2'-(Trimethylammonio)ethyl 4-(Hexadecyloxy)-3(S)-methoxybutanephosphonate: A Novel Potent Antineoplastic Agent

Robert Bittman,*,† Hoe-Sup Byun,†
Brenda Mercier,‡ and Hassan Salari‡,§

Department of Chemistry and Biochemistry, Queens College of The City University of New York, Flushing, New York 11367, Department of Medicine, University of British Columbia, and I. P. I., Vancouver, British Columbia, Canada V6H 3Z6

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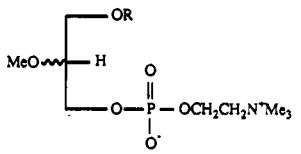
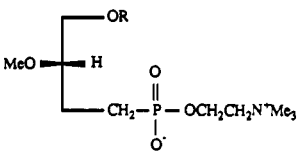
Synthetic ether lipids that have a 16- or 18-carbon alkyl chain at the *sn*-1 position of glycerol, a methoxy group or similar nonmetabolizable group at the *sn*-2 position, and a phosphocholine moiety or quaternary ammonium moiety at the *sn*-3 position inhibit tumor cell growth.[1] Clinical trials are currently in progress with various alkyl phospholipids, including 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (1, ET-18-OCH_3);[2] [3-(hexadecylthio)-2-(methoxymethyl)propyl]phosphocholine (BM 41.440), which has a thioether group at the *sn*-1 position and a methoxymethyl group at the *sn*-2 position;[3] the cyclic ether analogue SRI 62-834;[4] and hexadecylphosphocholine, which lacks the glycerol moiety.[5]

Unlike many other antitumor agents, alkyl phospholipids do not interact with DNA but instead are targeted to membranes, where they affect a variety of growth factor signaling pathways, modulate activities of enzymes involved in lipid metabolism, and induce perturbations in membrane structure and permeability.[6] Although alkyl phospholipids such as 1 accumulate in the membranes of



rac-ET-18-OCH_3 (1) (R = $C_{18}H_{37}$)



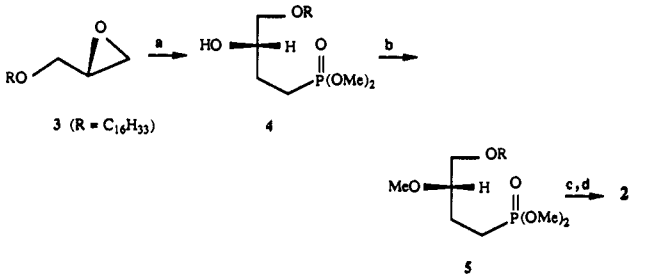
2 (R = $C_{16}H_{33}$)

some cytotoxically sensitive cells,[7] they may become degraded because of susceptibility to hydrolysis by phospholipase C or D, which cleave the phosphate bond on the glycerol or choline side, respectively. Therefore, an isosteric analogue of 1 that would be resistant to phospholipase C may represent a new antineoplastic ether lipid which is degraded only very slowly in neoplastic cells.

We outline here a convenient and novel method for the preparation of isosteric phosphonocholine 2 that can also be used to synthesize other phospholipid analogues; the method is based on nucleophilic opening of an epoxide by a phosphonate anion in the presence of BF_3·Et_2O. Note that 2 is an isostere of 1, since it has a methylene group substituted for the oxygen atom connecting phosphorus to glycerol at the *sn*-3 position. In a colonogenic assay with a monocytic leukemic cell line (WEHI-3B), 2 was found to have a cytotoxic potency comparable to that of 1, which is generally regarded as the prototype for the alkyl phospholipid analogues. Phosphonocholine 2 was

† Queens College of the City University of New York.
‡ University of British Columbia.
§ I.P.I.

Scheme I.[a] Synthesis of Phosphonolipid 2



[a] Reagents: (a) $LiCH_2P(O)(OMe)_2$, BF_3·Et_2O, THF, −78 to −20 °C; (b) CH_2N_2, SiO_2, 0 °C; (c) Me_3SiBr, CH_2Cl_2, room temperature, 2 h; then, THF–H_2O (8:1); (d) choline tosylate, Cl_3CCN, py, 50 °C, 48 h.

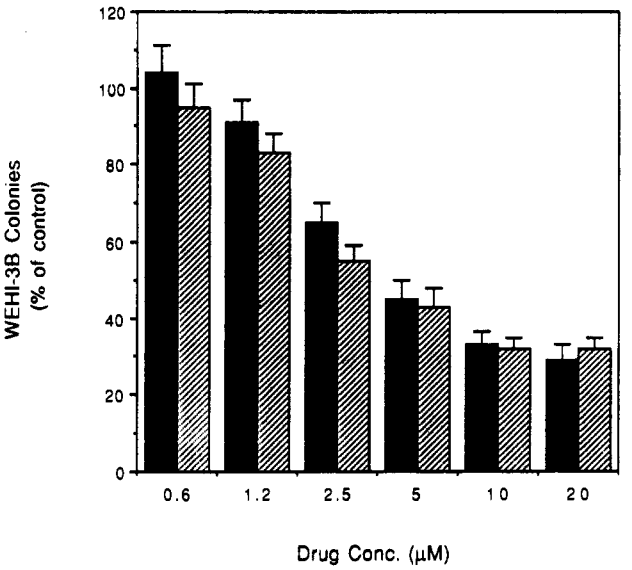


Figure 1. Effects of 1 (filled box) and 2 (hatched box) on WEHI-3B cell growth. Cells were cultured as reported elsewhere,[17] and the colonogenic assay was carried out using methyl cellulose/RPMI in the presence of various concentrations of 1 and 2 for 5 days. The number of colonies present after 5 days in 60-mm Petri dishes was counted and plotted as the percentage of the number of colonies detected in untreated (control) samples. The data are the mean of three experiments carried out in triplicate ± SD.

also found to be at least equally effective as 3-*O*-hexadecyl-2-*O*-methyl-*sn*-glycero-1-phosphocholine [(*S*)-ET-16-OCH_3)] with respect to delaying tumor growth implanted in BALB/C mice.[8]

Scheme I shows the synthetic route from hexadecyl (*S*)-2-oxiranylmethyl ether (3) to 2.[9,10] Lithium dimethyl methanephosphonate was prepared by adding a solution of *n*-butyllithium (40 mmol, 16 mL of a 2.5 M solution in hexane) to a solution of 4.97 g (40 mmol) of dimethyl methanephosphonate in 30 mL of dry THF at −78 °C. Then, BF_3·Et_2O (5 mL, 40 mmol) was added, followed by a solution of 5.24 g (20 mmol) of 3 in 100 mL of THF. The mixture was stirred for 3 h at −78 °C and for 1 h at −20 °C and then quenched with saturated aqueous ammonium chloride solution. Dimethyl 4-(hexadecyloxy)-3(*S*)-hydroxybutanephosphonate (4) was obtained in 89% yield after flash chromatography (elution with chloroform–methanol 25:1). Hydroxy phosphonate 4 was methylated by a modification of a method described previously.[11] Dimethyl 4-(hexadecyloxy)-3(*S*)-methoxybutanephosphonate (5) was isolated in 88% yield as a colorless oil after flash chromatography (elution with chloroform–methanol

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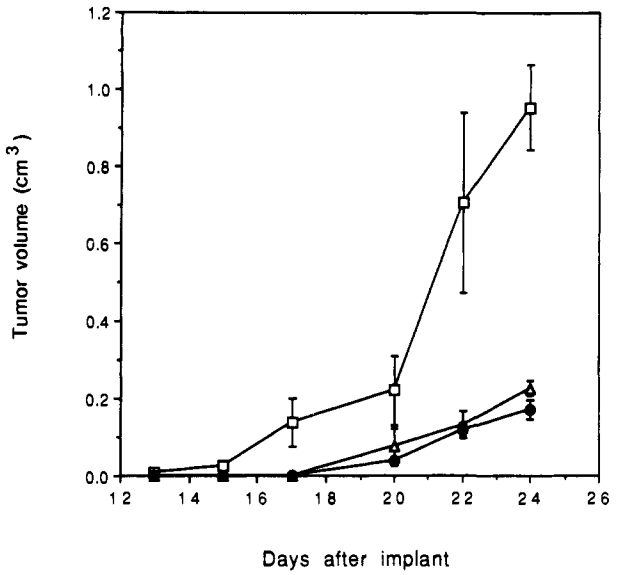

Figure 2. Effects of (*S*)-ET-16-OCH_3 and **2** on the growth of WEHI-3B tumors in BALB/C mice. Mice were injected subcutaneously in the back with 1×10^6 WEHI-3B cells. After 3 days of cell implantation, the mice received 200 μL of saline (□), 500 μg per 200 μL of **2** (●), or 500 μg per 200 μL of (*S*)-ET-16-OCH_3 (△) once per day intramuscularly in the leg. The tumors were measured using a caliper. Data are the mean of five mice ± SD. (*S*)-ET-16-OCH_3 was prepared as described previously.[11]

50:1). The methyl esters were removed by hydrolysis using trimethylsilyl bromide followed by THF–water 8:1, affording the corresponding phosphonic acid as a white solid in quantitative yield. The choline group was coupled to the phosphonic acid as described previously,[12] giving 2′-(trimethylammonio)ethyl 4-(hexadecyloxy)-3(*S*)-hydroxybutanephosphonate (**2**) in 69% yield after silica gel column chromatography (elution with chloroform–methanol–ammonium hydroxide–water 65:35:3:2).

We compared the in vitro and in vivo cytotoxicity of **1** and **2** to WEHI-3B cells. When antineoplastic activity was assessed by colonogenic assay[13] using WEHI-3B cells, **1** and **2** showed comparable cytotoxic properties ($IC_{50} \sim 2.5$ μM) (Figure 1). When BALB/C mice bearing WEHI-3B received once daily doses of (*S*)-ET-16-OCH_3 or **2**, tumor volume was reduced markedly; **2** was at least as effective as (*S*)-ET-16-OCH_3 (Figure 2).

In summary, phosphonolipid analogues of **1** are promising new antineoplastic agents.[14] Compound **2**, which was made via opening of epoxide **3** by $LiCH_2P(O)(OMe)_2$ using BF_3 catalysis, is representative of a new anticancer agent that may be more effective in vivo than many previously used ether-linked phospholipids. Its lack of susceptibility to degradation by cytosolic phospholipase C, which is widely distributed throughout the body,[15] is a potential advantage since the lipid product of the phospholipase C reaction (1-*O*-alkyl-2-*O*-methylglycerol) is known to affect the activities of important regulatory enzymes and may thus modulate cell function.[16]

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Supplementary Material Available: Listings of physical and analytical data (IR, ^1H NMR, specific rotations, and elemental analyses) of compounds **2**, **4**, and **5** (1 page). Ordering information is given on any current masthead page.

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(8) It has been shown previously that the stereochemistry at C2 does not affect the cytotoxicity of ET-16-OCH_3 against WEHI-3B cells, since (*R*)- and (*S*)-ET-16-OCH_3 inhibited [^3H]thymidine uptake into DNA to an equal extent: Guivisdalsky, P. N.; Bittman, R.; Smith, Z.; Blank, M. L.; Snyder, F.; Howard, S.; Salari, H. Synthesis and Antineoplastic Properties of Ether-linked Thioglycolipids. *J. Med. Chem.* **1990**, *33*, 2614–2621. Furthermore, the hexadecyl analogue of **1** has virtually identical behavior as the octadecyl compound (**1**) against WEHI-3B cells (unpublished results). It is not known, however, whether (*R*)- and (*S*)-ET-16-OCH_3 differ with respect to their susceptibility to phospholipase C catalyzed hydrolysis.
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