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An Efficient and Stereospecific Synthesis of 1-Stearoyl-2-[(z,z,z)-9,12,15-Linolenoyl]-sn-Glycerophosphocholine and Its Bioactivity

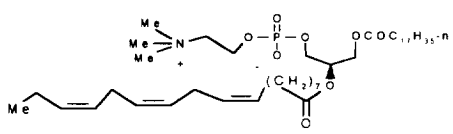
Jie Xia and YongZheng Hui*

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

Abstact: A polyunsaturated mixed-acid phosphatidylcholine, 1-stearoyl-2-linolenoyl-sn-glycerophosphocholine **(1)** prepared from D-mannitol as an optically active starting material and its bioactivity are described.

Current investigations of the structure of biological membrane require efficient methods for the preparation of phospholipids. In particular, there is a growing need for mixed acid phospholipids with defined fatty acid composition[1], for example, as (1) components of artificial membranes for physicochemical studies[2], fluorescent[3], radiolabelled[4] and spin-labelled probes[5] for the study of membrane motion, and (2) photoactivatable probes for investigation of protein-lipid interactions[4a,c,d,6]. A commonly employed method for synthesis involves specific deacylation of phospholipase A_2 and reacylation of the resulting 2-lysophospholipids with the desired acid.

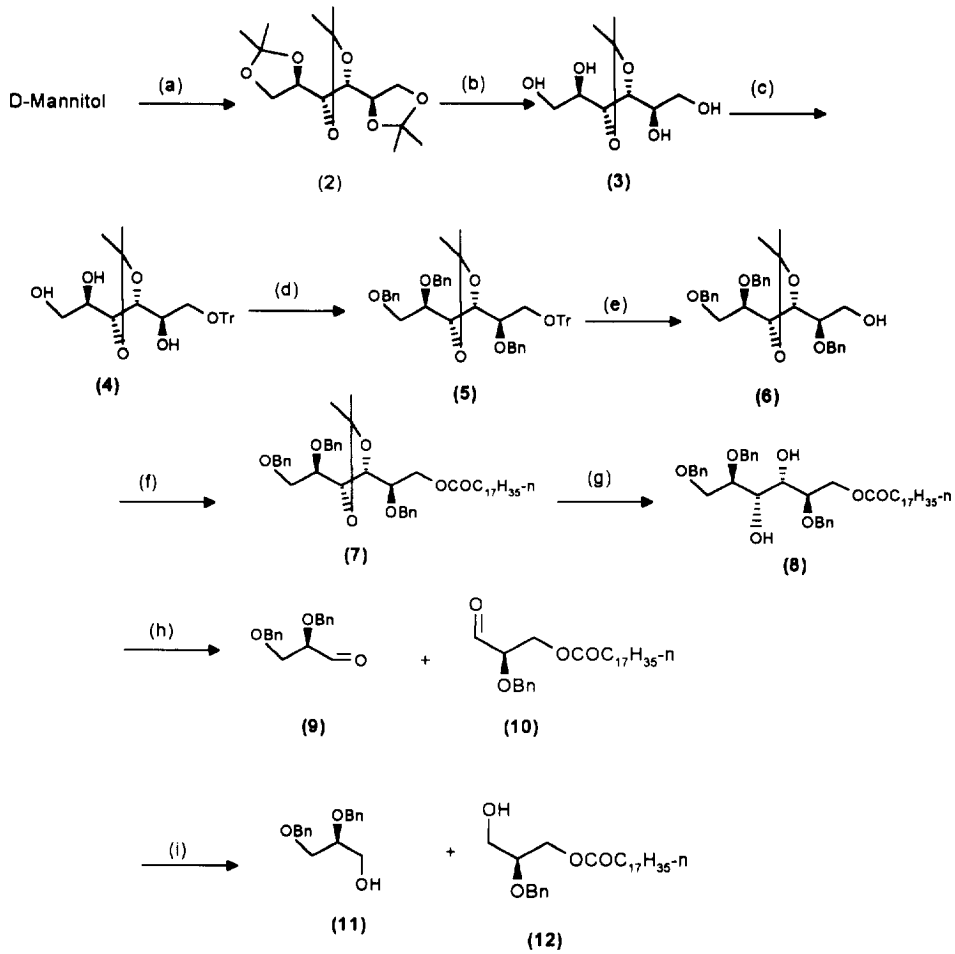
Liposome technology has provided a powerful tool for efficient drug delivery and targeting[7], as a number of pharmaceuticals have been encapsulated in liposome form or attached to the surface of liposomes by a labile bond[8], and (z,z,z)-9,12,15-linolenic acid has been known as an essential fatty acid for humans, its deficiency will alter membrane function, both in the brain as well as in the peripheral nerve[9,10]. Furthermore, it has also been reported capable of reducing the incidence and multiplicity of chemically-induced colon turmors[11,12,13], decrease the yield of chemically-induced mammary tumors[14,15,16] and inhibit the hairlessness of mice[17]. These exciting results inspired us to re-investigate the synthesis of 1-stearoyl-2-[(z,z,z)-9,12,15-linolenoyl]-sn-glycerophosphocholine in which α-linolenic acid is desired to be released by hydrolysis with phospholipase A_2 when it is inserted in the 2-position of glycerophospholipid containing glycerol. Although 1-stearoyl-2-linolenoyl-sn-glycerophosphocholine[18] was semisynthesized through reacylation of 2-lysophospholipid prepared from specific deacylation of DSPC by phospholipase A_2. In the present communication, we first described the total synthesis of 1-stearoyl-2-[(z,z,z)-9,12,15-linolenoyl]-sn-glycerophosphocholine from D-mannitol as the optically active starting material.



(1)

As the natural phospholipids are in general compounds with optical activity, we synthesized the key intermediate **(12)** through 9 steps reaction using D-mannitol as the optically active starting material. The synthesis of the intermediate **(12)**[19], 1-stearoyl-2-benzyl-sn-glycerol, was outlined in Scheme 1:

Scheme 1.

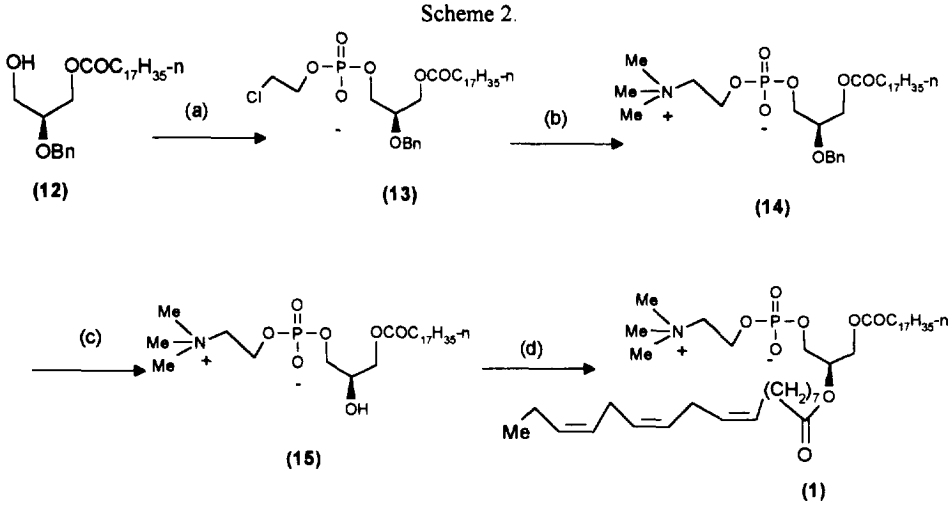


Reagents and Conditions:

(a).Acetone, con.H_2SO_4, r.t, 24h, yield 74%.
(b).HOAc(70%), 40-45 °C, 1.5h , yield 84.4%
(c).TrCl/pyridine, r.t, 4h, yield 73.2%.
(d).BnCl/KOH, 130-140 °C, 4h, yield 100%.
(e).H_2SO_4/i-C_3H_7OH-CH_3OH, r.t, 3h, yield 72.9%
(f).$C_{17}H_{35}COOH$/DCC-DMAP/CH_2Cl_2, r.t,12h, Yield 84.6%.
(g).CF_3COOH/70%$HClO_4$/CH_2Cl_2-CH_3OH, yield 70.0%
(h).$Pb(OAc)_4$/ethyl acetate, r.t, 4h, yield 85%
(i).$NaBH_4$/ CH_3OH, r.t,12h , yield 85-88%.

We attempted to ditritylation 3,4-isopropylidinene-D-mannitol **(3)**, which 1,2:3,4:5,6-triisopropylidene-D-mannitol **(2)** prepared from D-mannitol by reaction with acetone/H_2SO_4 was converted into **(3)** with trityl chloride in anhydrous pyridine, but the main product we obtained was monotrityl derivative **(4)**, which was then benzylated resulting in obtaining a derivative **(5)**. The trityl group was then selectively removed from **(5)** in methanol/2-propanol/H_2SO_4 to obtain 2,5,6-tribenzyl-3,4-isopropylidene-D-mannitol **(6)**. This was esterified in dichloromethane with stearic acid/dicyclohexylcarbodiimide(DCC)/4-(dimethylamino)pyridine to produce 2,5,6-tribenzyl-1-monostearoyl-D-mannitol **(7)**. Acidic deacetonation of **(7)** in dichloromethane/trifluoroacetic acid/70%$HClO_4$ afforded 2,5,6-tribenzyl-1-monostearoyl-D-mannitol **(8)**, the vicinal diol moiety of which, in positions 3 and 4, was cleaved with lead tetraacetate in dry ethyl acetate, and the resulting aldehyde **(10)** was then reduced with $NaBH_4$ to the key intermediate, 1-stearoyl-2-benzyl-sn-glycerol **(12)**. Phosphorylation of the intermediate **(12)**, with chloroethylphosphoric acid dichloride in dry dichloromethane/triethylamine, yielded the sn-3-phosphoric acid derivative **(13)** which had the natural confirguration , outlined in Scheme 2:

Scheme 2.



Reagents and conditions:
(a).i.$ClCH_2CH_2OP(:O)Cl_2/Et_3N$-$CH_2Cl_2$, 0-5 ^{0} C,4h, yield 86.3%. ii H_2O, r.t, 30min.
(b).Me_3N/EtOH/$CHCl_3$, sealed, 60-70 ^{0} C, 60h,yield 60%.
(c).Pd-C(10%)-H_2/CH_3OH-$CHCl_3$, r.t, 4h, yield 100%.
(d).Linolenic acid anhydride/DMAP, CH_2Cl_2, r.t, 3days, yield 65%.

The compound **(13)** was aminated by trimethylamine in chloroform/ethanol to obtain the compound **(14)**, which was debenzylated to lysophosphocholine **(15)** with Pd-C(10%)-H_2. Esterification of lysophosphocholine **(15)** in dichloromethane with linolenic acid anhydride/DMAP[20] afforded the target molecule **(1)**.

Finally, studies were completed on the bio-activity of 1-stearoyl-2-[(z,z,z)-9,12,15-linolenoyl]-sn-glycerophosphocholine **(1)**. The experimental results showed that this molecule could inhibit aggregation of rabbit platelets induced by platelet-activating factor(PAF).

In conclusion, we, for the first time, described a convenient method to totally synthesize the mixed-acid phosphatidylcholine **(1)** using D-mannitol as optically starting active material and detected its bioactivity of inhibiting aggregation of rabbit platelets induced by platelet-activating factor(PAF).

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