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A General Method for Synthesis of Ether Phospholipids with α-Linolenic Acid and Their Properties.

Jie Xia and Yongzheng Hui*

State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Science Shanghai 200032, China.

Abstract: *The synthesis of the novel ether phospholipids with α-linolenic acid and their properties including bioactivity are described*

Antitumor active ether phospholipids occupy a particularly important position as they represent a new class of chemotherapeutic agents with potent and selective anticancer activity *in vitro or in vivo*[1-4]. For example, the platelet-activating factor, PAF(1-octadecyl-2-acetyl-sn-glycerophosphocholine) and its analogues have been shown to exhibit a broad spectrum of biological activities[5-9]. Replacement of the sn-2-acetyl-moiety of PAF by a short chain alkyl (methyl or ethyl) group or introduction of an 2-acetamido function resulted in a series of highly potent and selective tumor-cytotoxic ether phospholipids[10]. The importance of these and related PAF derivatives is best illustrated by the fact that three such analogues have entered clinical trials recently as potential antileukemic and anticancer drugs[11].

On the other hand, α-linolenic acid has been known as an essential fatty acid for humans and its deficiency will alter membrane function, both in the brain as well as in pheripheral nerves[12-13]. Furthermore, it has been also reported to be capable of reducing the incidence and multiplicity of chemically induced colon tumors[14], decreasing the yield of chemically induced mammary turmors[15], and inhibiting the hairless mice[16].

In order to understand if there are any different properties shown for the novel ether phospholipids and the normal phospholipids, in the present communication, we, for the first time, describe a convenient method for the synthesis of the novel analogues of PAF, 1-hexadecyl-2-acyl-sn-glycero

-phosphocholine started from D-mannitol through a new deprotecting group approach when t-butyldimethylsily as a protecting group for hydroxy-functions without any acyl migration and describe their properies.

As the natural ether phospholipids are in general compounds with optical activity, we synthesize 2,3-di-O-benzyl-D-glyceritol *(1)* as a starting material from D-mannitol according to literature[17]. Thus, the alkoxide of compound*(1)* prepared using sodium hydride (60% conversion) in dry THF was alkylated with hexadecan-1-ol-O-methanesulphonate to afford 1-hexadecyl-2,3-di-O-benzyl-D-glyceritol *(2)*. Catalytic hydrogenolysis of compound *(2)* in chloroform-methanol with Pd-C(10%) led to the removal of the benzyl proup and formation of 1-hexadecylglyceritol *(3)*. The next step in the synthetic sequence was a reaction selective for the primary hydroxyl over the secondary hydroxyl group in the compound *(3)*. The trityl group had frequently been used as a selective protecting group in phospholipid synthesis[18], but as an alternative method we have re-examined a silylation-desilylation sequence. Silylation of compound *(3)* with tert-butyldimethylchlorosilane was very selective, giving the monosilyl ether *(4)* in nearly quantitative yield. Esterfication of the compound *(4)* with a series of fatty acids under esterification condition afforded the compound ***(5a-5d)***, the reaction conditions and results were given in Table 1:

Table 1. ***Esterification of 1-hexadecyl-3-t-butyldimethylsily-D-glycerol(4)with fatty acids***

No	Esterification Reagents	Temp.(°C)	R.t.(days)	Yield(%)
5a	α-linolenic acid anhydride, DMAP/CH_2Cl_2	22-25	3-4	70
5b	Palmitic acid, DCC, DMAP/CH_2Cl_2	22-25	3-4	60
5c	Oleic acid, DCC, DMAP/CH_2Cl_2	22-25	3-4	60.9
5d	Acrylic acid, DCC, DMAP/CH_2Cl_2	22-25	3-4	60

The tert-butyldimethylsilyl group had been considered unsuitable as a protecting group for diacyl glycerol because of the standard methods for removal accompanied by extensive acyl migration[19]. However, we had found that pyridinium p-toluenesulfonate(PPTS)which was a weaker acid (PH=3.0 in 1.0 M aqueous solution)than acetic acid(PH=2.4 in 1.0M aqueous solution)[20] in dichloromethane-methanol might be mild enough to be used for this deprotection without any acyl migration under carefully controlled conditions. Therefore, by using PPTS with compounds ***(5a-5d)*** , we were able to obtain key intermediate compounds ***(6a-6d)***, 1-hexadecyl-2-acyl-glycerol ***(6a-6d)***, without any acyl migration shown in Table 2:

Table 2. ***Deprotection of silyl of 1-hexadecyl-2-acyl-3-t-butyldimethylsilylglycerol(5a-d)***

No	Reagent	Temp.(°C)	R.t.(days)	Yield(%)
6a	PPTS	0- -5	4-7	80
6b	PPTS	0- -5	4-7	86.1
6c	PPTS	0- -5	4-7	66.7
6d	PPTS	0- -5	4-7	75

Alcohols ***(6a-6d)*** were converted to optically pure phosphocholine ether-esters by using the standard procedure shown in Table 3 and Scheme 1.

Table 3. ***The formation of phosphocholine(7a-7d)with standard procedure.***

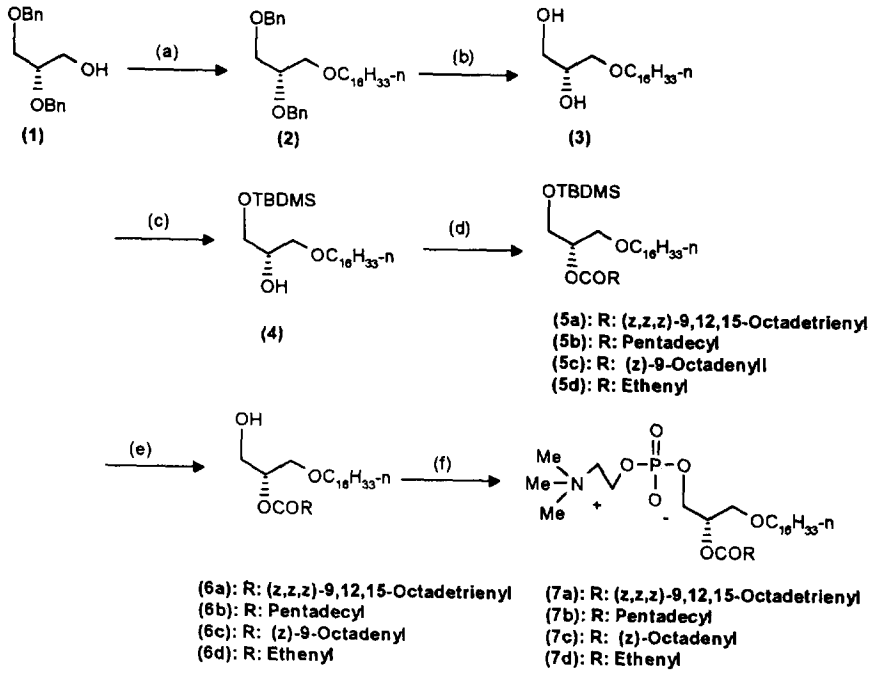
No	(i)Phospholation (ii) Amination	Yield(%)
7a	(i).$ClCH_2CH_2OP(:O)Cl_2$ (ii) Me_3N	65
7b	(i).$ClCH_2CH_2OP(:O)Cl_2$ (ii) Me_3N	64
7c	(i).$ClCH_2CH_2OP(:O)Cl_2$ (ii)Me_3N	60
7d	(i).$ClCH_2CH_2OP(:O)Cl_2$ (ii)Me_3N	63

The bilayer forming properties of phospholipids ***(7a-c)*** were studied by coating the lipid onto the wall of a round-bottomed flask(dichloromethane evaporation), dispersing the lipid into double distilled water, and irradiating the dispersion with ultrasound at 50 °C for about 10-20 minutes, TLC indicated that no lipid decomposition occured during sonication. Opalescent to optically clear aqueous dispersion were obtained. The average diameter of vesicles derived from phospholipids ***(7a-c)*** and the liposomes ***(8)*** formed by the mixture of lipid ***(7a)*** and cholesterol were shown in Table 4 and Figure 1.

Table 4. ***The average diameter of vesicles derived from phospholipids(7a-7c).***

Phospholipids	diameter(nm)	Thickness(A)
7a	1000-2300	100-150
7b	1100-1900	100-150
7c	1250-2750	100-100

Scheme 1.



Reagents and conditions:
(a).NaH/THF, refluxing, $C_{16}H_{33}OMs$, 8hr, Yield: 86%.
(b).H_2/Pd-C(10%)CH_3OH-CH_2Cl_2, r.t. 12hr, Yield: 86%.
(c).TBDMSCl/DMAP-Et_3N/CH_2Cl_2, r.t, 24hr, Yield: 93.3%.

The phase transition of phospholipids(7a-7c) shown in Table 5 was obtained by changes in absorbance at 400nm as a function of temperature which were used to monitor phase-transition behavior in the vesicular state[21].

Finally, studies were completed on the bioactivity of 1-hexadecyl-2-[α-linolenoyl]-glycerophosphocholine*(7a)*. The experimential results showed that this molecule had a ratio of inhibiting aggregation of rabbit platelete induced by platelet-activating factor(PAF) to 4.3%.

Table 5. ***The phase transition of phospholipids(7a-7c).***

Phospholipids	Temp.(°C)
7a	35
7b	37
7c	34

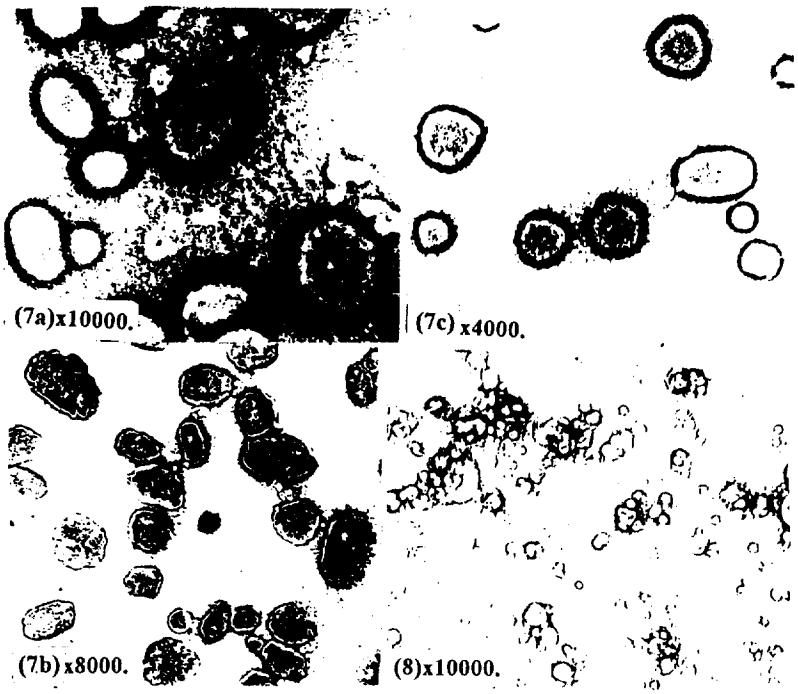


Figure 1. Electron micrographs of vesicles of phospholipids (7a-7c)and (8).

In conclusion, we, for the first time, described a convenient method for the synthesis of the novel analogues of PAF, 1-hexadecyl-2-acyl-sn-glycerophosphocholine started from D-mannitol through a new deprotecting group approach when t-butyldimethylsily as a protecting group for hydroxy-functions without any acyl migration and described their properies.

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