Synthesis of *cyclopentano*-N-methylphosphatidylethanolamines: aminolysis during the use of methylamine

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Summary Monomethylamine and N-benzyl-N-methylamine were used as nucleophiles in the amination of the bromoethylester of *cyclopentano*-phosphatidic acid. The former reagent led to extensive aminolysis and the quantitative formation of N-methylpalmitamide, rather than the desired *cyclopentano*-phosphatidyl-N-methylethanolamine. However, the method of Shapiro and Rabinsohn (1964. *Biochemistry.* **3:** 603–605), in which N-benzyl-N-methylamine was used as a nucleophile, was adapted for a successful synthesis.—**Pajouhesh, H, and A. J. Hancock.** Synthesis of *cyclopentano*-N-methylphosphatidylethanolamines: aminolysis during the use of methylamine. *J. Lipid Res.* 1984. **25:** 310–312.

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It has been reported that treatment of bromoalkyl esters of 1,2-diacyclglycerophosphoric acid with a variety of amines leads to good yields of nitrogenous phospholipids (1). We have evaluated an identical approach for the synthesis of nitrogenous cyclopentanoid phospholipids. This communication describes the serious drawbacks inherent in this synthetic approach when it is used for N-monomethyl-*cyclopentano*-phosphatidylethanolamine synthesis. We describe a modified procedure which gives good yield of N-monomethyl-*cyclopentano*-PE.

MATERIALS AND METHODS

Melting points were measured on a Thomas Hoover Unimelt capillary melting point apparatus and are uncorrected. Infrared spectra were measured for KBr dispersions with a Perkin-Elmer 621 spectrometer (Perkin-Elmer Corp., Norwalk, CT) and were calibrated with polystyrene. NMR spectra were obtained with a Bruker instrument (300 MHz). Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Reactions were monitored by thin-layer chromatography on silica gel G (adsorbent thickness, 250 μm; EM Laboratories, Inc., Elmsford, NY). Lipid products were purified by column chromatography on silicic acid buffered with triethylamine, essentially as described by Aneja, Chadha, and Davies (2). Phosphates were detected after analytical chromatography by the modified reagent (3) of Dittmer and Lester (4). Aqueous methylamine (40%) was obtained from Aldrich Chemical Co., Inc., Milwaukee, WI. The bromoethyl phosphoric ester of 1,3/2-dipalmitoyl cyclopentane-1,2,3-triol (**Scheme 1, 1**) was synthesized as described previously (5).

EXPERIMENTAL

Aminolysis reaction of 2-bromoethylester of all-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentane-1,2,3-triol-1-phosphoric acid with methylamine

A solution of *cyclopentano*-phosphatidic acid, bromoethylester (Scheme 1, **1**) (803 mg; 1.0 mmol), was dissolved in 60 ml of chloroform–isopropanol–dimethylformamide 1:1:4 (v/v/v) and the solution was treated at 40°C with methylamine (7 ml; 40% aqueous solution). The progress of the reaction was monitored by TLC; no phospholipid product was detected during the 16 hr reflux. Instead, a phosphate-negative product (R_f 0.70; $CHCl_3$–CH_3OH 8:1, v/v) was observed. This product was isolated after removal of solvents in vacuo, purified by silicic acid chromatography (eluting solvent $CHCl_3$–CH_3OH 96:4, v/v) and was found to be identical to an authentic sample of N-methylpalmitamide (Scheme 1, **2**). The yield of amide was >95% of the theoretical based on the weight of bromoethylester used.

Free acid form of bromoethylester of all-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentane-1,2,3-triol-1-phosphoric acid (Scheme 1, 1)

The sodium salt form of the 2-bromoethylester intermediate (Scheme 1, **1**) (1200 mg; 0.15 mmol) was converted into the free acid form by the modified Bligh and Dyer procedure as described previously (6). The acid product was precipitated from its chloroform solution by the addition of ten volumes of methanol, and used directly in the next step.

All-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentano-1-phosphoryl-N-benzyl-N-methylethanolamine (Scheme 1, 3)

This intermediate was prepared according to the procedure of Shapiro and Rabinsohn (7). To the free acid form of 2-bromoethylester of all-*trans*-2,3-dipalmitoyl-cyclopentane-1,2,3-triol-1-phosphoric acid (Scheme 1, **1**) (1000 mg; 1.28 mmol) in dry toluene (4 ml) was added

Abbreviations: PE, phosphatidylethanolamine; TLC, thin-layer chromatography.

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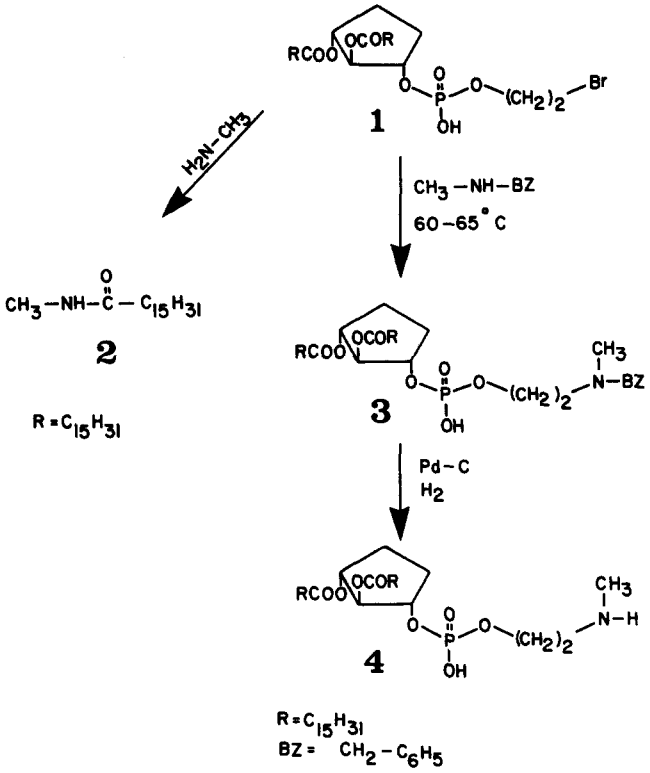

Scheme 1. Reaction scheme for synthesis of *cyclopentano*-phosphatidyl-N-methylethanolamine.

a 10-molar excess of benzylmethylamine (1.5 ml) and the mixture was maintained at 60–65°C (water bath) for 40 hr. The mixture was then cooled, the crystals of N-benzyl-N-methylamine hydrobromide were filtered off, and the filtrate was evaporated to dryness under reduced pressure.

The oily residue was dissolved in chloroform and the solution was agitated several times with aqueous HCl (0.5 N) and then washed with water (2 × 30 ml). To the separated chloroform layer were added 15 ml of methanol and 5 ml of distilled water, and the emulsion was stirred vigorously with 2.5 g each of Amberlite ion-exchange resins IR 45 and IRC 50 for 2 hr. The resin was removed by filtration and the clear filtrate was concentrated under reduced pressure to give a white solid. TLC analysis of the solid showed a major phosphate-positive spot (R_f 0.80 in $CHCl_3$–CH_3OH–H_2O 65:25:4, v/v/v). A solution of the product (800 mg) in chloroform–methanol 8:1 (v/v) containing 0.5% triethylamine was introduced to a column of silicic acid (35 g). Elution with the same solvent mixture yielded 550 mg (50%) of the N-benzyl derivative (Scheme 1, **3**), mp 65–67°C. Anal. Calc. for $C_{47}H_{84}O_8NP \cdot 1\frac{1}{2}H_2O$ (849.04) C, 66.48; H, 10.33; N, 1.64; P, 3.65. Found: C, 66.66; H, 10.61; N, 1.32; P, 3.86. IR(KBr) cm^{-1}: 3500–3550 (broad), 3040, 2900, 2840, 2650, 2500, 1735, 1455, 1410, 1365, 1215, 1165, 1055, 900, 925, 730, 695, 535, and 475.

All-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentano-1-phosphoryl-N-methylethanolamine (Scheme 1, 4)

The N-benzyl intermediate (Scheme 1, **3**) (500 mg; 0.59 mmol) was dissolved in glacial acetic acid (60 ml) at 45°C and the solution was hydrogenated at room temperature at a hydrogen pressure of 50 p.s.i. (4 hr) in the presence of palladium-charcoal catalyst 10% (500 mg). The suspension was diluted with $CHCl_3$ (15 ml), filtered over Celite, and evaporated under reduced pressure to give a yellow oil. TLC analysis showed a major phosphate-positive spot (R_f 0.65 in $CHCl_3$–CH_3OH–H_2O 65:25:4, v/v/v) and a minor spot near the solvent front. The product was purified by column chromatography (eluant $CHCl_3$–CH_3OH 4:1 (v/v) containing 0.5% triethylamine as a buffering agent). Acetone precipitation (10 vol) from a chloroform solution (1 vol) at 0°C yielded 350 mg (81%) of the pure phospholipid (Scheme 1, **4**); mp 69–70°C. Anal. Calc. for $C_{40}H_{78}O_8NP$ (731.91) C, 65.63; H, 10.74; N, 1.91; P, 4.23; N/P ratio 1.00. Found: C, 65.45; H, 10.48; N, 1.77; P, 4.14; N/P ratio 0.95. IR(KBr) cm^{-1}: 3340, 2925, 2850, 2700–2590 (broad), 1735, 1560, 1410, 1365, 1215, 1155, 1060, 900, 830, and 740.

RESULTS AND DISCUSSION

Our initial attempts to prepare an N-methyl-*cyclopentano*–PE homolog were based on a method described by Diembeck and Eibl (1) in which a phospholipid intermediate containing an ω-brominated head group is reacted with a nucleophilic reagent such as ammonia or an N-alkyl ammonia. Although our attempts to use this methodology in the amination of bromoalkyl *cyclopentano*-phospholipids with ammonia and dimethylamine were reasonably successful (5), the use of monomethylamine led to extensive deacylation of the *cyclopentano*-phospholipid intermediate. The only lipid product isolated was N-methyl palmitamide which accounted for more than 95% of the palmitate originally present in the diester. The characterization of the product was based on an empirical formula from elemental analysis, a singlet in the ^1H NMR spectrum [3.40 δ, 3H; measured in $CDCl_3$ solution], and absorption bands for secondary amide in the infrared spectrum (3500, 1655, and 1300 cm^{-1}). Diembeck and Eibl (1) have reported that the reaction of the bromoalkylester of *glycero*-phosphatidic acid with aqueous methylamine gives *glycero*-phosphatidyl-N-methylethanolamine in reasonable yield during a 6 hr reflux. In our study with the all-*trans* cyclopentanoid analog, the nucleophilic displacement of bromide ion did not proceed at a detectable rate, and longer reaction times (16 hr) at the same temperature led to the deacylation due to the general base characteristic of mono-

methylamine. We speculate that the stereochemical disposition of the ring substituents favors a particularly facile deacylation mechanism leading to the formation of N-methyl palmitamide, either from the bromoester or from the N-methylated *cyclopentano*-PE itself. Steric factors may also govern the rate at which deacylation occurs; we have observed that although tripalmitin (tripalmitoyl glycerol) also undergoes quantitative deacylation within 6 hr under the experimental conditions reported by Diembeck and Eibl (1), long chain cholesteryl esters are much less susceptible to methylamine, and even the short chain cholesteryl ester (hexanoyl) was deacylated only in about 20% yield.

Alternate route to N-methyl-*cyclopentano*-PE (Scheme 1, 4)

The procedure we adopted is based on that described by Shapiro and Rabinsohn (7). The free acid form of the bromoethyl ester of 1,3/2-dipalmitoyl-cyclopentane-1,2,3-triol (Scheme 1, **1**) was condensed with N-methylbenzylamine and the product (Scheme 1, 3) was debenzylated by catalytic hydrogenolysis using palladium-on-charcoal. It was important to remove sodium ion from the bromoethyl ester intermediate, for failure to free the compound from metallic ions inhibited the reaction, in accord with the observations of Shapiro and Rabinsohn (7) for the corresponding synthesis of the glycerolipid.

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