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Efficient synthesis of the cholinephosphate phospholipid headgroup

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Abstract

In search of an efficient method to prepare cholinephosphate headgroups in phospholipids under mild conditions (where the diacylglycerol moiety is not subject to oxidation), a method was developed for phosphorylation using a trialkyl phosphite and I_2. The active intermediate is a phosphoryl iodide formed by oxidation of the phosphite with I_2. 2-Bromoethanol, dimethyl chlorophosphite, and an alcohol (diglyceride) are converted to a phosphate triester in a one-pot reaction with high yield. In the second reaction, the phosphate triester is demethylated, and the ethyl bromide group is converted to choline by treatment with aqueous trimethylamine. This procedure is applied to the synthesis of hexadecylphosphocholine, and 1,2-didecanoyl-1-deoxy-1-thio-*sn*-glyceryo-3-phosphocholine. © 2001 Published by Elsevier Science Ireland Ltd.

Keywords: 2-Bromoethanol; Cholinephosphate; Hexadecylphosphocholine; Phosphatidylcholine; Phosphite triester; Phospholipid; Synthesis; Phosphoryl iodide

1. Introduction

Synthesis of the cholinephosphate headgroup in glycerophospholipids has been accomplished by a variety of methods (Bittman, 1993). Methods based on phosphate chemistry, involve phosphorylation of a diglyceride with phosphorus oxychloride, followed by coupling of the diacylglycerophosphoryl dichloride with choline tosylate (Brockerhoff and Ayengar, 1979), or 2-bromoethanol, and subsequent treatment with trimethylamine (Eibl and Woolley, 1988), or condensation with choline tosylate in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (Paltauf and Hermetter, 1991). Alternatively, the diglyceride is phosphorylated with 2-bromoethyl phosphoryldichloride (Hansen et al., 1982), or 2-chloro-2-oxo-1,3,2-dioxaphospholane (Chandrakumar and Hajdu, 1982; Fuji et al., 1997) followed by treatment with trimethylamine. A method based on phosphite chemistry involves treatment of a diglyceride with methyl dichlorophosphite followed by 2-bromoethanol, oxidation of the phosphite diester with H_2O_2, and quaternary amine formation with trimethylamine (Martin et al., 1994).

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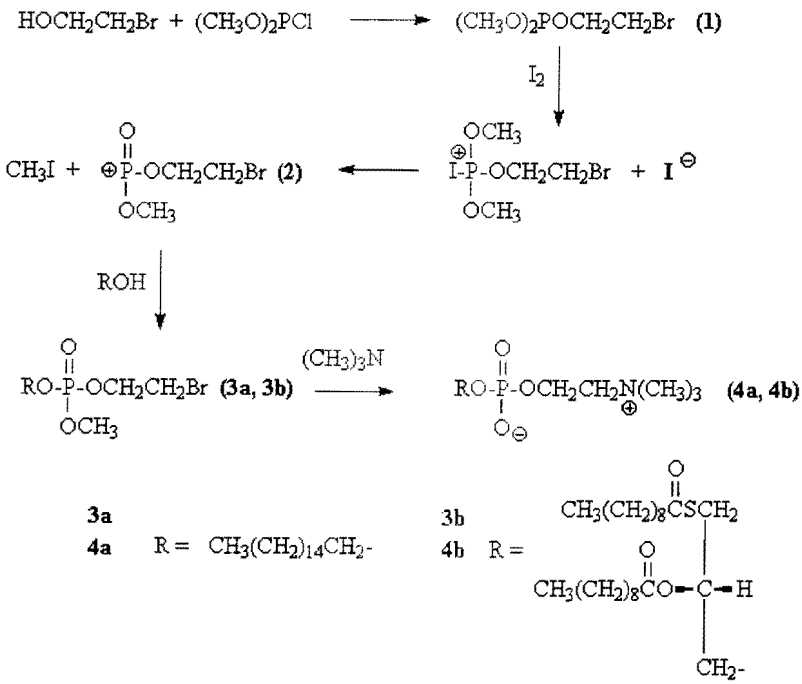
Phosphorylation based on phosphite chemistry (Beaucage and Iyer, 1992) has proven more reliable and gives higher yields than methods based on phosphate chemistry. Watanabe et al. (1993) reported a method involving activation of trialkylphosphites with pyridinium bromide perbromide in the presence of triethylamine. Later, Stowell and Widlanski (1995) published a method for phosphorylation using a trialkyl phosphite and I_2. They reported near-quantitative yields under exceptionally mild conditions. Since, the active intermediate is a phosphoryl iodide formed by oxidation of the phosphite with I_2, this method avoids subjecting the diacylglycerol moiety to oxidizing conditions. We have successfully used this method, to synthesize phosphate monoesters of inositol (Hendrickson and Hendrickson, 1998), and Gefflaut et al., 1997 used this method in an efficient synthesis of bromoacetol phosphate, and dihydroxyacetone phosphate. In search of an efficient method to prepare choline phosphate headgroups in phospholipids under mild conditions (where the diacylglycerol moiety is not subject to oxidation), we have developed the procedure reported here and shown in Scheme 1. 2-Bromoethanol, and dimethyl chlorophosphite are converted to the phosphate triester (**3a**, and **b**) in a one-pot reaction with high yield. In the second reaction, the phosphate triester is demethylated and the ethyl bromide group is converted to choline by treatment with aqueous trimethylamine. This procedure is applied to the synthesis of hexadecylphosphocholine and 1,2-didecanoyl-1-deoxy-1-thio-*sn*-glyceryo-3-phosphocholine (Hendrickson and Hendrickson, 1990).

2. Experimental

2.1. Materials and analytical procedures

1,2-Didecanoyl-1-deoxy-1-thio-*sn*-glycerol was synthesized according to the procedure of Hendrickson and Hendrickson (1990). Dimethylchlorophosphite was synthesized from phosphorus trichloride, and trimethylphosphite according to Nagai et al. (1989). 2-Bromoethanol, hexadecanol, and trimethylamine (40% in H_2O)



Scheme 1. Synthesis of cholinephosphate diesters via phosphite triester and I_2.

were obtained from Aldrich Chemical Co., St. Louis, MO. Methylene chloride and pyridine were dried by distillation from calcium hydride. Column chromatography was performed on silica gel 60 (75–150 μm: Analtech, Newark, DE), and TLC on silica gel plates. ^{1}H NMR spectra were determined at 200 MHz.

2.2. Hexadecyl methyl 2-bromoethylphosphate (3a)

2-Bromoethanol (redistilled, 0.37 g, 3 mmol) was added to a solution of 1.26 g of dry pyridine and 30 ml of dry CH_2Cl_2 with stirring in an ice bath. Dimethylchlorophosphite (0.40 g, 3.2 mmol) in 2 ml of CH_2Cl_2 was added drop-wise, and stirring was continued for 10 min. A stoichiometric amount of I_2 (about 0.55 g, titrated to just colorless by a small addition of dimethylphosphite, if necessary) was added. A copious precipitate formed, and after a few min the reaction mixture was filtered at room temperature and returned to an ice bath. Hexadecanol (0.36 g, 1.5 mmol) in 2 ml of CH_2Cl_2 and 0.2 ml of pyridine was immediately added drop-wise, and the reaction was followed by, TCL (silica gel; hexane-acetone, 7:3). The reaction was complete after about 45 min. The reaction mixture was diluted with an equal volume of CH_2Cl_2, washed with 10% $NaHSO_4$ (a small amount of methanol was added to help break the emulsion), 10% sodium phosphate buffer (pH 7), dried over Na_2SO_4, and rotovaped to dryness with additions of absolute ethanol to remove any water. The crude product was dissolved in hexane and chromatographed on a silica gel column with increasing amounts of acetone in hexane. Pure (by TLC) **3a** (0.54 g, 82% yield) eluted with 8% acetone. ^{1}H NMR ($CDCl_3$/CD_3OD) δ 0.88 (t, $J = 6.7$ Hz, 3H), 1.24–1.27 (m, 28H), 1.6–1.7 (m, 2H), 3.5–3.6 (m, 2H), 3.79 (d, $J = 11.4$ Hz, 3H), 4.0–4.1 (m, 2H), 4.25–4.35 (m, 2H).

2.3. Hexadecylphosphocholine (4a)

Hexadecyl methyl 2-bromoethylphosphate (**3a**) (0.22 g, 0.5 mmol) was dissolved in 2 ml CH_2Cl_2, 3.3 ml acetonitrile, 3.3 ml isopropanol, and 5 ml 40% aqueous trimethylamine. The reaction was stirred at room temperature overnight. TLC (silica gel; $CHCl_3$:CH_3OH:H_2O, 65:35:3) indicated the reaction was complete. The solvent was removed in vacuo, and the residue was shaken with a mixture of 9 ml H_2O, 9 ml $CHCl_3$, and 12 ml CH_3OH. The $CHCl_3$ layer was separated and the aqueous layer again extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried over Na_2SO_4, and then rotovaped. The crude product was dissolved in $CHCl_3$:CH_3OH (10:1), and chromatographed on a silica gel column with increasing amounts of CH_3OH in $CHCl_3$, followed by $CHCl_3$–CH_3OH–H_2O. Pure (by TLC) **4a** (0.16 g, 80% yield), eluted with $CHCl_3$:CH_3OH:H_2O, 65:35:4. ^{1}H NMR ($CDCl_3$/CD_3OD) δ 0.88 (t, $J = 6.5$ Hz, 3H), 1.35–1.45 (m, 26H), 1.75–1.85 (m, 2H), 3.22 (s, 9H), 4.0–4.1 (m, 2H), 4.15–4.25 (m, 2H).

2.4. Methyl 1,2-didecanoyl-1-deoxy-1-thio-sn-glycero-3-phospho-2-bromoethanol (3b)

Compound **3b** was synthesized in 64% yield from 2-bromoethanol and 1,2-didecanoyl-1-deoxy-1-thio-*sn*-glycerol by the same procedure as for **3a**. Pure (by TLC) **3b** eluted from a silica gel column with 13% acetone. ^{1}H NMR ($CDCl_3$/CD_3OD) δ 0.88 (t, $J = 7.0$ Hz, 6H), 1.2–1.3 (m, 24H), 1.6–1.7 (m, 4H), 2.32 (t, $J = 7.5$ Hz, 2H), 2.56 (t, $J = 7.5$ Hz, 2H), 3.01–3.34 (m, 2H), 3.54 (t, $J = 6.6$ Hz, 2H), 3.81 (dd, $J = 11.3$, 2.6 Hz, 3H), 4.13–4.20 (m, 2H), 4.26–4.38 (m, 2H), 5.08–5.19 (m, 1H).

2.5. 1,2-Didecanoyl-1-deoxy-1-thio-sn-glycero-3-phosphocholine (4b)

Compound **4b** was synthesized in 71% yield from **3b** by the same procedure as for **4a**. Pure (by TLC; R_f identical to same compound synthesized earlier, Hendrickson and Hendrickson, 1990) **4b** eluted from a silica gel column with $CHCl_3$:CH_3OH:H_2O, 65:35:2. The NMR spectrum was identical (under the same conditions), to that of the same compound synthesized earlier

(Hendrickson and Hendrickson, 1990). ^{1}H NMR ($CDCl_3/CD_3OD$) δ 0.88 (t, $J=6.9$ Hz, 6H), 1.2–1.3 (m, 24H), 1.5–1.7 (m, 4H), 2.30 (t, $J=7.3$ Hz, 2H), 2.54 (t, $J=7.5$ Hz, 2H), 2.9–3.0 (m, 1H), 3.23 (s, 9H), 3.25–3.35 (m, 1H), 3.5–3.6 (m, 2H), 4.2–4.3 (m, 2H), 5.0–5.1 (m, 1H).

3. Results and discussion

2-Bromoethanol, dimethyl chlorophosphite, and an alcohol (hexadecanol or 1,2-didecanoyl-1-deoxy-1-thio-*sn*-glycerol) were converted in high yield, in a one-pot reaction, to the corresponding methyl alkyl 2-bromoethylphosphate. Subsequent treatment of this product with aqueous trimethylamine, resulted in both demethylation of the methylphosphate and conversion of the 2-bromoethyl moiety to choline. The conditions of the first reaction are very mild and do not subject the diglyceride moiety to oxidative conditions. This is important in the synthesis of the thiolester analog **3b**, which is subject to oxidation at the sulfur atom and labile to hydrolytic cleavage. Conversion of the methyl alkyl 2-bromoethylphosphate (**3a**, and **b**) to the alkyl cholinephosphate (**4a**, and **b**) (see Woolley and Eibl, 1988), which involves both demethylation and quaternary amine formation, is accomplished in a single reaction with trimethylamine. The yield of hexadecylphosphocholine was high, whereas the yield of the thiolester analog (**4b**) was somewhat lower due to hydrolysis of the thiolester and ester groups under the basic aqueous conditions of the reaction. Nevertheless, the overall yield of **4b** was much higher than that achieved in the previous method with $POCl_3$ and choline tosylate (Hendrickson and Hendrickson, 1990).

This method can also be used for the synthesis of other phospholipids, such as, phosphatidyl–ethanolamine, and -serine, using suitably-protected alcohols in place of 2-bromoethanol. A simple high-yield synthesis of 1,2-dialkylphosphatidylmethanol was accomplished (Ghomaschi F., unpublished results), by reacting 1,2-dialkylglycerol with trimethylphosphite and I_2, followed by monodemethylation of the dimethyl alkylphosphate with LiBr.

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