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The efficient synthesis of mixed diacyl phospholipids with polyunsaturated fatty acid in sn-2 position of glycerol

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Abstract: A convenient method for synthesis of the mixed-diacyl phospholipid using boron trichloride as an efficient reagent for the removal of benzyl protecting group from polyunsaturated diacylglycerols without affecting the double bond and acyl migration is described. © 1997 Elsevier Science Ltd

Current investigations of the structure of biological membranes require efficient methods for the preparation of phospholipids. In particular, there is a growing need for the mixed-acid phospholipids with defined fatty acid composition,[1] for example, as components of artificial membranes for physiochemical studies,[2] fluorescent,[3] radio-labelled[4] and spin-labelled[5] probes for the study of membrane motion and as photoactivatable probes for investigation of protein–lipid interactions[6] and signal transduction in enzymatic processing of phospholipids.[7] A commonly employed method for synthesis of mixed-acid phospholipids involves specific deacylation of phospholipase A_2 and reacylation of the resulting 2-lysophospholipids with desired acid.[8] However, the applicability of this tactic is limited to the small scale synthesis of phospholipids. Therefore, efficient synthetic methods for the preparation of mixed diacyl phospholipids has continued to be one of the most timely problems in lipid chemistry and biochemistry today.[1d,3c,6b,9] The efficient synthesis of mixed diacyl phospholipids with polyunsaturated fatty acid in sn-2 position of glycerophospholipids is described in this communication. Natural mixed diacyl phospholipids are in general compounds with optical activity. The key intermediate compounds **6a–e** were synthesized through five reaction steps using commercially available L-2,3-O-isopropylidene-sn-glycerol **1** as the optically active starting material. The strategy for synthesis of the key intermediates **6a–e** and target mixed diacyl phospholipids is outlined in Scheme 1.

Scheme 1.

Thus, L-2,3-O-isopropylidene-sn-glycerol **1** was first benzylated under benzyl chloride–NaH (60% dispersion) system in dry THF to compound **2** in good yield. The resulting compound **2** was treated with HOAc–H_2O (50%) resulting in the removal of isopropylidene protecting group from 1-benzyl-2,3-isopropylideneglycerol. Primary hydroxyl group of 1-benzylglycerol could be selectively acylated with free fatty acid using dicyclohexylcarbodiimide (DCC) in the presence of DMAP.[10] Subsequent acylation of the monosters **4a,b** with a second carboxylic acid in the presence of DCC and DMAP proceeded without deleterious 1,2-acyl migration to furnish the mixed diacyl glycerol **5a–e**. The 3,4-dimethoxylbenzyl,[6b] TBDMS,[9,11] 9-phenylxanthen-9-ol (PxOH),[12] 2,2,2-trichloroethoxycarbonyl,[13]

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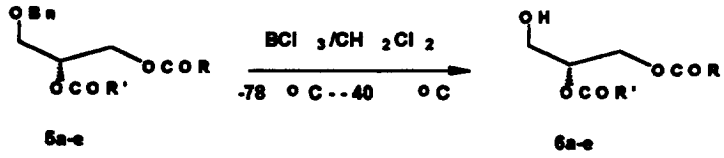
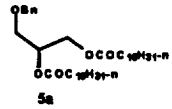
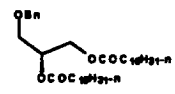
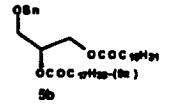
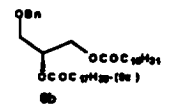
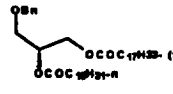
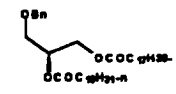
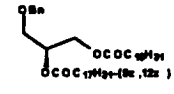
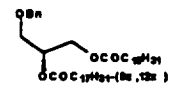
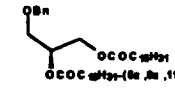
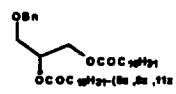
Table 1. The removal of the benzyl group from 1-benzyl-2,3-diacylglycerols with boron trichloride



5a-e 6a-e

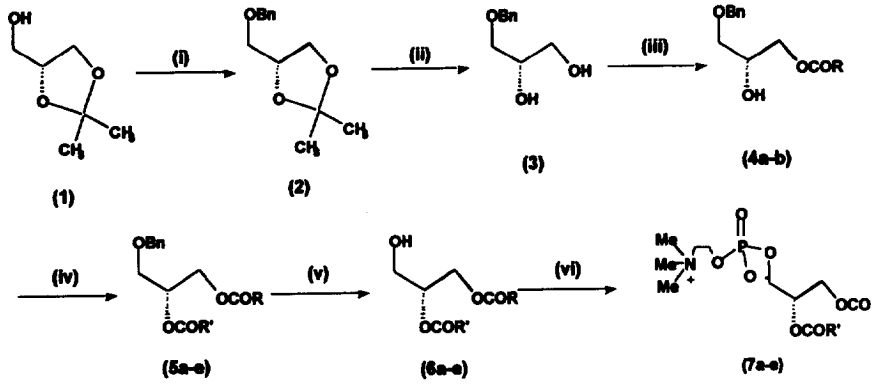
Entry	Substrate	Product	Yield(%)
1	 5a	 6a	88
2	 5b	 6b	98
3	 5c	 6c	95
4	 5d	 6d	93
5	 5e	 6e	96

trityl,[14] are widely employed protecting groups for preparation of diacylglycerols with polyunsaturated fatty acids chain instead of the benzyl protecting group because of the lack of an efficient method for removal of the benzyl protecting group without affecting the double bonds. Although there are several methods[15] in which dimethylboron bromide has been used at low temperature to deprotect acyl glycerols bearing benzyl, trityl, 4-methoxybenzyl, and isopropylidene groups, dimethylboron bromide was not an efficient reagent for the removal of the benzyl group from glycerols.[16] Therefore, the benzyl protecting group of 1-benzyl-2-3-diacylglyerols **5a–e** was efficiently removed by using boron trichloride in dry dichloromethane at −78°C to −40°C without acyl group migration which was confirmed by TLC, HPLC, ^{1}H NMR. The results are shown in Table 1.

Typical experimental: To a solution of 1-benzyl-2,3-diacylglycerol **5a–e** (1 mmol) in dry dichloromethane (6–8 ml) at −78°C to −40°C, trichloroborane (2.2 mmol) (1.0 M in dichloromethane) was added over 10 min. The reaction mixture was stirred for another 30 min under the protection of nitrogen. The reaction mixture was then poured into ice water. The dichloromethane was separated and washed with water, dried with anhydrous sodium sulfate and removed under reduced pressure at room temperature to a crude product which was purified on a silica gel column eluting with light petroleum ether–ethyl acetate 10:1 and 4:1 resulting in an oil.

Finally, the intermediate compounds **6a–e** were converted into target mixed diacyl phospholipids under the standard condition shown in Scheme 2.

In conclusion, a convenient method for synthesis of the mixed-diacyl phospholipids using boron



Reagents and Conditions: (i) BnCl/NaH-THF, rt, 12 h, Yield: 80%. (ii) HOAc-H_2O(50%), rt, 3-4 h, Yield: 85-90%. (iii) RCOOH/DCC-DMAP/CH_2Cl_2, 0 °C, 12 h, Yield: 80-85%. (iv) R'COOH/DCC-DMAP/ CH_2Cl_2, rt, 12 h, Yield: 88-90%. (v) BCl_3/CH_2Cl_2, -78 ° C - -40 ° C, 0.5-1 h, Yield: 88-98%. (vi). a) $ClCH_2CH_2OP(O)Cl_2/Et_3N$-$CH_2Cl_2$, 0-5 °C, 4-6 h, then, H_2O, 30 min, Yield: 80-90%. b) Me_3N-EtOH/$CHCl_3$, sealed, 70-80 ° C, 64-70 h, Yield: 60-65%.

Scheme 2.

trichloride as an efficient reagent for the removal of the benzyl protecting group from polyunsaturated diacylglycerols without affecting the double bond and acyl migration is described.

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