Pergamon

*Tetrahedron Letters*, Vol. 35, No. 32, pp. 5783-5784, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01179-6

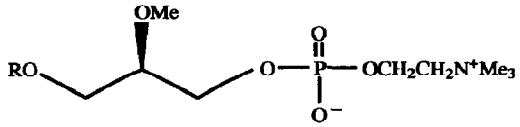
# Antitumor Phospholipids: A One-pot Introduction of a Phosphocholine Moiety into Lipid Hydroxy Acceptors

Ravi Kumar Erukulla, Hoe-Sup Byun, and Robert Bittman*

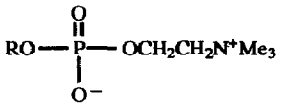
Department of Chemistry and Biochemistry, Queens College of The City University of New York, Flushing, New York 11367-1597 U.S.A.

**Abstract:** A high-yielding, 3-step, one-pot conversion of lipid hydroxy acceptors **2** into clinically useful alkylphosphocholines **1** is reported. Reaction of **2** with ethylene chlorophosphite gave phosphite **3**, which underwent oxidation and ring opening with bromine in $CH_2Cl_2$ to give (2-bromoethyl)phosphate ester **4**; hydrolysis of the P-Br bond and quaternization of **4** with aqueous trimethylamine generated **1**.

Alkylphosphocholines **1** represent a new class of antitumor agents.[1] In contrast to conventional anticancer drugs, the antineoplastic phospholipids exert their effects at the membrane level, where they interfere with many cellular functions, including signal transduction pathways, phospholipid metabolism, permeability, differentiation, and invasive activity.[2] Alkylphosphocholines are prepared by attaching a phosphocholine moiety onto lipid hydroxy acceptors by a multistep route that involves a phosphitylation[3] or phosphorylation[4] reaction and appropriate oxidation, coupling, and deprotection steps. Since the known methods have the drawbacks of multiple steps and low to moderate yields (45-60%),[5] a new method for the preparation of the clinically important[6] drugs **1a-c** would be desirable.



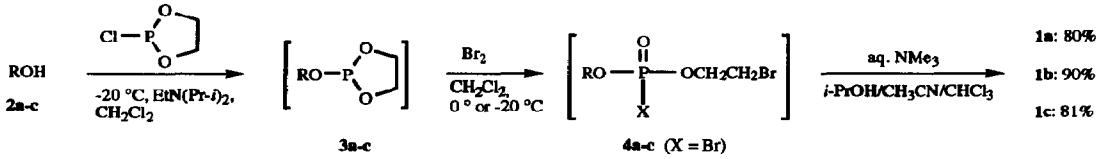

edelfosine, R = $C_{18}H_{37}$
**1a:** R = $C_{16}H_{33}$




**1b:** R = $C_{16}H_{33}$ (miltefosine, D-18506)
**1c:** R = *n*-$C_8H_{17}CH{=}CH(CH_2)_{12}$-

Here we report a simple one-pot method for insertion of a phosphocholine moiety into 1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol (**2a**), hexadecanol (**2b**), and erucyl alcohol (C22:1-13Δ-*cis*, **2c**), using commercially available ethylene chlorophosphite (Scheme 1). This procedure combines four operations in one pot. First, ethylene chlorophosphite reacts rapidly with alcohols **2**. Note that since ethylene chlorophosphite contains an ethylene unit, the second coupling reaction for choline insertion that is used with other phosphitylating agents[7] is not required. Second, the two operations of oxidation of phosphite **3** and opening of the dioxaphospholane are carried out by simply adding bromine[8] in methylene chloride to provide (2-bromoethyl)phosphate ester **4**. In this manner, the two-step procedure formerly used[5] to provide **4** (X = Cl) is avoided. Third, hydrolysis of the P-Br bond, and fourth quaternization of **4** are achieved by use of aqueous trimethylamine; previously, prolonged heating of cyclic phosphate triesters with anhydrous trimethylamine (gas) in a pressure tube has been used for quaternization.[4,5] Scheme 1 illustrates the application of this methodology for the synthesis of **1a-c** in high yields.[9] The method is applicable to unsaturated glycerol derivatives such as **2c** with minor modification.[10]

**Scheme 1**




In summary, a simple one-pot method involving five sequential reactions (phosphitylation, P(III) oxidation, ring opening, hydrolysis, and amination) in three operations for the preparation of antitumor alkylphosphocholines **1** is reported. The new methodology avoids the multistep route currently in use for insertion of the phosphocholine moiety into **2**.

**Acknowledgments.** This research was supported by National Institutes of Health Grant HL-16660. The FAB mass spectra were obtained at the Michigan State University of Mass Spectrometry Facility.

***References***

1. Hilgard, P.; Klenner, T.; Stekar, J.; Unger, C. *Cancer Chemother. Pharmacol.* **1993**, *32*, 90-95.
2. Houlihan, W. J.; Workman, P.; Lohmeyer, M.; Cheon, S. H. *Med. Chem. Reviews* **1994**, in press.
3. Garrigues, B. *Helv. Chim. Acta* **1988**, *71*, 274-276.
4. Guivisdalsky, P. N.; Bittman, R. *J. Org. Chem.* **1989**, *54*, 4637-4642.
5. For a recent review of methods for phosphitylation and phosphorylation of glycerol derivatives, see Bittman, R. In *Phospholipids Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, 1993; pp 141-232.
6. For references to clinical uses, see: **1a**: Khanavkar, B. et al. *Contrib. Oncol.* **1989**, *37*, 224-235. **1b**: (i) Unger, C. *Onkologie* **1990**, *13*, 56. (ii) Danhauser-Riedl, S. et al. *J. Lipid Mediators* **1990**, *2*, 271-280. (iii) Danhauser-Riedl, S. et al. *Onkologie* **1991**, *14*, 392. (iv) Sindermann, H.; Junge, K.; Burk, K. *Onkologie* **1994**, *17*, 21-26. **1c**: Berger, M. R.; Eibl, H. *Proc. Am. Assoc. Cancer Res.* **1992**, *33*, 41.
7. Loffredo, W. M.; Tsai, M.-D. *Bioorg. Chem.* **1990**, *18*, 78-84.
8. Attempts to utilize oxidation reagents other than bromine were not successful. Iodine oxidized and also opened the cyclic phosphite, but the resulting (2-iodoethyl)phosphate ester was converted into a primary alcohol during the attempted quaternization with aqueous trimethylamine. Pyridinium bromide perbromide was also tried, but a complicated mixture was obtained, and product isolation was difficult.
9. A general procedure is as follows. To a solution of alcohol (**2a-c**) (1 eq) in 5 mL of $CH_2Cl_2$ were added $EtN(Pr\text{-}i)_2$ (2 eq) and ethylene chlorophosphite (1.5 eq) at -20 °C. After the mixture had stirred for 40 min at -20 °C, $Br_2$ (3 eq) was added at 0 °C. The reaction mixture was stirred for another 10 min, then the solvent was removed under reduced pressure. The residue was dissolved in 6 mL of $CH_3CN$-*i*-PrOH-$CHCl_3$ (3.0/3.0/1.8), and 6 mL of 45% aqueous trimethylamine was added. After the mixture had stirred at room temperature overnight, the solvents were removed, the residue was passed through a TMD-8 ion exchange column. The fractions containing the crude product were pooled and concentrated, giving a residue that was purified by chromatography (elution with MeOH or 4:1 MeOH/$CHCl_3$). After silica gel was removed by filtration, the filtrate was lyophilized ($C_6H_6$) to give **1a-c** (80-90%) as white powders.[11]
10. To prepare **1c**, 4 eq of $EtN(Pr\text{-}i)_2$ and 2.25 eq of ethylene chlorophosphite were used. After the reaction proceeded at -20 °C for 30 min, the mixture was stirred at rt for 3.5 h. Bromine (3 eq) was added at -20 °C.
11. All compounds were characterized by MS and NMR. **1a**: HRMS [FAB, $(MH)^+$]: Calcd for $C_{25}H_{54}NPO_6$ 496.3767. Found 496.3764. **1b**: HRMS [FAB, $(MH)^+$]: Calcd for $C_{21}H_{47}NPO_4$ 408.3243. Found 408.3251. **1c**: HRMS [FAB, $(MH)^+$]: Calcd for $C_{27}H_{57}NPO_4$ 490.4025. Found 490.3993.

(*Received in USA* 12 *May* 1994; *accepted* 15 *June* 1994)