acids (**5**) in 67—91% yield (Scheme 2).

The glycerol backbone of compounds (**1**) and (**2**) may be derived from D-mannitol (**6**),[13] D-tartaric acid,[14] ascorbic acid[15] and L-serine.[16] We were interested in compound (**6**) as a starting compound for 1,2-di-*O*-substituted *sn*-glycerols (**13a**) and (**13b**) for economical reasons. However, the $ZnCl_2$-catalysed acetalization of compound (**6**) with acetone into 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**7**), which has been cited by virtually every previous paper, was found to be so delicate that only a yield in the range 0—40% was obtained (reported yield 72%). It was found that retroacetalization occurred during the work-up of the reaction mixture. Hence, the literature method was modified to synthesize compound (**7**) routinely in 65-82% yield (see the Experimental section).



Scheme 3. *Reagents:* i, Me_2CO–$ZnCl_2$; ii, KIO_4; iii, $NaBH_4$; iv, NaH; v, $PhCH_2Br$; vi, H_3O^+; vii, NaH; viii, Bu_2SnO



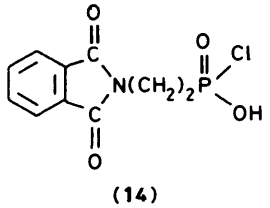
Scheme 4.

Compound (7) was then converted into 3-*O*-benzyl-*sn*-glycerol (**9**) *via* 1,2-*O*-isopropylidene-*sn*-glycerol (**8**) in an overall yield of 60%. Here, compound (**8**) was prepared in one pot—a periodate oxidation of compound (7) followed by a borohydride reduction of the resulting glyceraldehyde. Although similar reactions have beeen described as unsuitable to prepare compound (**8**) because of acid (trace of periodic acid)-catalysed racemization during distillation of the crude compound,[17] it was possible to prepare the optically active (**8**) by maintaining the pH of the reaction mixture at 7—8. The optical purity of compound (**8**) diminished when the pH was allowed to rise to 11 or above.

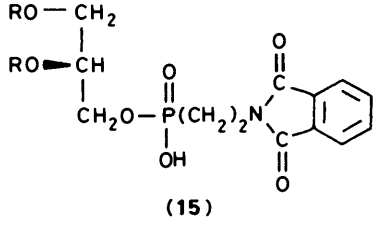
Etherification of the hydroxy groups of compound (**9**) is usually carried out according to the Williamson's ether synthesis.[18–21] For instance, treatment of (**9**) with sodium or potassium hydroxide in benzene or THF followed by refluxing the alkoxide solution with octadecyl iodide for 15—20 h afforded 1,2-di-*O*-octadecyl-3-*O*-benzyl-*sn*-glycerol (**12a**) in 42% yield. In order to improve this rather inefficient etherification, the disodium salt of 3-*O*-benzyl-*sn*-glycerol (**10**) was allowed to react with octadecyl toluene-*p*-sulphonate at *ca.* 190 °C in the absence of any solvent. The reaction was complete after only 0.5 h to provide (**12a**) in 67% with an optical activity equivalent to the literature value.

A new etherification reaction was also investigated. Compound (**9**) was transformed into a 1,2-*O*-dibutylstannylene derivative (**11**) a reaction prompted by the recent reports that alkoxy groups become activated towards electrophiles under co-ordination to a tin atom.[22–24] Indeed, (**11**) underwent smooth condensation with octadecyl toluene-*p*-sulphonate when the mixture was heated at 180—190 °C for 1 h under nitrogen to produce chiefly compound (**12a**) and 1-*O*-octadecyl-3-*O*-benzyl-*sn*-glycerol in yields of 26 and 40%, respectively.

On the other hand, the reaction of hexadecanoyl chloride with compound (**11**) generated 1,2-di-*O*-hexadecanoyl-3-*O*-benzyl-*sn*-glycerol (**12b**) in 50% yield when a benzene solution of the reactants was refluxed for 3 h. The result was comparable to a classic reaction of (**9**) with a long-chain acyl chloride in the presence of a base such as pyridine.[18,20,25]



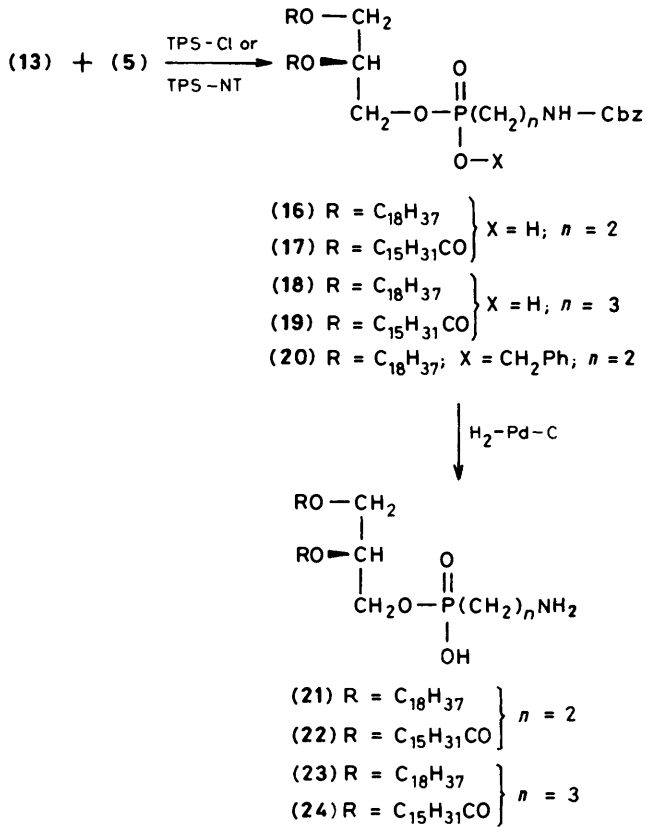
(**14**)



(**15**)

Construction of the phosphonoester linkage of the phosphonolipids (**1**) has been performed by treatment of 2-phthalimidoethylphosphonochloridic acid (**14**) with 1,2-di-*O*-substituted *sn*-glycerol to give compound (**15**) which was deprotected by means of hydrazine to generate the corresponding lipids.[9–12] The demasked lipids, however, were isolated after complex work-up of the reaction mixtures. In addition, hydrazine appeared to react not only with the phthalimido group but also the carboxylic and phosphonic esters.[26] The yields of the free lipids were thus decreased to 40—50%.

By contrast, we attempted to couple the benzyloxycarbonylaminophosphonic acids (**5a**) and (**5b**) with 1,2-di-*O*-substituted *sn*-glycerols (**13a**) and (**13b**) by the use of condensing reagents. It was found that 2,4,6-tri-isopropylbenzenesulphonyl chloride (TPS-Cl) or its 4-nitrotriazole (TPS-NT) caused efficient coupling to furnish the corresponding *N*-protected lipids (**16**)—(**19**) in yields of 70—88%. Subsequent hydrogenolysis of compounds (**16**)—(**19**) gave the respective phosphonolipids (**21**)—(**24**) quantitatively. Similar results were observed by the condensation of benzyl 2-benzyloxycarbonylaminoethylphosphonate (**5c**) with compound (**13a**) to afford (**20**) though an extra step for the conversion of (**4a**) into (**4c**) did not merit the use of compound (**5c**) in the preparation of compound (**21**) *via* (**20**).



Scheme 5.

A preliminary study has indicated that liposomes are not formed from (**21**)—(**24**) alone, but mixtures of the phosphonolipids and cholesterol are found to furnish multilayered vesicles. The details will be reported later.

Experimental

D-Mannitol (**6**), hexadecanoyl chloride, TPS-Cl and TPS-NT were commercially available. 2-Aminoethylphosphonic acid (**4a**) was kindly donated from Sogo-Yakko Co. Ltd., Sagamihara-shi 229, Japan. 2-(*N*-Benzyloxycarbonylamino)ethylphosphonic acid (**5a**),[26] its benzyl ester (**5c**)[26] and octadecyl toluene-*p*-sulphonate[27] were prepared according to the literature procedures cited. Column chromatography was carried out using silica gel (Merck 7734, 70—230 mesh). T.l.c. was performed on silica gel (Merck precoated sheets, 60 F_{254}) using the following solvent systems: *A*, hexane–ethanol (7—1 v/v); *B*, chloroform–methanol–concentrated ammonia (130—35—5 v/v); *C*, chloroform–methanol–concentrated ammonia (65—35—5 v/v). Compound-spots in t.l.c. were visualized under u.v. light (for compounds having a phenyl group), spraying with 0.0012% aqueous Rhodamine 6G (for compounds having long-chain alkyl groups) and the Dittmer-Lester reagent[28] (for phosphonic acid esters). M.p.s were measured with a Shibata hot-stage apparatus and uncorrected. I.r. spectra and optical rotations were measured by means of a JASCO A202 spectrometer and a JASCO DIP-4 automatic polarimeter, respectively. N.m.r. spectra were recorded on a JEOL PS-100 using dilute solution in $CDCl_3$ with $SiMe_4$ as internal standard. Generally, like normal lipids such as 1,2-di-*O*-hexadecanoyl-*sn*-glycero-3-phosphoethanolamine, compounds (**21**)—(**24**) gave n.m.r. spectra which were not well-resolved even at high temperature (60 °C) perhaps because of formation of organized assemblies of unknown structure in chloroform.

3-(*Benzyloxycarbonylamino*)*propylphosphonic Acid* (**5b**).—To a vigorously stirred solution of 3-aminopropylphosphonic acid[29] (6.4 g, 46 mmol) in 2M-sodium hydroxide (46 ml) was added simultaneously benzyloxycarbonyl chloride (30—35% toluene solution; 38 ml) and 2M-sodium hydroxide at *ca.* −10 °C over a period of 30 min. The mixture was stirred for an additional 1 h. The excess of the chloride reagent was removed by washing the reaction mixture with diethyl ether, and the aqueous solution was cooled in an ice–water bath and acidified to pH 2 with hydrochloric acid. The resulting solid was collected by suction-filtration and treated subsequently with a cation exchange resin (H^+ form) to afford *compound* (**5b**) as crystals (8.9 g, 91%), m.p. 98—102 °C (from chloroform); δ_H 1.4—2.0 (4 H, m, CH_2CH_2P), 3.12 (2 H, t, *J* 6 Hz, NCH_2), 5.02 (2 H, s, CH_2Ph), and 7.24 (5 H, s, Ph); $\nu_{max.}$(KBr) 3 250m, 1 660s, 1 440m, 1 350m, 1 335m, 1 245m, and 1 245m cm^{-1} (Found: C, 48.35; H, 5.6; N, 4.8. $C_{11}H_{16}NO_5P$ requires C, 48.35; H, 5.90; N, 5.12%).

1,2:5,6-*Di-O-Isopropylidene*-D-*mannitol* (**7**).—Anhydrous zinc chloride (120 g) was shaken with dry acetone (600 ml). Without removal of a small amount of insoluble substance, which appeared not to affect the next acetalization, the solution was stirred subsequently with D-mannitol (20 g, 0.11 mol) at 20 °C for 16 h or 22 °C for 13 h. The reaction mixture was then diluted with cold chloroform (500 ml), and to the vigorously stirred solution, in an ice–water bath, was added an aqueous potassium carbonate (140 g in *ca.* 150 ml of water) over a period of *ca.* 3 min. After the mixture had been stirred for an additional 30 min, the supernatant was separated from the resulting slurry, and the white precipitate was washed twice by vigorous stirring with chloroform (2 × 300 ml). The first supernatant was concentrated below 40 °C to *ca.* 300 ml and combined with the chloroform extracts; thereupon, the mixture was separated into two phases. The lower layer was dried (Na_2SO_4) and concentrated to afford compound (**7**) as crystals, which was recrystallized from hexane (22.5 g, 78%), m.p. 118—119 °C (lit.,[13] 119 °C).

1,2-*O-Isopropylidene*-sn-*glycerol* (**8**).—Potassium periodate was added in portions to a water (50 ml)–THF (5 ml) solution of the mannitol (**7**) (9.5 g, 36 mmol) at room temperature until the solution showed a blue reaction to KI–starch paper. Stirring was continued for an additional 30 min. Throughout the oxidation reaction, the pH of the solution was maintained at 7—8 by 1M-sodium hydroxide. The resulting solution was cooled in an ice–water bath and then carefully mixed with sodium borohydride (5 g, 0.13 mol) and stirred for 2 h at room temperature. During the reduction reaction, the pH of the solution was adjusted to 7—8 by 1M-hydrochloric acid. The reaction mixture was extracted with chloroform (70 ml × 5), and the combined solutions were concentrated to give the residue which, after drying (K_2CO_3) was distilled under reduced pressure to furnish the title compound as a colourless liquid (7.8 g, 82%), b.p. 85—86 °C at 15 mmHg (lit.,[13] 77—77.5 °C at 10 mmHg); $[\alpha]_D^{25}$ +13.8° (lit.,[13] +12.6°).

3-O-*Benzyl*-sn-*glycerol* (**9**).—A solution of the glycerol (**8**) (10.6 g, 80 mmol) in anhydrous THF (100 ml) was swirled with sodium hydride (*ca.* 60% in oil; 2.6 g, 175 mmol). After the evolution of hydrogen gas had subsided, benzyl bromide (13.8 g, 88 mmol) was added to the sodium alkoxide solution, and the mixture was refluxed for 15 h. The contents were then cooled, mixed with water and extracted with THF. The organic solution was concentrated to give a residue which was distilled to afford

1,2-*O*-isopropylidene-3-*O*-benzyl-*sn*-glycerol (15.4 g, 87%), b.p. 120—122 °C at 3 mmHg (lit.,[17] 95—97 °C at 0.3 mmHg), $[\alpha]_D^{25}$ +17.8° (lit.,[17] +16.8°).

The product (7.6 g, 34 mmol) was dissolved in 95% ethanol (50 ml) and acidified with 6M-hydrochloric acid (40 ml). The solution was allowed to stand at room temperature for 15 h, before being neutralized by sodium hydrogen carbonate and extracted with diethyl ether. The solvent was removed from the extract to afford compound (**9**) which was purified by distillation (5.6 g, 90%), b.p. 178—180 °C at 3 mmHg (lit.,[17] 138—139 °C at 0.3 mmHg); $[\alpha]_D^{25}$ +5.8° (lit.,[17,18] +5.3°, +6.1°, and +5.5°).

1,2-O-(*Dibutyl*)*stannylene*-3-O-*benzyl*-sn-*glycerol* (**11**).—A mixture of compound (**9**) (10 g, 55 mmol) and dibutyltin oxide (13.7 g, 55 mmol) in benzene (300 ml) was refluxed for 1.5 h in a flask equipped with a distillation apparatus. Water, generated during the reaction, was removed by azeotropic distillation with benzene. The cooled reaction mixture was filtered, concentrated to *ca.* 50 ml and mixed with hexane (300 ml) to furnish the title compound as colourless crystals (17.5 g, 77%), m.p. 124—125 °C, $[\alpha]_D^{25}$ +4.5° (*c* 12.0, in chloroform); δ_H *ca.* 0.90 (6 H, br t, *J ca.* 6 Hz, 2 × Me), 1.08—1.85 [12 H, m, 2 × $(CH_2)_3$], 3.0—4.0 (5 H, m, OCH_2CHCH_2O), 4.5 (2 H, m, CH_2Ph), and 7.27 (5 H, m, Ph); $\nu_{max.}$(KBr) 2 930s, 1 600w, 1 500w, 1 450m, 1 360m, 1 080m, 1 055s, and 995m cm^{-1} (Found: C, 52.0; H, 7.6. $C_{18}H_{30}O_3Sn$ requires C, 52.33; H, 7.32%).

1,2-*Di*-O-*octadecyl*-3-O-*benzyl*-sn-*glycerol* (**12a**).—*Method A*. A mixture of compound (**9**) (1.25 g, 6.9 mmol) and sodium hydride (*ca.* 60% in oil; 0.60 g, 15.1 mmol) in THF was warmed at 60 °C with stirring. Octadecyl toluene-*p*-sulphonate (7.03 g, 16.6 mmol) was added to the alkoxide solution, and the mixture was heated to remove the solvent. The resulting semi-solid residue was further heated in an oil-bath at 190—200 °C for 30 min. A chloroform solution of the cooled reaction mixture was washed with water and concentrated to provide a residue which was dissolved in ethanol (150 ml) and allowed to stand at *ca.* 15—20 °C overnight. The resulting solid was collected by suction-filtration and recrystallized from acetone to give the glycerol (**12a**) (3.17 g, 67%), m.p. 35—35.5 °C (lit.,[18] 35.5—36.0 °C, $[\alpha]_D^{25}$ −0.32° (*c* 6.2, in chloroform) (lit.,[18] −0.29°); R_F 0.81 (Solvent *A*).

Method B. Compound (**11**) (5.0 g, 12 mmol) was allowed to react with octadecyl toluene-*p*-sulphonate (10.3 g, 24.2 mmol) at 180—190 °C for 30 min with stirring under nitrogen. The reaction mixture was treated with hot hexane and filtered. The filtrate was concentrated to give a residue which was dissolved in ethanol. The alcoholic solution was kept at 15—20 °C, to give compound (**12a**) as crystals which were purified by silica gel column chromatography using a mixture of hexane and ethyl acetate (5:1 v/v) as a solvent (1.5 g, 18%). The mother liquor obtained above was concentrated and applied to a silica gel column using the same solvent. The first fraction gave compound (**12a**) (0.4 g, 8%), and the second fraction afforded 1-*O*-octadecyl-3-*O*-benzyl-*sn*-glycerol (2.1 g, 40%), m.p. 34.37.5 °C (from methanol–water), *m/z* 416 (dehydrated molecule ion). The mono-octadecyl ether was converted into 1-*O*-octadecyl-*sn*-glycerol by catalytic hydrogenolysis using palladium–carbon, which had the same physical and spectroscopic characteristics [including, m.p. (69—70 °C), i.r. and n.m.r.] as those of an authentic sample prepared according to a literature procedure.[30]

1,2-*Di*-O-*hexadecanoyl*-3-O-*benzyl*-sn-*glycerol* (**12b**).—Hexadecanoyl chloride (12 g, 44 mmol) in anhydrous chloroform (100 ml) was added dropwise to a refluxing chloroform solution of compound (**11**) (7.1 g, 17.2 mmol). When the addition was complete reflux was continued for a further 2 h. The cooled reaction mixture was mixed with pyridine (4 ml), washed with water, and concentrated to afford the residue which was extracted with diethyl ether. The organic solution was dried (Na_2SO_4) and concentrated to give the residue which was treated with a mixture of acetone and n-hexane to provide crude (**12b**) (5.7 g, 50%). Silica gel column chromatography of the product (2.0 g) using a mixture of n-hexane and ethyl acetate (5:1 v/v) gave pure (**12b**) (1.6 g), m.p. 42—43 °C (lit.,[17] 42—42.5 °C), $[\alpha]_D^{25}$ +6.6° (*c* 8.4, in chloroform) (lit.,[17] +6.3°); R_F 0.75 (solvent *A*).

1,2-*Di*-O-*octadecyl*-sn-*glycerol* (**13a**).—A solution of compound (**12a**) (1.4 g, 2.0 mmol) in a mixture of ethanol and acetic acid (60 ml; 5:1 v/v) was hydrogenated in the presence of 10% palladium–carbon (0.5 g) at an atmospheric pressure and 45 °C. The mixture was diluted with chloroform and filtered. The filtrate was concentrated to furnish crude (**13a**) which was purified by recrystallization from acetone, 1.1 g (90%), m.p. 65—66 °C (lit.,[18] 64 °C), $[\alpha]_D^{25}$ −7.3° (*c* 7.5, in chloroform) (lit.,[18] −6.85°), R_F 0.42 (solvent *A*).

1,2-*Di*-O-*hexadecanoyl*-sn-*glycerol* (**13b**).—The compound (**12b**) (5.0 g, 7.6 mmol) was hydrogenated in the presence of 10% palladium–carbon (0.35 g) in a manner similar to that mentioned above to furnish compound (**13b**) as crystals (3.8 g, 93%), m.p. 66—67 °C (from acetone) (lit.,[17] 67 °C), $[\alpha]_D^{25}$ −2.5° (*c* 8.0, in chloroform) (lit.,[17] −2.3°); R_F 0.35 (solvent *A*).

1,2-*Di*-O-*octadecyl*-sn-*glycerol* 3-(2-*Benzyloxycarbonylaminoethyl*)*phosphonate* (**16**).—To a cooled solution of compounds (**13a**) (0.51 g, 0.85 mmol) and (**5a**) (0.26 g, 1.0 mmol) in dry pyridine (15 ml) was added TPS-Cl (0.6 g, 2.0 mmol). The solution was stirred at room temperature for 3 h and then mixed with water (2 ml) and allowed to stand in a refrigerator overnight. The reaction mixture was concentrated below 40 °C to give a residue which was dissolved in chloroform and washed with water. The organic solution was dried (Na_2SO_4), concentrated, and applied to a silica gel column. Elution using a mixture of chloroform and methanol (20:1 v/v) afforded the *product* (**16**), which was recrystallized from ethyl acetate (0.43 g, 60%), m.p. 157—160 °C, $[\alpha]_D^{25}$ +5.5° (*c* 3.8, in chloroform); R_F 0.8 (solvent C); δ_H 0.89 (6 H, br t, 2 × Me), 1.25 [60 H, br s, 2 × $(CH_2)_{15}$Me], 1.37 (4 H, br, 2 × $CH_2C_{16}H_{33}$), 1.53—*ca.* 2.1 (2 H, m, CH_2P), *ca.* 3.0—4.0 (11 H, m, OCH_2CHCH_2O, 2 × $CH_2C_{17}H_{35}$ and CH_2CH_2P), 5.0 (2 H, s, CH_2Ph), 6.1 (1 H, br, NH), and 7.21 (5 H, m, Ph); $\nu_{max.}$(KBr) 2 930s, 1 695m, 1 540w, 1 475m, 1 260m, 1 200m, and 1 080s cm^{-1} (Found: C, 70.1; H, 10.9; N, 1.6. $C_{49}H_{92}NO_7P$ requires C, 70.21; H, 11.06; N, 1.67%).

1,2-*Di*-O-*octadecyl*-sn-*glycerol* 3-[*Benzyl*-(2-*benzyloxycarbonylaminoethyl*)*phosphonate*] (**20**).—The compound (**5c**) (0.6 g, 1.7 mmol) was condensed with compound (**13a**) (0.81 g, 1.4 mmol) in the presence of TPS-Cl (0.5 g, 1.7 mmol) in a manner similar to that described for the preparation of the phosphonate (**16**). The column chromatography of the crude reaction product using a mixture of chloroform and methanol (20:3 v/v) gave the *title compound* which was recrystallized from ethyl acetate (0.69 g, 55%), m.p. 180—182 °C, $[\alpha]_D^{25}$ +3.2° (*c* 6.0, in chloroform); R_F 0.80 (solvent *C*); δ_H 0.89 (6 H, br t, 2 × Me), 1.26 [60 H, br s, 2 × (CH_2)Me], 1.45 (4 H, br m, 2 × CH_2CH_2O), 1.74—2.1 (2 H, m, CH_2P), 3.2—3.7 (11 H, m, OCH_2CHCH_2O, 2 × OCH_2, and CH_2CH_2P), 5.0—5.2 (4 H, m, 2 × CH_2Ph), 7.08 (5 H, s, Ph), and 7.13 (5 H, s, Ph); $\nu_{max.}$(KBr) 3 350m, 2 950s, 1 480m, 1 240s, 1 160m, and 1 060—1 000m cm^{-1} (Found: C, 71.9; H, 11.0; N, 1.3. $C_{56}H_{98}NO_7P$ requires C, 72.45; H, 10.64; N, 1.51%).

1,2-*Di*-O-*hexadecanoyl*-sn-*glycerol* 3-(2-*Benzyloxycarbonylaminoethyl*)*phosphonate* (**17**).—Compound (**13b**) (1.26 g, 2.3 mmol) was condensed with (**4a**) (0.62 g, 2.4 mmol) using TPS-Cl (1.45 g, 4.8 mmol) according to the procedure described above. Silica gel chromatography of the chloroform extract of the reaction mixture using chloroform–ethanol (20:3 v/v) as the eluant provided the *phosphonate* (**17**) as crystals (1.2 g, 63%), m.p. 189—191 °C (from ethyl acetate), $[\alpha]_D^{25}$ +5.9° (*c* 13.4, in chloroform); R_F 0.77 (solvent *C*); δ_H 0.89 (6 H, br t, 2 × Me), 1.26 [48 H, br s, 2 × $(CH_2)_{12}$Me], *ca.* 1.3—1.6 (4 H, m, 2 × CH_2CH_2CO), 1.68—2.10 (2 H, m, CH_2P), 2.05—2.34 [4 H, m, 2 × C=OCH_2], *ca.* 3.0—4.8 (6 H, m, CH_2CH_2P, OCH_2CHCH_2O), 4.98 (2 H, s, CH_2Ph), 5.12 (1 H, m, CH), 5.9 (1 H, br, NH), and 7.21 (5 H, m, Ph); $\nu_{max.}$(KBr) 3 350w, 2 920s, 1 730s, 1 530m, 1 460m, 1 180s, and 1 060m cm^{-1} (Found: C, 66.3; H, 10.0; N, 1.5. $C_{45}H_{80}NO_9P$ requires C, 66.72; H, 9.95; N, 1.73%). The following lipid-precursors were similarly prepared.

1,2-*Di*-O-*octadecyl*-sn-*glycerol* 3-(3-*benzyloxycarbonylaminopropyl*)*phosphonate* (**18**): yield, 75%; m.p. 183—186 °C (from ethyl acetate), $[\alpha]_D^{25}$ +4.1° (*c* 1.7, in chloroform); R_F 0.47 (solvent *B*); δ_H 0.90 (6 H, br t, 2 × Me), 1.26 [60 H, s, 2 × $(CH_2)_{15}$Me], 1.40—1.85 (8 H, m, CH_2CH_2P and 2 × $CH_2CH_2CH_2O$), 2.8—4.0 (7 H, m, OCH_2CHCH_2O and CH_2N), 4.95 (2 H, s, CH_2Ph), and 7.24 (5 H, s, Ph); $\nu_{max.}$(KBr) 2 950s, 1 700m, 1 540m, 1 475m, and 1 270—1 150 cm^{-1} (Found: C, 70.2; H, 10.8; N, 1.6%).

1,2-*Di*-O-*hexadecanoyl*-sn-*glycerol* 3-(3-*benzyloxycarbonylaminopropyl*)*phosphonate* (**19**): yield, 63%; m.p. 178—184 °C (from ethyl acetate), $[\alpha]_D^{25}$ +3.1° (*c* 2.4, in chloroform); R_F 0.53 (solvent *C*); δ_H 0.90 (6 H, br t, 2 × Me), 1.26 [48 H, br s, 2 × $(CH_2)_{12}$Me], 1.3—1.6 (4 H, m, 2 × CH_2CH_2C=O), *ca.* 1.4—1.9 (4 H, m, CH_2CH_2P), 2.01—2.34 (4 H, m, 2 × CH_2C=O), *ca.* 3.0—4.7 (6 H, m, OCH_2CHCH_2O and CH_2N), 6.0 (1 H, br, NH), 4.98 (2 H, s, CH_2Ph), and 7.25 (5 H, s, Ph); $\nu_{max.}$(KBr) 3 350m, 2 920s, 1 735s, 1 540w, 1 460m, 1 180s, and 1 090—1 050 cm^{-1} (Found: C, 67.4; H, 9.9; N, 1.75. $C_{46}H_{82}NO_9P$ requires C, 67.04; H, 10.03; N, 1.70%).

1,2-*Di*-O-*octadecyl*-sn-*glycerol* 3-(2-*Aminoethyl*)*phosphonate* (**21**).—Compound (**16**) (0.23 g, 0.27 mmol) was dissolved in a mixture of ethanol and ethyl acetate (5:1 v/v), and 10% palladium–carbon (0.11 g) was dispersed in the solution. Hydrogen gas was then passed through the mixture at 40—50 °C for 3 h. The catalyst was removed by suction-filtration, and the filtrate was concentrated to give crude (**21**); which was purified by recrystallization from ethyl acetate (0.16 g, 84%), m.p. 179—182 °C (lit.,[12] 155—156 °C), $[\alpha]_D^{25}$ +2.5° (*c* 4.0, in chloroform–methanol 4:1 v/v) (lit.,[12] +1.53°), R_F 0.55 (solvent *C*); δ_H 0.89 (6 H, br t, 2 × Me), 1.25 [60 H, s, 2 × $(CH_2)_{15}$Me], 1.47 (4 H, m, 2 × CH_2CH_2O), 2.37—2.88 (2 H, m, CH_2P), *ca.* 2.9—3.5 (2 H, m, CH_2CH_2P), *ca.* 3.3—3.75 (11 H, m, OCH_2CHCH_2O, 2 × $CH_2C_{17}H_{35}$, and CH_2CH_2P), and *ca.* 8.6 (3 H, br, $\overset{+}{N}H_3$); $\nu_{max.}$(KBr) 3 400w, 2 930s, 2 850m, 1 480m, 1 380m, 1 200m, and 1 090s cm^{-1} (Found: C, 70.20; H, 12.1; N, 2.1. $C_{41}H_{86}NO_5P$ requires C, 69.94; H, 12.31; N, 1.99%). The following lipids were prepared similarly by catalytic hydrogenation of the corresponding *N*-benzyloxycarbonyl derivatives (**17**)—(**19**).

1,2-*Di*-O-*hexadecanoyl*-sn-*glycerol* 3-(2-*aminoethyl*)-*phosphonate* (**22**): yield, 88%; m.p. 207—208 °C (from ethyl acetate) (lit.,[10] 180.5—181.5 °C), $[\alpha]_D^{25}$ +8.5° (*c* 3,4, in chloroform–methanol, 4:1 v/v) (lit.,[10] +7.9°); R_F 0.54 (solvent *C*); δ_H 0.89 (6 H, br t, 2 × Me), 1.25 [48 H, s, 2 × $(CH_2)_{12}$Me], 1.43—1.68 (4 H, m, 2 × CH_2CH_2C=O), 1.77—2.07 (2 H, m, CH_2P), 2.1—2.34 (4 H, m, 2 × CH_2C=O), 2.91—3.45 (2 H, m, *J* 20 Hz, CH_2CH_2P), 3.80—4.53 (4 H, m, OCH_2CHCH_2O), 5.17 (1 H, m, CH), and *ca.* 5.6—6.6 (3 H, br, $\overset{+}{N}H_3$); $\nu_{max.}$(KBr) 3 360w, 2 920s, 1 735s, 1 460m, 1 380m, 1 195s, 1 060s, and 820m cm^{-1} (Found: C, 65.7; H, 11.4; N, 2.3. $C_{37}H_{74}NO_7P$ requires C, 65.74; H, 11.04; N, 2.07%).

1,2-*Di*-O-*octadecyl*-sn-*glycerol* 3-(3-*aminopropyl*)-*phosphonate* (**23**): yield, 62%; m.p. 163—164 °C (from ethyl acetate), $[\alpha]_D^{25}$ +2.4° (*c* 4.2, in chloroform); R_F 0.60 (solvent *B*); δ_H 0.90 (6 H, br t, 2 × Me), 1.26 [60 H, br s, 2 × $(CH_2)_{15}$Me], 1.4—1.65 (4 H, br m, 2 × CH_2CH_2O), *ca.* 1.7—2.3 (4 H, m, CH_2CH_2P), 3.09 (2 H, m, CH_2N), and *ca.* 3.3—3.66 (8 H, m, OCH_2CHCH_2O and 2 × $OCH_2C_{17}H_{35}$); $\nu_{max.}$(KBr) 3 400m, 2 930s, 1 480m, 1 195s, 1 090s, and 1 050m cm^{-1} (Found: C, 70.5; H, 12.0; N, 2.0. $C_{42}H_{88}NO_5P$ requires C, 70.24; H, 12.35; N, 1.95%).

1,2-*Di*-O-*hexadecanoyl*-sn-*glycerol* 3-(3-*aminopropyl*)-*phosphonate* (**24**): yield, 60%; m.p. 189—192 °C (from ethyl acetate), $[\alpha]_D^{25}$ +3.1° (*c* 2.6, in chloroform), R_F 0.54 (solvent *B*); δ_H 0.90 (6 H, br t, 2 × Me), 1.26 [48 H, br s, 2 × $(CH_2)_{12}$Me] 1.25—*ca.* 2.0 (8 H, m, 2 × CH_2CH_2O and CH_2CH_2P), *ca.* 2.1—2.4 (4 H, m, 2 × CH_2C=O), 5.12 (1 H, m, CH), and *ca.* 6.7 (3 H, br, $\overset{+}{N}H_3$); $\nu_{max.}$(KBr) 3 400m, 2 930s, 1 740s, 1 460s, 1 180s, and 1 160s cm^{-1} (Found: C, 66.5; H, 10.9; N, 2.0. $C_{38}H_{76}NO_7P$ requires C, 66.14; H, 11.10; N, 2.03%).

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