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# Modern Methods of Synthesis of Modified Glycerophospholipids

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The advances in the synthesis of modified glycerophospholipids of different structure are surveyed. An attempt has been made at a general classification of these compounds. Questions concerning the problem of whether or not various methods of synthesis can be used on a preparative scale are examined.
The bibliography includes 233 references.

CONTENTS

I. Introduction 382
II. Classification and strategy in the synthesis of modified glycerophospholipids 382
III. Modified aliphatic acids 383
IV. General procedures in the synthesis of modified glycerophospholipids 385

## I. INTRODUCTION

The vigorous development of bio-organic chemistry during the last decade has led to the creation of preparative methods of synthesis of virtually all principal classes of substances found in living organisms, including glycerophospholipids (PL).[1] However, the tasks faced by investigators have increasingly required the use of modified PL. The applications of modified PL are extensive. They serve as instruments for research into (a) the metabolism of individual representatives of PL,[2] (b) the molecular organisation of submolecular structures whose composition includes PL (membranes, blood plasma lipoproteins, etc.) and the processes incurring in them,[3-15] and (c) enzymes associated with the metabolism of PL;[16-25] furthermore, certain representatives of modified PL are of interest as potential therapeutic agents,[26-31] while a number of PL analogues, which can be immobilised on solid carriers, are of interest as ligands in affinity chromatography for the isolation of various proteins exhibiting an affinity for PL.[32-39]

In principle, traditional methods of synthesis of PL can be used to obtain modified PL,[1] but in most cases special procedures are required, taking into account the specific features of both the label and of the methods used in their subsequent application. The fact that the majority of the labels are relatively difficult to obtain is also reflected in the strategy of the synthesis. It is believed to be preferable to introduce the label in the last stages of the synthesis, which reduces the possibility of the modification of the label (which is as a rule labile) during synthesis and permits an economical utilisation of the labelled intermediates.

## II. CLASSIFICATION AND STRATEGY IN THE SYNTHESIS OF MODIFIED GLYCEROPHOSPHOLIPIDS

As the term PL embraces a large group of compounds with extremely varied structures, it is essential to deal with the classification of such PL before considering the methods of their synthesis. PL can be classified in terms of various features: in terms of the aims of the modification (and hence the way in which they are subsequently used), in terms of the site of the modification, and in terms of the type of modification (the type of label).

In terms of the aims of the modification, it is possible to distinguish two types of modified PL: labelled PL (as a rule PL having the natural structure and containing the label or reporter group; they are normally employed as lipid-specific probes or labels closely imitating the corresponding lipids in terms of their properties) and structural analogues of PL (PL in which the structure of the hydrophobic or polar component of the molecule and/or the type of the chemical bond between them has been modified in order to impart to them properties different from those of the natural PL).

Labelled PL can be subdivided further in terms of the type of label into radioisotope-labelled, spin-labelled (those whose composition includes stable nitroxy-radicals), fluorescence-labelled, and photoreactively labelled PL (containing photoreactive groups), PL with NMR-labels (detected with the aid of NMR), and PL with electronically dense labels.

In terms of the site of the modification, all the modified PL can be divided into two classes: (1) derivatives of lipids having the natural structure (this class includes the majority of the labelled PL) and (2) PL with an altered "skeleton". The former are subdivided in their turn into PL modified in the hydrophobic or polar components (Fig.1) and the latter into derivatives of 2-glycerophosphate,[25, 40-42] PL modified in the glycerol residue,[16,20,21,43-54] and PL modified in the phosphoester group.[55-71]

In the general case, the synthesis of modified PL reduces to the synthesis of the initial compounds and their interaction (key stage). The starting materials for the synthesis of the modified PL can be subdivided into two types: structures carrying the label (or the modified fragment), i.e. modifying compounds, and "skeletal" structures which determine the type of the required PL and the site of the label in its molecule.

As regards strategy, the methods of synthesis of modified PL can be arbitrarily divided into synthetic (the assembly of the PL molecule from elementary components: glycerol, aliphatic acids, etc.), semisynthetic (modification of natural PL with the aid of chemical and enzymic reactions), and biological. The semisynthetic methods are the most effective and for this reason they have been used recently with increasing frequency for the modification of PL. However, in a number of instances, for example, in the synthesis of PL modified in the glycerol residue[16,20,21,43-54] and in the phosphoester group[16, 22-24,44,55-72] or isomeric PL,[16,25,40-42,44] total chemical synthesis is essential. The biological methods are more rarely used preparatively[73] and we shall therefore confine ourselves to the consideration of the first two most often used methods for the modification of PL.

To facilitate the use of the present review and to reduce its size, we propose the following procedure for indexing the references. A four-digit number indicating the structure and method of synthesis of the modified PL (see Table 1 and the list of literature references) is assigned to each reference to the synthesis of modified PL (in the list of literature references, it is written at the end of the reference in angular brackets). The first digit indicates the class of the PL, the

second the type of modification (vertical columns in Table 1 with numbers ranging from 0 to 9), and the third the site of the modification (in conformity with Fig.1). We introduce the fourth digit in order to reflect as far as possible the position of the key stage (usually the reaction involving the introduction of the modifying fragment) in the synthesis of the modified PL (Fig.2).

For example, the designation <2044> corresponds to the synthesis of phosphatidylethanolamine (PE) fluorescence-labelled in the polar component, the key stage in the synthesis of which involves a reaction in the polar head (evidently *N*-alkylation or *N*-acylation). Thus it is possible to find readily from Table 1 the reference to a given feature and the list of literature references contains coded information about the contents of a particular communication.

## III. MODIFIED ALIPHATIC ACIDS

One of the main types of initial compounds carrying a modified fragment (or label) are modified (labelled) aliphatic acids (ALA). In the synthesis of modified PL, fluorescence-labelled, spin-labelled, and photoreactively labelled ALA (Table 2), as well as isotope-labelled ALA are most widely used.

One of the general and widely employed methods for the introduction of isotope labels ($^2H$, $^3H$) into the hydrocarbon chain of the ALA involves the hydrogenation of unsaturated acids in a $^2H_2$ or $^3H_2$ atmosphere. It is then possible to obtain both saturated ALA [145-147] and fully *cis*-monoene or *cis*-polyene ALA from the corresponding acetylenic acids by hydrogenating the latter in the presence of the Lindlar catalyst.[134-145]

The $^1H$ atoms can be substituted by the radioactive isotope $^3H$ also by heterogeneous catalytic isotope exchange with gaseous tritium[148,149] or by the method involving thermal activation of tritium.[132] Randomly tritium-labelled ALA are then obtained. In the case of deuterium, the employment of the heterogeneous catalytic isotope exchange method makes it possible to obtain perdeuteriated ALA with an isotopic purity in excess of 99%.[150,151]

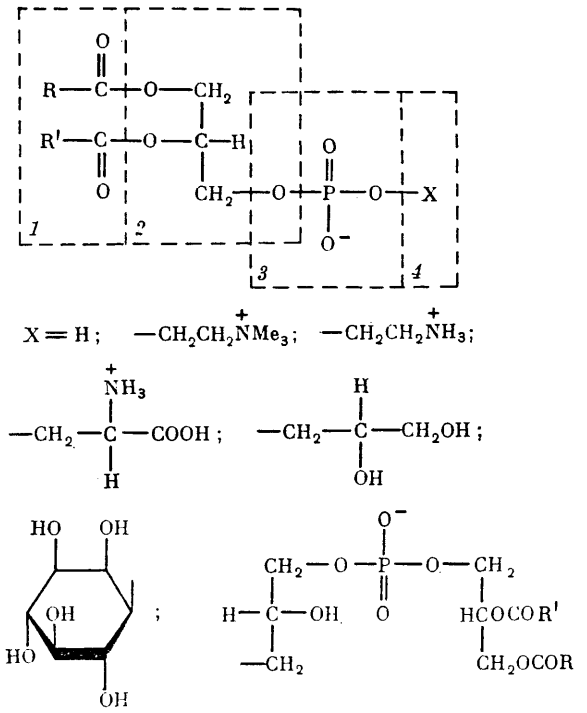



**Figure 1.** The possible sites of the modification of PL: 1) hydrophobic component (acyl residues); 2) glycerol residue; 3) phosphodiester/phosphomonoester group; 4) polar component.

Apart from the methods listed above, there exist also various procedures which make it possible to introduce the $^{13}C$, $^{14}C$, $^2H$, or $^3H$ labels at the end,[143,145,152,153] beginning,[154] or middle of the

**Table 1.** Notation for the references to the synthesis of the modified PL and ALA.

| PL | No. | Labels | | | | | | Structural analogues | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | fluorescent | spin | photoreactive | radioisotope | NMR | electron-dense | 3-glycerophosphate derivatives | 2-glycerophosphate derivatives | modified in glycerol residue | modified in phosphoester group |
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| PC | 1 | 3, 5, 7—11, 19, 74—76, 78—81, 85, 87, 88, 90, 92—99, 101—104, 106—111, 182, 213 | 17, 113, 114, 117, 121, 122, 125, 127, 129, 186—188 | 4, 11—14, 131—142, 144, 182, 202 | 4, 11, 18, 23, 104, 132, 134, 136, 138, 139, 146, 147, 182, 189, 191, 202, 225, 227, 228 | 144, 151, 158—160, 190, 209, 224, 226, 227 | 161 | 2, 32—38, 55, 162—169, 183, 185, 192, 217, 218 | 16, 25, 40—42, 44 | 16, 20, 21, 43—48, 51, 53 | 15, 16, 24, 44, 55, 61, 63, 66, 67, 70, 71, 231,232 |
| PE | 2 | 6, 78 87, 88, 99, 104, 109, 170—172, 174, 177, 180, 181, 193, 200, 201 | 115, 116, 118, 119, 129, 175, 188, 199, 207 | 12, 131, 135, 137, 178 | 22, 72, 146, 189, 211, 212 | — | 204 | 26, 27, 39, 164, 165, 176, 179, 214,215, 216—219, 222 | 40 | 20, 21, 53, 54 | 60, 62, 63, 67, 71 |
| PS | 3 | 18, 99, 173 | 129, 175, 187, 188 | — | 18, 189, 197, 212 | 210 | — | 176 | — | 52 | — |
| PG | 4 | 7, 99 | 188 | 194 | — | — | — | — | — | — | — |
| PI | 5 | 77, 88, 99, 195, 196 | 17, 120, 175, 196 | — | 73 | — | — | — | — | — | — |
| DPG | 6 | 88 | 123, 184, 198 | 203 | — | — | — | — | — | — | — |
| PA | 7 | 7, 99, 105, 109, 170 | 186—188, 205—207 | — | 189 | — | — | 28—31, 58,59, 164, 165, 220, 221, 223 | — | 49, 50, 65, 68 | 56—59, 64, 65, 67—69, 231 |
| ALA | 8 | 3, 5, 74, 82—84, 86, 89—96, 100—102, 110, 111 | 17, 112, 113, 124—128, 130, 207 | 131—134, 136—140, 142—144 | 132, 134, 136, 138, 139, 142, 143, 145, 148—150, 152, 156, 157 | 144, 151, 152—155, 158 | 161 | — | — | — | — |

hydrocarbon chain of the ALA [144,153,155] and also to obtain ALA labelled at the carbonyl carbon atom.[142,145,156,157]

Apart from isotope labels, certain heteroatoms, for example $^{19}F$, whose chemical shift is sensitive to differences between the outer and inner surfaces of vesicles, can be introduced as NMR-labels.[158-160]

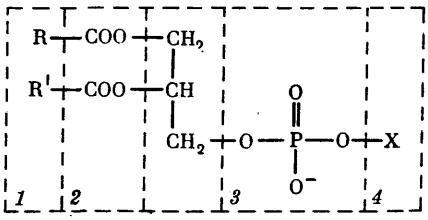



**Figure 2.** The types of key reactions in the synthesis of modified PL as a function of their location: 1) reactions in the hydrocarbon chains of the acyl residues of the PL; 2) formation of ester (thioester, amide, or others) bonds; 3) phosphorylation and reactions of phosphorus(III) derivatives; 4) reactions in the polar head of the PL.

**Table 2.** Labelled carboxylic acids most promising for the synthesis of modified PL.

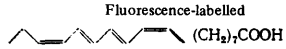
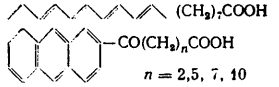
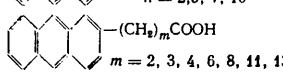
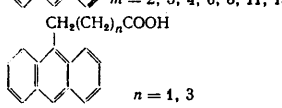
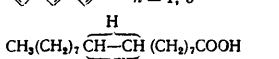
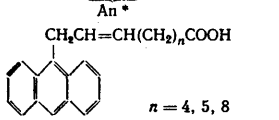
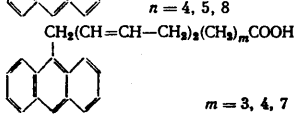
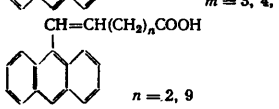
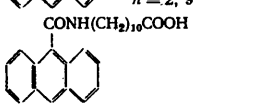
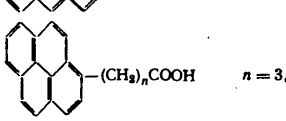
| Name | Formula | Refs. |
|---|---|---|
| | Fluorescence-labelled | |
| α-Parinaric |  | [74–79] |
| β-Parinaric |  | [74–76, 78, 80] |
| ω-(2-Anthroyl)-carboxylic |  $n = 2,5, 7, 10$ | [81–84] |
| ω-(2-Anthryl)-carboxylic |  $m = 2, 3, 4, 6, 8, 11, 13, 16$ | [7, 82, 89–91] |
| ω-(9-Anthryl)-carboxylic |  $n = 1, 3$ | [89] |
| 9(10)-Anthryl-stearic |  An * | [85–88] |
| ω-(9-Anthryl)-*cis*-monoenoic |  $n = 4, 5, 8$ | [92] |
| ω-(9-Anthryl)-*cis*-dienoic |  $m = 3, 4, 7$ | [92] |
| ω-9-(Anthrylvinyl)-carboxylic |  $n = 2, 9$ | [93, 94] |
| *N*-(9-Anthroyl)-11-amino-undecanoic |  | [93] |
| ω-(1-Pyrenyl)-carboxylic |  $n = 3, 5, 9$ | [5, 95–100] |

**Table 2** (contd.).

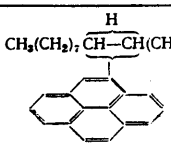
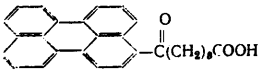
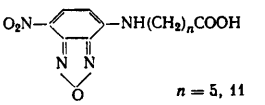
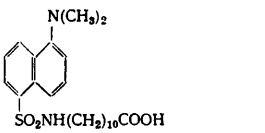
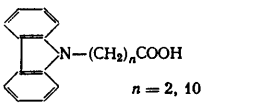
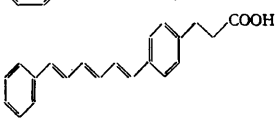
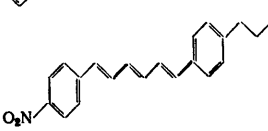
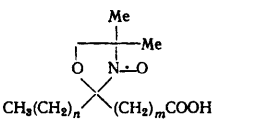
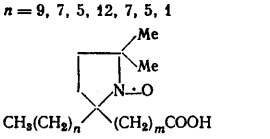
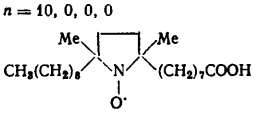
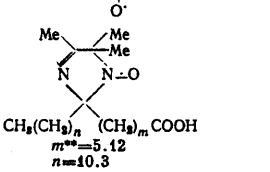
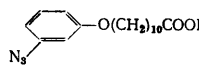
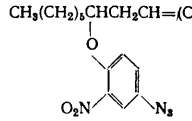
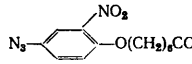
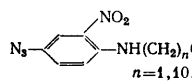
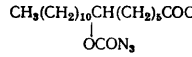
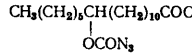
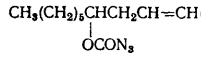
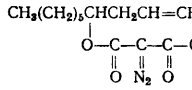
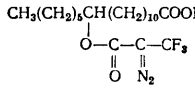
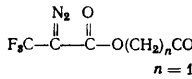
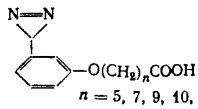
| Name | Formula | Refs. |
|---|---|---|
| 9(10)-(4-Pyrenyl)-stearic |  | [88] |
| 9-(3-Perylenoyl)-nonanoic |  | [101] |
| *N*-NBD-amino-carboxylic |  $n = 5, 11$ | [102–106] |
| *N*-Dns-11-amino-decanoic |  | [107] |
| *N*-(9-Carbazolyl)-carboxylic |  $n = 2, 10$ | [108, 109] |
| β-[4-(Phenylhexatrienyl)phenyl]-propionic |  | [110] |
| β-[4-(4-Nitrophenyl-hexatrienyl)-phenyl]-propionic |  | [111] |
| | Spin-labelled | |
| Doxylcarboxylic |  $m^{**} = 4, 6, 8, 3, 8, 10, 14$ $n = 9, 7, 5, 12, 7, 5, 1$ | [112–123] |
| Proxyl-carboxylic |  $m^{**} = 5, 7, 14, 15$ $n = 10, 0, 0, 0$ | [124, 125] |
| *cis*(*trans*)-10-Azetoxyeicosanoic |  | [126, 127] |
| Iminoxycarboxylic |  $m^{**}=5.12$ $n=10.3$ | [128–130] |
| | Photoreactively-labelled | |
| 16-Azido-palmitic | $N_3(CH_2)_{15}COOH$ | [4] |
| 12-Azido-stearic | $CH_3(CH_2)_5CH(CH_2)_{10}COOH$ <br> $N_3$ | [131, 132] |
| 12-Azido-oleic | $CN_3(CH_2)_5CHCH_2CH=CH(CH_2)_7COOH$ <br> $N_3$ | [4, 131, 133] |
| 18-Azidolinoleic | $N_3(CH_2)_5(CH=CHCH_2)_2(CH_2)_6COOH$ | [4, 134] |

**Table 2.** Labelled carboxylic acids most promising for the synthesis of modified PL.

| Name | Formula | Refs. |
|---|---|---|
| 11-(*m*-Azidophenoxy)undecanoic |  | [135] |
| 12-(4-Azido-2-nitrophenoxy)-oleic |  | [131] |
| 7-(4-Azido-2-nitrophenoxy)-heptanoic |  | [135, 136] |
| N-(4-Azido-2-nitrophenyl)-ω-aminocarboxylic |  $n$=1,10,11 | [132, 137—139] |
| 7-Azidocarbonyloxystearic |  | [170] |
| 12-Azidocarbonyloxystearic |  | [140] |
| 12-Azidocarbonyloxyoleic |  | [133] |
| 12-(Ethyl-2-diazomaloyloxy)-oleic |  | [131, 133] |
| 12-(2-Diazo-3,3,3-trifluoropropionyloxy)-stearic |  | [140] |
| ω-(2-Diazo-3,3,3-trifluoropropionyloxy)-carboxylic |  $n$ = 10, 11, 15 | [135, 141, 142] |
| ω-(*m*-3*H*-diazirinophenoxy)-carboxylic |  $n$ = 5, 7, 9, 10, 11, 13 | [12, 13, 135, 142—144] |

*The significance of the symbol An is indicated in Scheme 1.
**$m$ and $n$ are inter-related.

In order to obtain probes for the investigation of the outer structure of biological membranes by electron microscopy, an organotin analogue of ALA, namely 12,12-dimethyl-12-stannahexadecanoic acid, has been synthesised[161] and introduced into the composition of phosphatidylcholine (PC).

Since 1980, a series of syntheses of photopolymerisable ALA, containing the methacryloyloxy-group[162] or conjugated diacetylene or diene groups,[163-165] have been synthesised. Membranes made up of photopolymerised PL exhibit the properties of both the usual phospholipid double layers and certain properties (for example, resistance to the action of detergents) more characteristic of polymer membranes;[166,167] in 1985, the synthesis of PC incorporating ALA containing the thiol group at the end or beginning of the hydrocarbonchain was reported.[168,169] The liposomes consisting of such PL are capable of reversible polymerisation and depolymerisation via the thiol—disulphide oxidation-reduction cycle.

## IV. GENERAL PROCEDURES IN THE SYNTHESIS OF MODIFIED GLYCEROPHOSPHOLIPIDS

The synthesis of modified PL can be conveniently considered in most cases in accordance with the site of the modification of the PL, which makes it possible to differentiate the general methods of synthesis regardless of the type of modification.

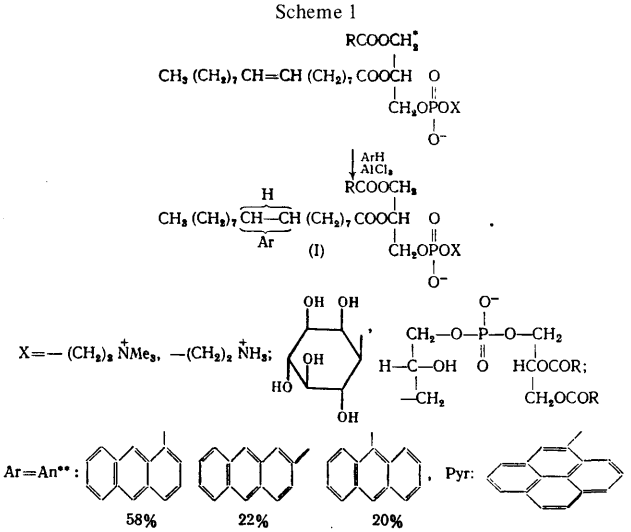
### 1. Synthesis of Glycerophospholipids Modified in the Hydrophobic Component

The most general feature influencing the properties of modified PL is the location of the label (modified fragment)—the hydrophobic or polar part of the molecule. Since the polar part of the molecule as a rule determines the majority of the physicochemical properties of the PL, its modification can alter significantly the behaviour of the PL molecule. For example, acylation of PE or phosphatidylserine (PS) at the amino-group[131,170-181] alters the charge and size of the polar head of the lipid molecule. Therefore, in the synthesis of labelled PL which should possess properties close to those of the corresponding natural PL, it is more desirable to modify the aliphatic acid part of the PL molecule; this is particularly justified in view of the fact that this region is relatively large and introduction of the label in this way therefore has less effect on the behaviour of the PL molecule.

#### *(a) Addition to the C=C Bonds of the Acyl Residues in Glycerophospholipids*

The introduction of the label at the double bonds of the acyl residues of the PL is the simplest method of obtaining PL modified in the hydrophobic component. The interaction of natural PL with polynuclear aromatic hydrocarbons (anthracene or pyrene) under the conditions of the Friedel—Crafts reaction yields in a single stage PL fluorescence-labelled in the hydrophobic component[85,87,88] (Scheme 1). The label in the PL (I) is then located mainly in the 2-position in the glycerol residue of the molecule (in conformity with the distribution of unsaturated ALA in natural PL).[87,88]

Scheme 1



*Oleic acid is indicated in the 2-position of the glycerol residue, since it represents more than 50% of the unsaturated acids contained in the majority of natural PL.[182]
**The ratio of the anthracene isomers was determined by $^1$H NMR.[86]

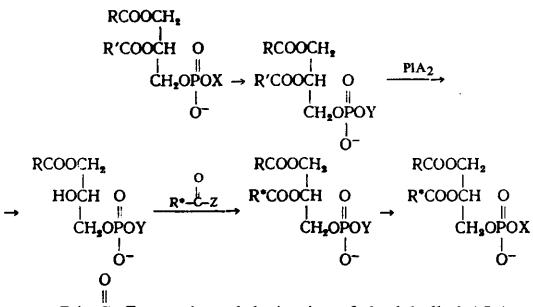
An undoubted advantage of this method is its universality: aromatic fluorophores can be introduced into the majority of natural PL.

Addition to the double bonds of the acyl residues of PL is also used to introduce isotope labels ($^2H$, $^3H$) and certain heteroatomic labels (Br) into PL.[147,183]

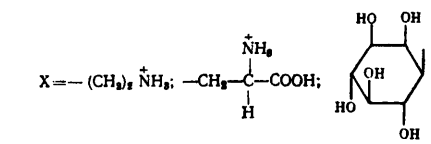
*(b) Acylation of Glycerophosphates and Lysophospholipids*

The commonest version of the semisynthetic method involves the introduction of a labelled ALA into lyso-PL, which is obtained by the enzymic hydrolysis of the PL using phospholipase $A_2$(Pl $A_2$) [74-79,81,90,92-95,99,101,102] (Scheme 2):

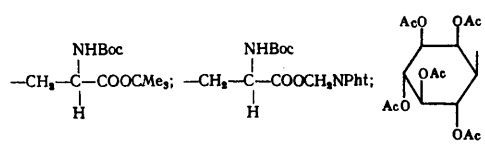
Scheme 2



R*-C-Z = activated derivative of the labelled ALA



Y = — $(CH_2)_2$NHNPS; —$(CH_2)_2$NHBoc; —$(CH_2)_2$NPht;

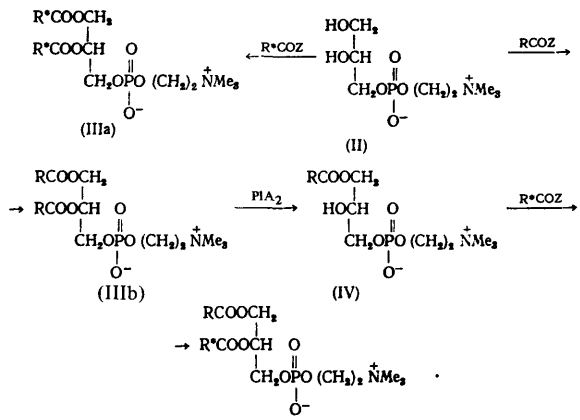


NPS = *o*-nitrobenzenesulphenyl; Boc = t-butoxycarbonyl; Pht = phthaloyl

For all the PL, except PC, it is then as a rule necessary to carry out a preliminary blocking of the functional groups in the polar component of the PL and their deblocking after the acylation of the lyso-PL (Scheme 2). The amino-groups of PE and PS are blocked by *o*-nitrobenzenesulphenyl, t-butoxycarbonyl, and phthaloyl protecting groups,[1,115,116,118,119] the carboxy-group of the PS is blocked by t-butyl and phthalimidomethyl groups,[1] the hydroxy-groups of myoinositol are protected by acetylation,[1,77,99,120] while the hydroxy-group of the glycerol residue of diphosphatidylglycerol (DPG) is not as a rule blocked[123,184] because of its insignificant reactivity.

The labelled PL obtained by this method consist of a mixture of molecular types differing in the acids in the 1-position in the glycerol residue. A procedure consisting in the alkaline hydrolysis of PC and the subsequent acylation of the ALA-labelled glycerophosphocholine (II) is used for the synthesis of the individual substances[79,80,96,135,140,158,162] (Scheme 3):

Scheme 3

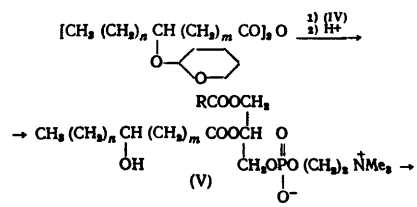


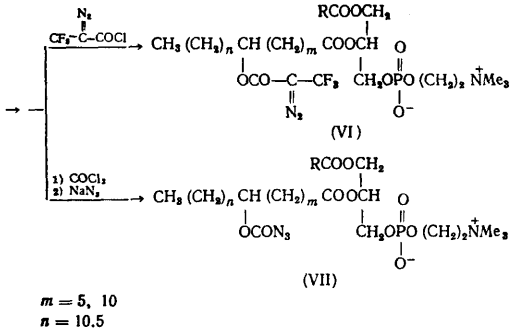
In order to obtain heteroacid PL, glycerophosphocholine (II) is acylated initially with the natural ALA, after which compound (IIIb) is hydrolysed by Pl $A_2$ and the lyso-PC (IV) is acylated with the labelled ALA [18,99,135,142,144,162] (Scheme 3), the reverse acylation sequence leading to PC with the label in the first acyl residue.[79,80,140,158,162]

Lyso-PL (IV) and the glycerophosphates (II) are acylated by traditional methods in the chemistry of PL.[1] The acylating agents employed are the chlorides of the modified acids[77,92,93,95,96,99,133] and also the more effective (especially in the case of ALA which are difficult to obtain and labile labelled ALA) anhydrides of the ALA in the presence of readily dissociating alkali metal salts of the ALA,[74,102,115,119,158,185] alkali metal oxides,[113,114,123,161,186,188] 4-*NN*-dimethylaminopyridine (DMAP),[79,90,99,110,111,132,135,137,140,142,144,162,189,190] or 4-pyrrolidinopyridine;[106] also used are ALA in the presence of dicyclohexylcarbodiimide (DCC) and DMAP [94,101,138,139,168] or trifluoroacetic anhydride and pyridine,[191] as well as ALA imidazolides.[80,81,93,104,105,116-118,120-122,125,127,129,136]

In many cases (especially when labile labels are used), it is useful to obtain initially the PL containing in one of the acyl residues (and sometimes in both) an active functional group (usually the hydroxy- or amino-group)[103,104,106,107,140,189] with the subsequent introduction of the label into the resulting PL molecule. Thus the photoreactively labelled PC (VI) and (VII) have been obtained by acylating the hydroxylated PC (V) with 2-diazo-3,3,3-trifluoropropionyl chloride or chloroformylated phosgene with subsequent replacement of the chlorine atom by the azido-group[140] (Scheme 4):

Scheme 4





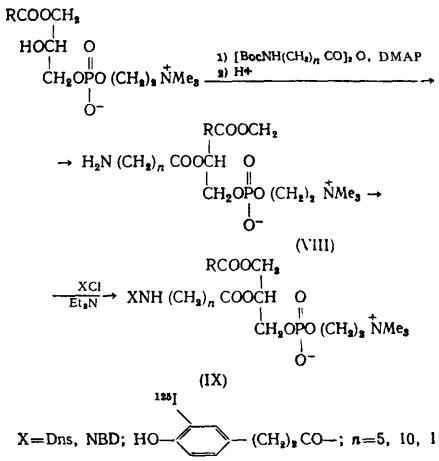
(VI)

(VII)

$m = 5, 10$
$n = 10.5$

Fluorescence-labelled PC [103,104,106,107] as well a a PC analogue containing the $^{125}I$ isotope (IX) [189] have been obtained by acylating (or alkylating) the amino-groups in the ω-aminoacyl residues of PC (VIII) (Scheme 5):

Scheme 5



(VIII)

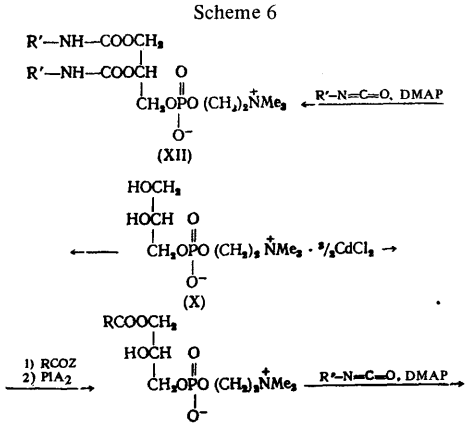
(IX)

X=Dns, NBD; HO—⟨I⟩—$(CH_2)_2CO$—; $n$=5, 10, 11;

Dns = 5-dimethylaminonaphthalene-1-sulphonyl (dansyl);
NBD = 7-nitrobenzo-2-oxa-1,3-diazol-4-yl

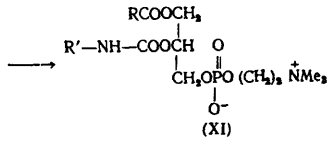
The interaction of lyso-PC (IV) or the cadmium complex of glycerophosphocholine (X) with alkyl isocyanates results in the formation of PC analogues non-hydrolysable by phospholipase $A_2$, in which the ester group at the C(2) atom of the glycerol residue [as in compound (XI)] or both ester groups, at C(1) and C(2) [as in compound (XII)], have been substituted by the carbamoyloxy-group[192] (Scheme 6):

Scheme 6



(XII)

(X)

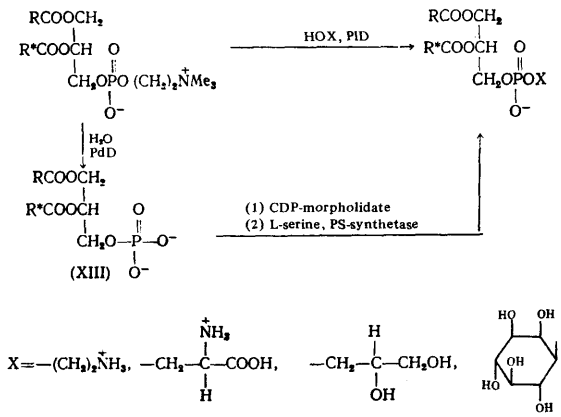


(XI)

*(c) Transphosphatidylation of PC by phospholipase D*

In connection with the discovery of the reaction involving the transfer of the phosphatidyl residue from one alcohol component to another, catalysed by phospholipase D, the possibilities in the synthesis of modified PL have expanded greatly. Thus conditions have been found in which phospholipase D effects the exchange of the choline group in PC for ethanolamine,[20,63,78,99,129,137,188,189,193] glycerol,[7,99,188,194] serine,[99,189] and myoinositol.[195,196] This makes it possible to unify the scheme for the preparation of modified PL: the synthesis of the labelled PC and its transphosphatidylation by phospholipase D in the presence of ethanolamine, serine, glycerol, and myoinositol or hydrolysis with formation of phosphatidic acid (PA) (Scheme 7):

Scheme 7



(XIII)

CDP = cytidine diphosphate

Fluorescene-labelled PE, PS, phosphatidylglycerol (PG), phosphatidylinositol (PI), and PA,[7,99,193,195,196] spin-labelled PE, PS, PG, PI, and PA,[129,186,188] photoreactively labelled PE and PG,[137,194] and also radioactive analogues of PE, PS, and PA labelled with $^{125}I$ [189] have been obtained by this method.

Another version of enzymic synthesis has been used to obtain PS spin-labelled in the hydrophobic component[187] or radioisotope-labelled in the polar component:[197] the conversion of PA (XIII) into CDP-diglyceride followed by its interaction with L-serine in the presence of PS-synthetase (Scheme 7).

## 2. The Synthesis of Glycerophospholipids Modified in the Polar Head Groups

The presence in the polar components of many PL of active functional groups (hydroxy- or amino-groups) has led to a convenient and relatively simple way of modifying them.[131,170-181,198-204] However, at the same time taditional synthetic methods have continued to be used for the preparation of PL modified in the polar component.[1,28,31,58,59,94,205-223]

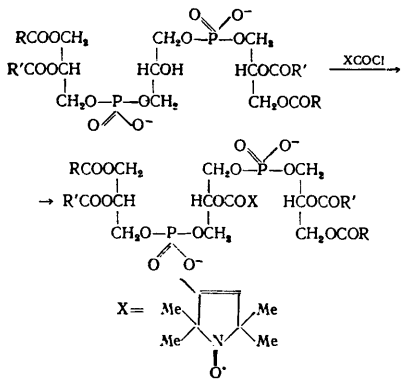
The modification of PL in the polar head groups is used to obtain not only membrane probes[131,170-178,180,198-213] but in recent years has been employed with increasing frequency for the synthesis of various lipophilic physiologically active substances, for example, antioxidants

(phosphatidylhydroquinones),[58,59] the antiviral preparation acyclovir,[31] immunologically active PL analogues containing various haptens in the polar component,[179,181,220,221] nucleotido-PL with antitumour activity,[28-30] etc.

*(a) Acylation of the Hydroxy-groups in the Polar Head of the PL*

Only a few examples of the direct introduction of the label into the polar component of DPG and PI are known. In the former case,[198] the natural DPG was acylated with the chloride of 2,2,5,5-tetramethyl-*N*-oxypyrroline-3-carboxylic acid in the presence of pyridine (Scheme 8):

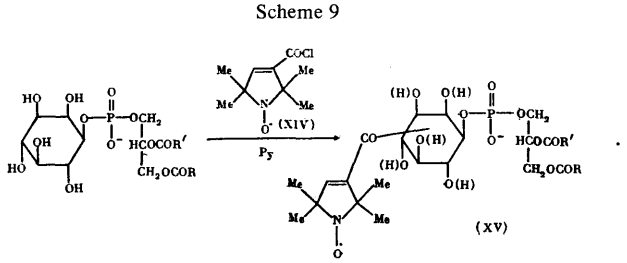
Scheme 8



However, it is known that the hydroxy-group of DPG is difficult to acylate under these conditions[123] and the attainment of high yields in the acylation reaction is therefore unlikely in the given instance. The synthesis of photoreactive analogues of DPG containing the 4-azido-2-nitrophenyl group in the polar component of the molecule has also been reported.[203]

The acylation of PI with an equimolar amount of the acid chloride (XIV) yielded compound (XV) with an indefinite position of the label in the cyclitol ring[175] (Scheme 9):

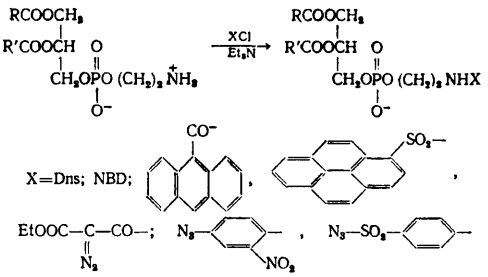
Scheme 9



*(b) N-Acylation and N-Alkylation of PE and PS*

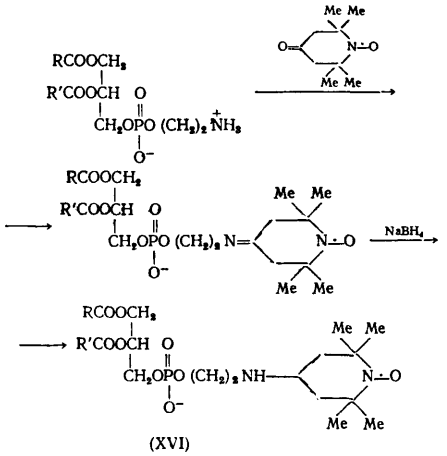
The reactions involving the acylation and alkylation of the amino-groups are used very often for the direct introduction of the label into the polar component of PE or PS. *N*-Dns-PE,[170-173] *N*-NBD-PE,[200] *N*-(9-anthroyl)-PE,[174] *N*-(1-pyrenesulphonyl)-PE,[177] *N*-(ethyl-2-diazomalonyl)-PE,[131,178] *N*-(azido-2-nitrophenyl)-PE,[131] and *N*-(4-sulphonylazidophenyl)-PE [202] (Scheme 10), as well as *N*-Dns-PS,[173] *N*-NBD-PS,[18] and *N*-biotinyl-PE and *N*-biotinyl-PS [176] have been obtained by this method:

Scheme 10



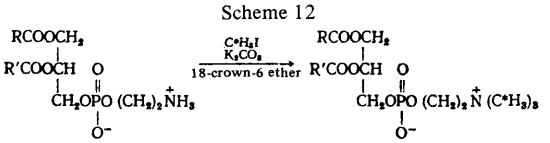
The method involving the reductive alkylation of the amino-group in PE has also been used to obtain the paramagnetic analogue (XVI) of PE [199] (Scheme 11):

Scheme 11



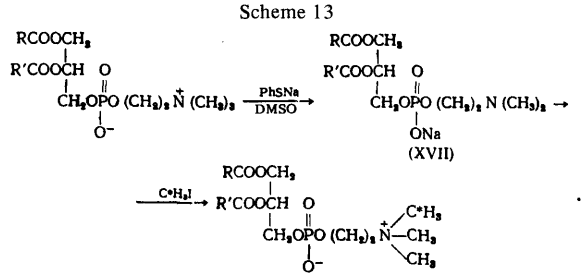
One of the methods of synthesis of PC, isotopically-labelled in the choline group, consists in the *N*-methylation of PE with labelled methyl iodide in the presence of crown-ether and anhydrous potassium carbonate[224] (Scheme 12):

Scheme 12



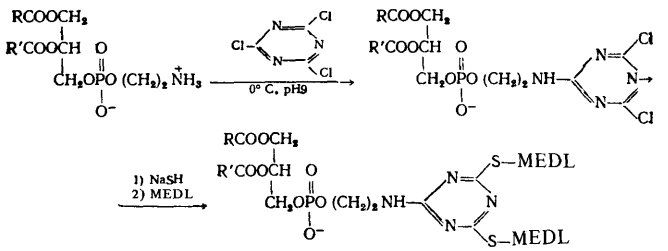
Partial demethylation of PC in the presence of sodium thiophenoxide in dimethyl sulphoxide (DMSO) followed by the methylation of the dimethylamino-derivatives (XVII) with labelled methyl iodide likewise leads to isotope-labelled PC [225-228] (Scheme 13):

Scheme 13



One of the few examples of the synthesis of PL with an electronically dense label is based on the arylation of PE with cyanuric chloride and subsequent replacement of the Cl atoms by SH groups and the reaction with MEDL† [204] (Scheme 14):

Scheme 14



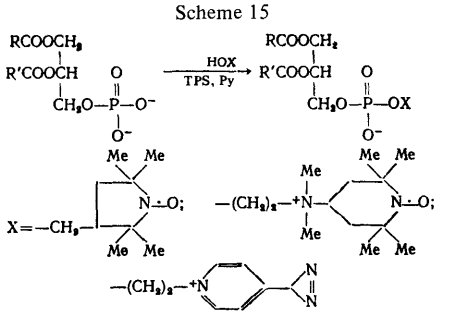
*(c) Phosphorylation*

PL modified in the polar component are frequently obtained using classical methods for the phosphorylation of glycerol derivatives (1,2-diglycerides, 1,2-*O*-isopropylidene-*sn*-glycerol, or halogenohydrins of 1,2-diglycerides): the phosphorochlorinate method, the silver salt method, and the interaction of phosphomonoester derivatives (usually PA) with the alcohol components in the presence of various activating agents.[28,31,205-223]

Thus, PC, PE, *N*-methyl-PE, and *NN*-dimethyl-PE analogues with different distances between the P and N atoms have been obtained by the phosphorochloridate method;[217-219] the silver salt method has been used to obtain a fluorescent analogue of PC containing the 2-[3,6-bis(dimethylamino)-10-acridino]ethyl group,[213] two immunoactive analogues of PL containing 3β-cholesterol and 3β-hydroxynorpregnen-5-ene,[220,221] several isotope-labelled PL,[211] and a series of PE analogues with different distances between P and N atoms.[214,215]

In the method using activating agents, the phosphomonoester component is usually activated with 2,4,6-triisopropylbenzenesulphonyl chloride (TPS).[109,142,205-210] The free-radical analogues of PA [205,207] and PC,[208] the photoreactive analogue of PC [142] (Scheme 15), and a series of isotope-labelled PC [209] and PS [210] have been obtained by this method:

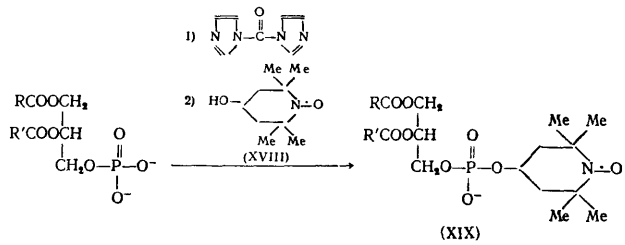
Scheme 15



†MEDL is the electron-dense mercarbide label (organomercury compound having not less than four C—Hg—X groups, where X is an acid anion).[204]

In the interaction of PA with secondary alcohols, this method proved to be relatively unsuitable owing to the low reactivity of these compounds under phosphorylation conditions, which in fact accounts for the difficulty of obtaining the nitroxy-derivative of PA of type (XIX).[205] In order to increase the efficiency of the phosphorylation of secondary alcohols, in particular of the alcohol (XVIII) with an iminoxy-group, use was made of the highly reactive PA imidazolides obtained without isolation by treating the reaction mixtures with carbonyl bisimidazole[206] (Scheme 16):

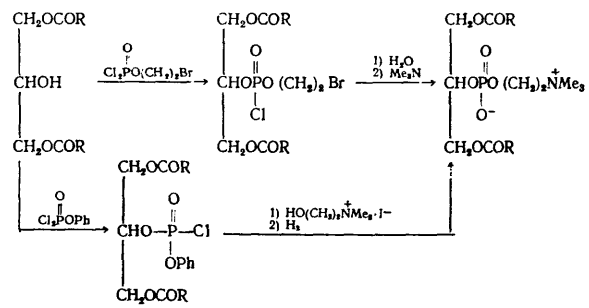
Scheme 16



## 3. Synthesis of Isomeric PL—Derivatives of 2-Glycerophosphate

A general method of synthesis of structural isomers of PL involves the phosphorylation of 1,3-diacylglycerols[40] by the phosphorylating agents used in the synthesis of PL having the natural structure[41,42] (Scheme 17)

Scheme 17



Isomeric PC and PE have been obtained in this way,[40-42] but the yields are as a rule lower in this instance than in the phosphorylation of the primary hydroxy-group.[40]

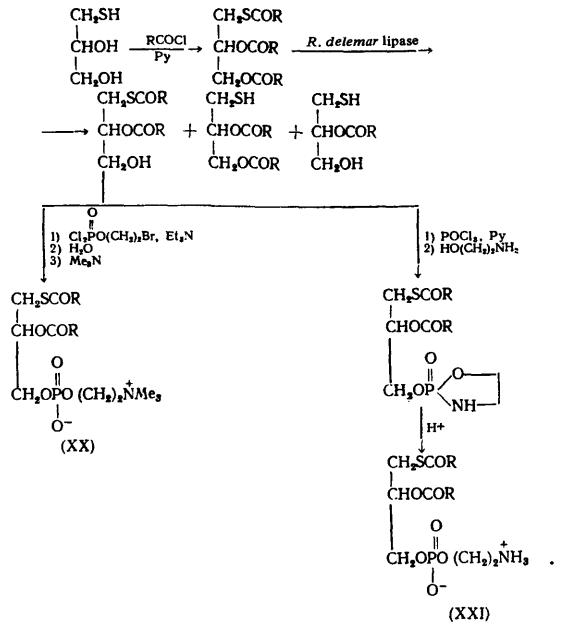
Isomeric PL are analogues of substrates of phospholipases which are hydrolysed much more slowly than the natural PL.[16]

## 4. Synthesis of PL Modified in the Glycerol Residue

PL analogues in which the ester linkage in one of the positions in the glycerol residue of the molecule has been replaced by the thioester linkage[21] or a phosphoester linkage has been replaced by a thiophosphoester linkage[20-43] are convenient substrates for spectrophotometric studies on phospholipases.

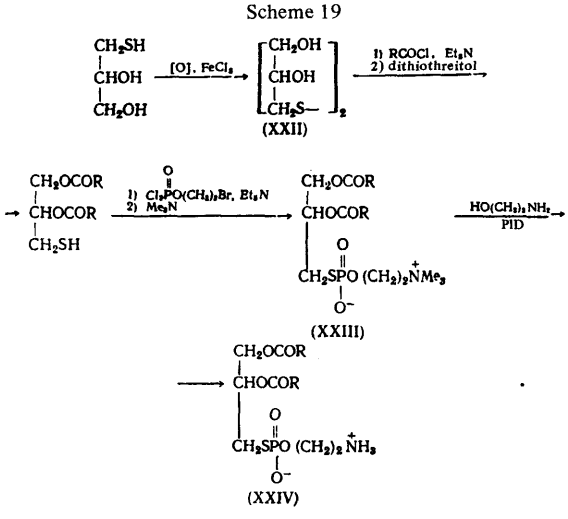
The thioester analogues of PC [compound (XX)] and PE [compound (XXI)] [21] have been obtained from 1-mercapto-*rac*-glycerol[229] by classical methods for the synthesis of PL [230] (Scheme 18):

Scheme 18



In the synthesis of the thio-PL (XXIII) and (XXIV), the temporary protection of the mercapto-group in the acylation of mercaptoglycerol was achieved by oxidising it in air in the presence of iron(III) chloride to the disulphide (XXII) and its subsequent reduction with dithiothreitol[20] (Scheme 19):

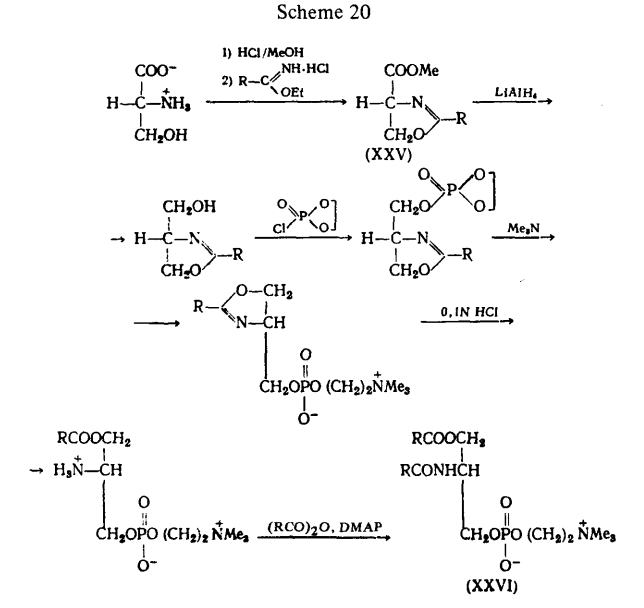
Scheme 19



In the $^{31}P$ NMR spectra, the chemical shifts of the $^{31}P$ nuclei in the thio-PL (XXIII) and (XXIV) and in the PL having the natural structure differ by 19 p.p.m.,[43] which makes it possible to observe separately, with the aid of the selective saturation method, the signals of the thio-PL forming part of the composition of liposomal membranes.

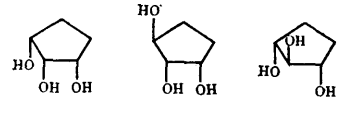
PC analogues non-hydrolysable by phospholipase $A_2$ have been obtained by replacing the ester linkage in the 2-position in the glycerol residue of PC by the amide linkage. The first synthesis of the acylamide analogue of PC, carried out starting with L-serine,[16,44] led to a racemic compound. Later methods based on the use of D-serine[46] or L-serine[47] were developed and made it possible to obtain the optically active compound. In both cases the key stage of the synthesis is the formation of the oxazoline (XXV) which which makes it possible to reduce the ester group to the hydroxy-group and blocks the amino- and hydroxy-groups in the stages involving the phosphorylation and subsequent alkylation of trimethylamine by the phosphate obtained[46,47] (Scheme 20):

Scheme 20



The amido-PC (XXVI) is a specific inhibitor of phospholipase $A_2$, exhibiting an even greater affinity for the enzyme than the natural substrate.[46]

PL analogues in which the glycerol residue has been replaced by another triol residue—an alkanetriol (1,2,4-butanetriol[44] or 1,2,5-pentanetriol[48]) or one of the three diastereoisomeric cyclopentane-1,2,3-triols (XXVII)–(XXIX)[49-54]—have been synthesised by traditional methods:



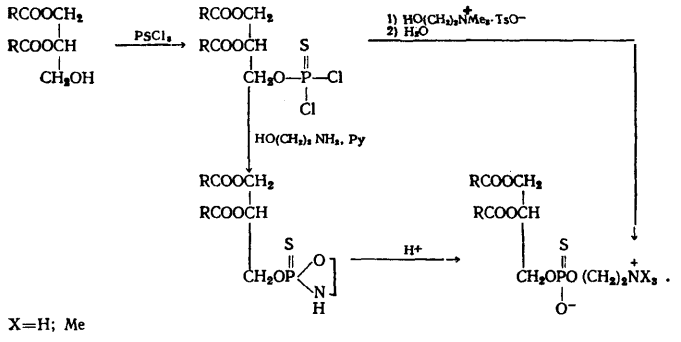
(XXVII) (XXVIII) (XXIX)

## 5. Synthesis of PL Modified in the Phosphoester Group

The thiono-analogues of PL containing the thiophosphoryl group instead of the phosphoryl group are convenient objects for the study of the behaviour of individual classes of PL in membranes by $^{31}P$ NMR, since the chemical shifts of the $^{31}P$ nuclei in thiono-PL and PL having the natural structure differ by 57 p.p.m.,[15,231] which makes it possible to differentiate the thiono-PL using $^{31}P$ NMR even when these substances are part of the composition of liposomal and biological membranes where the difference in chemical shift reaches 50 p.p.m.[231]

One of the methods for the introduction of the phosphorothioate group into the PL molecule involves the phosphorylation of 1,2-diacyl-*sn*(or *rac*)-glycerol with thiophosphoryl trichloride[62,63] (Scheme 21):

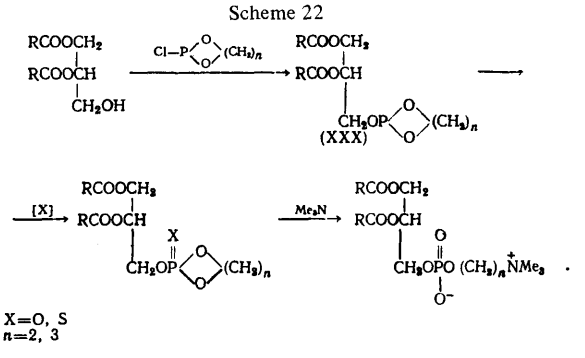
Scheme 21



X=H; Me

Another convenient method of synthesis of thiono-analogues of PC as well as PC analogues with different distances between the P and N atoms involves the oxidation (or sulphurisation) of alkylene phosphites derived from glycerol (XXX) and the subsequent interaction of the cyclic phosphates or phosphonothioates with triethylamine[55,61,232] (Scheme 22):

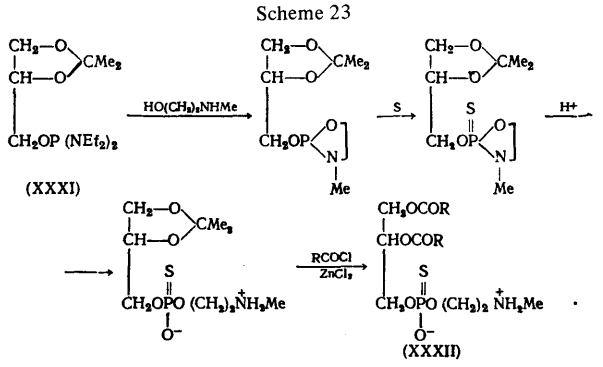
Scheme 22



X=O, S
*n*=2, 3

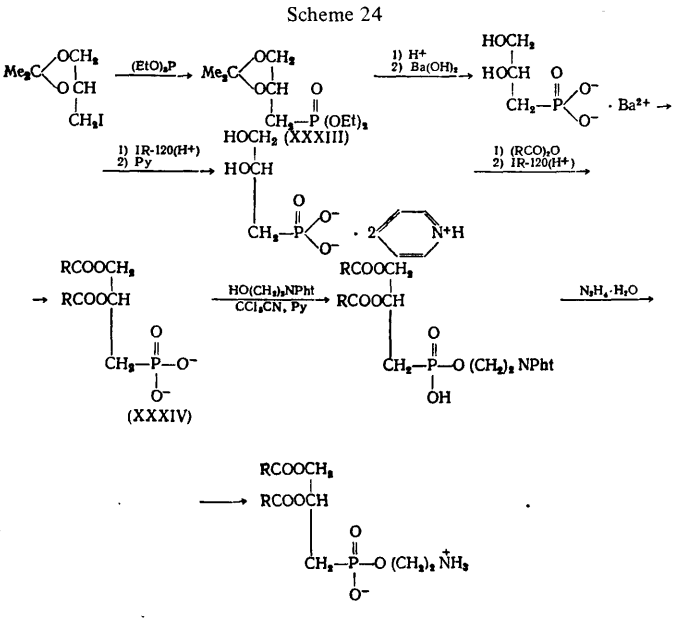
*N*-Methylthio-PE (XXXII) [60] (Scheme 23), thio-PA,[56,57] and also thiono-analogues of phosphatidylhydroquinones[58,59] have been obtained using various phosphoramidites derived from 1,2-*O*-isopropylidene-*rac*-glycerol (XXXI):

Scheme 23



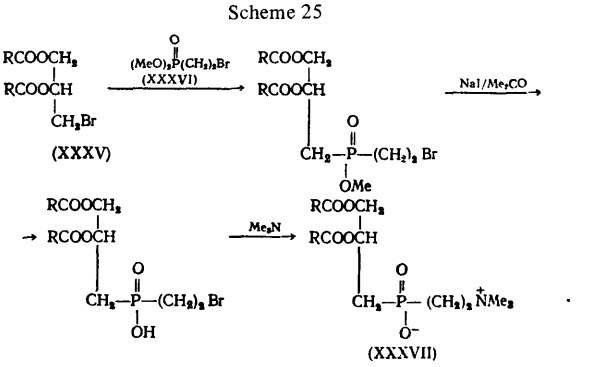
The reaction of the halides of 1,2-disubstituted glycerols with phosphorous acid triesters (Arbuzov alkylation) has been used to synthesise PL analogues in which the polyol is linked to the phosphorus atom by a C–P bond[64,65,67-69] (Scheme 24). The hydrolysis of the resulting diester by the substituted dihydroxypropylphosphonic acid (XXXIII) and subsequent acylation lead to the phosphonic analogue of PA (XXXIV), which can be readily converted into the analogues of other PL [66,67] (Scheme 24):

Scheme 24



The PC analogue (XXXVIII), in which the phosphodiester group has been replaced by the C–P–C linkages (the phosphine analogue of PC), has been obtained by the Arbuzov reaction between the bromohydrin of 1,2-dipalmitoyl-*sn*-glycerol (XXXV) and dimethyl 2-bromoethylphosphonate (XXXVI) with subsequent elimination of the methyl group and quaternisation by reaction with triethylamine[70]‡ (Scheme 25):

Scheme 25

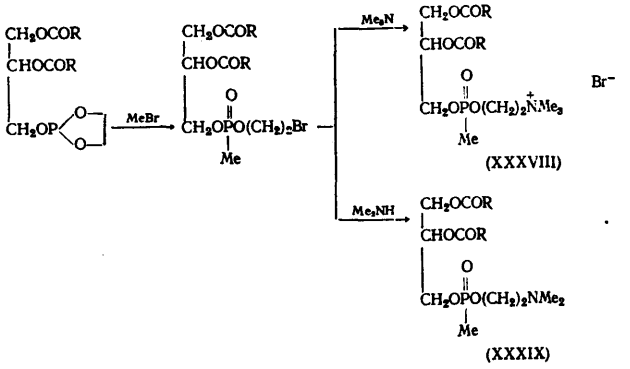


Analogues of PC (XXXVIII) and *NN*-dimethyl-PE (XXXIX), which are neutral esters of methylphosphonic acid, have been obtained by the analogous reaction of ethylene phosphites derived

‡See also Rosenthal.[67]

from 1,2-disubstituted glycerols with methyl bromide[71] (Scheme 26):

Scheme 26



The phosphonic and phosphinic analogues of PL are used as inhibitors of various phospholipases.[16,44]

Thus the modern methods of synthesis of modified PL considered in the review§ make it possible to obtain substances with virtually any structure, necessary both for the study of the structures and functioning of model and biological membranes and for the solution of the ever expanding range of other problems in molecular biology and biochemistry.

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