



(86) Date de dépôt PCT/PCT Filing Date: 1997/02/17
(87) Date publication PCT/PCT Publication Date: 1997/08/21
(45) Date de délivrance/Issue Date: 2007/08/07
(85) Entrée phase nationale/National Entry: 1998/08/14
(86) N° demande PCT/PCT Application No.: EP 1997/000749
(87) N° publication PCT/PCT Publication No.: 1997/030058
(30) Priorités/Priorities: 1996/02/16 (DE196 05 833.3);
1996/06/03 (DE196 22 224.9)

(51) Cl.Int./Int.Cl. *C07F 9/10* (2006.01),
A61K 47/24 (2006.01), *A61K 48/00* (2006.01),
A61K 9/127 (2006.01), *C07C 43/13* (2006.01),
C07C 43/178 (2006.01), *C07D 317/22* (2006.01),
C07D 407/12 (2006.01), *C07F 9/09* (2006.01),
C12N 15/87 (2006.01)

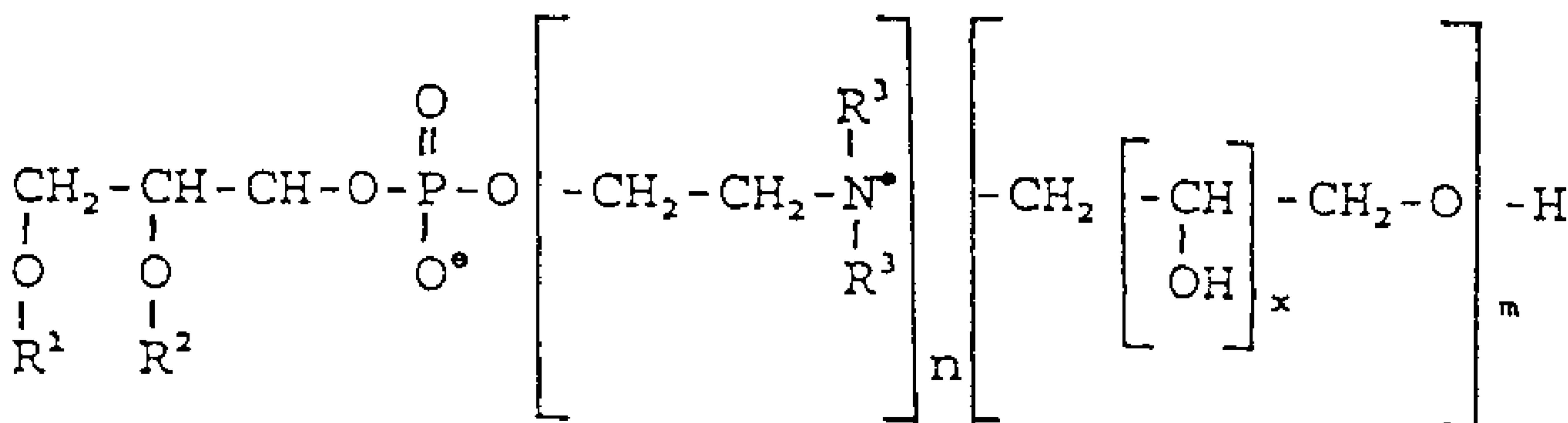
(72) Inventeur/Inventor:
EIBL, HANS-JORG, DE

(73) Propriétaire/Owner:
MAX PLANCK GESELLSCHAFT ZUR FORDERUNG
DER WISSENSCHAFTEN E.V., DE

(74) Agent: OGILVY RENAULT LLP/S.E.N.C.R.L.,S.R.L.

(54) Titre : PHOSPHATIDYLOLIGOGLYCERINES

(54) Title: PHOSPHATIDYL OLIGOGLYCEROLS



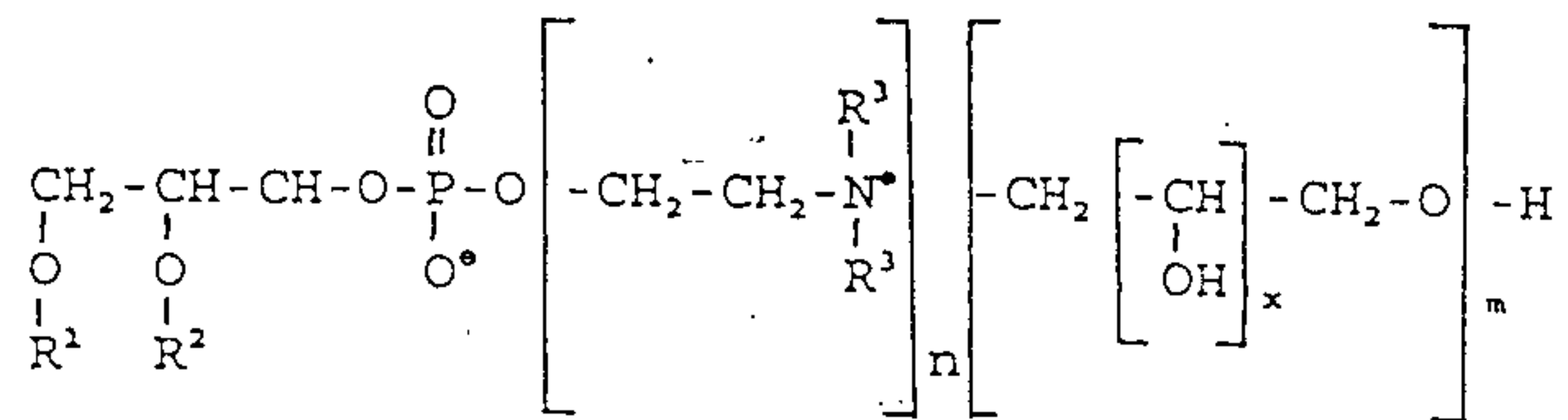
(57) Abrégé/Abstract:

In order to form liposomes with a longer half-life in blood, use is made of defined compounds with the general formula (A) (see above formula)



Abstract

In order to form liposomes with a longer half-life in blood, use is made of defined compounds with the general formula (A)



Phosphatidyl oligoglycerols

Specification

5 The invention relates to phosphatidyl compounds which contain a defined hydrophilic residue, and to long-circulating liposomes.

Conventional liposomes circulate in the serum for up to 5 hours. However, especially when liposomes are used as a means of drug delivery, it is desirable that they circulate in the bloodstream for as long as possible.

To this end, the so-called "stealth liposomes" were developed, which are not destroyed in the bloodstream so quickly. These "stealth liposomes" are built up on the basis of phosphatidyl compounds which have an extended polyethylene glycol residue. The polyethylene glycol residue proved to be most effective in producing the desired increase in liposome survival duration when the molecular weight was between 2000 and 3000. A serious disadvantage, however, of these "stealth-liposomes", ie, of these phosphatidyl compounds with a polyethylene glycol residue, is that the compounds are not exactly defined, since the polyethylene glycol residues display different chain lengths.

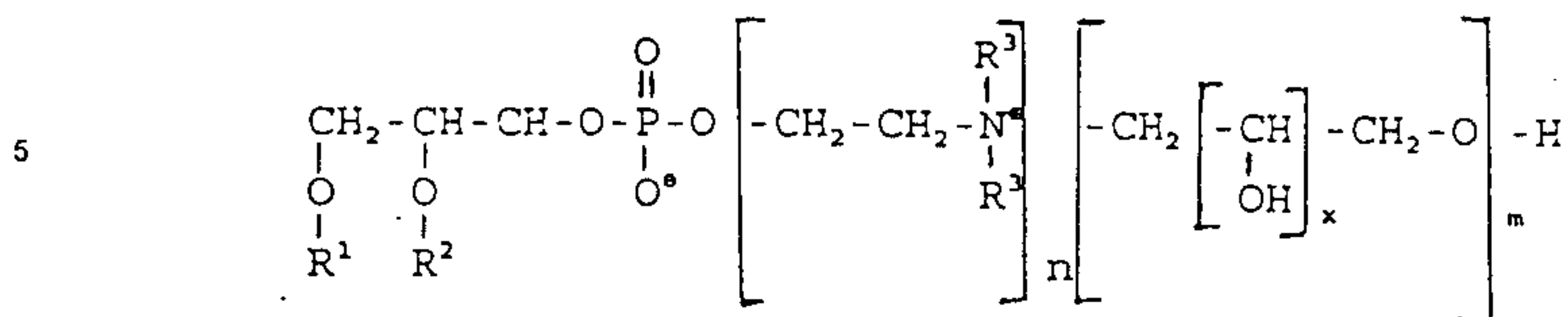
Maruyama et al. (Int. J. Pharmac. 111 (1994), 103-107) suggested the use of dipalmitoyl phosphatidyl polyglycerols to lengthen the duration of liposome circulation. However, since technical-grade polyglycerols were used as starting material, no uniform products were obtained here either. Technical polyglycerols, which consist of a mixture of polyglycerols with different chain lengths and monoglycerol, and which are characterized by their average molecular weight, were phosphatidylated by means of phospholipase D. The resulting products only led to a small increase in the survival duration of liposomes in the blood.

30

The object of this invention was thus to provide compounds which increase the survival duration of liposomes and which are of exactly definable composition.

This objective is established according to the invention by means of a compound with the general formula (A)

35



where R^1 and R^2 , independent of each other, stand for hydrogen or a saturated or unsaturated alkyl or acyl residue, which may be branched and/or substituted, R^3 stands for hydrogen or an alkyl residue,

$n = 0$ or 1 ,

x is a whole number from 1 to 4 and

m is a whole number from 2 to 10 if $n = 0$, or a whole number from 1 to 10 if $n = 1$, or 1 if x is greater than 1 ,

and where, in the case that $n = 0$, the compound is more than 90% uniform with respect to the value of m .

The stepwise synthesis - upon which this invention is based - of the hydrophilic residues of the phosphatidyl compounds of formula (A) makes it possible to obtain compounds of an exactly defined composition.

The compound of the invention, with the formula (A), is thus not a mixture of various molecules of indefinite composition and chain length, but can be synthesized to have precisely the desired structure. If, for example, the desired product is a triglycerol derivative, ie, $x = 1$ and $m = 3$ in formula (A), the content of monoglycerol, diglycerol, tetraglycerol and higher oligoglycerol derivatives will be low. It is preferable if a glycerol derivative of a certain chain length is obtained that is largely free of glycerol derivatives of other chain lengths. The content of monoglycerol derivatives, in particular, is low, amounting to less than 5% , preferably less than 1% or, even more preferably, less than 0.1% relative to the desired oligoglycerol derivative.

According to the invention, the compound of formula (A) is a uniform compound of defined structure. It is of advantage if, with respect to the value of m , the compound is greater than 95% uniform. It is to greater advantage if it is more than 99% uniform. It

is even possible to provide a compound which is more than 99.9 % uniform with respect to the value of m.

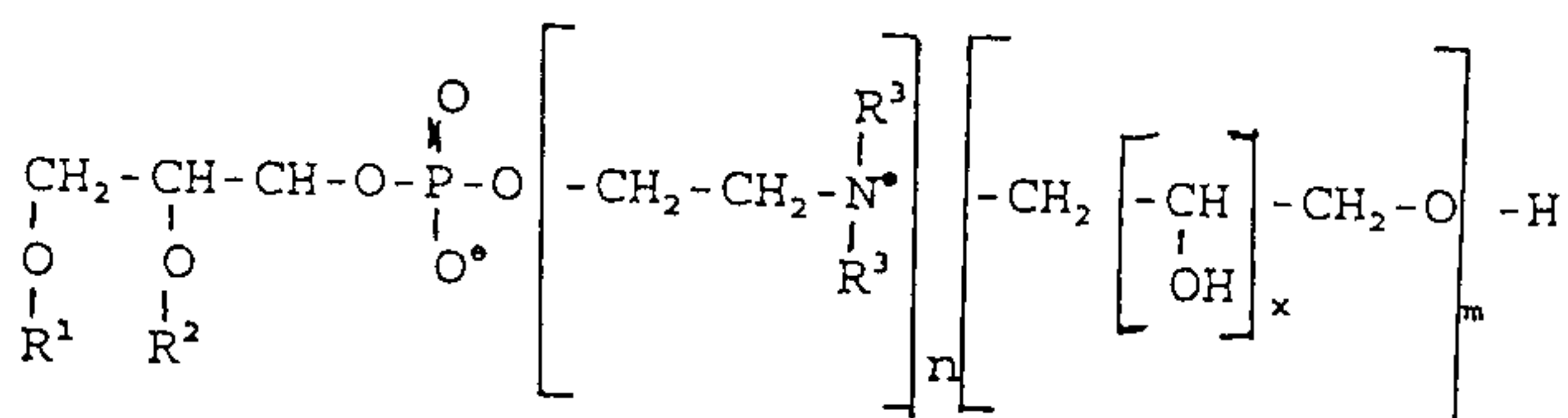
The compound is preferably an oligoglycerol derivative with 2 to 5 glycerol units, more preferably with 2 to 4 glycerol units. It is to advantage if these are 1,3-linked linear oligoglycerol residues.

According to the invention, the residues R^1 and R^2 , independent of each other, stand for hydrogen, a saturated or unsaturated C_1 - C_{24} alkyl or C_1 - C_{24} acyl residue, preferably hydrogen or a saturated or unsaturated C_8 - C_{24} alkyl or C_8 - C_{24} acyl residue, it being beneficial if at least one of the residues R^1 and R^2 is an acyl residue.

The residue R^3 is preferably hydrogen or an alkyl residue with 1 to 4 carbon atoms.

The compound of formula (A) can be a racemic compound which contains a phospho-rac-(1 or 3)-oligoglycerol linkage, or it can be in the form of a stereospecific isomer. The stereoisomers can have a phospho-sn-1-oligoglycerol linkage or a phospho-sn-3-oligoglycerol linkage. The formation of the stereospecific linkage can be carried out in a manner analogous to those described in the literature (DE 31 30 867 A1; H. Eibl et al., Chem. Phys. Lipids, 28 (1981), 1- 5, 41 (1986), 53-63 and 47 (1988, 47-53).

The subject matter of the invention also includes liposomes which contain phospholipids and/or alkyl phospholipids, maybe cholesterol, and 1 to 50 mol % of a compound with the general formula (A),



or salts thereof, where the cholesterol, the phospholipids, the alkyl phospholipids and the compound of formula (A) together make up 100 mol %, and R^1 and R^2 , independent of each other, stand for hydrogen, a saturated or unsaturated alkyl or acyl residue which may be branched and /or substituted,

R^3 stands for hydrogen or an alkyl residue,

$n = 0$ or 1 ,

x is a whole number from 1 to 4 and

m is a whole number from 2 to 10 if $n = 0$, or a whole number from 1 to 10 if $n = 1$,
 5 or 1 if x is greater than 1 , and where, in the case that $n = 0$, the compound (A) is more than 90% uniform in respect of the value of m .

The liposomes of the invention have a half-life in serum of up to 18 to 20 hours. Surprisingly, the liposome concentration in blood was found to decrease linearly.

10

It is beneficial according to the invention if compound (A) displays a uniformity of more than 95% or, even better, more than 99% with respect to the value of m . It is also possible, however, to use compound (A) in practically pure form, ie, more than 99.9% uniform with respect to the value of m .

15

The liposomes preferably contain a compound of formula (A), in which $x = 1$ and m is a whole number from 2 to 5 ; it is even more preferable if m is a whole number from 2 to 4 .

20

The residues R^1 and R^2 of the compound of formula (A) contained in the liposomes can stand, independent of each other, for hydrogen or a saturated or unsaturated C_1 - C_{24} alkyl or C_1 - C_{24} acyl residue, preferably hydrogen or a saturated or unsaturated C_8 - C_{24} alkyl or C_8 - C_{24} acyl residue. The substituent is a residue which does not interfere during the preparation. R^3 is preferably hydrogen or a C_1 - C_4 alkyl residue.

25

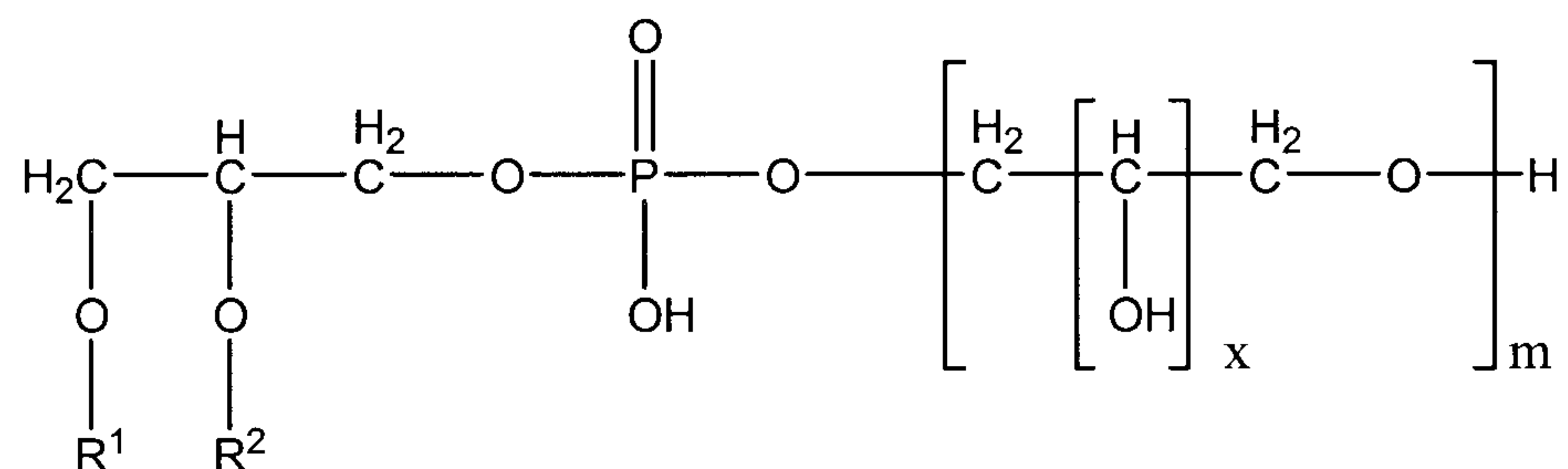
The compound of formula (A) can be present in the liposomes as a racemic mixture, ie, with a phospho-rac-(1 or 3)-oligoglycerol linkage. It is preferable if it is present in stereospecific form with a phospho-sn- 1 -oligoglycerol linkage or a phospho-sn- 3 -oligoglycerol linkage.

30

It is to advantage if at least one of the residues R^1 and R^2 of formula (A) is an acyl group.

35

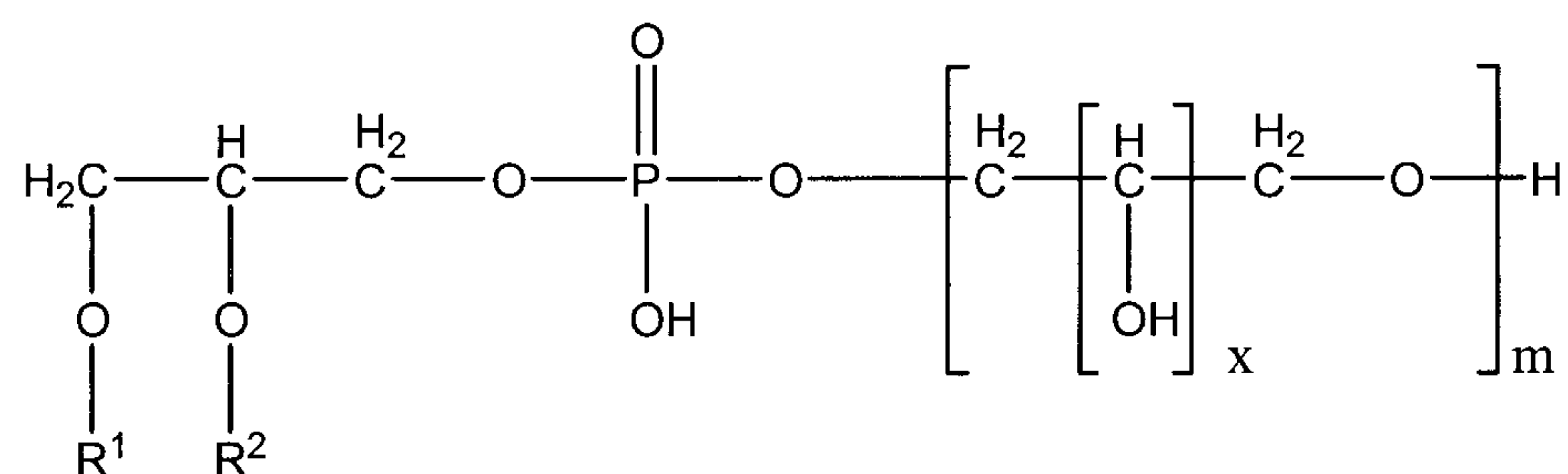
It is beneficial if liposomes containing the compound of formula (A) with $n = 0$ exhibit an excess negative charge. However, liposomes can also be prepared from



- 5a -

where R^1 and R^2 are independently hydrogen or a saturated or unsaturated, branched or unbranched aliphatic or acyl residue, x is a whole number from 1 to 4; and m is a whole number from 2 to 5, wherein the compounds are more than 90% uniform with respect to the value of m .

In accordance with another aspect of the present invention there are liposomes containing phospholipids or alkyl phospholipids or cholesterol, and 1 to 50 mol % of compounds with the formula (A):



or salts thereof, where the cholesterol, the phospholipids, the alkyl phospholipids and the compounds of formula (A) together make up 100 mol %, and R^1 and R^2 are independently hydrogen, a saturated or unsaturated, branched or unbranched aliphatic or acyl residue; x is a whole number from 1 to 4; and m is a whole number from 2 to 5; wherein compound (A) comprised in the liposomes are more than 90% uniform with respect to the value of m , wherein said phospholipid and compound of formula (A) contain the same fatty acid residues.

In accordance with a further aspect of the present invention there is a pharmaceutical composition comprising liposomes previously described and, entrapped in the liposomes, at least one pharmaceutical drug.

In accordance with yet another aspect of the present invention there is a method of preparing the pharmaceutical composition of the preceding paragraph, comprising preparing liposomes by transforming 1 to 50 mol % of a compound of said general formula (A), together with the other components of the liposomes being cholesterol phospholipids or alkyl phospholipids in a quantity which, together with the compound of formula (A) makes up 100 mol %, and at least one soluble or insoluble pharmaceutical drug into a lipid suspension, and transforming said lipid suspension into liposomes, wherein when said drug is a water-insoluble drug it is

- 5b -

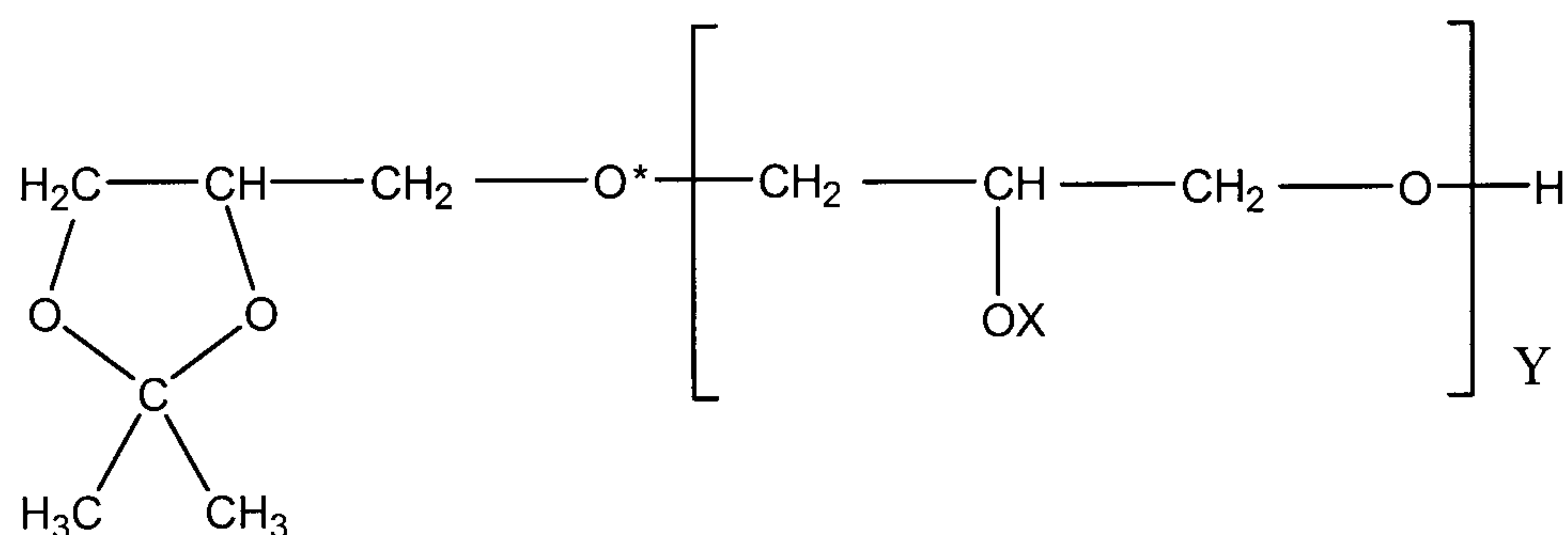
entrapped in said liposomes by dissolving the drug together with lipid constituents, and wherein when said drug is a water-soluble drug it is entrapped in the liposomes by adding an aqueous solution which contains the water-soluble drug to lipid film.

In accordance with yet a further aspect of the present invention there is a method of preparing the compounds of formula (A), as defined previously, comprising linking an oligoglycerol having a uniform chain length or a C₄-C₆ sugar alcohol with an alcohol of the formula:



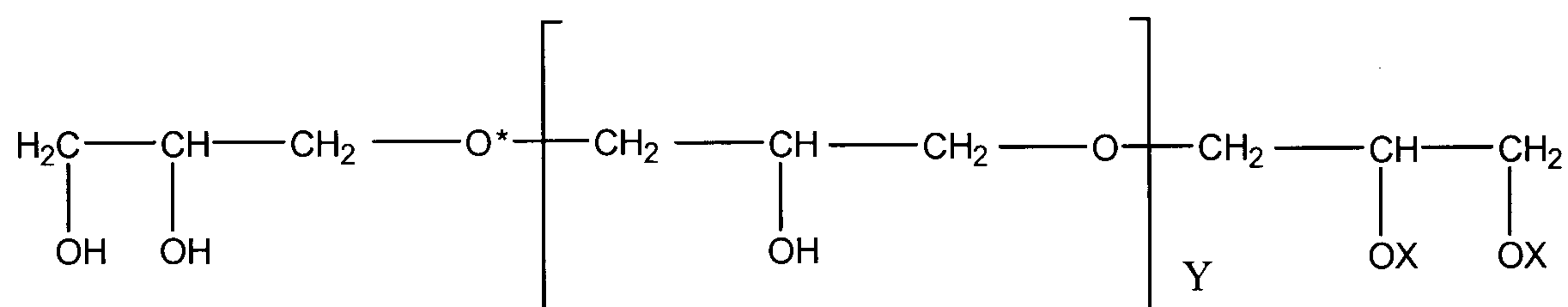
through use of a phosphorylation agent.

In accordance with one embodiment of the present invention there is a protected oligoglycerol with the formula (B):



in which Y is a whole number from 1 to 9 and X is a benzyl, alkyl or tetrahydropyranyl group, provided that when X is methyl then Y is 2 to 9.

In accordance with another embodiment of the present invention there is an alkyl oligoglycerol with the formula (C):



- 5c -

in which Y is a whole number from 1 to 8 and one of the residues X or Z is a saturated or unsaturated aliphatic residue, while the other residue is hydrogen.

The liposomes of the invention have a markedly longer half-life in the blood stream. Their half-life is preferably at least 10 hours, better still, more than 12 hours. Half-lives of 18 to 20 hours have been measured for the liposomes of the invention. Surprisingly, the decrease in blood lipid concentration with time was found to be absolutely linear. It is preferable according to the invention if, after 6 hours, more than 50 % of the liposomes added are still present in the blood; it is even more preferable if more than 60 % are still present.

A particularly surprising property of the liposomes of the invention is their preferred tendency to accumulate in the spleen. Depending on the composition and size of the

liposomes, enrichment thereof in the spleen has been found which exceeds enrichment in the liver by a factor of 25. Enrichment in the spleen compared with that in the liver increases with increasing value of m in formula A and with increasing size of the liposomes. With the transition from SUVs (Small Unilamellar Liposomes; diameter about 60 nm) to LUVs (Large Unilamellar Liposomes; diameter about 190 nm), the degree of enrichment in the spleen increases many times over. The preferential accumulation in the spleen also increases as the number of carbon atoms in R^1 and R^2 increases.

It was found, in addition, that the liposomes of the invention also accumulate in certain tumour tissues. This was observed to be the case, for example, with breast carcinomas induced by nitrosomethylurea (MNU carcinoma).

The liposomes of the invention can also contain one or more pharmaceutical drugs.

Generally speaking, all drugs can be used that can be introduced into the plasma by means of liposomes. Preferred groups of drugs are, on the one hand, cytostatic agents, especially anthracycline antibiotics such as doxorubicin, epirubicin and daunomycin, with doxorubicin being especially preferred. Other preferred anti-tumour drugs are idarubicin, hexadecylphosphocholine, 1-octadecyl-2-methyl-rac-glycero-3-phosphocholine, 5-fluoruracil, cis-platinum complexes such as carboplatin and novantron, and mitomycins.

Other preferred groups of drugs are immunomodulating substances such as cytokines, of which interferon and, in particular, α -interferon are given special preference, antimycotic substances (eg, amphotericin B), and drugs to combat protozoan diseases (malaria and trypanosome and leishmania infections). Taxol is another preferred drug.

Yet another group of preferred drugs is the group of lytic drugs, as are described in the DE 41 32 345 A1. The content of this patent application is thus included by way of reference. Preferred drugs are miltefosin, edelfosin, ilmofosin and SRI62-834.

The subject matter of the invention thus includes use of the liposomes according to the invention for preparing an anti-tumour agent, with the drug doxorubicin being given special preference.

- 5 The subject matter of the invention also includes use of the liposomes according to the invention for preparing an agent to influence cell proliferation, with the drug preferably being a cytokine, in particular α -interferon.

10 The subject matter of the invention includes, in addition, a pharmaceutical composition which contains the liposomes described above and, entrapped in the liposomes, one or more pharmaceutical drugs, combined if necessary with standard pharmaceutical diluents, adjuvants, carrier media and fillers.

15 The liposomes of the invention are prepared using methods which are known per se and with the usual equipment. Typically, a solution containing the various components of the liposome and 1 to 50 mol % of a compound of formula (A) is converted into a lipid suspension which is then pressed under high pressure through nozzles or a perforated disk; the size of the liposomes can be regulated by means of the size of the perforations in the disk. Suitable measures for converting a lipid suspension into
20 liposomes are familiar to persons versed in the art. Preferably, 5 to 15 mol % of a compound of the general formula (A), 35 to 43 mol % cholesterol and 42 to 60 mol % phospholipids and/or alkyl phospholipids are converted into a lipid suspension, which in turn is converted into liposomes by means of suitable measures and in a manner known per se.

25

These known methods can also be used to make a pharmaceutical formulation which contains the liposomes of the invention and one or more pharmaceutical drugs. To entrap water-insoluble drugs, the drug is dissolved together with the lipid components, while to entrap water-soluble drugs, an aqueous solution which contains the water-
30 soluble drug is added to the lipid film.

The compounds of the invention, having the formula (A), can be prepared in cases where $n = 1$ by linking a defined oligoglycerol with a phosphatidyl ethanolamine by way of the amino group. This results in neutral compounds, ie, compounds without an

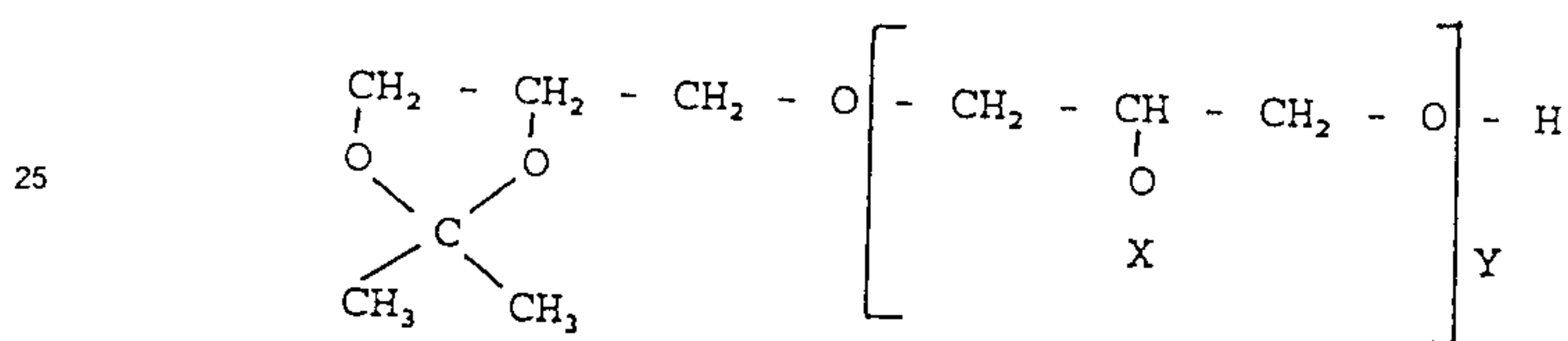
excess charge. The defined oligoglycerols used for linking are compounds with the formula (B).

In cases where $n = 0$, compounds with the general formula (A) are made by linking a defined oligoglycerol with a phosphatidylglycerol. When $n = 0$, compounds with the general formula (A) can also be made - using a phosphorylation agent - by linking a defined oligoglycerol or a C_4 - C_6 sugar alcohol with an alcohol of the formula $CH_2-OR^1-CHOR^2-CHOH$. As phosphorylation agent, use is made preferably of $POCl_3$.

The preparation of phospholipids from diacyl glycerols is described in the literature (DE 32 39 817 A1; P. Woolley et al., Chem. Phys. Lipids 47 (1988), 55-62; H. Eibl et al., Chem. Phys. Lipids 47 (1988), 63-68), and this method can be applied here.

Using the above-described methods, a racemic compound is formed which contains a phospho-rac-(1 or 3)-oligoglycerol linkage. It is to advantage if stereospecific compounds are formed, which exhibit a phospho-sn-1-oligoglycerol linkage or a phospho-sn-3-oligoglycerol linkage. To make a compound of formula (A), it is preferable to use a linear oligoglycerol of defined chain length.

The subject matter of the invention also includes a protected oligoglycerol of the formula (B),

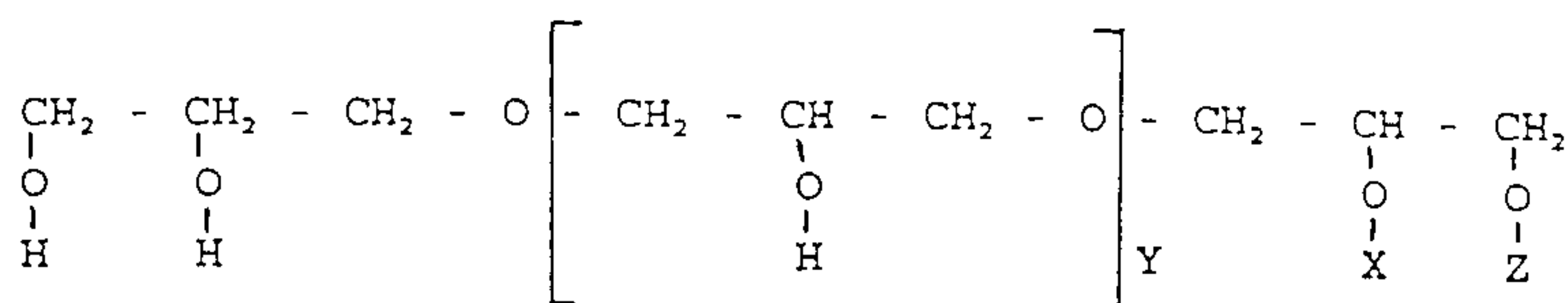


where Y is a whole number from 1 to 9 and X is a benzyl, alkyl or tetrahydropropanyl group. It is beneficial if Y is a whole number from 1 to 3. It is possible according to the invention to obtain 1,3-linked oligoglycerols in practically pure form. Oligoglycerols of a predefined chain length can be prepared which contain hardly any impurities in the form of oligoglycerols with different chain lengths. In addition, these oligoglycerols of the

invention are practically free of monomeric glycerol. In other words, uniform compounds are obtained, which have a defined structure.

In the oligoglycerol, X can also stand for a different suitable protective group. It is also possible to replace the acetone with another protective group, in particular another ketone.

The invention comprises, in addition, alkyl oligoglycerols of formula (C)



where Y is a whole number from 0 to 8, preferably a whole number from 1 to 3, and one of the residues X or Z is a saturated or unsaturated alkyl residue and the other residue is hydrogen. These alkyl oligoglycerols are also uniform compounds of defined structure.

The production of oligoglycerols, protected oligoglycerols and alkyl oligoglycerols is of particular interest, because with the help of these starting materials a number of important and novel adjuvants serving as solubilizers and to improve membrane permeation are obtained. Of particular interest with respect to increasing the period for which the liposomes survive in the blood stream is the production of phosphatidyl oligoglycerol derivatives of formula (A), which carry additional hydroxyl groups in the polar area.

Due to the preferred enrichment of the liposomes of the invention in the spleen, these liposomes are suitable generally for the selective introduction of substances into the spleen. These substances may be medicinal products, contrast agents or the like. This is especially important with regard to improving the quality of vaccines, since the spleen plays a major role in the formation of antibodies for the immune system. In the same way, the enrichment of the liposomes according to the invention such as was

observed in tumour tissue is of importance with regard to delivering drugs, contrast agents and the like specifically to such tissue.

The following examples, together with the enclosed drawings, explain the invention in more detail. In the drawings:

Figure 1 shows the total-organ distribution of liposomes according to the invention in the spleen and in the liver.

Figure 2 shows the per-gram distribution of liposomes according to the invention in the spleen and in the liver.

Figure 3 shows how the blood levels of different liposomes of the invention vary as a function of time.

Example 1

In an animal experiment, liposomes were used which consisted of 40 mol % cholesterol, 10 mol % phosphatidylglycerol and 50 % dipalmitoyl lecithin. The liposomes had a half-life in serum of 4 hours, with a typical persistence characteristic, ie, a rapid decrease to start with, followed by a slower decrease.

Liposomes of the same composition were prepared, in which the phosphatidylglycerol was replaced by a phosphatidylglycerol G2 of the invention. A half-life in serum of 18 to 20 hours was measured, the decrease with time being absolutely linear. This linear relation was observed irrespective of the size of the liposomes. The same linear reduction in serum liposome concentration was found with 50 nm liposomes and with 150 nm liposomes. The linear reduction in blood liposome concentration was also observed for different starting concentrations.

Example 2

Percentage of liposomes in the blood stream after 6 hours

Liposomes according to the invention were prepared, consisting of dipalmitoyl-sn-G-3-PC/cholesterol/dipalmitoyl-sn-G-3-PG₂ in a molar ratio of 45 : 45 : 10. The percent-

ages of liposomes still in the blood after 6 hours are listed in Table 1. For comparison, the percentages measured by Maruyama et al. under the same conditions for the system distearyl-sn-G-3-PC/cholesterol/dipalmitoyl-sn-G-3-PG_Y, 45 : 45 : 10 are listed too. Compared to the prior art, the example of the invention shows a pronounced increase in the quantity of liposomes found.

Table 1:

	Y	Comparative example	Y	Example of the invention
10	0	18 %	0	21 %
	2	19 %	2	80 %
	3	-	3	82 %
	4	20 %	4	56 %

15 Example 3

Liposomes consisting of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, 1,2-dipalmitoyl-sn-glycero-3-phosphoglyceroglycerol (PG_n) and cholesterol in a molar ratio of 4:1: 5 were doped with tritium-labelled inulin. These liposomes were administered to rats in a dosage of 100 µmol lipid per kg rat, and after 72 hours the distribution of these liposomes in the spleen and the liver was determined by measuring the radioactivity. Liver weights varied between 9 and 10 g, those of the spleen between 0.6 and 0.7 g. Figure 1 of the enclosed drawings shows that for a liver weight which is about 15 times higher than that of the spleen, the distribution of liposomes (SUVs) increases substantially in favour of the spleen as the number of glycerol units increases (x = 1; m = 1 to 4 in formula A).

In Figure 2, the liposome uptake by the spleen and the liver is depicted as uptake per gram of the organ. For n = 4, the spleen is seen to have a liposome concentration which is about 9 times higher than that of the liver; for n = 1, the enrichment factor equals 4. In the last column, Figures 1 and 2 show the effect which the size of the liposomes has. For LUVs with a diameter of 190 nm, liposome enrichment is even more in favour of the spleen: even when n only equals 2, the enrichment factor equals 24. In practical terms, this means that it is no longer possible to target the liver with these liposomes.

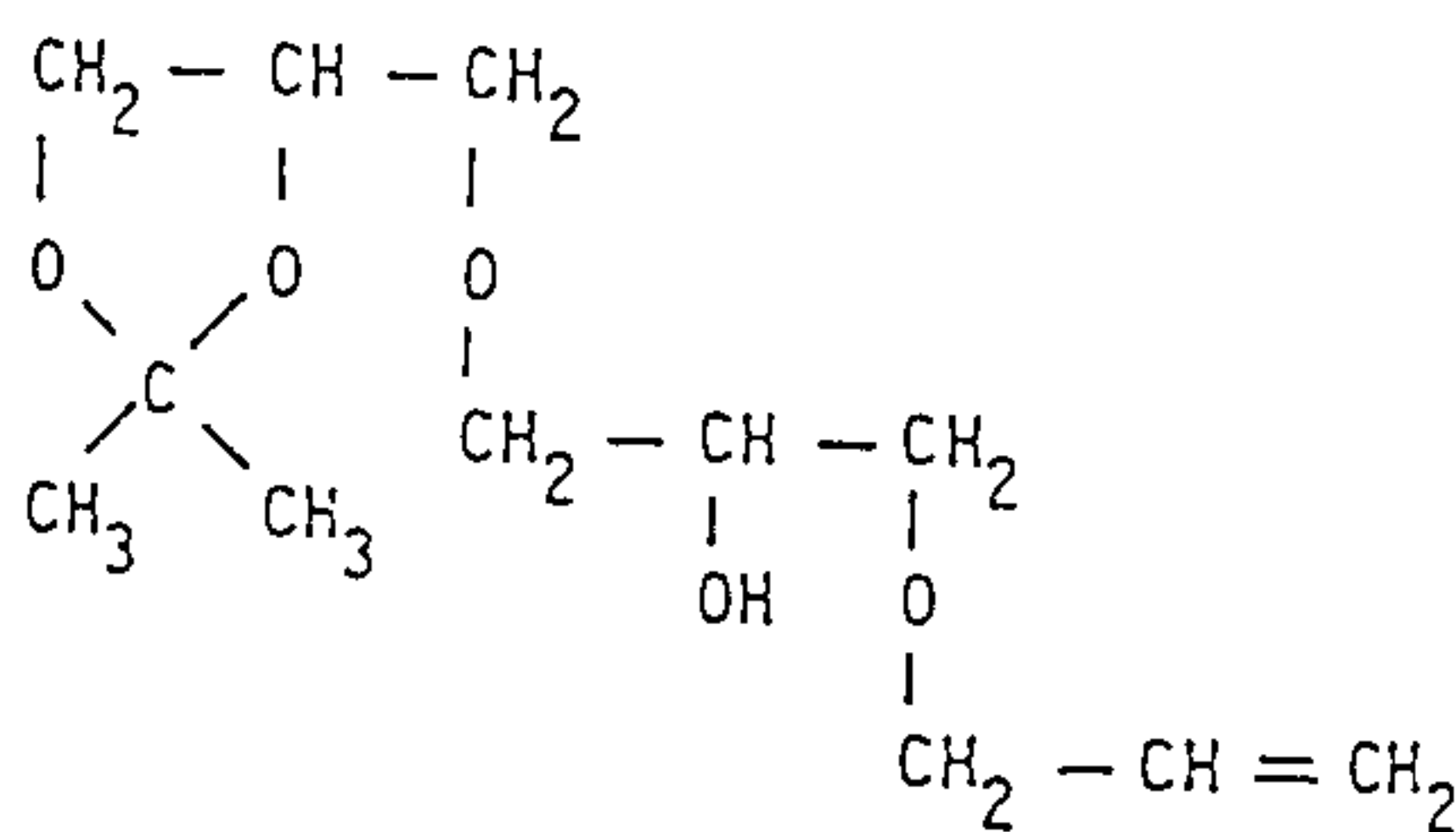
Example 4**Preparation of compounds with the formula (A)**

Example 4a: Key intermediate with the formula I

5

The oligoglycerols diglycerol (G_2), triglycerol (G_3) and tetraglycerol (G_4) can be prepared from an easily obtained key intermediate with the formula I,
1.2-isopropylidene-rac-glycero-3.1-rac-glycero-3-allylether, (see model A).

10



15

I

20

- 1) Rearrangement allyl/propenyl
- 2) Acidic cleavage

 G_2

- 1)1) Epoxidation
- 2)2) Acidic cleavage

 G_3

- 1) Epoxidation
- 2) Opening with allyl alcohol
- 3) Epoxidation
- 4) Acidic cleavage

 G_4

25

30

Model A: oligoglycerols from formula I

The intermediate product described by formula I can be obtained in large quantities from commercial allyl glycidyl ether by NaOH-catalyzed ring opening with 1.2-isopropylidene-rac-glycerol, which is likewise available in the chemicals trade:

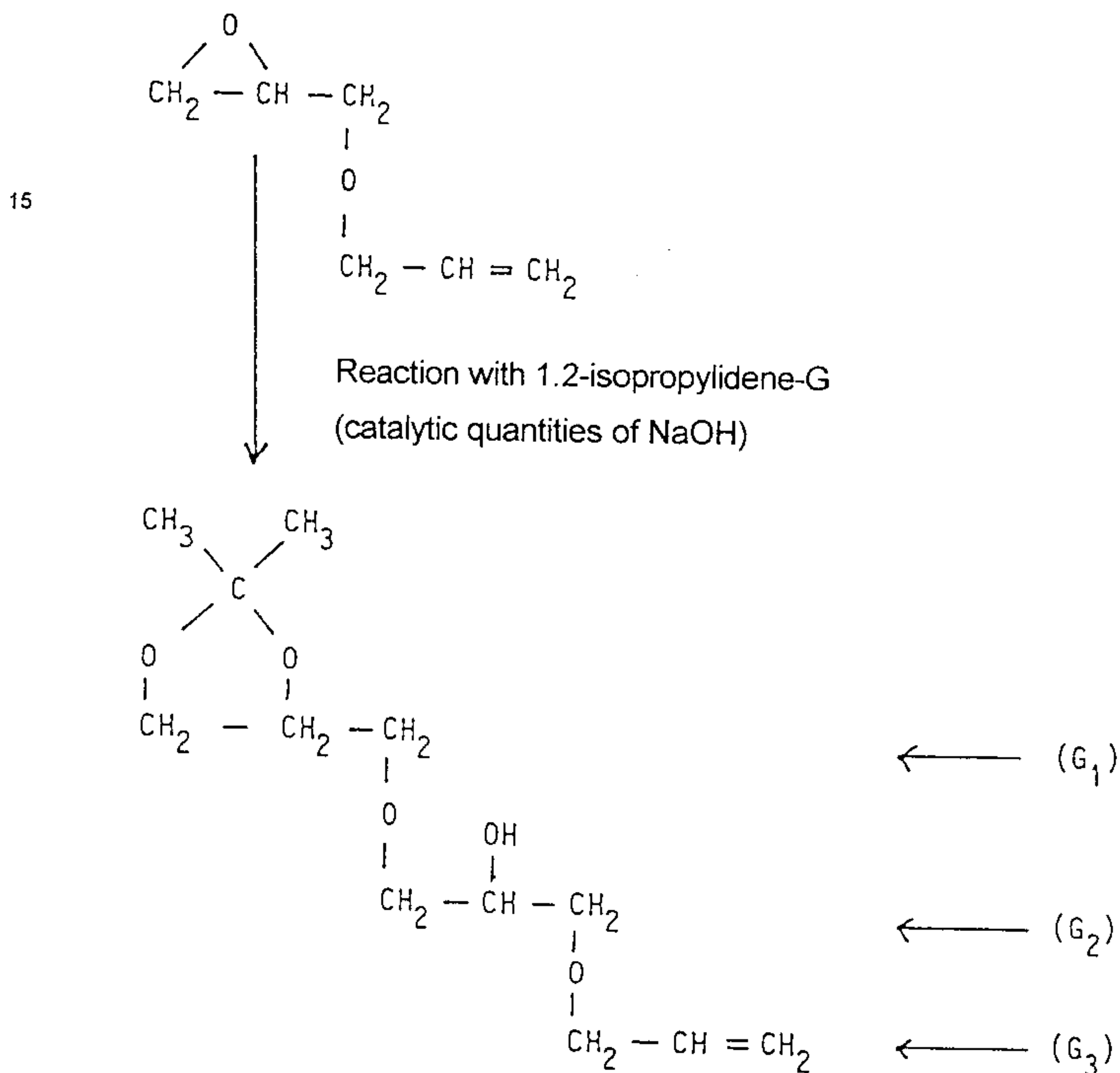
5

Epoxide opening with alcohols (general example)

Production of the key intermediate with the formula I:

1.2-isopropylidene-rac-G₁-3.1-0.0-3-0-allyl-rac-G₂

10



A catalytic quantity of NaOH (MW 40.00; 0.6 mol - 24 g) is added to 1.2-isopropylidene-rac-glycerol (MW 132.16; 16 mol - 2115 g), which is rendered a solution by stirring and heating to 80°C. At 80°C, allyl glycidyl ether (MW 114.14; 6 mol - 685 g) is added dropwise over a period of 2 hours, and the reaction mixture stirred for another 2 hours at 80°C. By this point in time the epoxide (R_f in ether = 0.8) has reacted completely to form the G_3 constituent (R_f in ether = 0.6). The excess isopropylidene-rac-glycerol has an R_f of 0.65 in ether and is removed from the reaction mixture at 75°C / 10 mbar. The residue has 1 l diisopropyl ether added to it and is extracted twice with 1 l NaCl (1 % solution in H_2O) in each case. The organic phase is rotated in an evaporator and distilled ($K_{pi_{10}}$ -1 mbar 125°C).

The yield of the pure product 1.2-isopropylidene-rac- G_1 -3.10.0-3-0-allyl-rac- G_2 (MW 246.30) is 1025 g (ca. 70 %).

Instead of 1.2-isopropylidene-rac-glycerol, it is possible to react other primary alcohols and also allyl alcohol and benzyl alcohol under the given conditions. In the same way, it is also possible to use other epoxides.

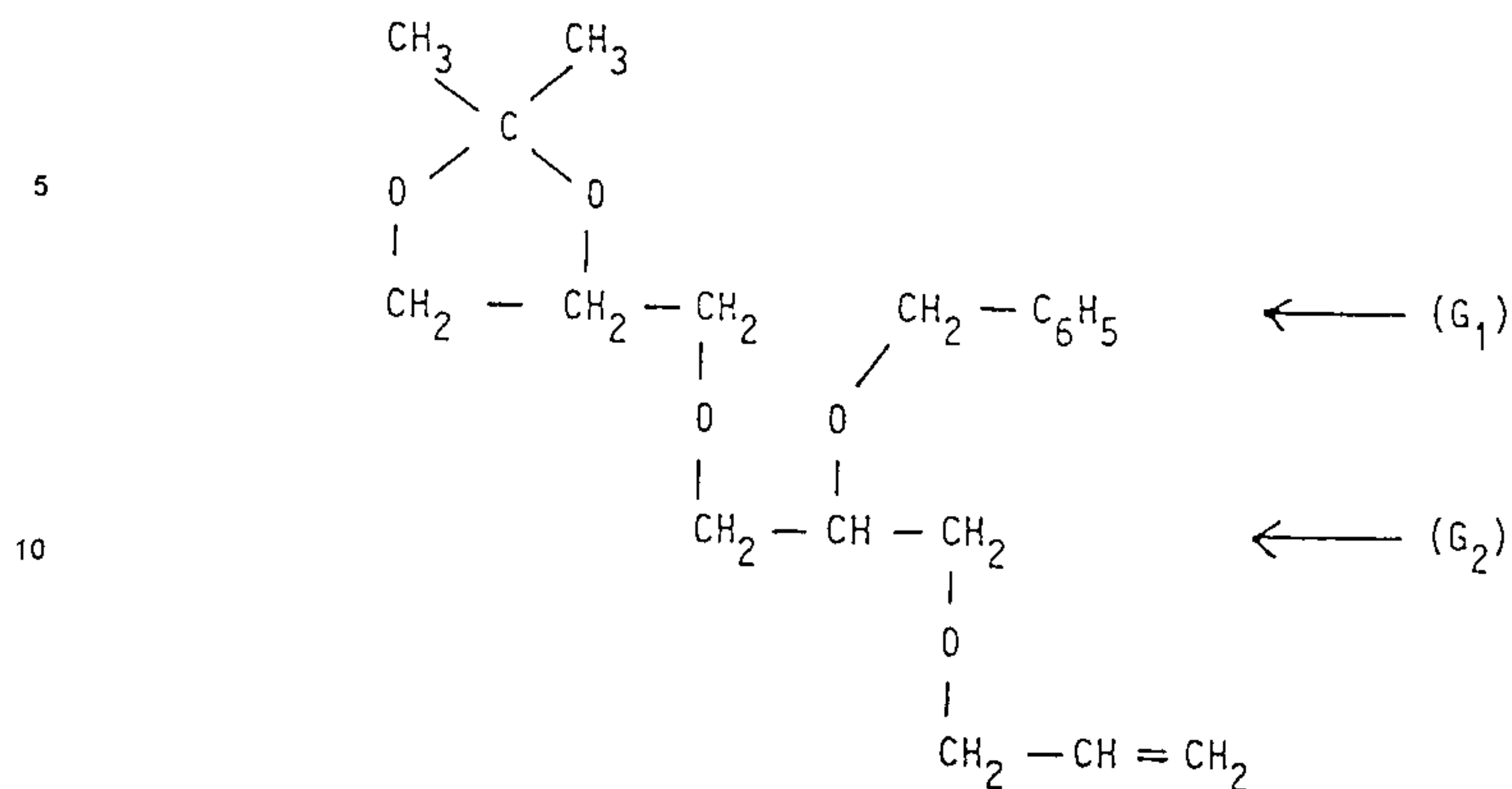
The intermediate product with the formula I can also be obtained from 1.2-isopropylidene-rac-glycero-3-glycidyl ether by means of NaOH-catalyzed ring opening with allyl alcohol. In this case, 1.2-isopropylidene-rac-glycero-3-glycidyl ether must first be made from allyl glycerol.

Example 4b:

Alkylation of primary or secondary hydroxyl groups (general example)

Preparation of a key intermediate:

1.2-isopropylidene-rac- G_1 -3.1-0.0-2-0-benzyl-3-0-allyl-rac- G_2



15

The key intermediate, 1,2-isopropylidene-rac-G₁-3,1-rac-G₂-0-allyl ether (MW 246.30; 0.5 mol - 123 g) is dissolved in 500 ml tetrahydrofuran, has benzyl chloride (0.6 mol - 76 g) added to it and is reflux-boiled. K-tert. butylate (0.7 mol - 79 g) dissolved in 500 ml tetrahydrofuran is added dropwise. The reaction is completed after 30 minutes of

20 reflux-boiling (TLC check - R_f in ethyl ether: educt, R_f = 0.1; product, R_f = 0.4). The reaction mixture has 1 l diisopropyl ether and 1 l 1% NaCl solution added to it, is shaken, and the upper phase rotated in an evaporator. The product can either be used directly, or recovered in pure form in approximately 90% yield by means of chromatography on silica gel.

25

Empirical formula C₁₉H₂₈O₅ (MW 336.42)

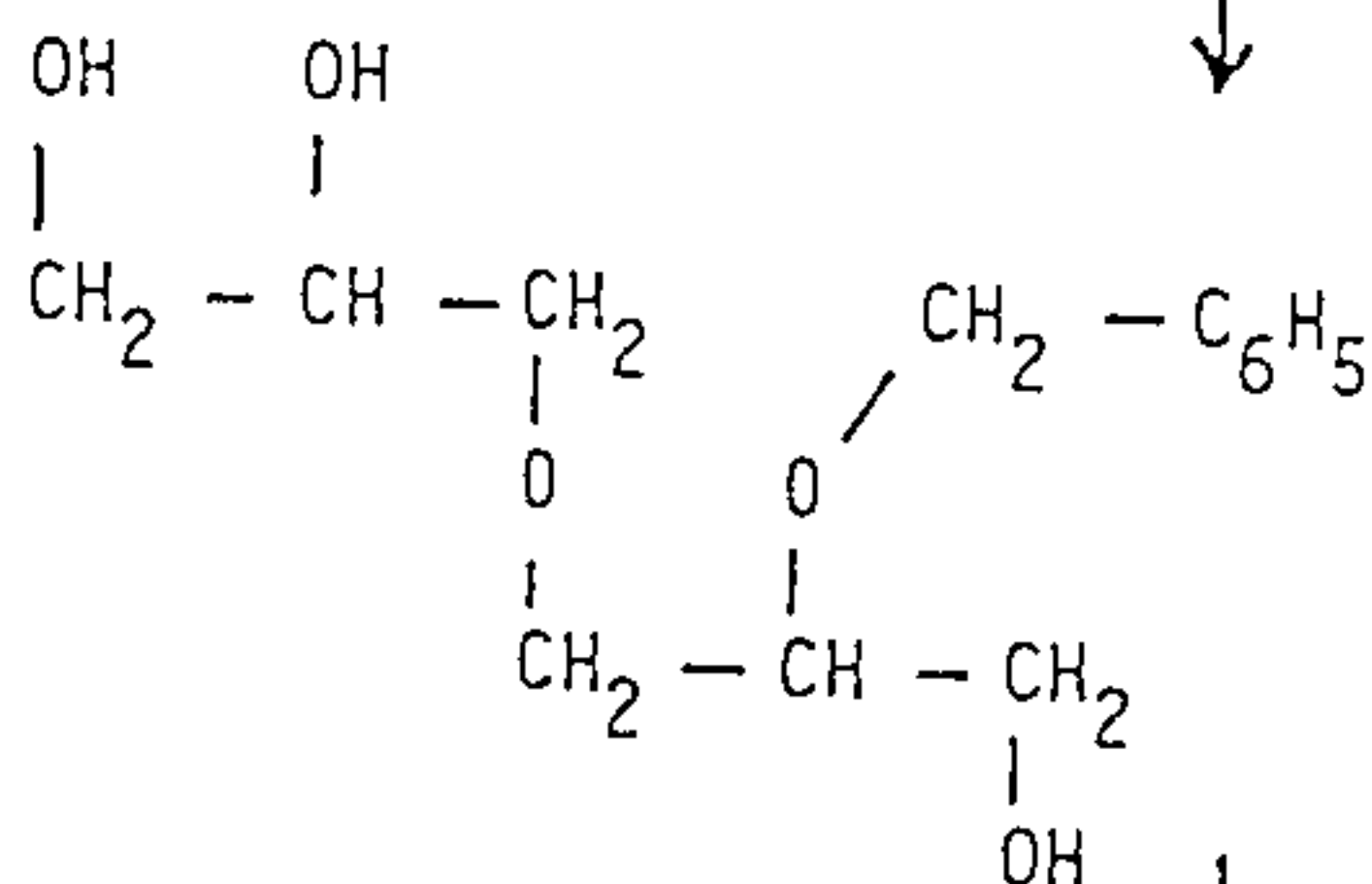
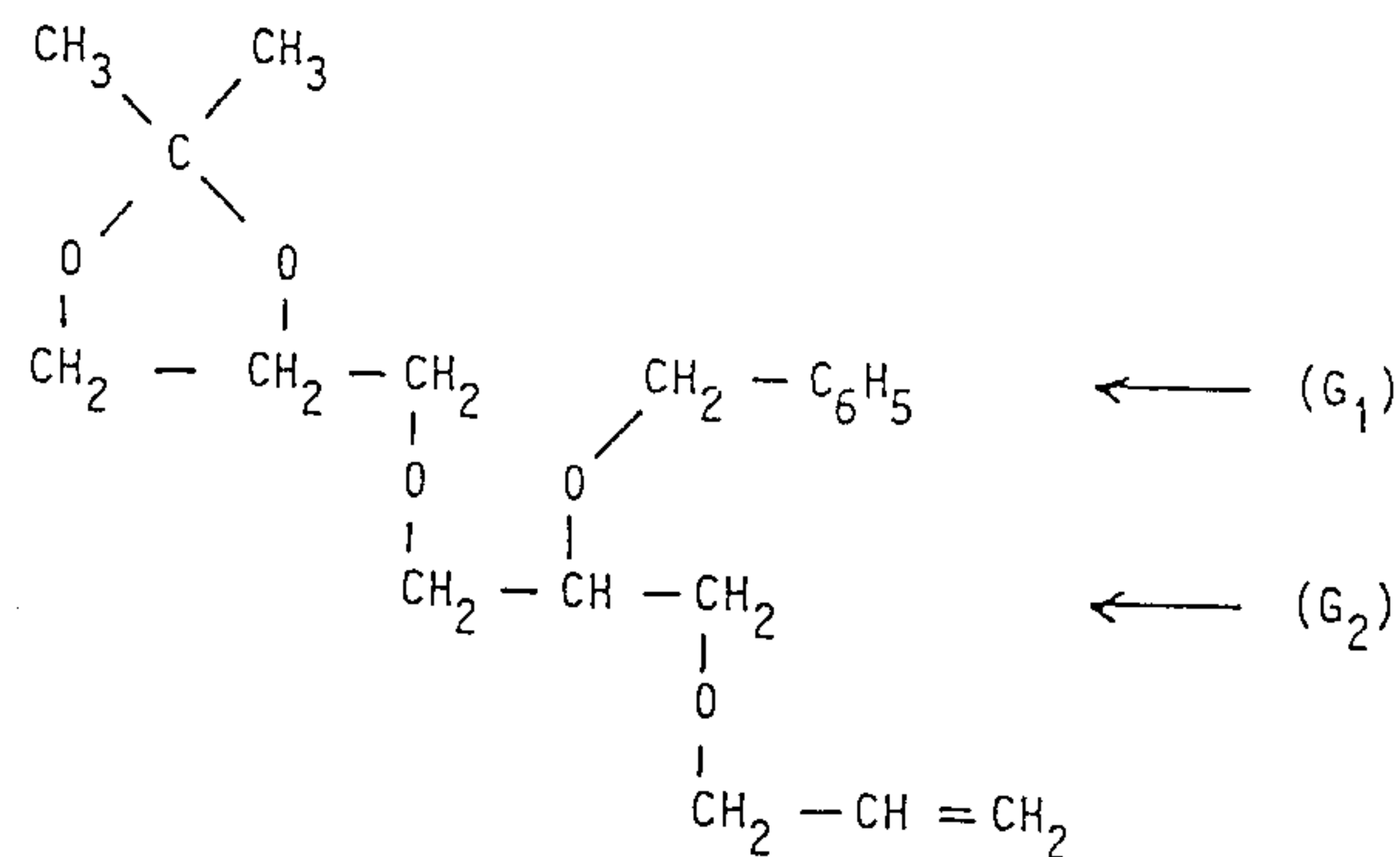
calculated: C, 67.83; H, 8.39; O, 23.79

measured: C, 67.78; H, 8.34; O, -

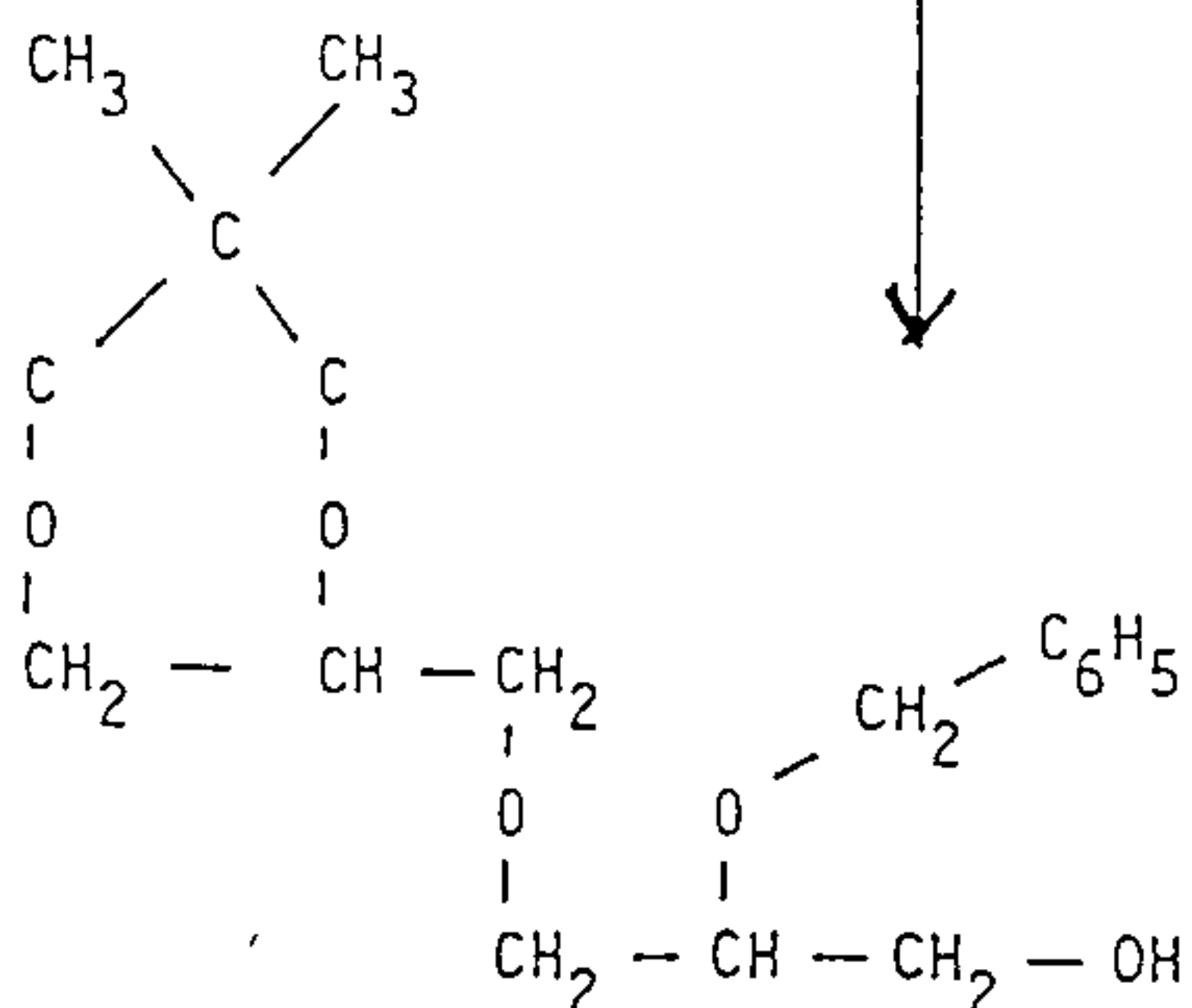
30

Instead of benzyl chloride, use can also be made of benzyl bromide, allyl chloride or allyl bromide, or of the halogenides or mesylates of primary alcohols. The products of the reaction between primary or secondary hydroxyl groups and alkyl mesylates, in particular, lead to high yields (> 90%) of the desired target compounds.

Example 4c:

Synthesis sequence 0-allyl ether \rightarrow 0-propenyl ether \rightarrow alcohol (general example)Preparation of 2-0-benzyl-rac-G₁-1.3-0.0-1.2-isopropylidene-rac-G₂

- 1) K-tert. butylate in dimethyl-formamide
- 2) HCl / H₂O in 1-propanol



- 3) 2.2 dimethoxypropane (H₂SO₄)

Rearrangement

1.2-isopropylidene-rac-G₁-3.1-0.0-2-0-benzyl-3-0-allyl-rac-G₂ (0.5 mol - 168 g) is dissolved in 500 ml DMF, to which k-tert. butylate (0.7 mol - 79 g) is then added. The reaction mixture is heated to 110 to 115°C with continuous stirring, left for 15 minutes at this temperature and then cooled to 20 °C. Following the addition of 500 ml diisopropyl ether and 500 ml 1% NaCl, the upper, diisopropyl ether phase is removed and the solvent eliminated under vacuum (TLC check - R_f in hexane/diisopropyl ether (1:1): educt, R_f = 0.2; product, R_f = 0.4).

Cleavage of the propenyl protective group

The residue from the above reaction, approximately 168 g, is dissolved in 500 ml methanol and, following addition of 50 ml 1 M HCl, is reflux-boiled. The reaction is complete after 60 minutes (TLC check in hexane/diisopropyl ether (1:1): educt, R_f = 0.4; product, R_f = 0). The yield of rac-G₁-3.1-rac-G₂-2-0-benzyl ether is > 90%. Under the acidic conditions prevailing during propenyl cleavage, the isopropylidene protective group is likewise removed. If necessary, it can be reintroduced in the 1.2 position.

Introduction of the isopropylidene protective group

The residue from the above reaction (approx. 0.5 mol) is dissolved in 300 ml THF to which, in succession, 2.2-dimethoxypropane (0.5 mol - 52 g) and 0.2 g H₂SO₄ in 10 ml THF are added, and then stirred for 2 hours at 25°C. The reaction mixture is neutralized with saturated Na₂CO₃ solution, the precipitate removed under suction and the filtrate rotated with xylol under vacuum to free it of water. The product is purified chromatographically on silica gel 60 (Merck, grain size 0.2 - 0.5 mm) (R_f in diethyl ether: educt, R_f = 0.0; product, R_f = 0.4). One obtains 121 g of the important intermediate needed for the preparation of phosphatidyl-diglycerols (G₂ parent substance).

G₂ parent system: 2-0-benzyl-rac-G₁-1.3-0.0-1.2-isopropylidene-rac-G₂ (yield 82%).

Empirical formula: C₁₆H₂₄O₅ (MW 296.36)

calculated: C, 64.85; H, 8.16; O, 26.99

5 measured: C, 64.82; H, 8.14; O, -

Intermediates which have higher proportions of oligoglycerol and are likewise needed for the production of phosphatidyl oligoglycerols can be prepared analogously. Some analytical findings pertaining to key intermediates are summarized below:

10

G₃ parent system: 2-0-benzyl-rac-G₁-1.3-0.0-2-0-benzyl-rac-G₂-1.3-0.0-1.2-isopropylidene-rac-G₃

Empirical formula: C₂₄H₃₆O₇ (MW 460.56)

calculated: C, 67.81; H, 7.88; O, 24.32

15 measured: C, 67.75; H, 7.85; O, -

G₄ parent system:

2-0-benzyl-rac-G₁-[1.3-0.0-2-0-benzyl-G]₂-1.3-0.0-1.2-isopropylidene-rac-G₄

Empirical formula: C₃₆H₄₈O₉ (MW 624.77)

20 calculated: C, 69.21; H, 7.74; O, 23.05

measured: C, 69.17; H, 7.69; O, -

G₆ parent system:

2-0-benzyl-rac-G₁[1.3-0.0-2-0-benzyl-rac-G]₄-1.3-0.0-1.2-isopropylidene-rac-G₆

25 Empirical formula: C₅₆H₇₂O₁₃ (MW 953.172)

calculated: C, 70.57; H, 7.61; O, 21.82

measured: C, 70.56; H, 7.54; O, -

G₈ parent system:

30 2-0-benzyl-rac-G₁-[1.3-0.0-2-0-benzyl-rac-G]₆-1.3-0.0-1.2-isopropylidene-rac-G₈

Empirical formula: C₇₆H₉₆O₁₇ (MW 1281.58)

calculated: C, 71.23; H, 7.55; O, 21.22

measured: C, 71.15; H, 7.53; O, -

Example 4d:

Substances which bear the tetrahydropyranyl protective group (instead of benzyl)

5 (Preparation of phosphatidyl oligoglycerols which contain unsaturated fatty acids)

For this variant, 1.2-isopropylidene-rac-glycero-3-O-allyl ether is prepared and epoxidized as described by H. Eibl and P. Woolley (Chem. Phys. Lipids 41 (1986) 53-63).

10 Epoxidation (general example)

1.2-isopropylidene-rac-glycero-3-O-allyl ether (MW 172.22; 1 mol - 172 g) is dissolved in 1 l CH₂Cl₂. 3-chloroperoxybenzoic acid (1.1 mol) is added portion-wise and the reaction mixture stirred for 6 hours at 25 - 30°C. The educt (R_f 0.5 in diethyl ether /
15 pentane 1:1) is by then transformed completely into the desired product (R_f 0.2 in the above system). After removing the precipitate by suction filtration, 100 g Na₂CO₃ is added to the filtrate and the mixture stirred for another 3 hours at 20°C. The precipitate is removed and the solvent eliminated under vacuum. The yield of epoxide (MW 188.22) is 170 g (90%). As described under "epoxide opening with alcohols" (Example
20 4a), the epoxide is now converted with benzyl alcohol into 1-O-benzyl-rac-G₁-3.1-0.0-2.3-isopropylidene-rac-G₂ and the free -OH groups converted with 3.4-dihydro-2H-pyran into the tetrahydropyran derivative.

Introduction of the tetrahydropyran protective group (general example)

25

1-O-benzyl-rac-G₁-3.1-0.0-2.3-isopropylidene-rac-G₂ (MW 296.36; 1 mol - 296 g) is dissolved in 1 l THF, to which 1.4 mol 3.4-dihydro-2H-pyran and 0.1 mol toluene sulfonic acid are added. The reaction is complete after 1 hour (educt, R_f 0.65; product, R_f 0.90 in diethyl ether). 1 l 0.2 mol Na₂CO₃ solution and 1 l diisopropyl ether are added,
30 and the mixture shaken thoroughly in a separating funnel. The upper phase is rotated in an evaporator and the product converted by means of hydrogenolysis with H₂ in the presence of a PD/C catalyst (5 % Pd based on the alcohols) into the G₂ constituent with free hydroxyl group.

G₂ parent system: 2-0-tetrahydropyranyl-rac-G₁-1.3-0.0-1.2-isopropylidene-rac-G₂
(Yield 80% expressed in terms of the epoxide)

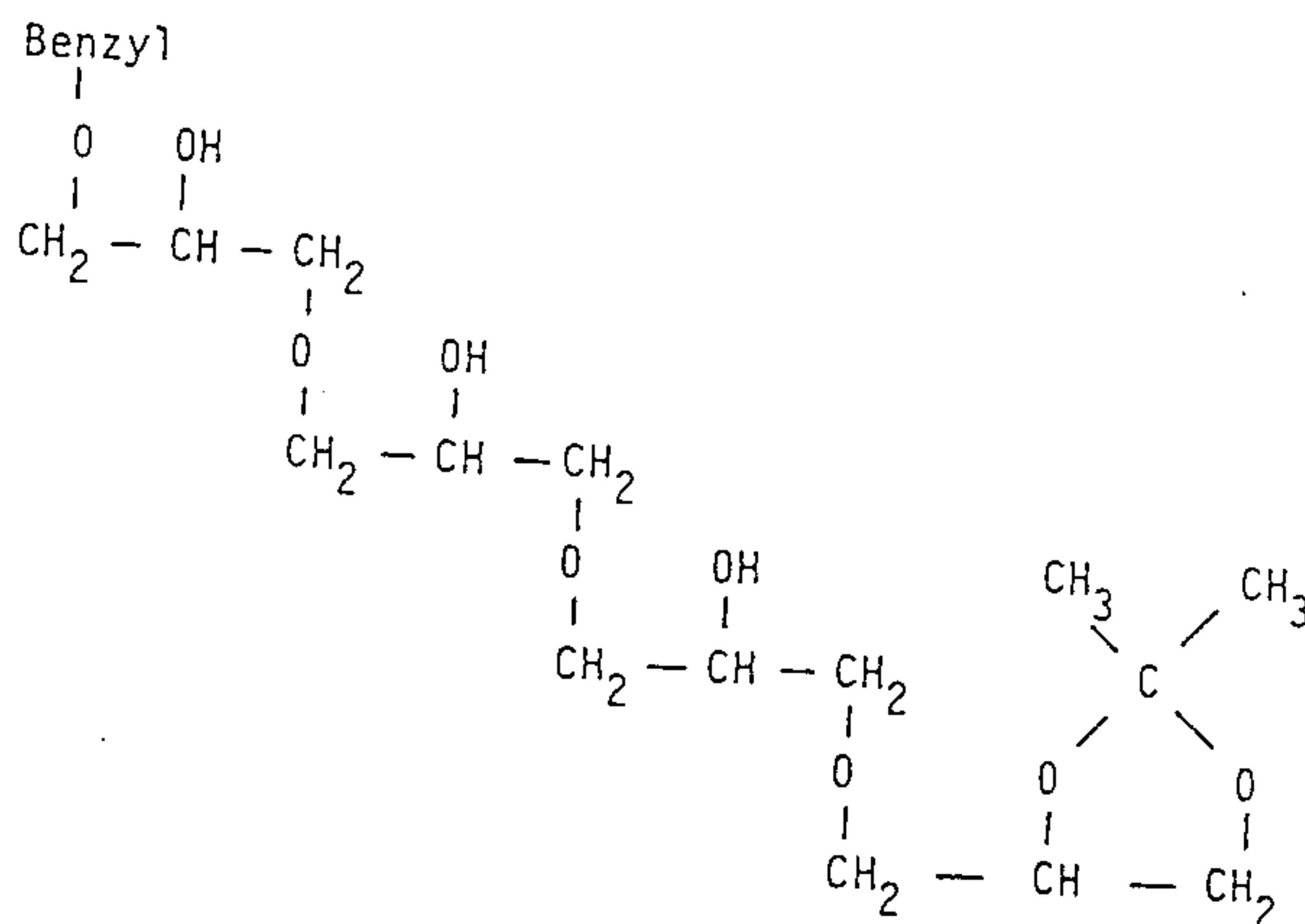
- 5 Empirical formula: C₁₄H₂₇O₆ (MW 291.36)
calculated: C, 57.71; H, 9.34; O, 32.95
measured: C, 57.59; H, 9.29; O, -

10 Compounds with other parent systems can be converted into THP-protected structures in the same way. For example, the 3-0-allyl ether of example 4a can be converted to an epoxide and opened with allyl alcohol. Again, a 3-0-allyl ether is formed, which is epoxidized and opened with benzyl alcohol to form the product below, which, through introduction of 3 THP protective groups and catalytic hydrogenolysis, can be converted to an intermediate with the G₄ parent system.

15

20

25



G₄ parent system:

2-O-THP-rac-G₁[1.3-O-2-O-THP-rac-G]₂-1.3-O-1.2-isopropylidene-rac-G₄

Empirical formula: C₃₀H₄₅O₂ (MW 607.75)

calculated: C, 59.29; H, 9.12; O, 31.59

5

measured: C, 59.24; H, 9.08; O, -

Example 4e:

Further processing of the intermediate with the formula I

10 G₂ parent system (racemic)

From formula I, a key intermediate for the preparation of the G₂ parent system is obtained (see model B). To this end, the secondary -OH function in formula I is alkylated, benzylated, or protected with tetrahydropyran.

15

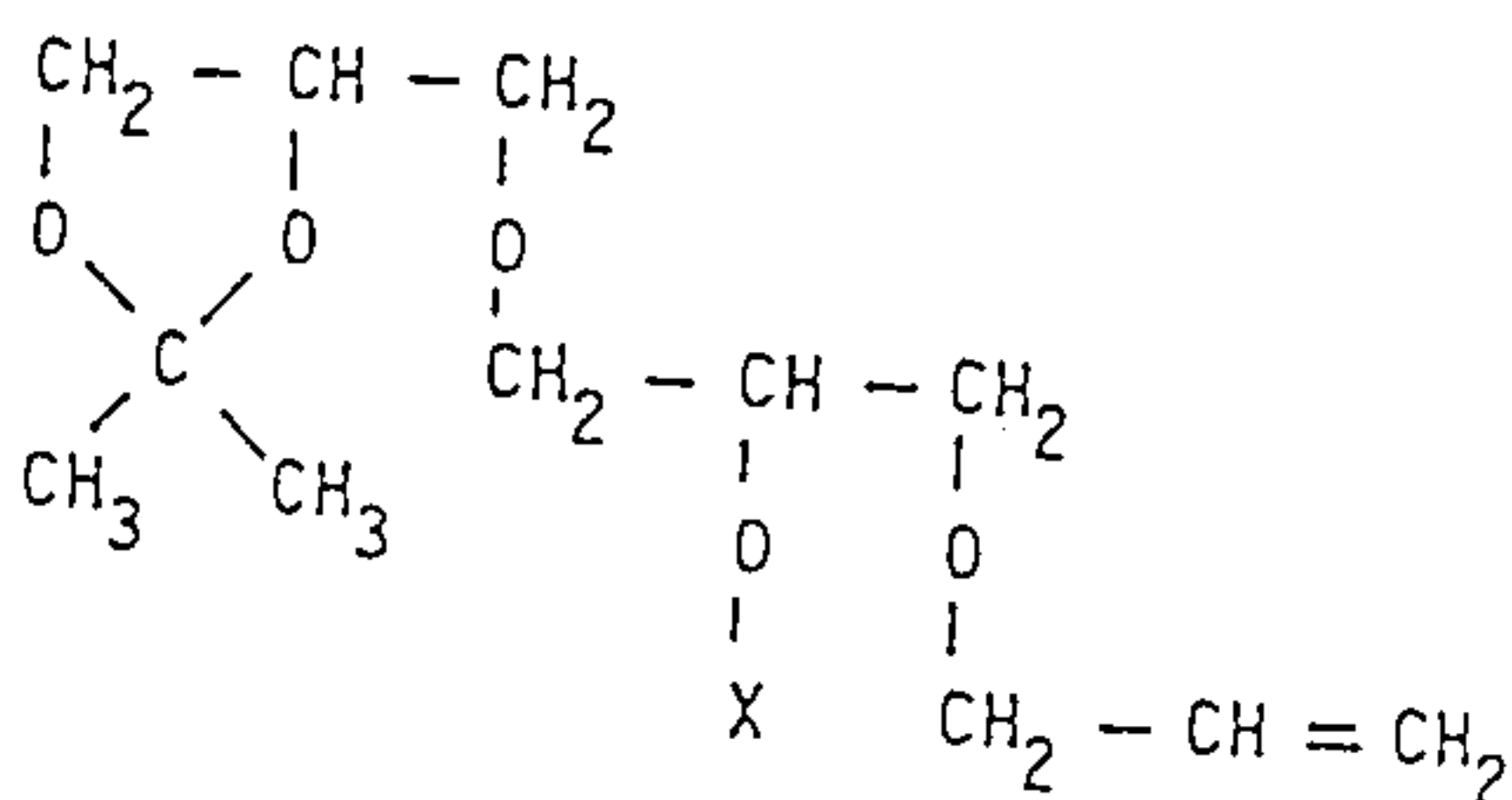
Formula I

a) Alkylation

b) Benzylation

20

c) Introduction of THP protective group



25

30

II

Model B: Key intermediate for preparing the G₂ parent system:

X = saturated or unsaturated alkyl, benzyl or THP

Alkyl-G₂ compounds1) 2-O-alkyl-rac-G₁-1.3-O.0-rac-G₂

- 5 The intermediate compound of formula II in which X = alkyl is freed of the protective groups. The following compounds were isolated:

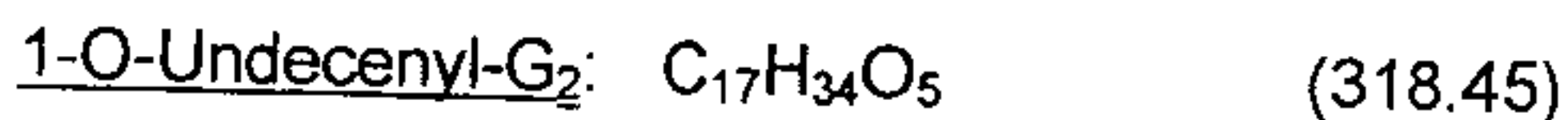
	<u>2-O-ethyl-G₂:</u>	C ₈ H ₁₈ O ₅	(194.23)
	<u>2-O-hexyl-G₂:</u>	C ₁₂ H ₂₆ O ₅	(250.33)
10	<u>2-O-undecenyl-G₂:</u>	C ₁₇ H ₃₄ O ₅	(318.45)
	<u>2-O-dodecyl-G₂:</u>	C ₁₈ H ₃₈ O ₅	(334.49)
	<u>2-O-octadecyl-G₂:</u>	C ₂₄ H ₅₀ O ₅	(418.65)
	<u>2-O-erucyl-G₂:</u>	C ₂₈ H ₅₆ O ₅	(472.75)

15 2) 1-O-alkyl-rac-G₁-3.1-O.0-rac-G₂

In the intermediate compound of formula II in which X = benzyl, allyl is removed from the 1-position and the corresponding alkyl chain incorporated in the 1-position. Following removal of the protective groups, the following compounds were obtained:

20	<u>1-O-methyl-G₂:</u>	C ₇ H ₁₆ O ₅	(180.20)
	<u>1-O-propyl-G₂:</u>	C ₉ H ₂₀ O ₅	(208.25)
	<u>1-O-nonyl-G₂:</u>	C ₁₅ H ₃₂ O ₅	(292.41)
	<u>1-O-undecyl-G₂:</u>	C ₁₇ H ₃₆ O ₅	(320.47)
25	<u>1-O-dodecyl-G₂:</u>	C ₁₈ H ₃₈ O ₅	(334.49)
	<u>1-O-octadecyl-G₂:</u>	C ₂₄ H ₅₀ O ₅	(418.65)

Unsaturated 1-O-alkyl diglycerols can also be obtained directly by way of epoxide opening of 1.2-isopropylidene-glycero-glycidyl ether (Model D, formula IV) with alcohols, eg,



However, this path is only suitable for shorter-chain alcohols, since the yields for long-chain alcohols such as oleyl alcohol are low. To prepare 1-oleyl-G₂, therefore, a synthetic pathway via 2-O-THP-glycero-1.3-O.0-(1.2-isopropylidene)-glycerol is preferred

35 (model D, formula V)

1-O-Oleyl-G₂: C₂₄H₄₈O₅ (416.64)

Intermediates for the synthesis of phospholipids which contain diglycerols in the polar area

5

Compounds with good protective groups for these syntheses contain a 2-O-benzyl ether or a 2-O-tetrahydropyranyl ether group in G₁.

1) 2-O-benzyl-rac-G₁-1.3-0.0-(1.2-isopropylidene)-rac-G₂:

10

C₁₆H₂₄O₅ (296.36)

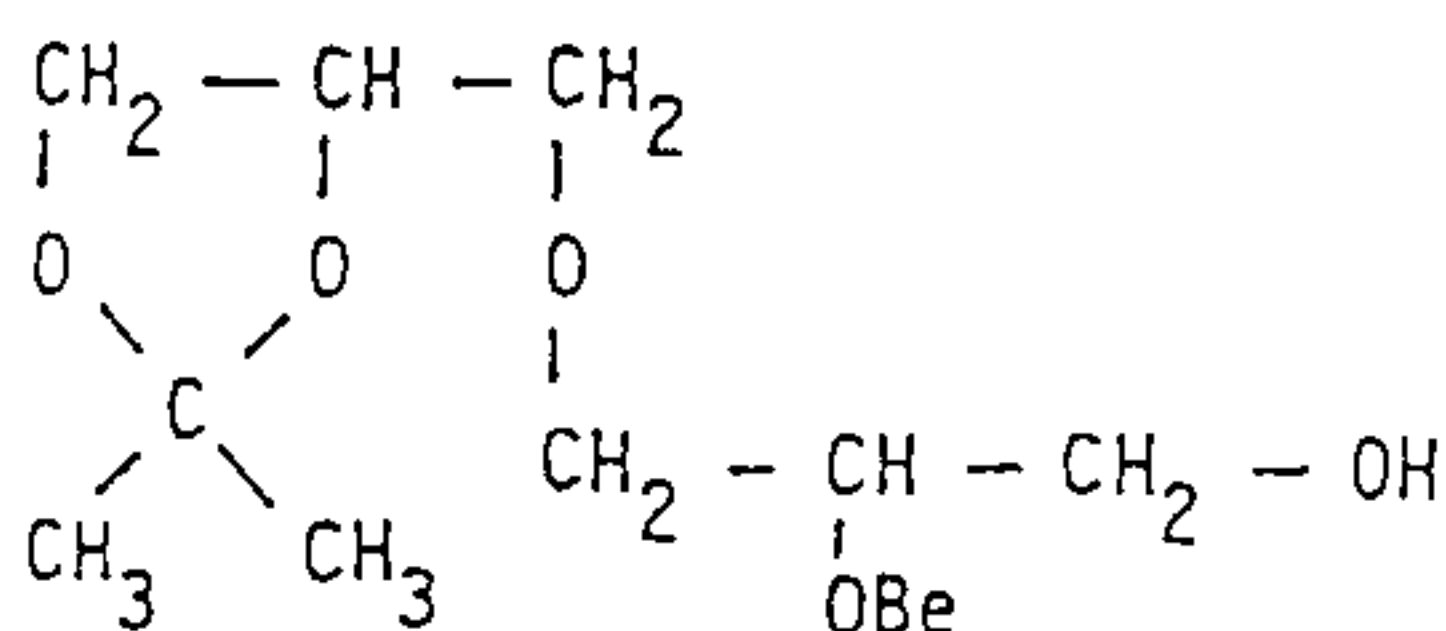
The compound with the formula III is obtained by alkaline allyl/propyl rearrangement, benzylation of the secondary -OH group and subsequent acidic cleavage of the propenyl protective group.

15

Formula I

- 1) Rearrangement allyl/propenyl
- 2) Benzylation
- 3) Acidic cleavage

20



25

Model C: Starting product for phosphatidyl diglycerols with saturated fatty acid residues.

30

2) 2-O-tetrahydropyranyl-rac-G₁-1.3-0.0-(1.2-isopropylidene)-rac-G₂:

C₁₄H₂₇O₆ (291.36)

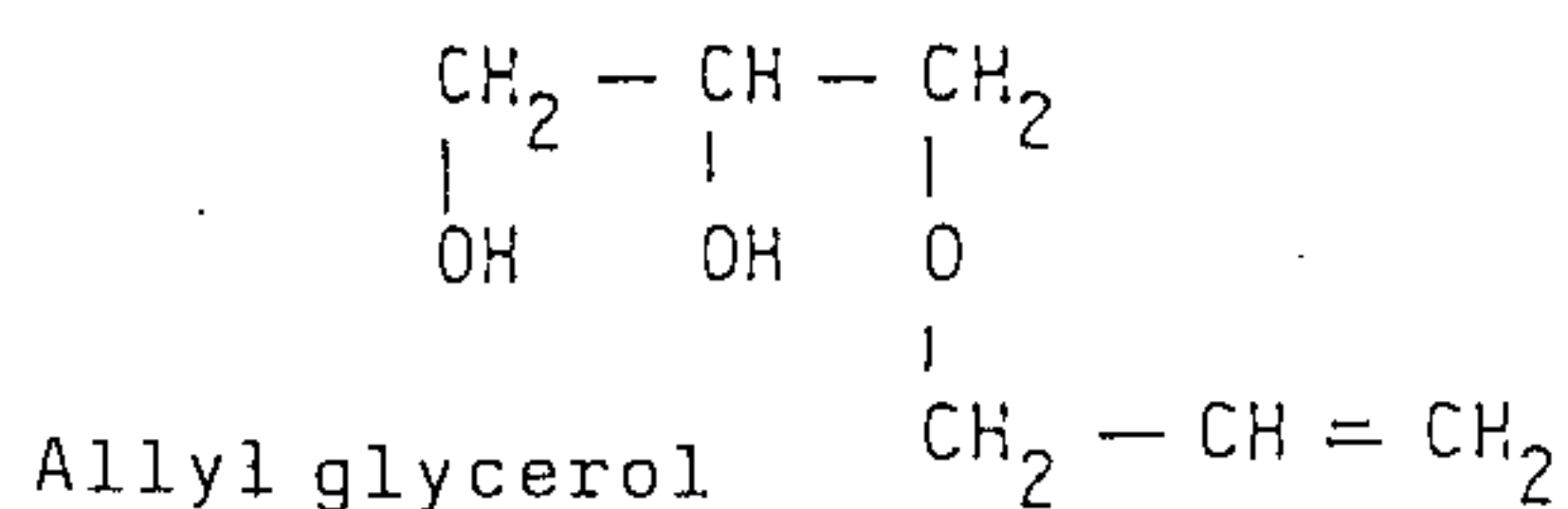
The compound with the formula V is made from allyl glycerol. The intermediate IV is obtained by way of addition of isopropylidene followed by epoxidation. After opening

35

the epoxide with benzyl alcohol, the THP protective group is introduced and the benzyl group removed.

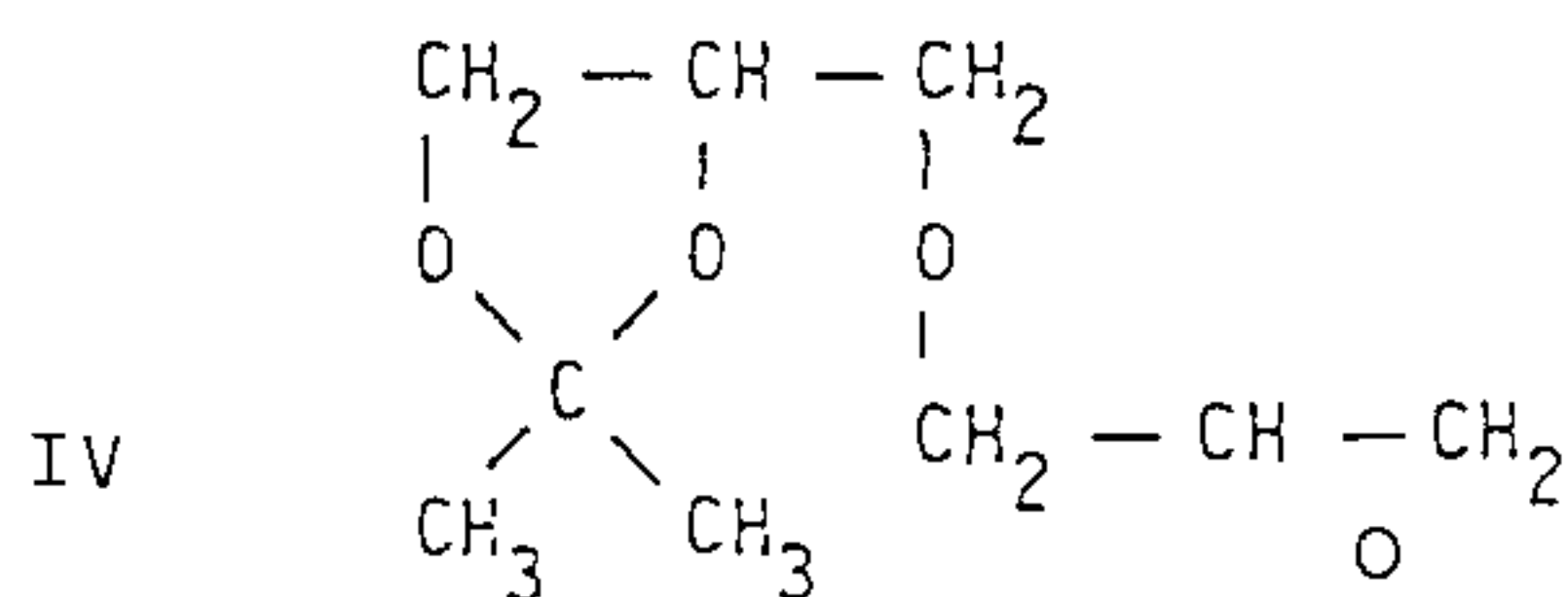
5

10

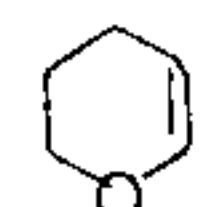


- 1) H^+ ; 2.2 dimethoxy propane
2) Epoxidation

15

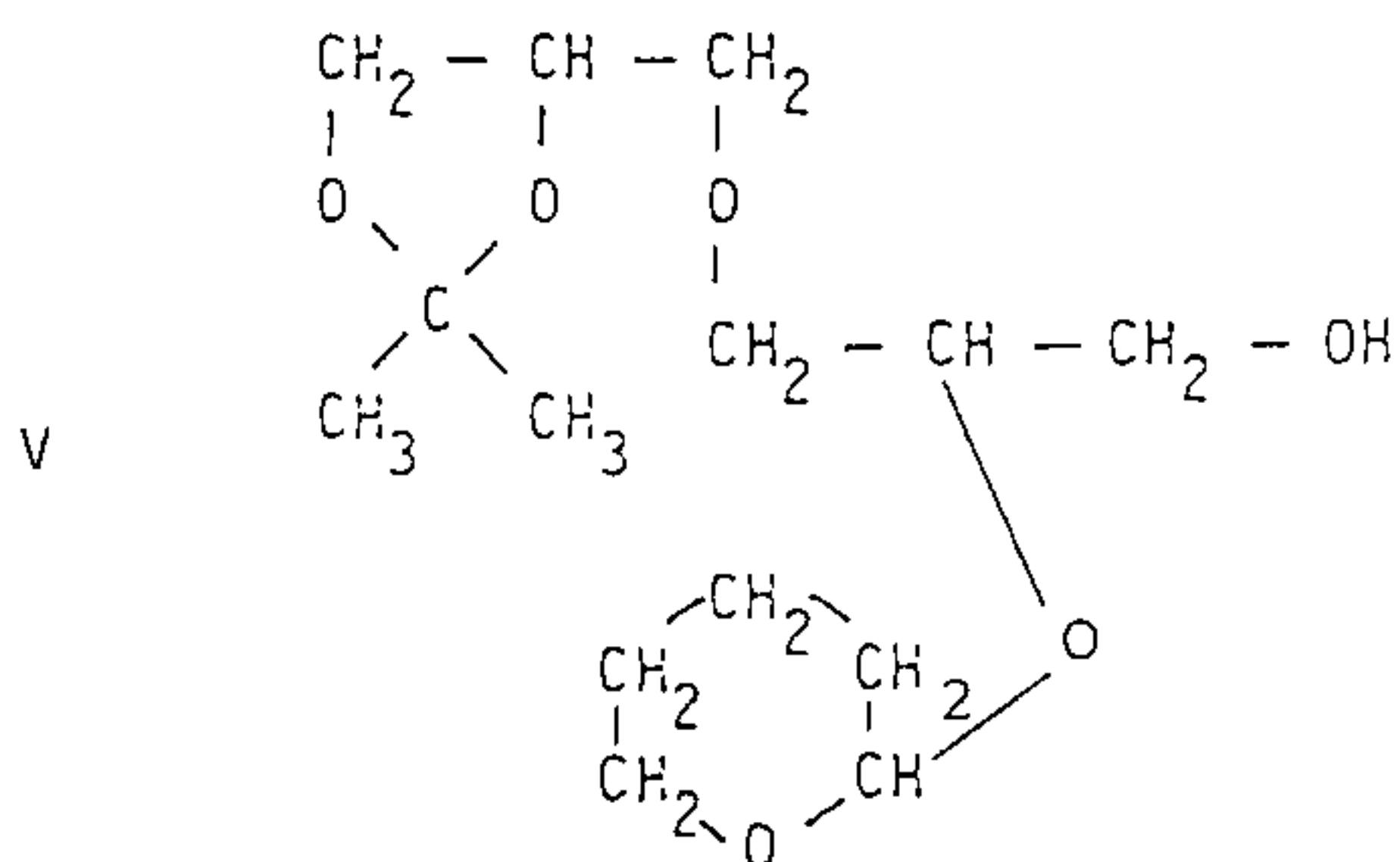


- 3) Opening with benzyl alcohol

- 4) H^+ ; 
5) H_2 ; Pd/C

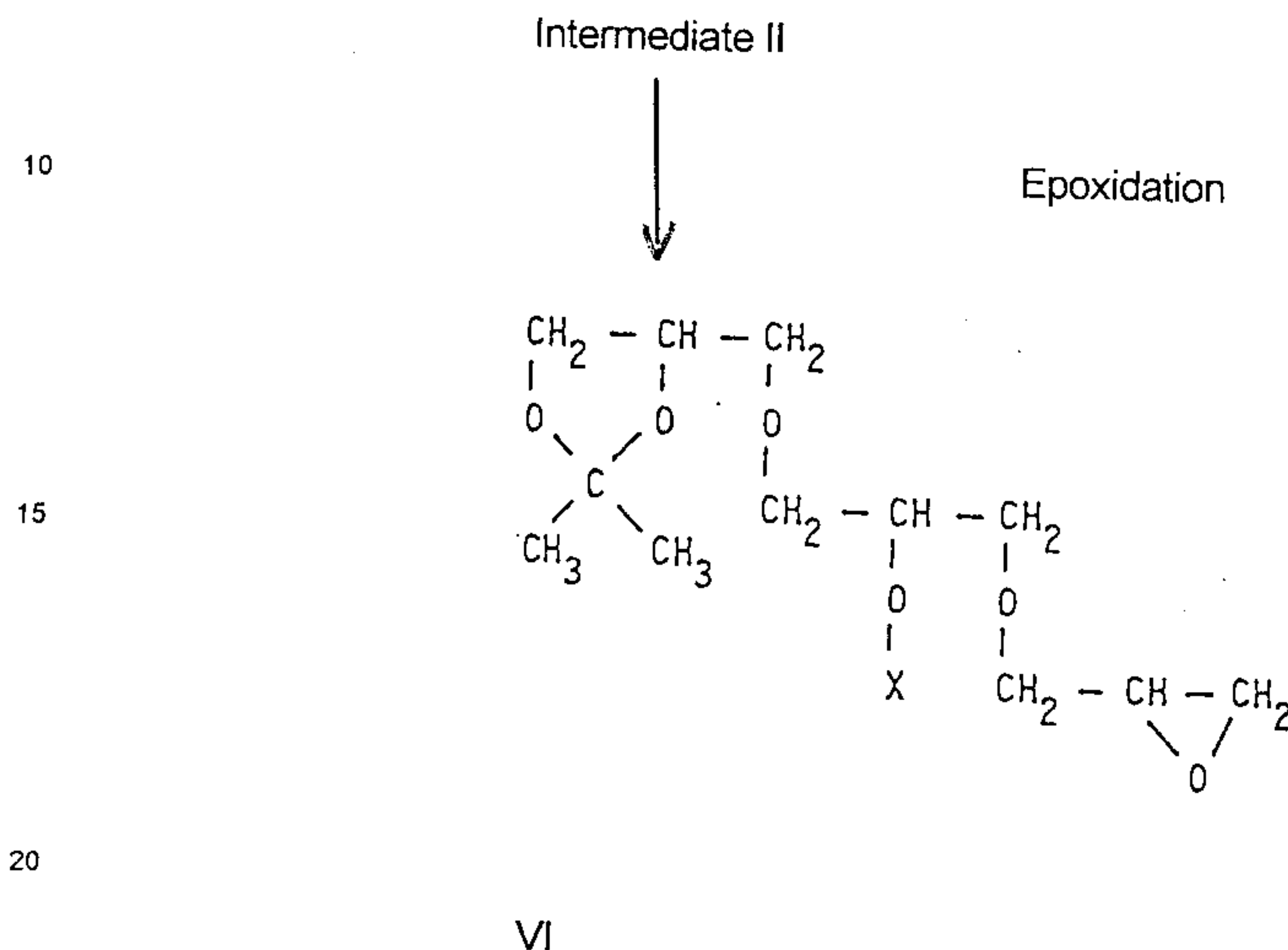
20

25



Model D: Starting product for phosphatidyl diglycerols with unsaturated fatty acid residues
G₃ parent system (racemic)

From the key intermediate with the formula II it is possible, with inclusion of the allyl
 5 group, to develop triglycerols. Following epoxidation, various intermediates and end products of pharmaceutical interest can be made from the epoxide.



Model E: Starting products for making G₃ parent systems.

25 Triglycerols can be prepared from the key intermediate with the formula VI; the intermediate is also used for making G₄ parent systems. In formula VI, X stands for hydrogen, a saturated alkyl, a benzyl or a THP residue.

Alkyl-G₃ compounds

30 1) 1-O-alkyl-rac-G₁-1.3-0.0-rac-G₂-1.3-0.0-rac-G₃

The epoxide with the formula VI (X = H) is opened directly with alcohols and, after the isopropylidene protective group has been split off, results in the following compounds:

	<u>1-O-ethyl-G₃</u> :	C ₁₁ H ₂₄ O ₇	(268.30)
	<u>1-O-hexyl-G₃</u> :	C ₁₅ H ₃₂ O ₇	(324.41)
	<u>1-O-nonyl-G₃</u> :	C ₁₈ H ₃₈ O ₇	(366.491)
5	<u>1-O-undecenyl-G₃</u> :	C ₂₀ H ₄₀ O ₇	(392.53)
	<u>1-O-dodecyl-G₃</u> :	C ₂₁ H ₄₄ O ₇	(408.57)

For longer-chain alcohols, direct opening results in poor yields. For this reason, the oleyl and erucyl compounds of G₃ were prepared by opening of VI (X = THP) with benzyl, THP-protection of the secondary hydroxyl groups formed, catalytic debenzyla-
 10 tion, alkylation in the 1 position and removal of the protective groups.

	<u>1-O-oleyl-G₃</u> :	C ₂₇ H ₅₄ O ₇	(490.72)
	<u>1-O-erucyl-G₃</u> :	C ₃₁ H ₆₂ O ₇	(456.82)

15

2) 2-O-alkyl-rac-G₁-1.3-0.0-rac-G₂-1.3-0.0-rac-G₃

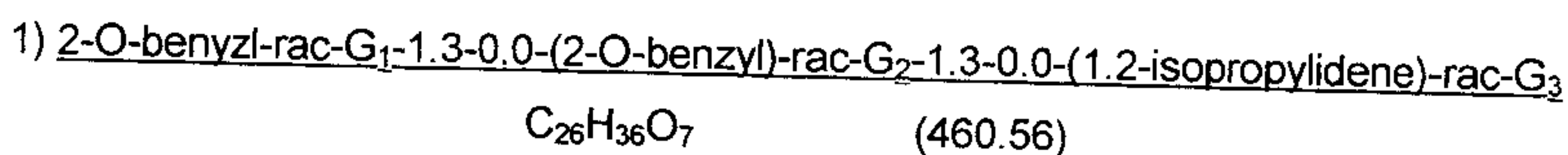
The epoxide of formula VI (X = benzyl or THP) is opened with allyl alcohol and alkylated in the 2 position. The protective groups are removed in the usual way. For the
 20 preparation of the unsaturated 2-O-alkyl compounds, rearrangement of the allyl protective groups must precede alkylation. In addition, only the THP protective group and not benzyl can be used in G₂ here. The following compounds were prepared:

	<u>2-O-methyl-G₃</u> :	C ₁₀ H ₂₂ O ₇	(111.99)
25	<u>2-O-propyl-G₃</u> :	C ₁₂ H ₂₆ O ₇	(282.33)
	<u>2-O-nonyl-G₃</u> :	C ₁₈ H ₃₈ O ₇	(366.49)
	<u>2-O-undecenyl-G₃</u> :	C ₂₀ H ₄₀ O ₇	(392.53)
	<u>2-O-dodecyl-G₃</u> :	C ₂₁ H ₄₄ O ₇	(408.57)
	<u>2-O-hexadecyl-G₃</u> :	C ₂₅ H ₅₂ O ₇	(464.68)
30	<u>2-O-oleyl-G₃</u> :	C ₂₇ H ₅₄ O ₇	(490.72)
	<u>2-O-erucyl-G₃</u> :	C ₃₁ H ₆₂ O ₇	(546.82)

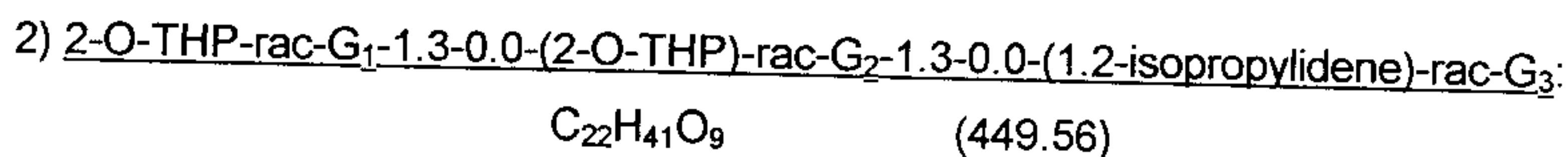
Intermediates for the synthesis of phospholipids which contain triglycerides in the polar area

5 Benzyl and tetrahydropyranyl (THP) residues are convenient protective groups for synthesizing phospholipids which have G₃ residues in the polar area. Benzyl residues are readily removed under mild conditions, provided that only saturated fatty acids are used. THP residues are of particular interest because they can be removed in a single step together with isopropyl protective groups.

10



15 This compound is obtained from the key intermediate VI (X = benzyl) by opening with allyl alcohol, benzylation of the 2 position and cleavage of the allyl protective group. In the text, the compound is referred to as formula VII.



20

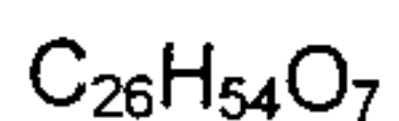
To prepare unsaturated G₃-phospholipids, the residue X = THP is used in VI. The epoxide VI is opened with benzyl alcohol, the secondary hydroxyl group thus exposed protected with THP, and the benzyl residue removed catalytically with H₂/Pt. The compound made in this way is referred to in the text as formula VIII.

25

Additional remarks

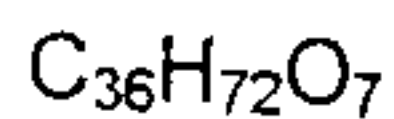
30 In the description so far we have not made use of the fact that in formula VI for X = saturated alkyl, compounds of the following structure can readily be prepared: 1-O-alkyl-rac-G₁-3.1-0.0-(2-O-alkyl)-rac-G₂-3.1-rac-G₃. The representatives of these new structures were made by opening the epoxide VI (X = hexadecyl) with CH₃OH or undecenyl alcohol and splitting off the isopropylidene protective group.

1-O-methyl-rac-G₁-3.1-O.O-(2-O-hexadecyl)-rac-G₂-3.1-rac-G₃:



(478.71)

1-O-Undecenyl-rac-G₁-3.1-O.O-(2-O-hexadecyl)-rac-G₂-3.1-O.O-rac-G₃:



(616.958)

G₄ parent system (racemic)

G₄ parent systems can be prepared from the key intermediate with the formula IX.

Intermediate VI

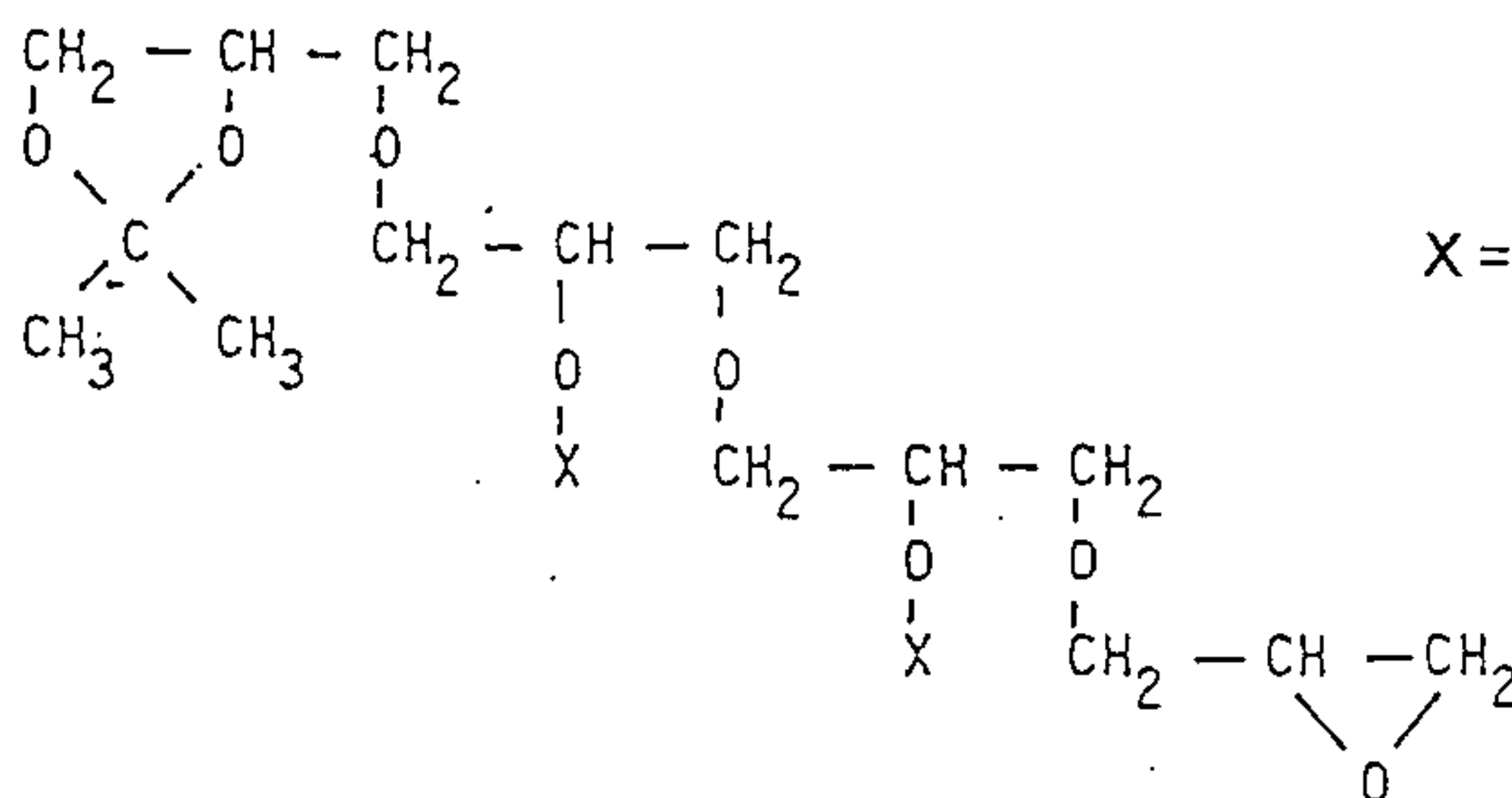
X = H, saturated alkyl,
benzyl or THP

1) Opening with allyl alcohol

2) X = H: epoxidation

X = Be: benzylation of
2-OH, followed by
epoxidation

X = THP: Introduction of
THP in 2-OH,
followed by
epoxidation



IX

Model F: Starting products for the preparation of G₄ parent systems.

Tetraglycerols can be made from the key intermediate with the formula IX. They can also be used to prepare pentaglycerols.

5

Oligoglycerols with two or more alkyl residues can be made from the intermediates, too. Suitable starting compounds here are molecules with the formula IX, in which X is a saturated alkyl residue.

10 Alkyl-G₄ compounds1) 1-O-alkyl-rac-G₁-1.3-0.0-rac-G₂-1.3-0.0-rac-G₃-1.3-0.0-rac-G₄

The epoxide of formula IX, X = H, is opened directly with alcohols. After the isopropylidene protective group has been split off, the following substances are obtained:

1-O-ethyl-G ₄ :	C ₁₄ H ₃₀ O ₉	(342.38)
1-O-hexyl-G ₄ :	C ₁₈ H ₃₈ O ₉	(398.49)
1-O-undecyl-G ₄ :	C ₁₉ H ₄₀ O ₉	(412.52)
20 1-O-undecenyl-G ₄ :	C ₁₉ H ₃₈ O ₉	(410.50)
1-O-dodecyl-G ₄ :	C ₂₀ H ₄₂ O ₉	(426.54)

This path is only suitable for shorter-chain alcohols, since the yields are much lower with long-chain alcohols.

25

For long-chain, saturated alcohols it is therefore necessary, as with G₂ and G₃, to select a synthetic pathway via the key intermediate with X = benzyl. One opens with allyl alcohol, benzylates the thus exposed 2-OH group, removes the allyl group in the 1 position and alkylates the 1 position. After removing the protective groups one obtains:

30 The important intermediate for the synthesis of phospholipids with G₄ residues in the polar area is made from formula IX, X = benzyl by opening the epoxide with allyl alcohol, benzylating the thus exposed 2-OH group and removing allyl. The compound is referred to as formula X in the text.

2. 2-0-THP-rac-G₁-1.3-0.0-(2-0-THP)-rac-G₂-1.3-0.0-(2-0-THP)-rac-G₃-1.3-0.0-(1.2-isopropylidene)-rac-G₄:

C₃₀H₄₅O

(607.75)

- 5 To make this compound, which is suitable for obtaining unsaturated phospholipids with G₄ parent systems in the polar area, one proceeds analogously as for the preparation of the G₃ compound. One opens the epoxide VII, X = THP with benzyl alcohol, protects the thus exposed 2-OH group with THP, and removes benzyl with H₂ (Pd/C catalysis.). The compound is referred to in the text as formula XI.

10

INTERMEDIATES FOR THE SYNTHESIS OF PHOSPHOLIPIDS WHICH CONTAIN OLIGOGLYCEROLS IN THE POLAR AREA AND PERMIT AN SN-1 LINKAGE TO THE PHOSPHATE (NATURAL CONFIGURATION)

- 15 In the preparation of compounds suitable for incorporation in the polar area of phospholipids (formula III and V for G₂, formula VII and VIII for G₃, formula X and XI for G₄), no attention was paid so far to the fact that in natural phosphatidylglycerol, ie, in 1.2-diacyl-sn-glycero-3-phospho-sn-1-glycerol, the link between phosphate and the non-acylated glycerol is an sn-1 linkage. Since the liposome components, as carriers of medicinal products, should be used in the most natural configuration possible, synthetic pathways were developed which also permit an sn-1 configuration of the polar oligoglycerol (model G).

20

sn-1-G₁-G₂ linkage

25

The stereospecific linkage can be obtained using methods analogous to those described in the literature (DE 31 30 867 A1; H. Eibl, Chem. Phys. Lipids 28 (1981) 1-5; H. Eibl et al., Chem. Phys. Lipids 41 (1986) 53-63; H. Eibl et al., Chem. Phys. Lipids 47 (1988) 47-53).

30

- The starting product for this linkage is 2-0-benzyl-3-0-allyl-sn-glycerol, which, following epoxidation, is hydrolysed to the diol. Following reaction with H⁺/2.2-dimethoxypropane, 2-0-Be-sn-G₁-3.1-0.0-(1.2-isopropylidene)-rac-G₂ is obtained, a molecule with the formula XII, which permits an sn-1 linkage to the phosphate group in phospholipids and corresponds to the racemate of formula III.

35

sn-1-G₁-G₂-G₃ linkage

5 The starting product for this linkage is again 2-0-benzyl-3-0-allyl-sn-glycerol. Protection of the sn-1 position with THP is followed by epoxidation, and then the epoxide ring opened with 1.2-isopropylidene glycerol. The thus-exposed -OH function in G₂ is benzylated, and the THP protective group removed. One obtains a molecule with the formula XIII, which permits an sn-1 linkage to the phosphate group in phospholipids. The molecule XIII corresponds to the racemate of formula VII.

10

sn-1-G₁-G₂-G₃-G₄ linkage

15 The starting product is again 2-0-benzyl-3-0-allyl-sn-glycerol, in order to ensure the sn-1 linkage. Incorporation of the THP protective group is followed by epoxidation, and the epoxide then opened with allyl alcohol. Following epoxidation of the intermediate, the epoxide is opened with isopropylidene glycerol, the two exposed -OH groups benzylated, and THP removed. One obtains XIV, which permits an sn-1 linkage to the phosphate and corresponds to the racemate of formula X.

20 If desired, compounds with an sn-3-G₁-G₂, sn-3-G₁-G₂-G₃ or sn-3-G₁-G₂-G₃-G₄ linkage with the phosphate can be made analogously. In this case, the same sequence of reactions is required, but instead of 2-0-benzyl-2-0-allyl-sn-glycerol, use is made of the enantiomeric 2-0-benzyl-1-0-allyl-sn-glycerol.

5

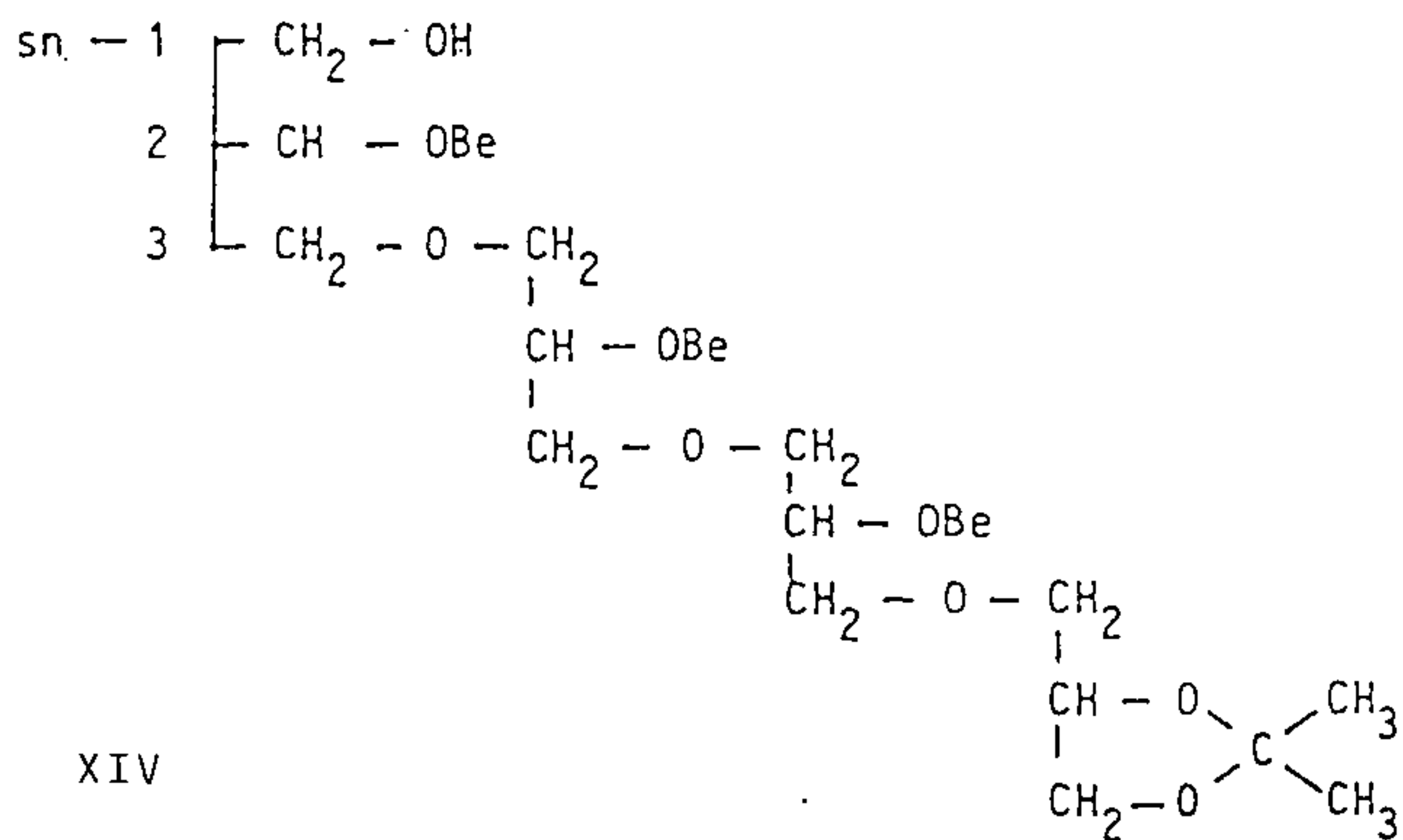


15



25

30



Model G: Phospholipid constituents which permit an sn-1-G_x linkage
(x = 2 - 4). Starting product is 2-O-benzyl-3-O-allyl-sn-glycerol.

5 Example 4f:

Intermediates which contain sugar alcohols (general examples)

Important intermediates here are, in particular, such sugar alcohols as are obtainable
at a reasonable price or can be made from these by means of simple reactions (see
10 enclosed table). Of special interest are D-mannitol as open form of inositol, xylitol,
which, when the middle carbon atom is phosphorylated, shows no optical activity and
which is readily obtained as 1,2; 4,5-diisopropylidene xylitol, and meso-erythritol. The
protective groups chiefly employed here are isopropylidene, trityl in combination with
benzyl, or allyl. Tetrahydropyranyl is also of some importance as protective group.
15 Some alternatives will now be described which serve as examples.

1,2;4,5-diisopropylidene-xylitol (general example for introduction of the isopropylidene
protective group)

20 Xylitol (1.0 mol - 152 g) is slurried with 500 ml 2-propanol, and mixed with dimethoxy-
propane (3.0 mol - 312 g). Following addition of 6 g H₂SO₄ in 100 ml 2-propanol, the
mixture is heated to 50 °C. After 30 minutes everything has dissolved. Sufficient con-
centrated ammonia is added to adjust the reaction mixture to a pH of about 8. The
solvent is removed in a rotary evaporator, and the residue taken up in hexane and
25 cooled to -20 °C. White crystals precipitate, which are sucked up and used for the
phosphorylation.

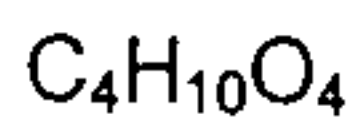
Empirical formula: C₁₁H₁₉O₅ (MW 231.27)

calculated: C, 57.13; H, 8.28; O, 34.59

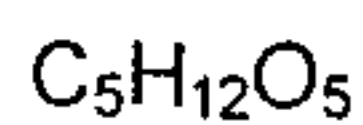
30

measured: C, 57.01; H, 8.27; O, -

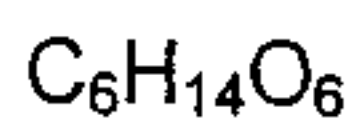
Structural formulae of some sugar alcohols:



5	meso-Erythritol	D-Threitol	L-Threitol
	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{CH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{CH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{CH}_2\text{OH} \end{array}$
10			



15	Adonite (Ribitol)	D(+)-Arabitol	L(-)-Arabitol	Xylitol
	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{CH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{CH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{CH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{CH}_2\text{OH} \end{array}$
20				



	Dulcitol (Galactitol)	D-Mannitol	D-Sorbitol
	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{CH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{CH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{CH}_2\text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{HO} - \text{C} - \text{H} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{H} - \text{C} - \text{OH} \\ \\ \text{CH}_2\text{OH} \end{array}$

1.2;3.4-diisopropylidene-5-benzyl-D-mannitol (general example for the use of trityl protective groups combined with benzyl protective groups)

Starting with 1.2;3.4;5.6-trisopropylidene-D-mannitol (MW 302.36), which is prepared
 5 analogously to the xylitol derivative, one obtains - by means of carefully splitting off the
 protective group - a 1.2.3.4-diisopropylidene-D-mannitol yield of about 30 %. Triiso-
 propylidene-D-mannitol (1.0 mol - 302 g) is dissolved in 600 ml CH₃OH, to which 15 (5
 g) Amberlyst® and 70 g H₂O are then added. The reaction mixture is heated to 50 °C,
 the solution stirred at this temperature for 40 minutes (educt, R_f 0.9; 1.2;3.4-derivative,
 10 R_f 0.7; 3.4-derivative, R_f 0.1 in CHCl₃/CH₃OH 1:1), cooled to 20 °C and filtered to 7.5
 ml 25 % ammonia in 25 ml 2-propanol (pH ~ 8). Cooling to 4 °C causes the starting
 product to precipitate, which can thus be recovered (ca. 120 g, ~ 40 %). The filtrate is
 rotated in an evaporator and purified chromatographically on silica gel 60 (Merck,
 Darmstadt). One obtains 84 g (~ 32 %) of 1.2;3,4-diisopropylidene-D-mannitol, which
 15 is retrieved in crystalline form from hexane.

Empirical formula: C₁₂H₂₂O₆ (MW 262.30)

calculated: C, 54.95; H, 8.45; O, 36.60

measured: C, 54.89; H, 8.34; O, -

20

Reaction of 1.2;3.4-diisopropylidene-D-mannitol with trityl chloride and benzyl chloride (general example for tritylation and subsequent alkylation)

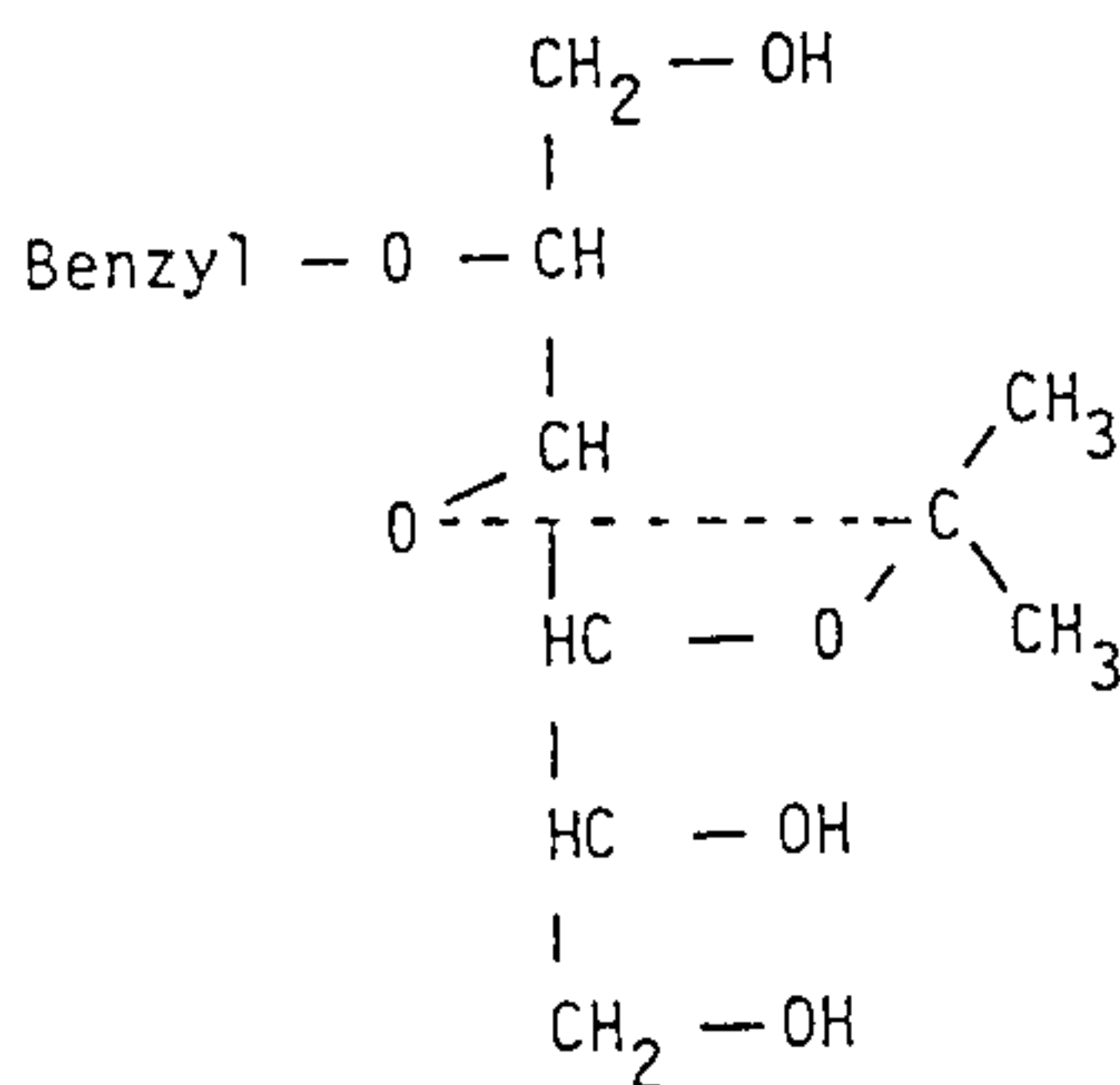
1.2;3.4-diisopropylidene-D-mannitol (0.2 mol - 52 g) is dissolved in 300 ml toluene,
 25 mixed with triethylamine (0.30 mol - 30 g) and reflux-boiled. Trityl chloride (MW
 278.78; mol - 64 g) in 200 ml toluene is added dropwise, and the mixture reflux-boiled
 for another 60 minutes (educt, R_f 0.7; product, R_f 0.90 in CHCl₃/CH₃OH 10:1). The
 reaction is then complete. The mixture is cooled to 20 °C, precipitated triethylamine
 hydrochloride filtered off, and the filtrate rotated in an evaporator. The residue is taken
 30 up in 400 ml THF, mixed with benzyl chloride (0.3 mol - 38 g) and reflux-boiled. K-tert.
 butylate (0.25 mol - 28 g) - dissolved in 200 ml THF - is added dropwise and the reac-
 tion mixture left to stand for 1 hour (educt, R_f 0.90; product, R_f 1.00 in CHCl₃/CH₃OH
 10:1). Following addition of 300 ml diisopropyl ether, the reaction mixture is extracted

with 600 ml H₂O, the upper phase taken off and the solvent removed under vacuum. The residue can be used directly.

Cleavage of the trityl protective group while retaining the 3,4-isopropylidene protective group (general example)

The oily residue from the preceding reaction (~0.2 mol) is dissolved in 600 ml acetone/CH₃OH 1:1, to which 3 ml H₂SO₄ are then added. The mixture is stirred at 40 °C for 40 minutes, which results in complete removal of the trityl- and the 1,2-isopropylidene protective groups (educt, R_f 0.95; product, R_f 0.15 in ether). The reaction mixture is adjusted to pH ~ 8, filtered and rotated in an evaporator. The residue is purified chromatographically on silica gel, and crystallized from hexane.

2-O-benzyl-3,4-isopropylidene-D-mannitol



Empirical formula: C₁₆H₂₄O₆ (MW 312.36)

calculated: C, 61.52; H, 7.74; O, 30.73

Measured : C, 61.44; H, 7.72; O, -

5 Empirical formula: $C_{19}H_{28}O_6$ (MW 352.42)
calculated: C, 64.75; H, 8.01; O, 27.24
measured: C, 64.68; H, 7.94; O, -

1.2:3.4-diisopropylidene-D-mannitol (0.2 mol - 26 g) is dissolved according to the method of H. Eibl (Chem. Phys. Lipids 28 (1981) 1-5) in 200 ml CH₃OH and added to a solution of 0.2 mol sodium metaperiodate in 500 ml water. The temperature should not exceed 30 °C. The reaction is complete after 15 minutes. The pH of the reaction mixture is raised to pH = 8 with 5 M KOH in water. The precipitated salts are filtered off, and the aldehyde reduced with sodium borohydride(0.25 mol). One obtains a > 90% yield of 1.2:3.4-diisopropylidene-D-lyxitol, which is extracted with 600 ml of chloroform. The chloroform phase is rotated in an evaporator and the product crystallized from hexane.

Empirical formula: C₁₁H₁₉O₅ (MW 231.27)
calculated: C, 57.13; H, 8.28; O, 34.59
measured: C, 57.07; H, 8.21, O, -

Preparation of simple ester and ether derivatives from the oligoglycerols and sugar alcohols portrayed (general description)

Methods of esterification and etherification, followed by cleavage of the protective groups, have been described in various publications. The articles listed below include different methods of phosphorylation. These methods can be employed here analogously.

5

Eibl, H.

Synthesis of glycerophospholipids

Chem. Phys. Lipids 26 (1980) 405-429

10

Eibl, H.

Phospholipid Synthesis

In: Liposomes: From Physical Structure to Therapeutic Applications (C.G. Knight, editor) Elsevier, Amsterdam (1981) 19-50

15

Eibl, H. and Kovatchev, S.

Preparation of phospholipids and of their analogues by phospholipase D. In: Methods of Enzymology. Vol. 72. Ed. J.M. Lowenstein, Academic Press, New York (1981) 632-639

20

Eibl, H.: Phospholipids als funktionelle Bausteine biologischer Membranen Angew. Chemie 96 (1984) 247-262

Eibl, H.: Phospholipids as functional constituents of biomembranes Angew. Chem. Int. Ed. Engl. 23 (1984) 257-271

25

Eibl, H. Phospholipid synthesis: Oxazaphospholanes and dioxaphospholanes as intermediates. Proc. Natl. Acad. Sci. USA 75 (1978) 4074-4077

Eibl, H. and Wooley, P.: Synthesis of enantiomerically pure glyceryl esters and ethers.

30

I. Methods employing the precursor 1,2-isopropylidene-sn-glycerol. Chem. Phys. Lipids 41 (1986) 53-63

Eibl, H. and Wooley, P.: Synthesis of enantiomerically pure glyceryl esters and ethers.

II. Methods employing the precursor 3,4-isopropylidene-D-mannitol. Chem. Phys.

35

Lipids 47 (1988) 47-53

Eibl, H. and Wooley, P.: A general synthetic method for enantiomerically pure ester and ether lysophospholipids. Chem. Phys. Lipids 47 (1988) 63-68

- 5 Wooley, P. and Eibl, H.: Synthesis of enantiomerically pure phospholipids including phosphatidylserine and phosphatidylglycerol. Chem. Phys. Lipids 47 (1988) 55-62

Example 4g:

- 10 Intermediates for the synthesis of phospholipids which contain sugar alcohols in the polar area

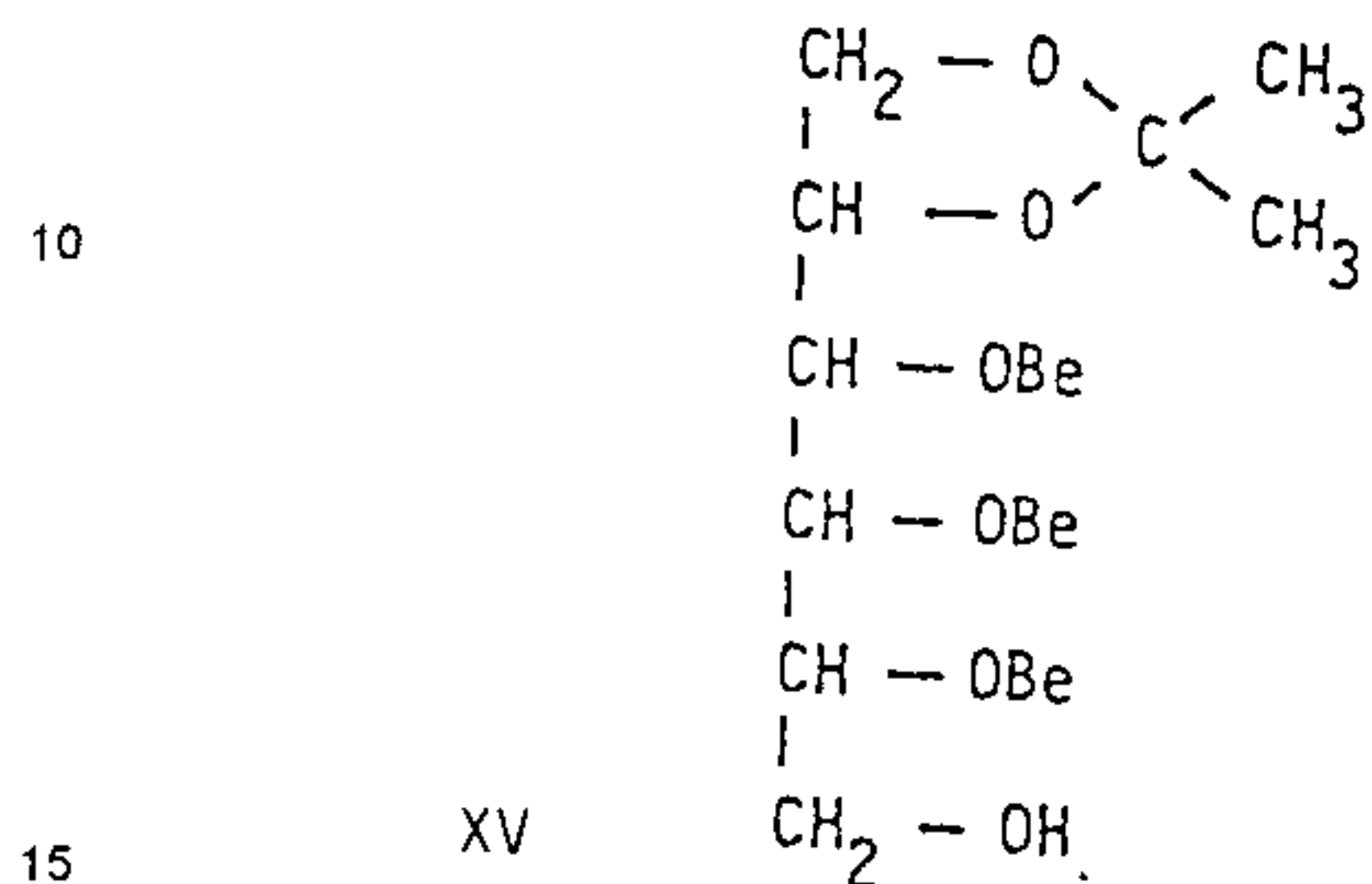
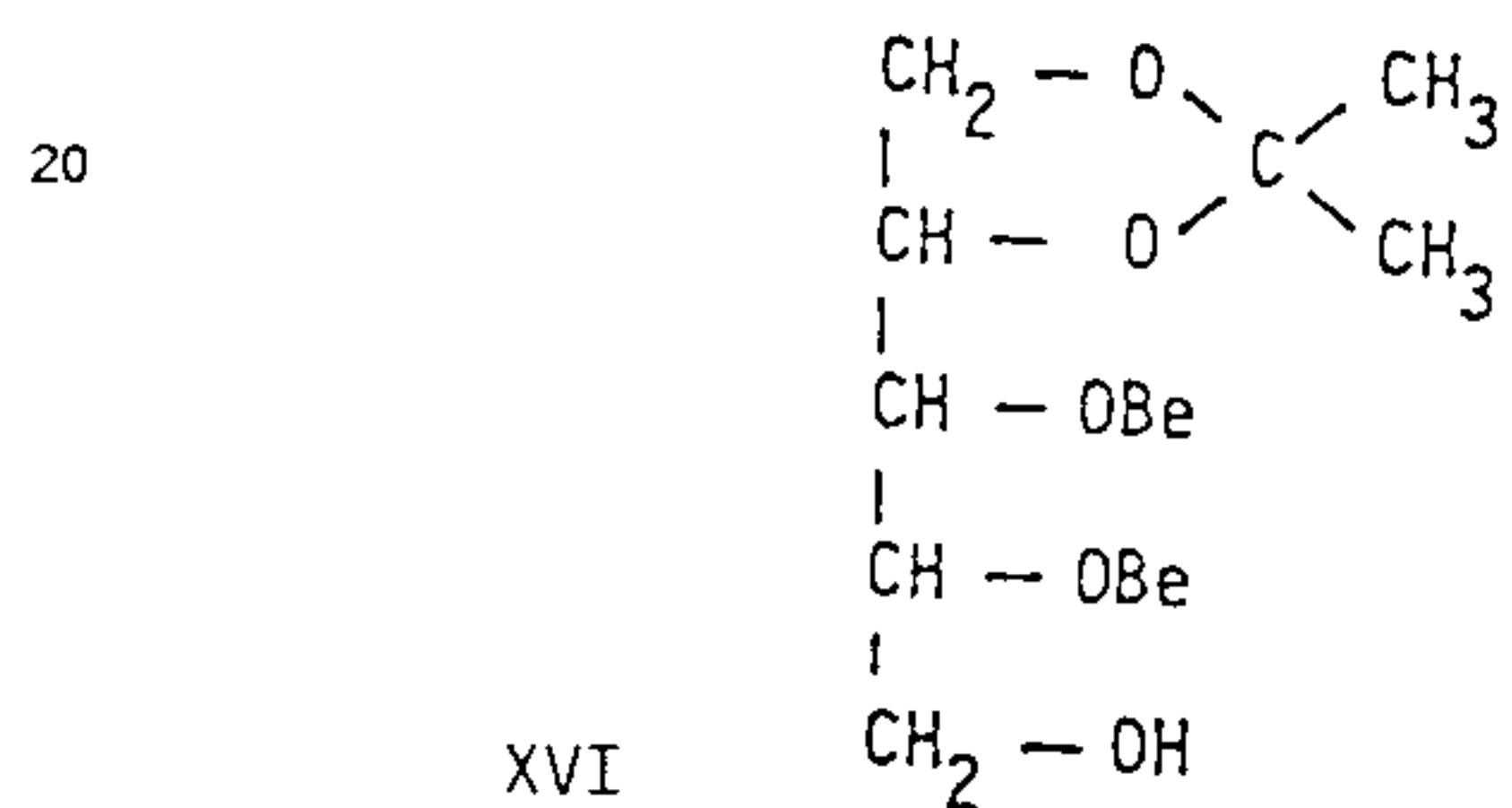
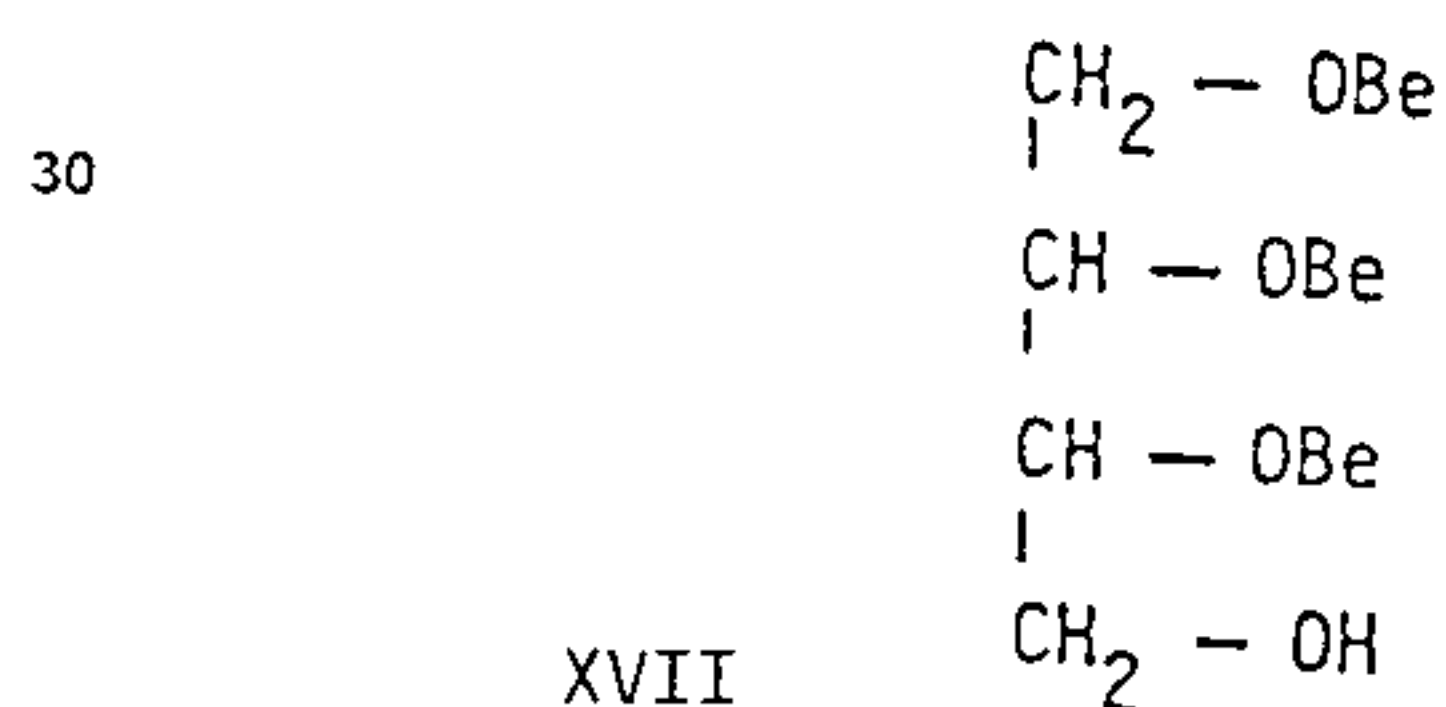
As already described, the introduction via oligoglycerols of substances in the polar area of phospholipids has a pronounced effect on the blood circulation if these substances are used as liposome components. The same result can be obtained if, instead of the oligoglycerols, use is made of sugar alcohols, eg, phosphoric acid esters of D-mannitol, D-lyxitol and D-threitol. These compounds can be introduced with suitable protective groups (see model H) into phospholipids in the manner described for oligoglycerols. With the derivatives described, coupling with phospholipids again leads to an sn-1 linkage between the phosphoric acid and the sugar alcohol.

D-mannitol derivative

3.4-0.0-dibenzyl-D-mannitol is prepared from 1.2.6.5-diisopropylidene-D-mannitol by benzylating in the 3.4 position and splitting off the isopropylidene protective groups. After introducing the isopropylidene protective group in the 1.2 position, tritylation and benzylation of the exposed -OH group, one obtains, following cleavage of the trityl group, the compound XV, which can be incorporated in the polar area of phospholipids.

D-lyxitol derivative

1.2-isopropylidene-3,4-O-benzyl-D-mannitol (see above) is cleaved with periodic
 acid and reduced with NaBH₄ to the alcohol XVI. This compound can be incorporated
 5 into the polar area of phospholipids.

D-mannitol derivative (6 hydroxyl groups)D-lyxitol derivative (5 hydroxyl groups; from D-mannitol)D-threitol derivative (4 hydroxyl groups; from D-mannitol)

Model H: Polyhydric alcohols with at least 4 hydroxyl groups for incorporation into the polar area of phospholipids

5 D-threitol derivative

The compound XVI is converted by way of benzylation into 1.2-isopropylidene-3.4.5-0.0.0-tribenzyl-D-lyxitol. After splitting off the isopropylidene protective group, periodic acid cleavage and reduction to the alcohol, one obtains XVII. This compound can be
10 incorporated into the polar area of phospholipids in the usual way.

Phospholipids which contain oligoglycerols in the polar area

In earlier publications we have described how phospholipids can be easily prepared
15 from diacyl glycerols with saturated and unsaturated fatty acid chains, with two identical or two different fatty acid chains (DE 32 39 817 Ar; P. Woolley et al. Chem. Phys. Lipids 47 (1988) 55-62; H. Eibl et al., Chem. Phys. Lipids 47 (1988) 63-68). It is also possible to use acyl/alkyl or alkyl/acyl glycerols as starting product. However, phospholipids which contain dialkyl glycerols are metabolically extremely stable and re-
20 sorption is negligible.

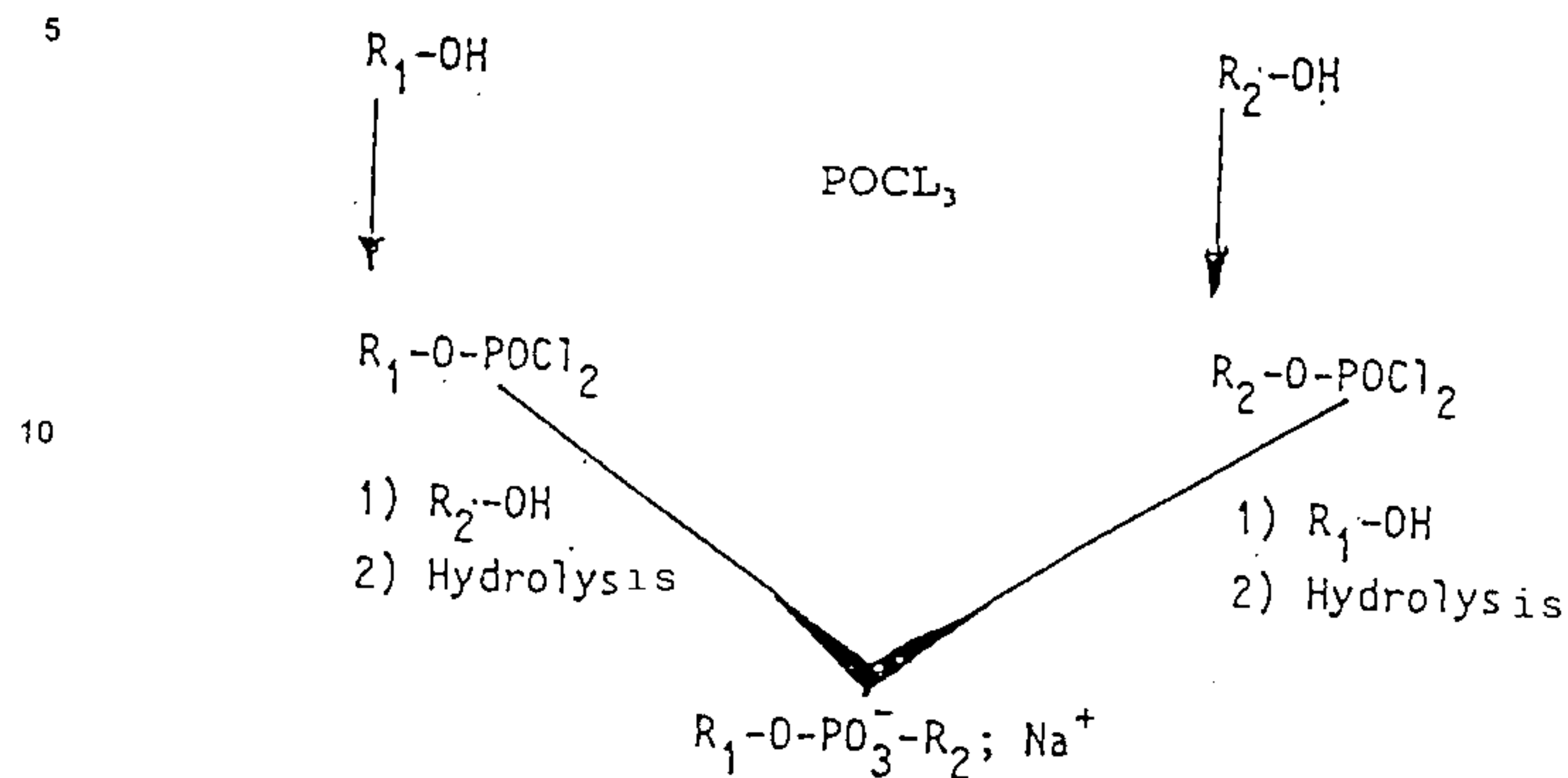
Basically, the compounds referred to can be prepared according to two different methods. This derives from the fact that a phosphoric acid diester is to be prepared from two alcohols, R_1 -OH and R_2 -OH.

25

The R_1 -OH alcohols are alcohols which contain a glycerol backbone with two fatty acid chains and a free hydroxyl group. They can also have just one fatty acid chain and an additional protective group, usually benzyl, for the preparation of monoacrylic phospholipids; R_1 -OH can, however, also stand for an alcohol with a simple alkyl
30 group with one or two cis double bonds.

The R_2 -OH alcohols are alcohols which have so far been designated as G_2 , G_3 and G_4 in the text. They are described by the structural formulae III and V (for G_2), VII and VIII (for G_3) and X to XIV (for G_4). In like manner, use can also be made of the sugar
35 alcohol derivatives XV to XVII.

Model G describes how two alcohols $R_1\text{-OH}$ and $R_2\text{-OH}$ can be utilized to prepare good yields of phosphoric acid diesters.

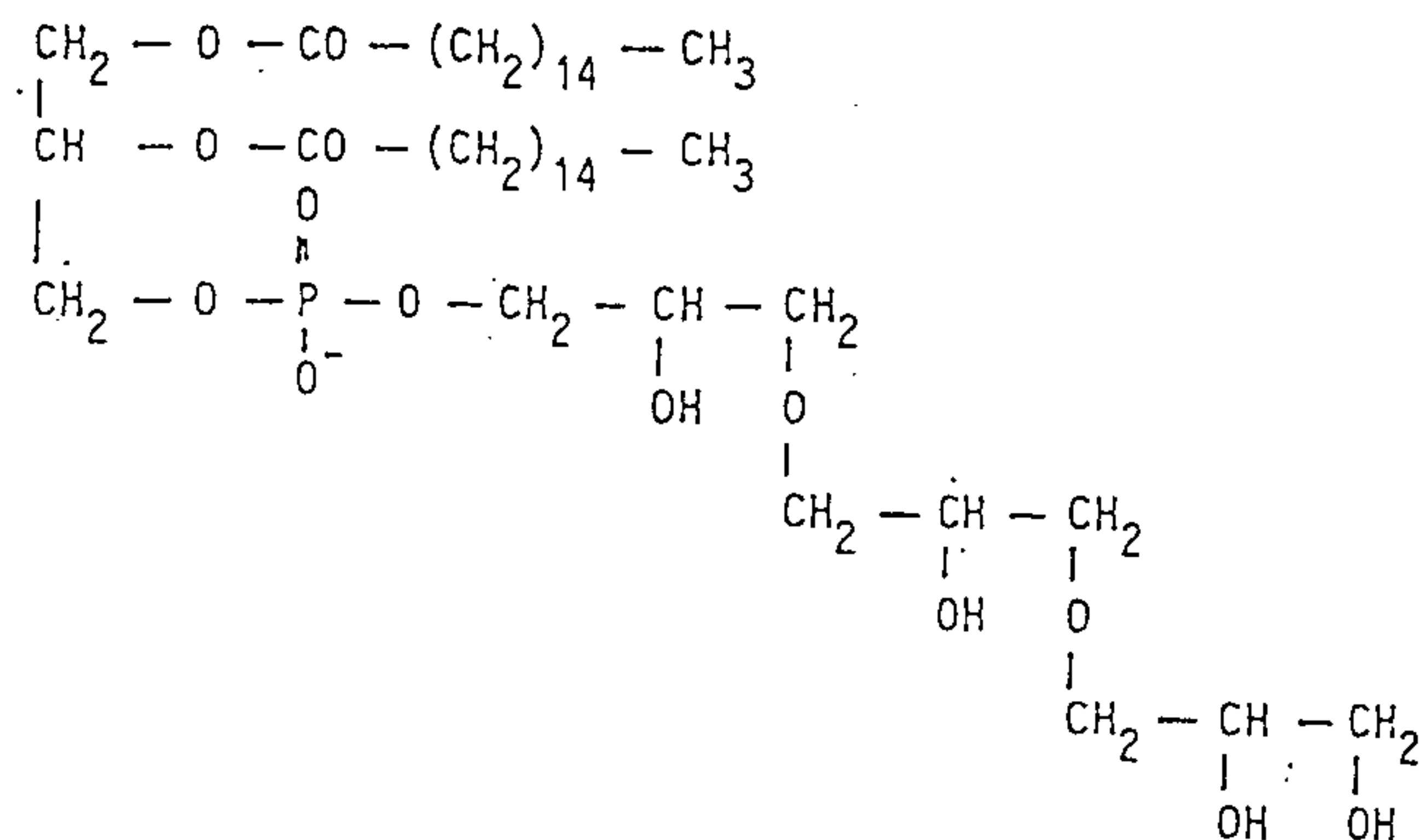


Where, for example, $R_1 = 1,2\text{-dipalmitoyl-sn-G}$ and $R_2 = \text{formula XI}$, the following structure is obtained after removal of the protective groups:

20

25

30



Model G: Phosphoric acid diester with the formula $R_1O-PO_3^- - R_2; Na^+$

Phosphorus oxychloride is used as phosphorylation agent. From the two alcohols R_1OH and R_2OH to be linked via phosphate, one first of all prepares the corresponding phosphoric acid dichloride; this is reacted in each case with the other alcohol to phosphoric acid monochloride. Slightly acid hydrolysis then leads to the phosphoric acid diesters, which, after the protective groups have been split off, form, eg, the salt XVIII, 1.2-dipalmitoyl-sn-glycero-3-phospho- $G_1-G_2-G_3-G_4; Na^+$.

The following list of examples can be extended at will by using different combinations of fatty acid chains or by introducing additional fatty acids, both of synthetic and natural origin. If necessary in order to obtain particular properties, the phosphatidyl-oligoglycerols can contain additional alkyl chains or fatty acid residues in the oligoglycerol part.

The oligoglycerol-based methods described here can thus be used and modified in manifold ways in order to vary and influence the properties of liposomes. By analogy with the hexadecylphosphocholines and the erucylphosphocholines, however, these substances may also be important biologically active molecules which influence signal transduction and thus functional pathways in the cells.

Examples of phospho - $G_1 - G_2$ compounds

1.	1.2-dipalmitoyl-sn-glycero-3-phospho- $G_1-G_2;$
Na ⁺ salt:	$C_{41}H_{80}NaO_{12}P$ (819.04)
2.	1.2-dimyristoyl-sn-glycero-3-phospho- $G_1-G_2;$
Na ⁺ salt:	$C_{37}H_{72}NaO_{12}P$ (762.93)
3.	1.2-distearoyl-sn-glycero-3-phospho- $G_1-G_2;$
Na ⁺ salt:	$C_{45}H_{88}NaO_{12}P$ (875.14)
4.	1-palmitoyl-2-lauroyl-sn-glycero-3-phospho- $G_1-G_2;$
Na ⁺ salt:	$C_{37}H_{72}NaO_{12}P$ (762.93)

5.	1-stearoyl-2-lauroyl-sn-glycero-3-phospho-G ₁ -G ₂ ;	
	Na ⁺ salt:	C ₃₉ H ₇₆ NaO ₁₂ P (790.98)
5 6.	1.2-dioleoyl-sn-glycero-3-phospho-G ₁ -G ₂ ;	
	Na ⁺ salt:	C ₄₅ H ₈₄ NaO ₁₂ P (871.11)
10 7.	1.2-dierucyl-sn-glycero-3-phospho-G ₁ -G ₂ ;	
	Na ⁺ salt:	C ₅₃ H ₁₀₀ NaO ₁₂ P (983.32)
8.	1-stearoyl-2-oleoyl-sn-glycero-3-phospho-G ₁ -G ₂ ;	
15	Na ⁺ salt:	C ₄₅ H ₈₆ NaO ₁₂ P (873.13)
9.	1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-G ₁ -G ₂ ;	
20	Na ⁺ salt:	C ₄₃ H ₈₂ NaO ₁₂ P (845.07)
10.	1-stearoyl-2-myristoyl-sn-glycero-3-phospho-G ₁ -G ₂ ;	
	Na ⁺ salt:	C ₄₁ H ₈₀ NaO ₁₂ P (819.04)
25 11.	1-stearoyl-2-palmitoyl-sn-glycero-3-phospho-G ₁ -G ₂ ;	
	Na ⁺ salt:	C ₄₃ H ₈₄ NaO ₁₂ P (847.09)
30 12.	1-myristoyl-sn-glycero-3-phospho-G ₁ -G ₂ ;	
	Na ⁺ salt:	C ₂₃ H ₄₆ NaO ₁₁ P (552.57)
13.	1-palmitoyl-sn-glycero-3-phospho-G ₁ -G ₂ ;	
35	Na ⁺ salt:	C ₂₅ H ₅₀ NaO ₁₁ P (580.62)
14.	1-stearoyl-sn-glycero-3-phospho-G ₁ -G ₂ ;	
40	Na ⁺ salt:	C ₂₇ H ₅₄ NaO ₁₁ P (608.68)
15.	Erucyl-phospho-G ₁ -G ₂ ;	
	Na ⁺ salt:	C ₂₈ H ₅₆ NaO ₈ P (574.71)
45 16.	Octadecyl-phospho-G ₁ -G ₂ ;	
	Na ⁺ salt:	C ₂₄ H ₅₀ NaO ₈ P (520.62)
50 17.	Hexadecyl-phospho-G ₁ -G ₂ ;	
	Na ⁺ salt:	C ₂₂ H ₄₆ NaO ₈ P (492.56)

18. Tetradecyl-phospho-G₁-G₂;

Na⁺ salt: C₂₀H₄₂NaO₈P (464.51)

19. Oleyl-phospho-G₁-G₂;

Na⁺ salt: C₂₄H₄₈NaO₈P (518.60)

20. 1-O-octadecyl-2-O-methyl-sn-glycero-3-phospho-G₁-G₂;

Na⁺ salt: C₂₈H₅₈NaO₁₀P (608.72)

Examples of phospho-G₁-G₂-G₃ compounds

1. 1,2-dipalmitoyl-sn-glycero-3-phospho-G₁-G₂-G₃;

Na⁺ salt: C₄₄H₈₆NaO₁₄P (893.12)

2. 1,2-distearoyl-sn-glycero-3-phospho-G₁-G₂-G₃;

Na⁺ salt: C₄₈H₉₄NaO₁₄P (949.22)

3. 1-palmitoyl-2-lauroyl-sn-glycero-3-phospho-G₁-G₂-G₃;

Na⁺ salt: C₄₀H₇₈NaO₁₄P (837.01)

4. 1-stearoyl-2-lauroyl-sn-glycero-3-phospho-G₁-G₂-G₃;

Na⁺ salt: C₄₂H₈₂NaO₁₄P (865.06)

5. 1,2-dioleoyl-sn-glycero-3-phospho-G₁-G₂-G₃;

Na⁺ salt: C₄₈H₉₀NaO₁₄P (945.19)

6. 1,2-dierucyl-sn-glycero-3-phospho-G₁-G₂-G₃;

Na⁺ salt: C₅₆H₁₀₆NaO₁₄P (1057.40)

7. 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-G₁-G₂-G₃;

Na⁺ salt: C₄₈H₉₂NaO₁₄P (947.21)

8. 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-G₁-G₂-G₃;

Na⁺ salt: C₄₆H₈₈NaO₁₄P (919.148)

9. 1-stearoyl-sn-glycero-3-phospho-G₁-G₂-G₃;

Na⁺ salt: C₃₀H₆₀NaO₁₃P (682.76)

10. Erucyl-phospho-G₁-G₂-G₃;5 Na⁺ salt: C₃₁H₆₂NaO₁₀P (648.79)11. Octadecyl-phospho-G₁-G₂-G₃;10 Na⁺ salt: C₂₇H₅₆NaO₁₀P (594.69)12. Hexadecyl-phospho-G₁-G₂-G₃;Na⁺ salt: C₂₅H₅₂NaO₁₀P (566.64)15 13. 3-O-octadecyl-2-O-methyl-sn-glycero-1-phospho-G₁-G₂-G₃;Na⁺ salt: C₃₁H₆₄NaO₁₂P (682.80)20 Examples of phospho-G₁-G₂-G₃-G₄ compounds1. 1.2-dipalmitoyl-sn-glycero-3-phospho-G₁-G₂-G₃-G₄;25 Na⁺ salt: C₄₇H₉₂NaO₁₆P (967.20)2. 1.2-distearoyl-sn-glycero-3-phospho-G₁-G₂-G₃-G₄;30 Na⁺ salt: C₅₁H₁₀₀NaO₁₆P (1023.30)3. 1-stearoyl-2-lauroyl-sn-glycero-3-phospho-G₁-G₂-G₃-G₄;Na⁺ salt: C₄₅H₈₈NaO₁₆P (939.14)35 4. 1.2-dioleoyl-sn-glycero-3-phospho-G₁-G₂-G₃-G₄;Na⁺ salt: C₅₁H₉₆NaO₁₆P (1019.27)40 5. 1.2-dierucyl-sn-glycero-3-phospho-G₁-G₂-G₃-G₄;Na⁺ salt: C₅₉H₁₁₂NaO₁₆P (1131.48)6. 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-G₁-G₂-G₃-G₄;45 Na⁺ salt: C₅₁H₉₈NaO₁₆P (1021.29)7. Erucyl-phospho-G₁-G₂-G₃-G₄;50 Na⁺ salt: C₃₄H₆₈NaO₁₂P (722.87)

Examples of phospho-sn-G₁ compoundssn-1-G₁-G₂:

1. 1.2-dipalmitoyl-sn-glycero-3-phospho-sn-1-G₁-G₂;

Na⁺ salt: C₄₁H₈₀NaO₁₂P (819.04)

2. 1.2-distearoyl-sn-glycero-3-phospho-sn-1-G₁-G₂;

Na⁺ salt: C₄₅H₈₈NaO₁₂P (875.14)

3. 1-stearoyl-2-lauroyl-sn-glycero-3-phospho-sn-1-G₁-G₂;

Na⁺ salt: C₃₉H₇₆NaO₁₂P (790.98)

4. 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-sn-1-G₁-G₂;

Na⁺ salt: C₄₅H₈₆NaO₁₂P (873.13)

sn-1-G₁-G₂-G₃:

1. Dipalmitoyl-sn-glycero-3-phospho-sn-1-G₁-G₂-G₃;

Na⁺ salt: C₄₄H₈₆NaO₁₄P (893.12)

2. 1.2-distearoyl-sn-glycero-3-phospho-sn-1-G₁-G₂-G₃;

Na⁺ salt: C₄₈H₉₄NaO₁₄P (949.22)

sn-1-G₁-G₂-G₃-G₄:

1. 1.2-dipalmitoyl-sn-glycero-3-phospho-sn-1-G₁-G₂-G₃-G₄;

Na⁺ salt: C₄₇H₉₂NaO₁₆P (967.20)

2. 1.2-distearoyl-sn-glycero-3-phospho-sn-1-G₁-G₂-G₃-G₄;

Na⁺ salt: C₅₁H₁₀₀NaO₁₆P (1023.30)

Examples of linkages with sugar alcoholsPhospho-D-mannitol compounds

1. 1.2-dipalmitoyl-sn-glycero-3-phospho-D-mannitol;

Na⁺ salt: C₄₁H₈₀NaO₁₃P (835.03)

2.	1.2-distearoyl-sn-glycero-3-phospho-D-mannitol;
5	Na ⁺ salt: C ₄₅ H ₈₈ NaO ₁₃ P (891.13)
3.	1-palmitoyl-2-lauroyl-sn-glycero-3-phospho-D-mannitol;
10	Na ⁺ salt: C ₃₇ H ₇₂ NaO ₁₃ P (788.92)
4.	1-stearoyl-2-lauroyl-sn-glycero-3-phospho-D-mannitol;
	Na ⁺ salt: C ₃₉ H ₇₆ NaO ₁₃ P (806.97)
15	5. 1-stearoyl-2-myristoyl-sn-glycero-3-phospho-D-mannitol;
	Na ⁺ salt: C ₄₁ H ₈₀ NaO ₁₃ P (835.03)
20	6. 1-stearoyl-sn-glycero-3-phospho-D-mannitol;
	Na ⁺ salt: C ₂₇ H ₅₄ NaO ₁₂ P (624.67)
7.	Octadecyl-phospho-D-mannitol;
25	Na ⁺ salt: C ₂₄ H ₅₀ NaO ₉ P (536.61)
8.	1-O-octadecyl-2-O-methyl-sn-glycero-3-phospho-D-mannitol;
30	Na ⁺ salt: C ₂₈ H ₅₈ NaO ₁₁ P (624.71)

Phospho-D-lyxitol compounds

35	1. 1.2-dipalmitoyl-sn-glycero-3-phospho-D-lyxitol;
	Na ⁺ salt: C ₄₀ H ₇₈ NaO ₁₂ P (805.00)
2.	1.2-distearoyl-sn-glycero-3-phospho-D-lyxitol;
40	Na ⁺ salt: C ₄₄ H ₈₆ NaO ₁₂ P (861.10)
3.	1-palmitoyl-2-lauroyl-sn-glycero-3-phospho-D-lyxitol;
45	Na ⁺ salt: C ₃₆ H ₇₀ NaO ₁₂ P (758.89)
4.	1-stearoyl-2-lauroyl-sn-glycero-3-phospho-D-lyxitol;
	Na ⁺ salt: C ₃₈ H ₇₄ NaO ₁₂ P (776.94)
50	5. 1-stearoyl-2-myristoyl-sn-glycero-3-phospho-D-lyxitol;
	Na ⁺ salt: C ₄₀ H ₇₈ NaO ₁₂ P (805.00)

Phospho-D-threitol compounds

1. 1.2-dipalmitoyl-sn-glycero-3-phospho-D-threitol;

Na⁺ salt: C₃₉H₇₆NaO₁₁P (774.97)

2. 1.2-distearoyl-sn-glycero-3-phospho-D-threitol;

Na⁺ salt: C₄₃H₈₄NaO₁₁P (831.07)

3. 1-stearoyl-2-lauroyl-sn-glycero-3-phospho-D-threitol;

Na⁺ salt: C₃₇H₇₂NaO₁₁P (746.91)

4. 1-stearoyl-2-myristoyl-sn-glycero-3-phospho-D-threitol;

Na⁺ salt: C₃₉H₇₆NaO₁₁P (774.97)

Example 4h:

Phosphorylation steps (general directions) based, by way of example, on the isolation of 1.2-dipalmitoyl-sn-glycero-3-phospho-glyceroglycerol, Na⁺ salt

POCl₃ (0.1 mol - 15.3 g) in 15 ml THF is introduced into a three-necked flask. While vigorously stirring the contents of the ice-cooled flask, one adds - dropwise - 1.2-dipalmitoyl-sn-glycerol (0.1 mol - 57 g) in 100 ml THF and, separately, triethylamine (0.11 mol - 11 g) in such manner that there is always a slight excess of triethylamine compared to 1.2-dipalmitoyl-sn-glycerol, which takes up the HCl as it forms. The temperature of the reaction mixture should not exceed 16 °C. On completion of the addition, the reaction mixture is left to stand for a further 30 minutes at 16 °C and then subjected to a TLC check to make sure that the reaction is complete (1.2-dipalmitoyl-sn-glycerol, R_f 0.8; 1.2-dipalmitoyl-sn-glycero-3-phosphoric acid dichloride is converted by way of methanolysis to the corresponding phosphoric acid dimethyl ester, R_f 0.4 in ether.)

The second phosphorylation step is carried out with a protected oligoglycerol. Here, the conversion with 2-O-benzyl-rac-G₁-1.3-0.0-1.2-isopropylidene-rac-G₂ is described.

To the reaction mixture with 1.2-dipalmitoyl-sn-glycero-3-phosphoric acid dichloride

one adds - dropwise - the above alcohol (0.105 mol - 31 g) and triethylamine (0.13 - 13 g) in 100 ml THF in such manner that the temperature of the reaction mixture does not exceed 40 °C. After 3 hours at 40 °C the reaction is complete (starting product phosphoric acid dimethyl ester, R_f 0.4; product methyl ester, R_f 0.7 in ether). One removes the triethylamine hydrochloride precipitate by filtration and hydrolyses the reaction mixture, mainly 1,2-dipalmitoyl-sn-glycerol-3-phospho-2-O-benzyl-rac-glycerol-1,3-0,0-1,2-isopropylidene-rac-glycerol-monochloride together with incompletely reacted 1,2-dipalmitoyl-sn-glycerol-3-phosphoric acid dichloride, with 26 g Na_2CO_3 dissolved in 260 ml H_2O . After 4 hours, 400 ml diisopropyl ether are added and the upper phase, which contains the product, rotated in an evaporator until crystals begin to form. 500 ml acetone are now added, and the crystals formed removed under suction at 20 °C. The filtrate contains the protected phosphatidylglyceroglycerol, Na^+ salt (R_f 0.6 in $\text{CHCl}_3/\text{CH}_3\text{OH}/\text{glacial acetic acid}/\text{H}_2\text{O}$ 600:60:20:5). After removal of the solvent, one obtains 48 g crude product, which is heated in 140 ml acetic acid and 60 ml H_2O for 30 minutes to 60 - 70 °C (cleavage of the isopropylidene protective group). One then adds 500 ml CHCl_3 , 600 ml CH_3OH and 400 ml H_2O and shakes thoroughly. The lower CHCl_3 phase is washed again with 600 ml CH_3OH and 500 ml H_2O , with addition of sufficient Na_2CO_3 to obtain a pH of 6 in the aqueous phase. The lower chloroform phase is rotated in an evaporator and the residue taken up in 400 ml THF. To remove the benzyl protective group, the solution has 6 g Pd/C added to it and is debenzylated in a H_2 atmosphere. The reaction is complete after about 4 hours. The catalyst is separated off by filtration, the solvent removed and the residue (~ 30 g) taken up in 100 ml CHCl_3 . 900 ml acetone are added, and the crystals formed removed under suction. One obtains a white powder, 1,2-dipalmitoyl-sn-glycerol-3-phospho-glyceroglycerol, Na^+ salt, yield: 26 g (~ 32 %).

Empirical formula: $\text{C}_{41}\text{H}_{80}\text{NaO}_{12}\text{P}$ (MW 819.04)

calculated: C, 60.13; H, 9.85; Na, 2.81; O, 23.44; P, 3.78

measured: C, 60.01; H, 9.79; Na, - ; O, - ; P, 3.69

1,2-dipalmitoyl-sn-glycerol-3-phospho-glycerol-glycerol, Na^+ salt

Empirical formula: $\text{C}_{44}\text{H}_{86}\text{NaO}_{14}\text{P}$ (MW 893.12)

calculated: C, 59.17; H, 9.71; Na, 2.57; O, 25.08; P, 3.47

measured: C, 59.11; H, 9.62; Na, - ; O, - ; P, 3.45

1.2-dipalmitoyl-sn-glycero-3-phospho-glycero-glycerol, Na⁺ salt

Empirical formula: C₄₇H₉₂NaO₁₆P (MW 967.20)

calculated: C, 58.37; H, 9.59; Na, 2.38; O, 26.47; P, 3.20

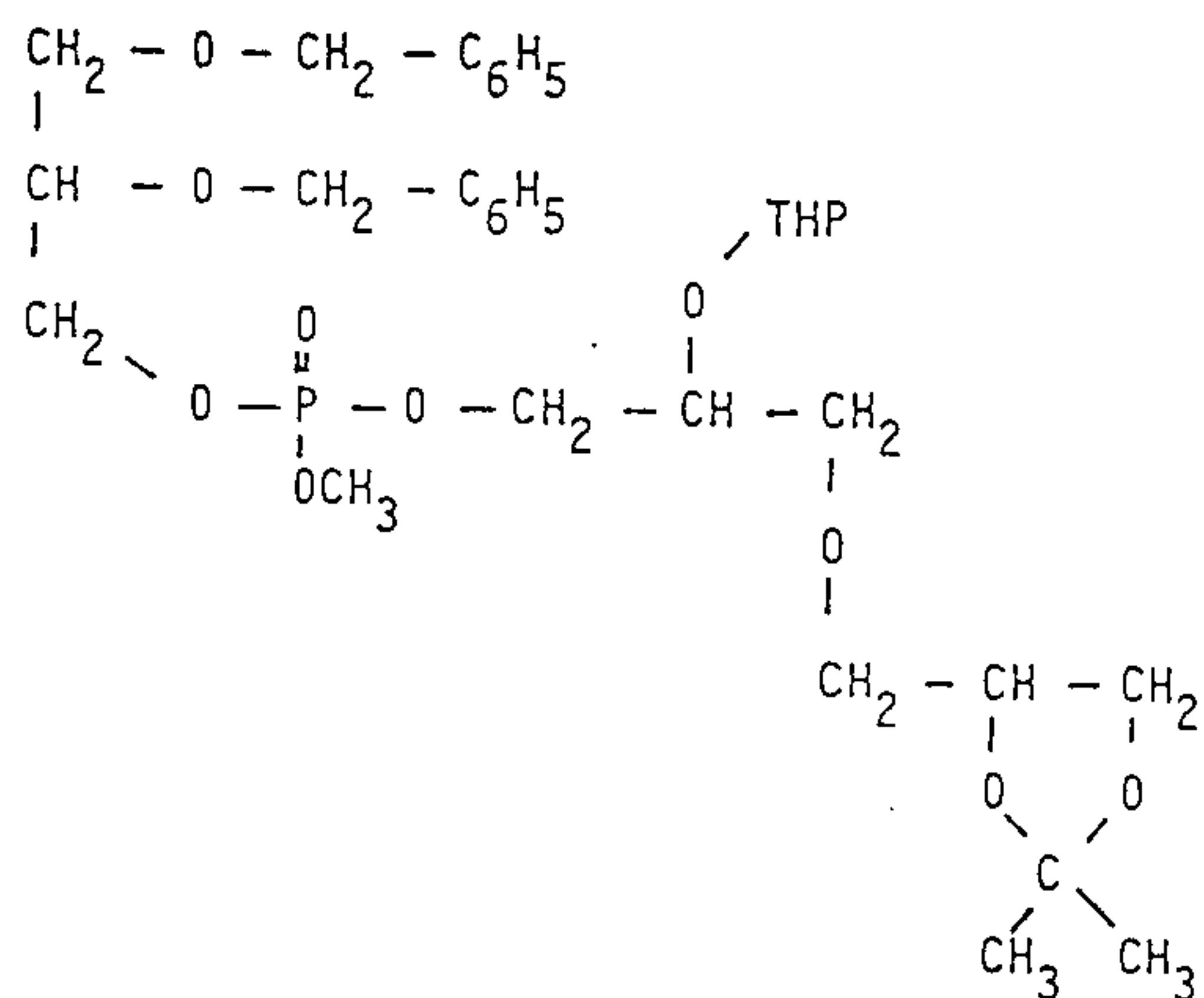
5 measured: C, 58.29; H, 9.53; Na, - ; O, - ; P, 3.19

To prepare phosphatidyl-oligoglycerols with an aliphatic chain, the so-called lysophosphatidyl-oligoglycerols, one can start with compounds which have a benzyl ether group in the sn-2 position of the glycerol, eg, 1-palmitoyl-2-O-benzyl-sn-glycerol, 1-stearoyl-2-O-benzyl-sn-glycerol, 1-O-hexadecyl-2-O-benzyl-sn-glycerol, 1-O-octadecyl-2-O-benzyl-sn-glycerol etc. We have described the preparation of these compounds in the publications H. Eibl and P. Woolley, Chem. Phys. Lipids 41 (1986) 53-63 and Chem. Phys. Lipids 47 (1988) 55-62. They are phosphorylated in the manner described for the preparation of 1.2-dipalmitoyl-sn-glycero-3-phosphoglycerol, Na⁺ salt, and reacted with the protected oligoglycerols. The protective groups are split off analogously. In the last step, by means of catalytic hydrogenolysis with Pd/C (5 % on activated charcoal) the benzyl groups are split off both the oligoglycerol part and the glycerol, which carries an acyl or alkyl group (see the examples).

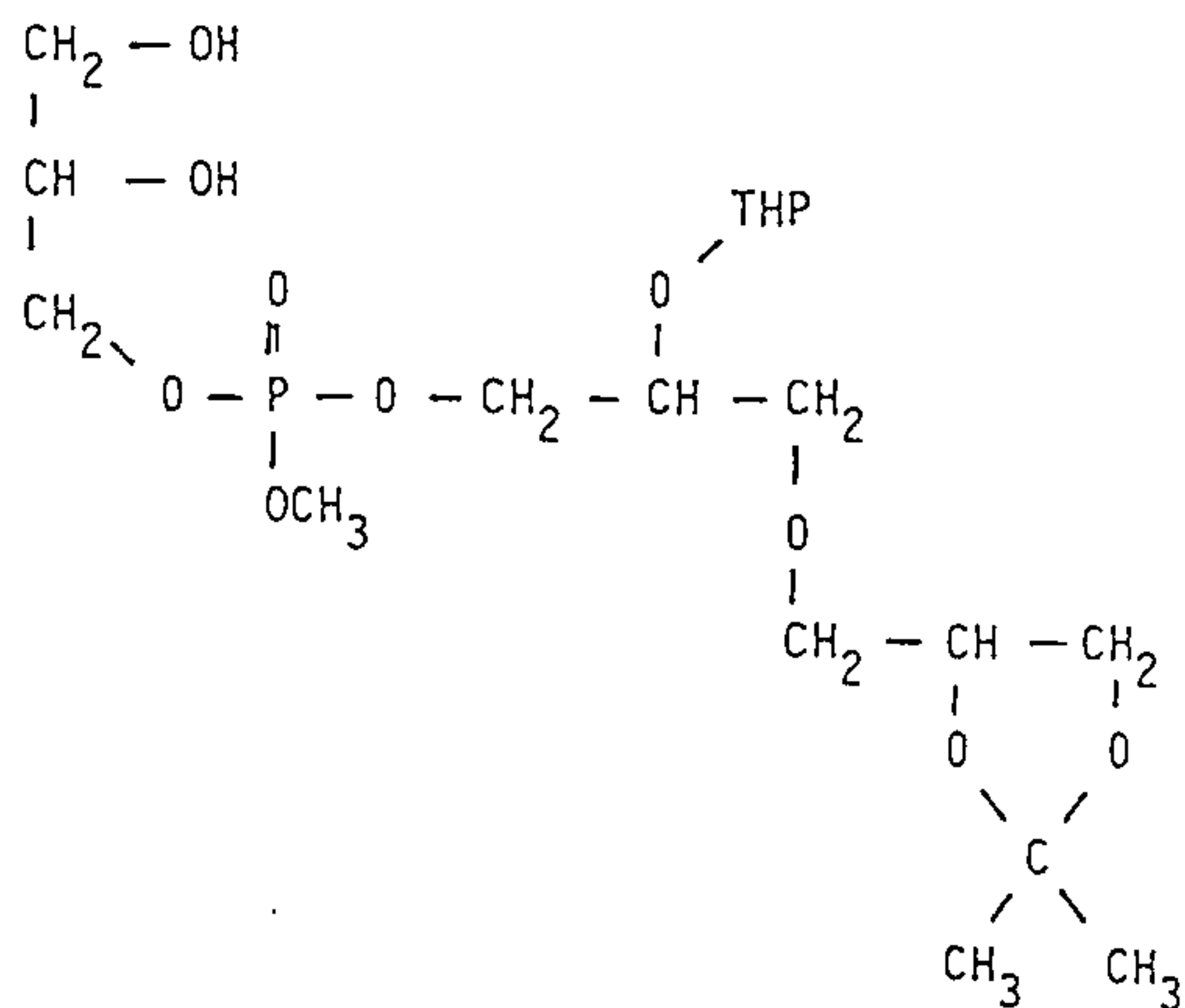
20 Preparation of the alkylphospho-oligoglycerols is easy by comparison, as in this case the corresponding alcohols are reacted according to the given phosphorylation pattern. To obtain the unsaturated sorts, however, one must use tetrahydropyranyl instead of benzyl as protective group.

25 A different strategy altogether is employed to synthesize the unsaturated representatives of this substance group. 1.2-dibenzyl-sn-glycerol is phosphorylated in the described manner (see, in addition, German patent application DE 32 39 817), then reacted with a tetrahydropyranyl-protected oligoglycerol. Instead of the hydrolysis, a methanolysis is performed and phosphoric acid triesters obtained, eg, for 2-O-tetrahydropyranyl-rac-G₁-1.3-0.0-1.2-isopropylidene-rac-G₂;

30

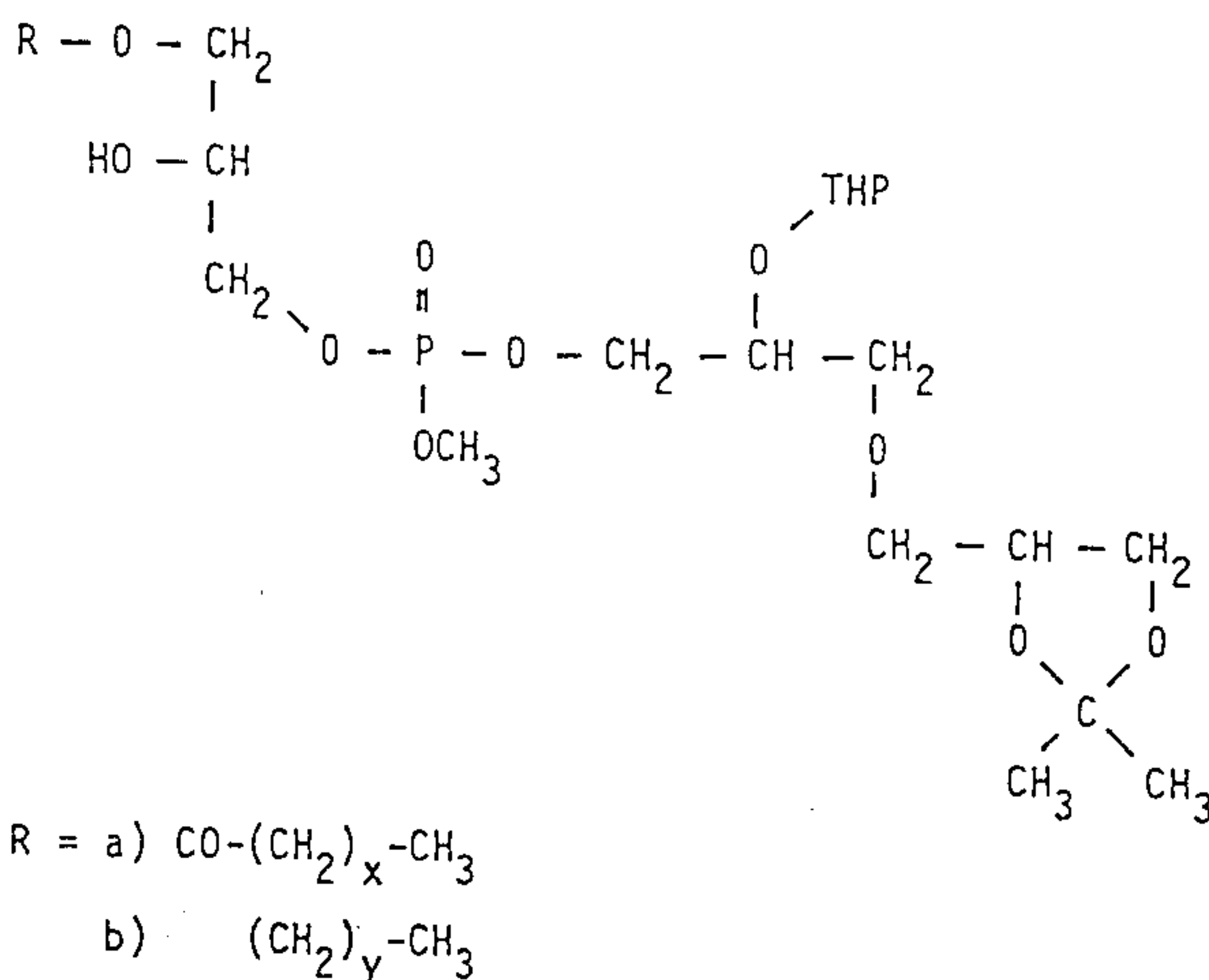


This key intermediate is now subjected to hydrolysis with Pd/C (5 % on activated charcoal). One obtains:



It is now possible to introduce arbitrary unsaturated and saturated fatty acids at the sn-1 and sn-2 positions of the glycerol molecule. This step is followed, as described in our earlier patent application, by splitting off the methyl group with LiBr and then hydrolysing the isopropylidene and tetrahydropyranyl protective groups in 70 % acetic acid at 60 to 70 °C. The dioleoyl compounds do not crystallize readily and must therefore be purified chromatographically; the dierucyl compounds, by contrast, are obtained easily in crystalline form.

The phosphoric acid triester strategy is also recommended for preparing mixed-chain phosphatidyl-oligoglycerols. In this case, as with the synthesis of lysophosphatidyl-oligoglycerols, 1-acyl-2-O-benzyl-sn-glycerols or 1-O-alkyl-2-O-benzyl-sn-glycerols are used as starting products and reacted analogously to 1,2-dibenzyl-sn-glycerol. Following catalytic debenzylation one obtains as intermediates for the G₂ compound:



Unsaturated fatty acids can now be introduced at the free sn-2 position, and the molecule freed from its protective groups as described. It is convenient that molecules with the fatty acid combination 1-palmitoyl-2-oleoyl- or 1-stearoyl-2-oleoyl crystallize readily.

5

Example 5: Additional properties

As the number of glycerol molecules increases, the number of free hydroxyl groups and thus also the polarity increases. For a proportion of 5 % in water, PP-G-PG₄ is the only PP-G-PG_n to form a clear isotropic solution. The lipids PP-G-PG₁, PP-G-PG₂ and PP-G-PG₃ dissolve when heated above 40 °C in water and form superstructures.

10

When the temperature falls below 40 °C, lamellar structures with differently pronounced hystereses are formed, which are recognizable on account of their anisotropy (birefracton in polarized light). The PP-G-PG₁ solution becomes cloudy most quickly, and excess lipid precipitates when the solution is left to stand at room temperature.

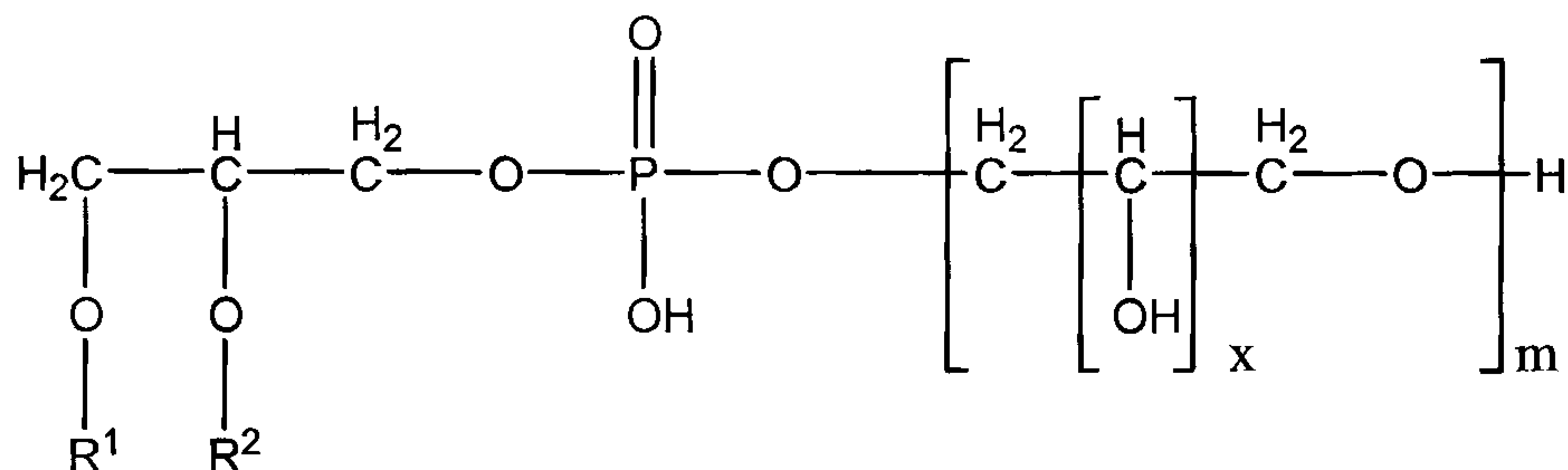
15

The lamellar phases of PP-G-PG₂ and PP-G-PG₃ remain stable even at low temperatures (down to 4 °C). Whereas the transition from the isotropic to the anisotropic phase at room temperature is recognizable after a few minutes with PP-G-PG₂, it takes several hours for PP-G-PG₃. The differences in polarity are also evident from the different retention factors (R_f) in the thin-layer chromatogram on silica gel.

20

CLAIMS:

1. A composition comprising compounds with the general formula (A):



where R¹ and R² are independently hydrogen or a saturated or unsaturated, branched or unbranched aliphatic or acyl residue,

x is a whole number from 1 to 4; and

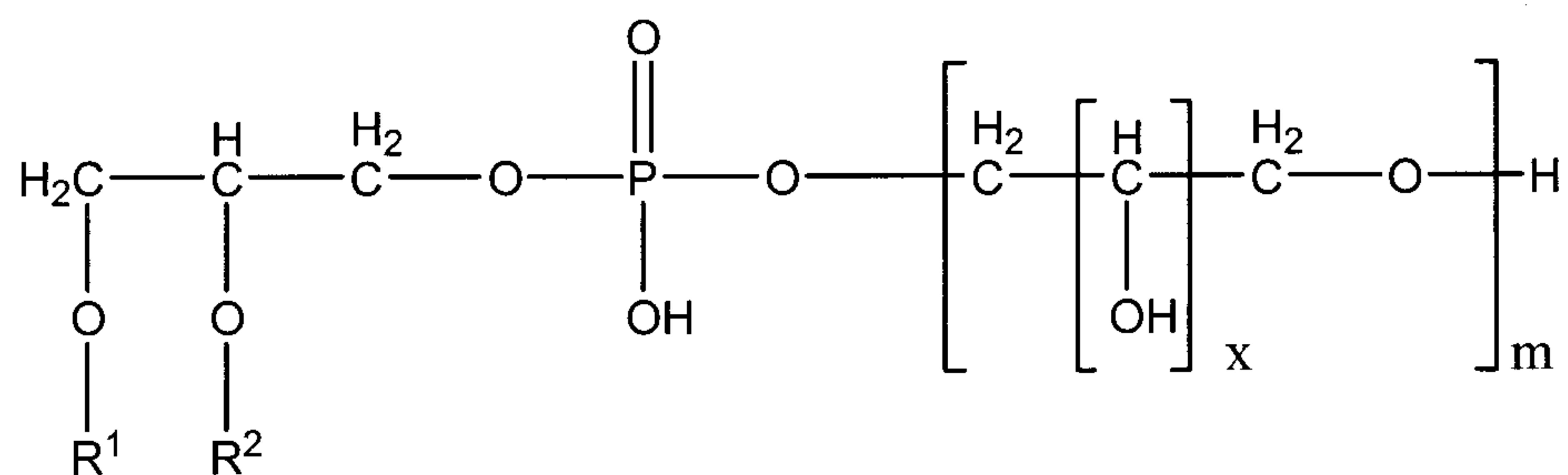
m is a whole number from 2 to 5.

wherein the compounds are more than 90% uniform with respect to the value of m.

2. The composition of claim 1, wherein the compounds are more than 95% uniform with respect to the value of m.
3. The composition of claim 1, wherein the compounds are more than 99% uniform with respect to the value of m.
4. The composition according to claim 1, 2 or 3, wherein in the compounds of formula (A) $x=1$ and m is a whole number from 2 to 4.
5. The composition according to claim 1, 2 or 3, wherein in the compounds of formula (A), R^1 and R^2 independently are hydrogen, a saturated or unsaturated C_1 - C_{24} aliphatic or C_1 - C_{24} acyl residue.
6. The composition according to any one of claims 1 to 5, wherein said compound contains a phospho-rac-(1 or 3)-oligoglycerol linkage.

- 57 -

7. The composition according to any one of claims 1 to 5, wherein said compound contains a phospho-sn-1-oligoglycerol linkage.
8. The composition according to any one of claims 1 to 5, wherein said compound contains a phospho-sn-3-oligoglycerol linkage.
9. The composition according to any one of claims 1 to 8, wherein at least one of the residues R^1 and R^2 is an acyl residue.
10. Liposomes containing phospholipids or alkyl phospholipids or cholesterol, and 1 to 50 mol % of compounds with the formula (A):



or salts thereof, where the cholesterol, the phospholipids, the alkyl phospholipids and the compounds of formula (A) together make up 100 mol %, and R^1 and R^2 are independently hydrogen, a saturated or unsaturated, branched or unbranched aliphatic or acyl residue;

x is a whole number from 1 to 4; and

m is a whole number from 2 to 5;

wherein compound (A) comprised in the liposomes are more than 90% uniform with respect to the value of m , wherein said phospholipid and compounds of formula (A) contain the same fatty acid residues.

11. The liposomes of claim 10, wherein the compounds (A) are more than 95% uniform with respect to the value of m .
12. The liposomes of claim 10, wherein the compounds (A) are more than 99% uniform with respect to the value of m .

13. Liposomes according to claim 10, 11 or 12, wherein said liposomes contain 5 to 15 mol % of compounds of formula (A), with the cholesterol, the phospholipids, the alkyl phospholipids and the compound of formula (A) together making up 100 mol %.
14. Liposomes according to claim 10, 11, 12 or 13, wherein $x=1$.
15. Liposomes according to claim 14, wherein m is a whole number from 2 to 4.
16. Liposomes according to any one of claims 10 to 15, wherein R^1 and R^2 are hydrogen, or a saturated or unsaturated C_1 - C_{24} aliphatic or C_1 - C_{24} 4 acyl residue.
17. Liposomes according to any one of claims 10 to 15, wherein the compounds of formula (A) have a phospho-rac-(1 or 3)-oligoglycerol linkage.
18. Liposomes according to any one of claims 10 to 15, wherein the compounds of formula (A) have a phospho-sn-1-oligoglycerol linkage.
19. Liposomes according to any one of claims 10 to 15, wherein the compounds of formula (A) have a phospho-sn-3-oligoglycerol linkage.
20. Liposomes according to any one of claims 10 to 15, wherein, in formula (A), at least one of R^1 and R^2 is an acyl group.
21. Liposomes according to any one of claims 10 to 20, wherein said liposomes have an excess negative charge.
22. Liposomes of any one of claims 10 to 20, wherein said liposomes have no excess charge.
23. Liposomes of any one of claims 10 to 20, wherein said liposomes have an excess positive charge.

24. Liposomes according to any one of claims 10 to 23, wherein said liposomes contain 0 to 70 mol % cholesterol, 1 to 50 mol % of said compounds of formula (A), and phospholipids or alkyl phospholipids, and these components together make up 100 mol %.

25. Liposomes according to any one of claims 10 to 23, wherein said liposomes contain 0 to 70 mol % cholesterol, 1 to 15 mol % of said compounds of formula (A), and phospholipids or alkyl phospholipids, and these components together make up 100 mol %.

26. Liposomes according to any one of claims 10 to 23, wherein said liposomes contain 25 to 43 mol % cholesterol, 5 to 15 mol % of said compounds of general formula (A), and phospholipids or alkyl phospholipids, and these components together make up 100 mol %.

27. Liposomes according to any one of claims 10 to 23, wherein said liposomes contain 38 to 42 mol % cholesterol.

28. Liposomes according to any one of claims 10 to 27, wherein $x = 2$, $x = 3$ or $x = 4$.

29. Liposomes according to any one of claims 10 to 28, wherein said liposomes contain one or more pharmaceutical drugs.

30. A pharmaceutical composition comprising liposomes according to any one of claims 10 to 28 and, entrapped in the liposomes, at least one pharmaceutical drug.

31. A pharmaceutical composition according to claim 30, comprising a pharmaceutical diluent, adjuvant, carrier media or filler.

32. A method of preparing the liposomes of claim 10, comprising transforming 1 to 50 mol % of compounds of general formula (A), as defined in claim 10, together with other components of the liposomes being cholesterol phospholipids or alkyl phospholipids in a quantity which, together

- 60 -

with the compound of formula (A) makes up 100 mol % into a lipid suspension and transforming said lipid suspension into liposomes.

33. The method of claim 32, wherein 5 to 15 mol % of said compounds of general formula (A), together with 35 to 43 mol % cholesterol and 42 to 60 mol % phospholipids or alkyl phospholipids are transformed into said lipid suspension.

34. A method of preparing the pharmaceutical composition of claim 30, comprising preparing liposomes by transforming 1 to 50 mol % of a compound of said general formula (A), together with the other components of the liposomes being cholesterol phospholipids or alkyl phospholipids in a quantity which, together with the compound of formula (A) makes up 100 mol %, and at least one soluble or insoluble pharmaceutical drug into a lipid suspension, and transforming said lipid suspension into liposomes, wherein when said drug is a water-insoluble drug it is entrapped in said liposomes by dissolving the drug together with lipid constituents, and wherein when said drug is a water-soluble drug it is entrapped in the liposomes by adding an aqueous solution which contains the water-soluble drug to lipid film.

35. A method of preparing the compounds of formula (A), as defined in claim 1, comprising linking an oligoglycerol having a uniform chain length with a phosphatidylglycerol.

36. A method of preparing the compounds of formula (A), as defined in claim 1, comprising linking an oligoglycerol having a uniform chain length or a C₄-C₆ sugar alcohol with an alcohol of the formula:



through use of a phosphorylation agent.

37. The method of claim 36, wherein said phosphorylation agent is POCl₃.

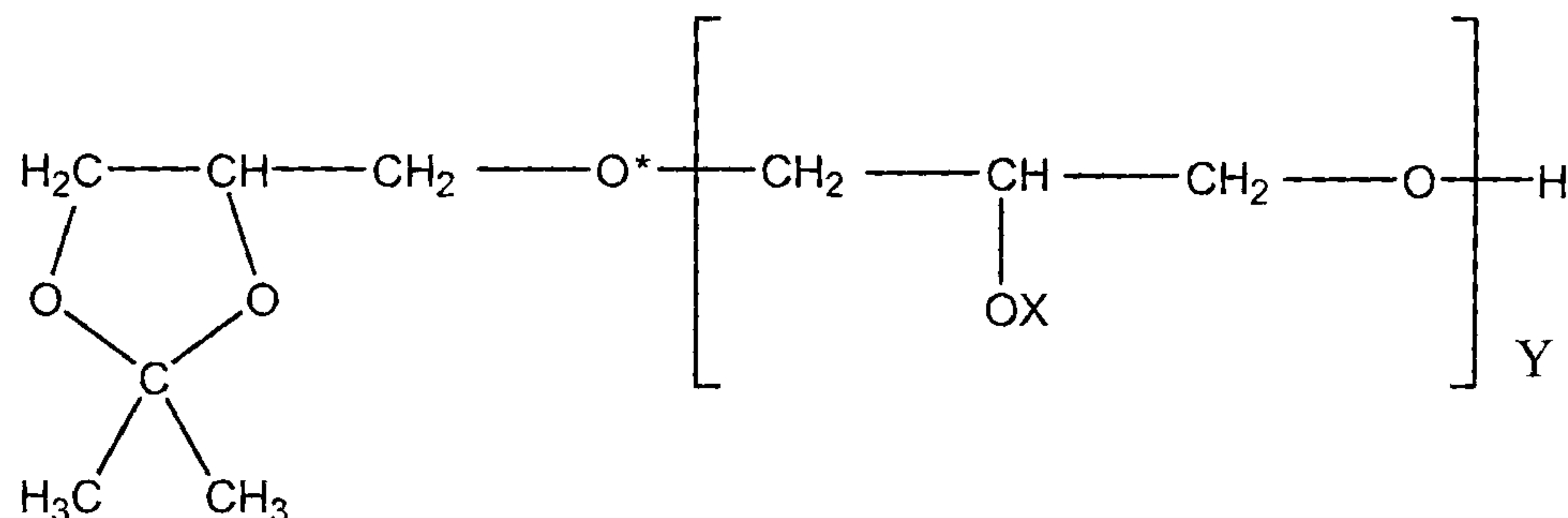
- 61 -

38. The method according to claim 35 or 36, wherein a phospho-rac-(1 or 3)-oligoglycerol linkage is formed.
39. A method according to claim 35 or 36, wherein a stereospecific compound is formed.
40. A method according to claim 35 or 36, wherein a phospho-sn-1-oligoglycerol linkage is formed.
41. A method according to claim 35 or 36, wherein a phospho-sn-3-oligoglycerol linkage is formed.
42. A method according to claim 35 or 36, wherein a linear oligoglycerol of uniform chain length is used.
43. A composition comprising compounds with the general formula (A), as defined in claim 1, the compound being prepared by the process of linking an oligoglycerol having a uniform chain length or a C₄-C₆ sugar alcohol with an alcohol of the formula:



through use of a phosphorylation agent.

44. A protected oligoglycerol with the formula (B):

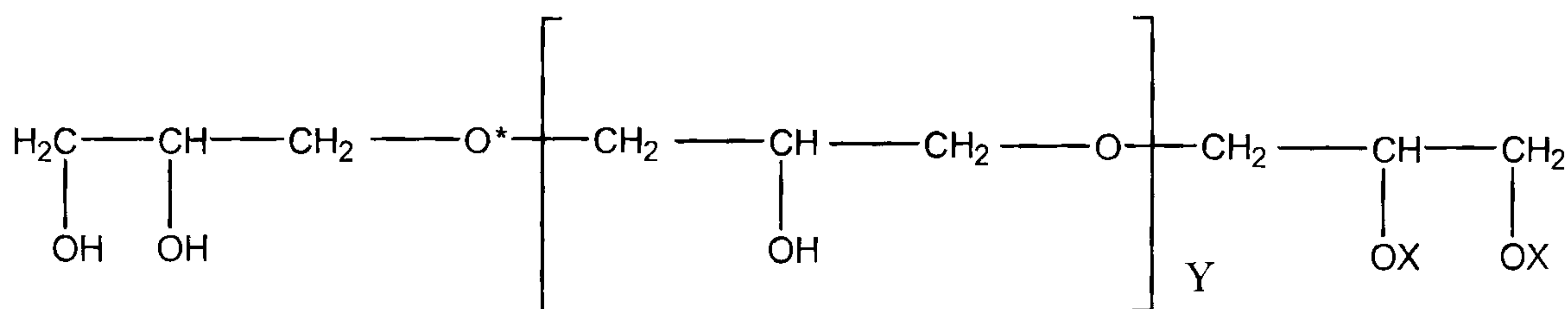


in which Y is a whole number from 1 to 9 and X is a benzyl, alkyl or tetrahydropyranyl group, provided that when X is methyl then Y is 2 to 9.

45. The protected oligoglycerol of claim 44, wherein Y is a whole number from 1 to 3.

46. Use of a protected oligoglycerol as claimed in claims 44 or 45 for preparing the compounds of formula (A), as defined in claim 1.

47. An alkyl oligoglycerol with the formula (C):



in which Y is a whole number from 1 to 8 and one of the residues X or Z is a saturated or unsaturated aliphatic residue, while the other residue is hydrogen.

48. The alkyl oligoglycerol of claim 47, wherein Y is a whole number from 1 to 3.

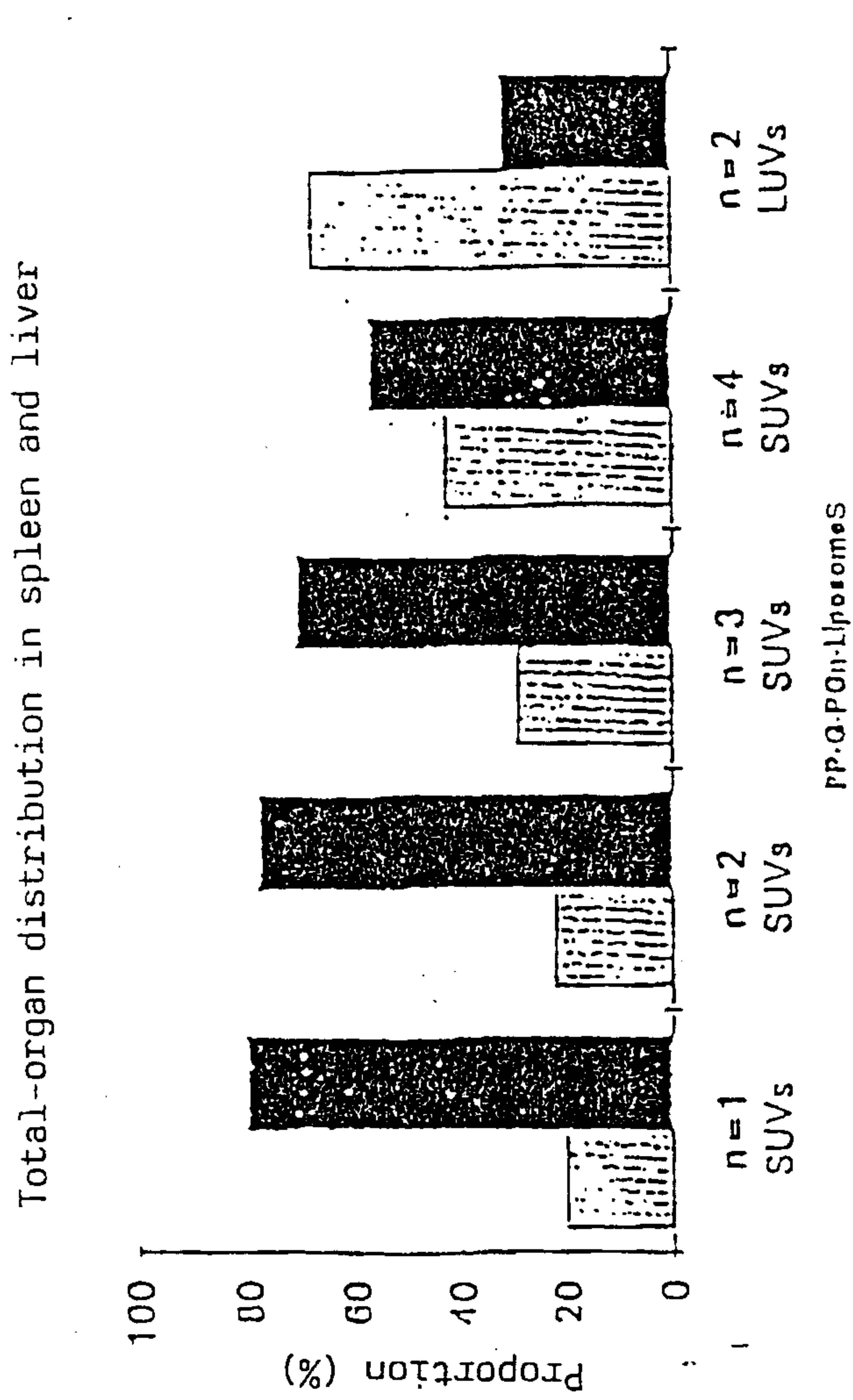


Figure 1

The liposome distribution in the organs was determined 72 h after i.v. administration. Pale bars: spleen; dark bars: liver. SUVs: small unilamellar liposomes of approx. diameter 60 nm; LUVs: large unilamellar liposomes of approx. diameter 190 nm.

Per-gram distribution in spleen and liver

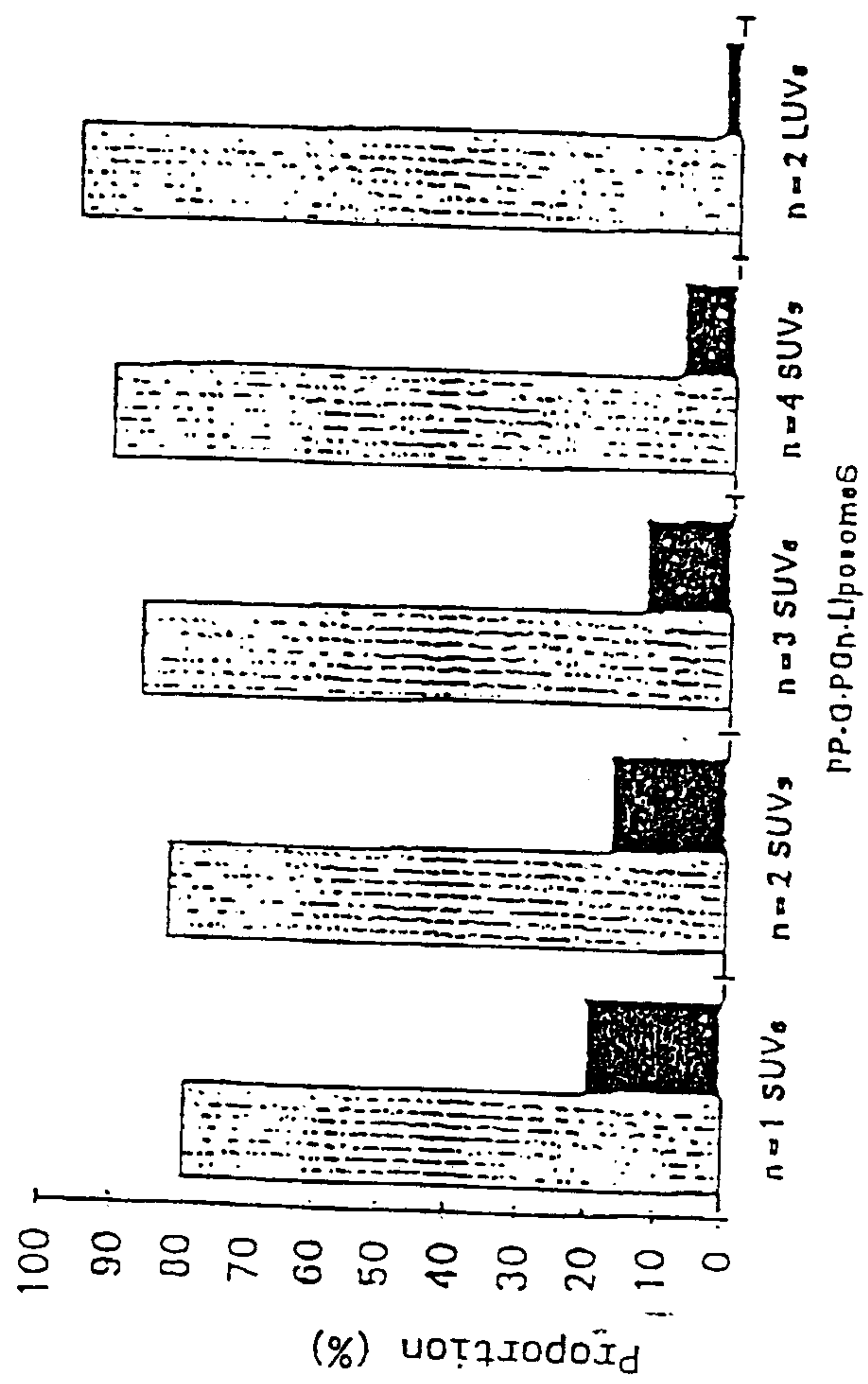


Figure 2

For key see Figure 1

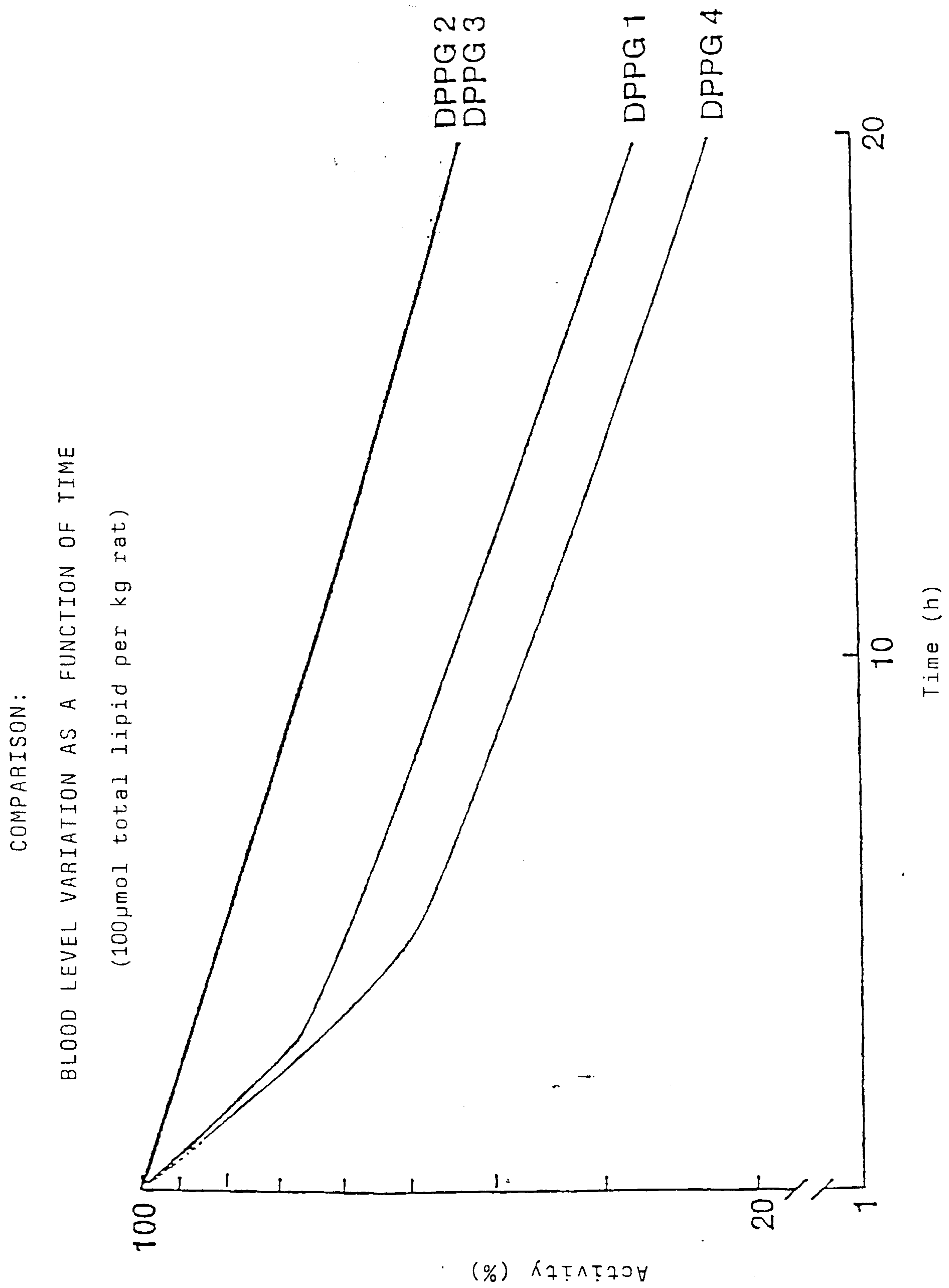


FIG. 3

