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Synthesis and Antitumor Activity of Ether Glycerophospholipids Bearing a Carbamate Moiety at the *sn*-2 Position: Selective Sensitivity Against Prostate Cancer Cell Lines

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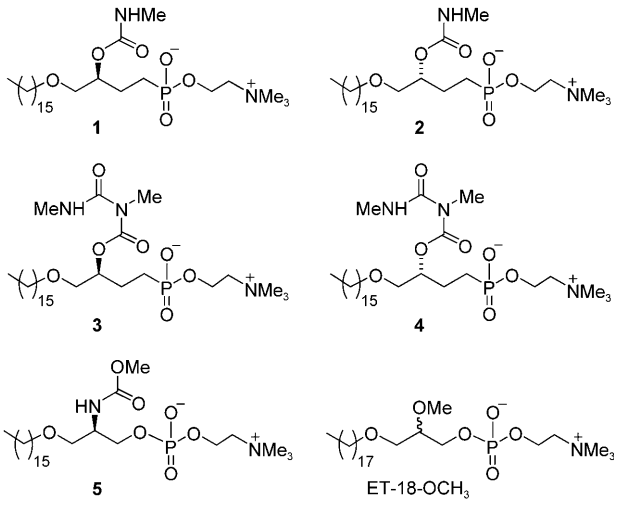
Analogues of 1-*O*-hexadecyl-*sn*-3-glycerophosphonocholine (compounds **1**–**4**) or *sn*-3-glycerophosphocholine (compound **5**) bearing a carbamate or dicarbamate moiety at the *sn*-2 position were synthesized and evaluated for their antiproliferative activity against cancer cells derived from a variety of tissues. Although all of the compounds are antiproliferative, surprisingly the carbamates (**1** and **2**) are more effective against the hormone-independent cell lines DU145 and PC3 than toward other cancer cell lines we examined. This selectivity was not observed with the dicarbamates (**3** and **4**). Phosphocholine carbamate analogue **5** is as effective against the prostate cancer cell lines as the corresponding phosphonocholine analogue **1**. Cell death induced by 2′-(trimethylammonio)ethyl 4-hexadecyloxy-3(*R*)-*N*-methylcarbamoyl-1-butanephosphonate (carbamate analogue **2**) appeared to be mediated by apoptosis, as assessed by caspase activation and loss of mitochondrial membrane potential. The in vivo activity of **2** was evaluated in a murine prostate cancer xenograft model. Oral and intravenous administration showed that **2** is effective in inhibiting the growth of PC3 tumors in Rag2M mice. Our studies show that the glycerolipid carbamates reported herein represent a class of prostate-cancer-selective cytotoxic agents.

Introduction

Prostate cancer is the most common male cancer and the second leading cause of cancer deaths in males.[1] Although androgen deprivation is initially effective, it does not cure the disease, and invariably the tumor recurs in an androgen-independent form that usually metastasizes primarily to bone.[2,3] The molecular events underlying the progression of the disease have yet to be elucidated; however, it is clear that once the disease progresses, it does not respond to the current array of chemotherapeutic agents.[4–6] There is, therefore, a clear need to develop new agents that kill hormone-independent prostate tumor cells and/or prevent their metastases.[7]

A group of antitumor compounds collectively known as antitumor ether lipids (AELs) act by perturbing intracellular signaling pathways, leading to cell death.[8–14] These compounds are long-lived analogues of the naturally occurring phospholipid lysophosphatidylcholine. Insertion of ether bonds into the *sn*-1 and *sn*-2 positions of lysophosphatidylcholine in place of the usual two ester bonds gives an analogue that is highly resistant to metabolism at sites other than in the vicinity of the phosphodiester linkage. AELs have the potential to deliver antitumor activity without any mutagenicity because, unlike many other anticancer agents, they do not interact directly with DNA. They possess cell-selective effects by inhibiting the proliferation and killing of cancer cells at concentrations that do not affect normal cells,[12,15–17] but the basis for this is unclear. The prototype or gold standard AEL is 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH_3), which inhibits a broad panel of tumor cell lines,[14,18–20] but exhibits no known selectivity against specific cancer cell types. We have



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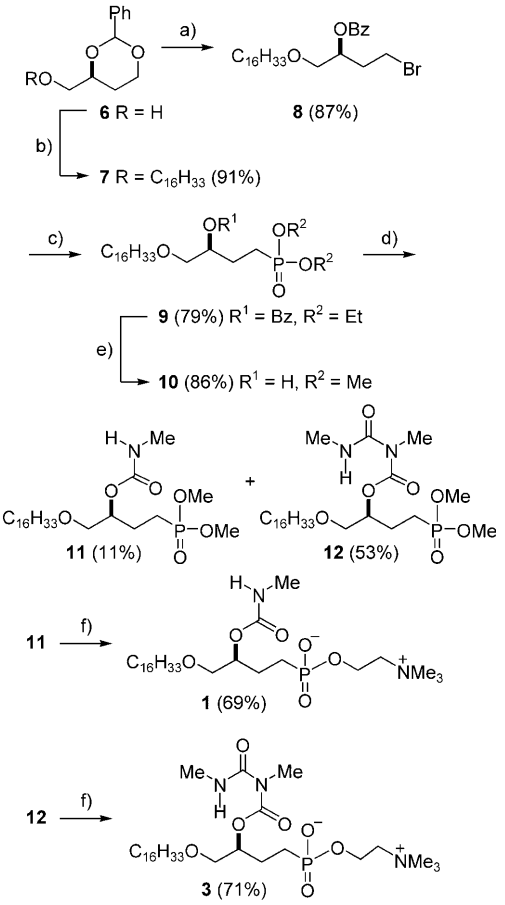
shown that replacing the phosphocholine moiety at the *sn*-3 position of ET-18-OCH_3 with a variety of glycosyl residues leads to differences in efficacy against various cell types and a difference in the mechanisms responsible for cell death.[21–23] In this study, we retained a long-chain *O*-alkyl group at the *sn*-1 position, but replaced the methoxy group at the *sn*-2 position of ET-18-OCH_3 with another hydrolytically stable group (carbamate), and we assessed the efficacy of these novel analogues against epithelial cancer cell lines. In addition, we replaced the phosphocholine head group, which is susceptible to hydrolysis catalyzed by phospholipase C, with a hydrolytically stable isosteric analogue bearing a carbon–phosphorus bond (i.e., a phosphonocholine head group). Herein we report the preferential effects of carbamate analogues **1**–**5** against hormone-independent prostate cancer cell lines. We also show that oral or intravenous delivery of the lead compound results in inhibition of the growth of prostate cancer xenografts.

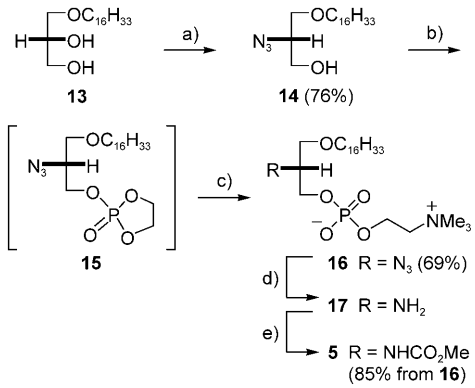
Results and Discussion

Chemistry

The synthesis of target compounds **1** and **3** commenced with (*S*)-(−)-1,2,4-butanetriol, which was converted into benzylidene acetal (*S*)-**6** as described previously[24] (Scheme 1). *O*-Alkylation of **6** under phase-transfer conditions afforded ether **7**,[24] which was treated with *N*-bromosuccinimide and barium carbonate to afford bromide **8**[24] in high yield. Arbuzov reaction of bromide **8** with neat triethyl phosphite at 150 °C gave benzoyl phosphonate **9** in 79% yield. Base-induced hydrolysis of the benzoyl group of phosphonate **9** provided hydroxyphosphonate **10** in 86% yield. During the deprotection of the benzoate group by methanolysis, the ethyl groups in phosphonate **9** were also converted into methyl groups by transesterification. Hydroxyphosphonate **10** was reacted with methyl isocyanate, which was generated in situ by the reaction of methyl iodide with potassium cyanate in tetrahydrofuran/*N*,*N*-dimethylformamide (10:1), to furnish a mixture of carbamates **11** and **12** in 11 and 53% yields, respectively. If the amount of DMF was increased, the rate of carbamate formation was faster but the yield of carbamate **11** decreased. After compounds **11** and **12** were separated by flash chromatography (eluted with a gradient of chloroform/methanol; see Experimental Section), the methyl groups in the phosphonate ester were removed by treatment of **11** or **12** with trimethylsilyl bromide in dichloromethane. The resulting phosphonates were then coupled to a choline group with choline toluenesulfonate promoted by trichloroacetonitrile in pyridine at 50 °C[25] to afford the target phosphonocholines **1** and **3** in good yield. Similarly, the target compounds **2** and **4** (the respective enantiomers of **1** and **3**) were prepared by starting from commercially available 3(*R*)-1,3-*O*-benzylidine-1,3,4-butanetriol (which is prepared from D-malic acid).[26]



Scheme 1. Synthesis of compounds **1** and **3**. *Reagents and conditions*: a) NBS, $BaCO_3$, $ClCH_2CH_2Cl$, reflux, 4 h; b) NaH, nBu_4NBr, THF, RT, 24 h; c) $(EtO)_3P$, 150 °C, overnight; d) MeI, KNCO, nBu_4NBr, $(iPr)_2NEt$, THF/DMF (10:1); e) NaOMe, MeOH, RT; f) 1. Me_3SiBr, CH_2Cl_2, RT, 2. choline toluenesulfonate, Cl_3CCN, py, 50 °C, 48 h.



Scheme 2. Synthesis of compound **5**. *Reagents and conditions*: a) Ph_3P, DIAD, Me_3SiN_3, CH_2Cl_2, 0 °C then RT; b) 2-chloro-2-oxo-1,3,2-dioxaphospholane, $(iPr)_2NEt$, CH_2Cl_2, 0 °C then RT; c) 1. Me_3SiBr, CH_2Cl_2, 2. $Me_3N_{(aq)}$, $CHCl_3$/MeCN/*i*PrOH (0.9:1.5:1.5), RT, 48 h; d) Pd/C, H_2, EtOH, RT, overnight; e) $ClCO_2Me$, Et_3N, $CHCl_3$, 0 °C then RT, overnight.

As depicted in Scheme 2, the preparation of carbamate phosphocholine **5** started with 3-*O*-hexadecyl-*sn*-glycerol (**13**), which was prepared from 1,2-*O*-isopropylidene-*sn*-glycerol via *O*-alkylation and deketalization, as described previously.[27] Regioselective and stereospecific azidation of glycerol **13** with azidotrimethylsilane under Mitsunobu conditions[28] gave azido alcohol **14** in 76% yield. Insertion of the phosphocholine head group into alcohol **14** provided *sn*-2-azidoglycerophosphocho-

line **16** in 69% overall yield by the following sequence of reactions: 1) phosphorylation of alcohol **14** with 2-chloro-2-oxo-1,3,2-dioxaphospholane, 2) ring opening of phospholane intermediate **15** with trimethylsilyl bromide, and 3) quaternization of the resulting ring-opened product with aqueous trimethylamine. Finally, reduction of the azido group of **16** by catalytic hydrogenation, followed by reaction of amine **17** with methyl chloroformate, furnished the target carbamate phosphocholine **5** in 85% overall yield.

Biology

Growth-inhibiting activity

The ability of compounds **1**–**5** to inhibit growth of a number of epithelial cancer cell lines was assessed using the CyQuant assay.[21] The cell lines were derived from breast (MCF-7, BT549, MDA-MB-231), lung (A549), cervix (HeLa), prostate (DU145, PC3), kidney (A498), and liver (HepG2). The compounds were added to exponentially growing cells, and the cells were incubated with the drugs for 48 h. The results are shown in figure 1 of the Supporting Information, and the IC_{50} values derived from these data are listed in Table 1. Compound **2** inhibited the proliferation of all of the cell lines with the exception of HepG2 cells. The most striking observation was an apparent higher sensitivity of the two hormone-insensitive prostate cancer cell lines, DU145 and PC3, to compound **2** relative to the concentrations required to inhibit the growth of most of the other cell lines. The IC_{50} value for **2** against DU145 and PC3 cells is 1.2 μM. Whereas compound **2** completely inhibited proliferation at 4 μM, a concentration of at least 12 μM was required to elicit a similar effect on the other cell lines with the exception of HeLa cells (Supporting Information figure 1). The dicarbamate analogue **4** was less active than the corresponding carbamate analogue **2** and, with the exception of BT549 cells, the IC_{50} value and concentrations needed to completely inhibit growth were greater than that obtained for compound **2**. In addition, the apparent preferential effect of **2** against prostate cancer cell lines relative to the other cells was not observed with **4**. To examine the influence of chirality on the activity, we synthesized compounds **1** and **3** (the respective enantiomers of **2** and **4**). Compound **1** was active in the same range as **2** and displayed a similar selectivity for DU145 and PC3 as compound **2**. Compound **3** was found to be less active than **4** and showed no selectivity for prostate cancer cells.

Table 1. Growth inhibitory potency of carbamate and dicarbamate compounds **1**–**5** against a panel of human cancer cell lines.

	IC_{50} [μM][a]				
Cell Line	**1**	**2**	**3**	**4**	**5**
DU145	2.1	1.2	8.2	4.8	2.4
PC3	2.5	1.2	4.1	2.8	1.9
BT549	6.8	4.4	6.6	4.9	13.4
MDA-MB-231	6.1	3.7	6.6	18.3	10.5
MCF-7	10.1	4	>20	13.4	9.1
HeLa	7.9	3.3	14.9	8.7	19.3
A549	>20	10.7	>20	>20	>20
HepG2	>20	>20	>20	>20	>20
A498	13.0	9.5	16.1	13.5	12.7

[a] IC_{50}: compound concentration that effects 50% growth inhibition; values were determined from growth curves (see Supporting Information) after 48 h incubation with **1**–**5** (0–20 μM); each sample is the mean of eight experiments.

Compound **5** differs from compounds **1** and **2** in two structural features: first, it has an isosteric phosphocholine moiety in place of the phosphonocholine head group; second, the carbamate connectivity to the glycerol backbone is reversed, as in this analogue there is an *N*-carbamoyl group in place of the *O*-carbamoyl group. The activity of compound **5** against the prostate cancer lines DU145 and PC3 was similar to the activity observed for compounds **1** and **2**, and was less active against cancer cells derived from other tissues (Table 1; figure 1, Supporting Information).

Toxicity

We compared the toxicity of the phosphonocholine carbamates **1** and **2** with that of phosphonocholine dicarbamate **4** against DU145 and PC3 cells (Figure 1). Compound **2** had LD_{50}

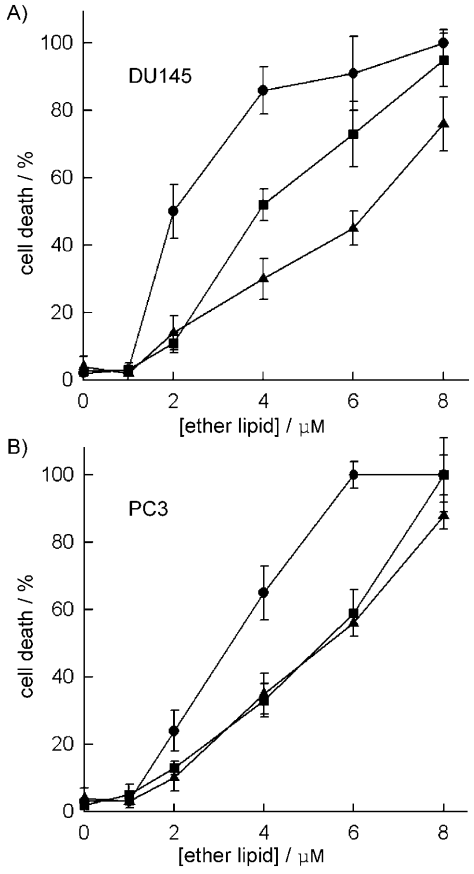


Figure 1. Effects of compounds **1** (■), **2** (●), and **4** (▲) on the viability of A) DU145 and B) PC3 prostate cancer cells. Cell viability was assessed with the ToxiLight assay in 96-well plates. Data represent the mean ± SD of eight replicates.

values of 3.3 and 2.0 μM for PC3 and DU145 cells, respectively, compared with LD_{50} values of 5.4 μM for PC3 and 3.9 μM for DU145 obtained with compound **1**. The values for dicarbamate **4** were 5.4 μM for PC3 and 6.3 μM for DU145. Compound **2** was more active than compound **4** against DU145 cells. Thus, glycerolipid carbamates **1**, **2**, and **5** and dicarbamates **3** and **4** are effective antiproliferative and cytotoxic compounds against epithelial cancer cells and represent novel analogues of AELs. Unexpectedly, the hormone-independent prostate cancer cell lines DU145 and PC3 appeared to be more affected by **1**, **2**, and **5** than the epithelial cancer cell lines derived from the other tissues investigated.

These two prostate cancer cell lines are well-known cellular models of the hormone-insensitive stage of prostate cancer,[29,30] the most deadly form of the disease that does not respond to current conventional chemotherapy. Because hormone-insensitive prostate cancer cells are highly sensitive to compounds **1**, **2**, and **5**, we postulate that it may be possible to selectively kill prostate cancer cells with low concentrations of the carbamate analogues of ET-18-OCH_3.

Carbamate 2 induces apoptosis in DU145 and PC3 cells

The alkyllysophospholipid class of AELs kill cells by apoptosis.[14,17,21] To investigate if compound **2** induces apoptosis, we examined the levels of activated caspases in the cells using a general caspase assay kit. Incubation of DU145 or PC3 cells with compound **2** at 5 μM for 8 h led to a 2.8- to 3.6-fold increase in the levels of activated caspases (Figure 2). We also

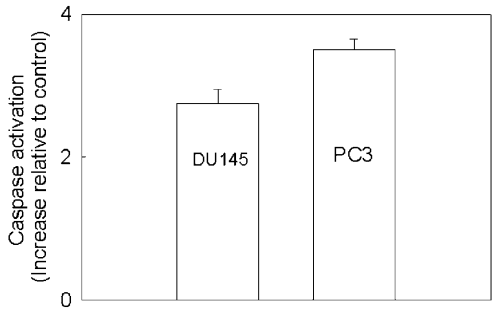


Figure 2. Effect of compound **2** on caspase activity in prostate cancer cell lines DU145 and PC3. Cells were incubated with compound **2** at 5 μM for 8 h. Caspase activity was measured by the CaspGlow assay[21,28] with FITC–VAD–FMK using a fluorescence plate reader. Data represent the mean ± SD of five replicates.

assessed the effect of compound **2** on the mitochondrial membrane potential in DU145 and PC3 cells by using the JC-10 assay, which determines the loss of mitochondrial membrane potential as a measure of irreversible apoptosis.[21,31] Incubation of DU145 cells with compound **2** at 5 μM for 8 h resulted in a change in the red color of the aggregates in the mitochondria to the monomeric green color, indicative of the loss of mitochondrial membrane potential in these cells (Figure 3). Similar results were obtained with PC3 cells (see figure 2, Supporting Information). These observations indicate that cell death by

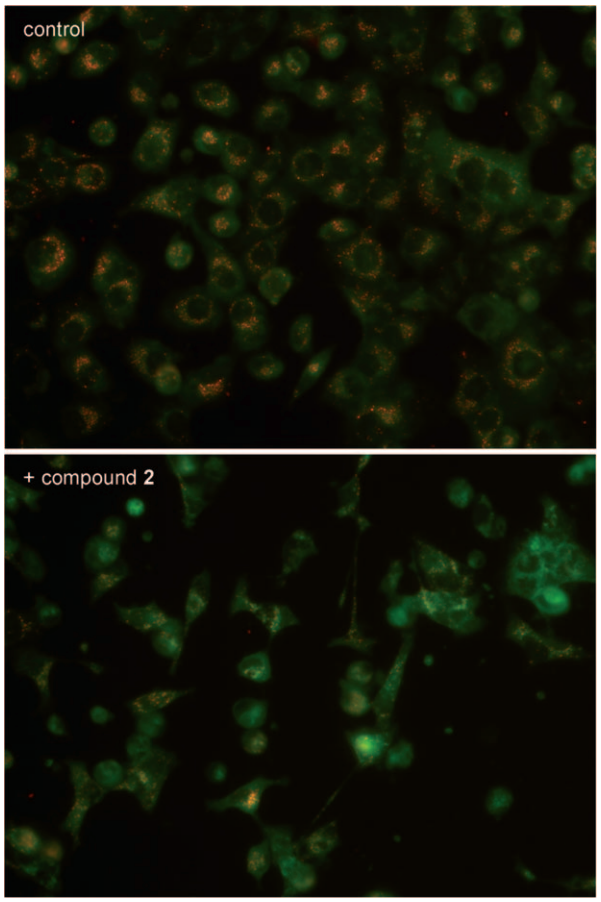


Figure 3. Effect of compound **2** on the mitochondrial membrane potential of DU145 cells. Cells were incubated with or without compound **2** (5 μM) for 8 h. The mitochondrial membrane potential was assayed with the JC-10 reagent.

carbamate **2** involves induction of apoptosis. However, further studies are required to establish why the DU145 and PC3 prostate cancer cells are more susceptible to these compounds relative to other cell types.

Effect of 2 on PC3 xenograft growth

To investigate the activity in vivo, we examined the efficacy of compound **2** in inhibiting tumor growth in Rag2M mice implanted with PC3 cells. Animals were given either 30 or 50 mg kg^{-1} orally or 10 or 15 μg kg^{-1} of compound **2** intravenously. These concentrations, which were established from tolerability studies, had no toxic effects on the animals. The animals were segregated into groups based on the sizes of the tumors at the start of the treatment. The experimental animals received compound **2** at the start of the treatment, and once a week for two weeks for a total of three treatments.

In the animals with a starting tumor size of 30 mm^3, oral treatment with 30 mg kg^{-1} of **2** delayed growth to 300 mm^3 by 9 days, while the delay for those receiving 50 mg kg^{-1} of **2** orally was 24 days. Growth to 500 mm^3 was delayed by 14 and 28 days, respectively, in animals given 30 and 50 mg kg^{-1} (Figure 4 A). At an initial tumor volume of 80 mm^3, a 12 day delay

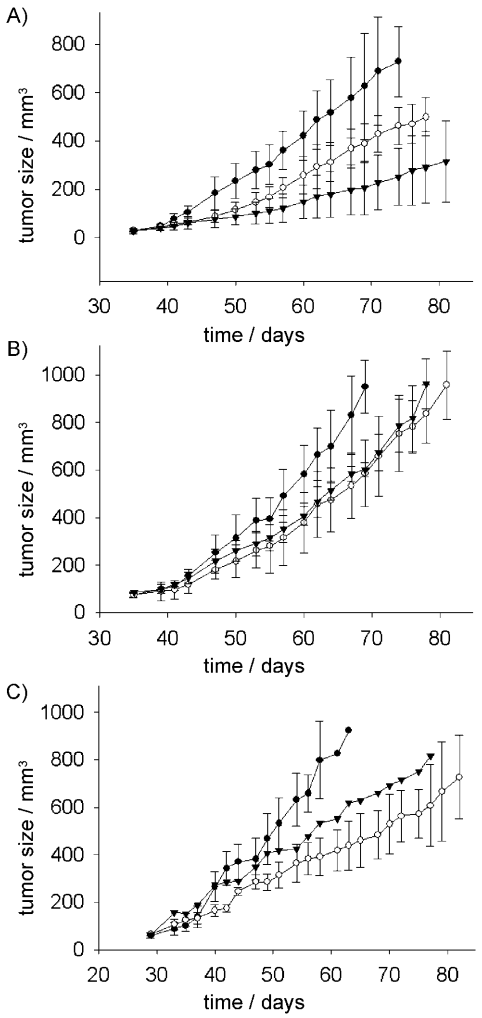


Figure 4. Effect of compound **2** on the growth of PC3 tumor xenografts in Rag2M mice. Mice bearing PC3 tumors were given three treatments of compound **2** orally or by intravenous injection at seven-day intervals. A) oral treatment of a 30-mm^3 tumor: control (●), 30 mg kg^{-1} (○), 50 mg kg^{-1} (▼); B) oral treatment of a 80-mm^3 tumor: control (●), 30 mg kg^{-1} (○), 50 mg kg^{-1} (▼); c) intravenous treatment of a 60-mm^3 tumor: control (●), 15 μg kg^{-1} (○), 10 μg kg^{-1} (▼). Data represent the mean ±SD of four animals; data points with no error bars represent the means of two animals.

was observed in the growth of the tumor to 950 mm^3 in animals given 50 or 30 mg kg^{-1} (Figure 4B). Not surprisingly, the effectiveness of the compound was dependent on the size of the tumor at the start of drug treatment, with the effect on smaller tumors being greater than that on larger tumors. The delays were shorter with increasing size of the tumor.

In animals receiving intravenous injections of **2**, growth of the tumor from 60 to 800 mm^3 was delayed by 14 days with three treatments of compound **2** at 15 μg kg^{-1} (Figure 4C). The delay with animals administered compound **2** at 10 μg kg^{-1} was 9 days.

The results of the PC3 xenograft studies (Figure 4) show the potential utility of carbamate **2** in inhibiting the growth of prostate tumors. The compound caused a significant decrease in tumor growth for both oral and intravenous administration.

Conclusions

In summary, we discovered a selective toxicity of the carbamate and dicarbamate glycerophospholipids and phosphonolipids against two hormone-independent prostate cancer cells lines, DU145 and PC3. In addition, compound **2** inhibited the growth of PC3 cells in an in vivo study using both oral and intravenous administration to Rag2M mice. In contrast to the present study in which compound **2** was administered only once per week, a recent study has indicated that frequent oral administration of ET-18-OCH_3 is needed to increase oral bioavailability.[32] The results reported herein warrant further investigation to optimize the frequency of dosing to achieve greater shrinkage and/or inhibition of tumor growth.

Experimental Section

Chemistry

THF was distilled from Na and benzophenone before use. Pyridine, DMF, $ClCH_2CH_2Cl$, $(iPr)_2NEt$, and CH_2Cl_2 were dried over CaH_2. ^1H, ^{13}C, and ^{31}P NMR spectra were recorded at 400, 100, and 161.9 MHz, respectively, and were referenced to the residual $CHCl_3$ at $\delta = 7.24$ (^1H) and $\delta = 77.00$ ppm (^{13}C) and to 85% H_3PO_4 (^{31}P). Optical rotations were measured in a cell with a path length of 1 dm on a digital polarimeter. TLC was carried out on Al-backed silica gel GF plates (250 μm thickness), and the compounds were visualized by charring with 10% H_2SO_4 in EtOH and/or short wavelength UV light. The products were purified by flash chromatography on silica gel 60 (230–400 ASTM mesh). HRMS data were obtained by electrospray ionization.

(3*S*)-4-*O*-Hexadecyl-1,3-*O*-benzylidine-1,3,4-butanetriol (7).[24] (3*S*)-1,3-*O*-Benzylidine-1,3,4-butanetriol (**6**)[24] (3.89 g, 20.0 mmol) in 20 mL THF was added to a suspension of NaH (1.60 g, 40.0 mmol; 60% in white oil, washed twice with dry hexane) in 100 mL dry THF at 0 °C. After the evolution of H_2 gas had stopped, 1-bromohexadecane (6.2 mL, 20.2 mmol) and nBu$_4$NBr (0.67 g, 2.0 mmol) were added. The mixture was stirred for 24 h at room temperature, and then the reaction was quenched with 10 mL MeOH. The volatile solvents were removed under reduced pressure, and the residue was diluted with 200 mL Et_2O and washed with H_2O. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel (elution with hexane/EtOAc 25:1) to afford **7** (7.62 g, 91%) as a white solid; mp: 54–55 °C; ^1H NMR ($CDCl_3$): $\delta =$ 0.88 (t, 3H, $J = 6.6$ Hz), 1.25 (br s, 26H), 1.54–1.60 (m, 3H), 1.80–1.91 (m, 1H), 3.44–3.52 (m, 3H), 3.62 (dd, 1H, $J = 5.8$, 10.3 Hz), 3.95–4.01 (m, 1H), 4.01–4.09 (m, 1H), 4.29 (dd, 1H, $J = 4.1$, 11.4 Hz), 5.53 (s, 1H), 7.31–7.38 (m, 3H), 7.49 ppm (dd, 2H, $J = 1.6$, 7.9 Hz); ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 22.7, 26.1, 28.3, 29.3, 29.5, 29.6, 29.7, 30.9, 31.9, 66.9, 71.9, 73.7, 76.3, 101.2, 126.1, 128.2, 128.7, 129.0, 129.7, 138.6 ppm; MS (ES) $[M+H]^+$ m/z calcd for $C_{27}H_{47}O_3$: 419.3, found: 419.2.

(3*R*)-4-*O*-Hexadecyl-1,3-*O*-benzylidine-1,3,4-butanetriol. The enantiomer of **7** was prepared in 90% yield from (3*R*)-1,3-*O*-benzylidine-1,3,4-butanetriol[33] by the procedure described above.

3(*S*)-Benzoyl-4-hexadecyloxy-1-bromobutane (8).[24] A mixture of **7** (5.19 g, 12.4 mmol), NBS (2.65 g, 14.9 mmol, and $BaCO_3$ (1.10 g, 5.57 mmol) in 100 mL CCl_4 (or $ClCH_2CH_2Cl$) was heated at reflux for 4 h. The reaction mixture was passed through a pad of silica gel,

which was rinsed with 100 mL hexane/EtOAc (10:1). The filtrate was concentrated to give a residue, which was purified by column chromatography on silica gel (elution with hexane/EtOAc 25:1) to provide bromide **8** (5.36 g, 87%) as a pale-yellow oil; $[\alpha]_D^{25} = -24.9$ ($c = 6.7$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.88$ (t, 3H, $J = 6.6$ Hz), 1.26 (br s, 26H), 1.50–1.56 (m, 2H), 2.31–2.41 (m, 2H), 3.42–3.50 (m, 4H), 3.62–3.65 (m, 2H), 5.35–5.41 (m, 1H), 7.42–7.46 (m, 2H), 7.54–7.59 (m, 1H), 8.05 ppm (dd, 2H, $J = 1.4$, 8.4 Hz); ^{13}C NMR ($CDCl_3$): $\delta =$ 14.1, 22.6, 26.0, 28.7, 29.3, 29.4, 29.5, 29.6, 31.9, 34.5, 71.2, 71.7, 71.7, 128.3, 129.6, 130.0, 133.0, 165.9 ppm; MS (ES) $[M+H]^+$ *m/z* calcd for $C_{27}H_{46}BrO_3$: 497.2, found: 497.2.

3(*R*)-Benzoyl-4-hexadecyloxy-1-bromobutane. The enantiomer of bromide **8** was prepared in 85% yield from (3*R*)-4-*O*-hexadecyl-1,3-*O*-benzylidine-1,3,4-butanediol by the procedure described above; $[\alpha]_D^{25} = +20.5$ ($c = 6.9$, $CHCl_3$).

Diethyl 3(*S*)-benzoyl-4-hexadecyloxy-1-butanephosphonate (9). A solution of bromide **8** (4.98 g, 10.0 mmol) in 25 mL $(EtO)_3P$ (150 mmol) was heated at 150 °C (oil bath temperature) overnight. After excess $(EtO)_3P$ was removed by using a stream of air, the residue was purified by column chromatography on silica gel (elution with $CHCl_3$/MeOH 25:1) to give benzoyl phosphonate **9** (4.39 g, 79%) as a colorless oil; $[\alpha]_D^{25} = -6.88$ ($c = 5.8$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.88$ (t, 3H, $J = 6.7$ Hz), 1.26 (br s, 26H), 1.28–1.33 (m, 6H), 1.70–2.00 (m, 2H), 3.43–3.62 (m, 4H), 4.06–4.12 (m, 4H), 5.23–5.27 (m, 1H), 7.42–7.46 (m, 2H), 7.54–7.59 (m, 1H), 8.04 ppm (d, 2H, $J = 7.0$ Hz); ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 16.4 (d, $J = 5.8$ Hz), 22.2 (d, $J = 143.2$ Hz), 22.6, 24.3 (d, $J = 4.0$ Hz), 29.3, 29.4, 29.6, 29.7, 31.9, 61.6, 71.3, 71.7, 72.8 (d, $J = 18.1$ Hz), 127.7, 128.3, 129.6, 130.1, 133.0, 166.0 ppm; MS (ES) $[M+H]^+$ *m/z* calcd for $C_{31}H_{56}O_6P$: 555.4, found: 555.3.

Diethyl 3(*R*)-benzoyl-4-hexadecyloxy-1-butanephosphonate. The enantiomer of benzoyl phosphonate **9** was prepared in 80% yield from 3(*R*)-benzoyl-4-hexadecyloxy-1-bromobutane by the procedure described above; $[\alpha]_D^{25} = +6.71$ ($c = 6.0$, $CHCl_3$).

Dimethyl 4-hexadecyloxy-3(*S*)-hydroxy-1-butanephosphonate (10).[25] Na^0 (0.18 g, 7.83 mmol) was added to 100 mL dry MeOH. After complete disappearance of Na^0, a solution of phosphonate **9** (3.91 g, 7.05 mmol) in 10 mL dry MeOH was added. After the mixture was stirred overnight, the reaction was quenched by the addition of 500 μL HOAc (8.73 mmol) and then concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel (elution with $CHCl_3$/MeOH 10:1) to provide hydroxyphosphonate **10** (2.56 g, 86%) as a colorless oil; $[\alpha]_D^{25} = -5.20$ ($c = 5.0$, C_6H_6); ^1H NMR ($CDCl_3$): $\delta = 0.88$ (t, 3H, $J =$ 6.8 Hz), 1.26 (br s, 26H), 1.54–1.58 (m, 2H), 1.70–1.90 (m, 4H), 2.78 (br s, 1H), 3.29 (dd, 1H, $J = 7.1$, 9.4 Hz), 3.40–3.48 (m, 3H), 3.74 (d, 6H, $J = 10.8$ Hz), 3.70–3.85 ppm (m, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 14.0$, 20.7 (d, $J = 142.1$ Hz), 22.6, 26.0, 26.1 (d, $J = 4.7$ Hz), 29.3, 29.4, 29.5, 29.6, 31.9, 52.3, 69.8 (d, $J = 14.8$ Hz), 71.6, 74.4; ESI-HRMS $[M+H]^+$ *m/z* calcd for $C_{22}H_{48}O_5P$: 423.3234, found: 423.3242.

Dimethyl 4-hexadecyloxy-3(*R*)-hydroxy-1-butanephosphonate. The enantiomer of hydroxyphosphonate **10** was prepared in 88% yield from diethyl 3(*R*)-benzoyl-4-hexadecyloxy-1-butanephosphonate by the procedure described above; $[\alpha]_D^{25} = +5.22$ ($c = 5.1$, C_6H_6).

Dimethyl 4-hexadecyloxy-3(*S*)-*N*-methylcarbamoyl-1-butanephosphonate (7) and dimethyl 4-hexadecyloxy-3(*S*)-[*N*-(*N*′-methylcarbamoyl)-*N*-methylcarbamoyl]-1-butanephosphonate (11). MeI (3.2 mL, 51.4 mmol) and $(iPr)_2NEt$ (1.8 mL, 10.4 mmol) were added to a mixture of KNCO (8.11 g, 100 mmol), nBu_4NBr (3.22 g, 10 mmol), and hydroxyphosphonate **10** (2.12 g, 5.02 mmol) in 25 mL THF/DMF (10:1). The mixture was stirred until compound **10** was completely consumed. The reaction mixture was diluted with $CHCl_3$ and washed with brine solution. The organic layer was dried over Na_2SO_4 and concentrated. The product was purified by column chromatography on silica gel (elution with a gradient of $CHCl_3$, $CHCl_3$/MeOH 100:1, $CHCl_3$/MeOH 50:1, then $CHCl_3$/MeOH 25:1) to give carbamoyl phosphonate **11** (265 mg, 11%) and dicarbamoyl phosphonate **12** (1.43 g, 53%) as colorless oils. Data for compound **11**: $[\alpha]_D^{25} = -5.82$ ($c = 5.5$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta =$ 0.88 (t, 3H, $J = 6.4$ Hz), 1.26 (br s, 26H), 1.48–1.62 (m, 2H), 1.15–2.00 (m, 4H), 2.87 (d, 3H, $J = 4.8$ Hz), 3.25–3.50 (m, 6H), 3.75 (d, 6H, $J =$ 10.8 Hz), 4.90–5.00 (m, 1H), 7.60–7.70 ppm (br s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 20.7 (d, $J = 151$ Hz), 22.6, 24.0 (d, $J = 5.0$ Hz), 26.0, 26.1, 26.4, 29.3, 29.4, 29.5, 29.6, 31.9, 52.4, 67.8, 74.4 ppm (d, $J =$ 6.0 Hz), 153.7; ^{31}P NMR ($CDCl_3$): $\delta = 35.5$; MS (ES) $[M+H]^+$ *m/z* calcd for $C_{24}H_{51}NO_6P$: 480.3, found: 480.3. Data for compound **12**: $[\alpha]_D^{25} =$ -4.70 ($c = 5.6$, $CHCl_3$); ^1H NMR ($CDCl_3$): $\delta = 0.88$ (t, 3H, $J = 6.0$ Hz), 1.25 (br s, 26H), 1.50–1.62 (m, 2H), 1.75–1.86 (m, 2H), 1.90–2.10 (m, 2H), 2.87 (d, 3H, $J = 4.4$ Hz), 3.22 (s, 3H), 3.42–3.52 (m, 4H), 3.51 (t, 2H, $J = 5.2$ Hz), 3.75 (d, 6H, $J = 10.8$ Hz), 4.90–5.00 (m, 1H), 8.40–8.50 ppm (br s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 14.1$, 20.7 (d, $J = 143$ Hz), 22.4, 24.0 (d, $J = 4.3$ Hz), 26.0, 27.1, 29.3, 29.4, 29.5, 29.6, 30.7, 31.9, 52.5, 71.1, 71.7, 75.0 (d, $J = 17.5$ Hz), 155.2, 155.9 ppm; ^{31}P NMR ($CDCl_3$): $\delta = 33.6$; ESI-HRMS $[M+H]^+$ *m/z* calcd for $C_{26}H_{54}N_2O_7P$: 537.3663, found: 537.3667.

Dimethyl 4-hexadecyloxy-3(*R*)-*N*-methylcarbamoyl-1-butanephosphonate and dimethyl 4-hexadecyloxy-3(*R*)-[*N*-(*N*′-methylcarbamoyl)-*N*-methylcarbamoyl]-1-butanephosphonate. The enantiomers of carbamoyl phosphonate **11** and dicarbamoyl phosphonate **12** were prepared in 12 and 50% yields, respectively, from 4-hexadecyloxy-3(*R*)-hydroxy-1-butanephosphonate by the procedure described above. Data for the enantiomer of **11**: $[\alpha]_D^{25} = +5.49$ ($c = 5.6$, $CHCl_3$); MS (ES) $[M+H]^+$ *m/z* calcd for $C_{24}H_{51}NO_6P$: 480.3, found: 480.3. Data for the enantiomer of **12**: $[\alpha]_D^{25} = +4.55$ ($c = 5.7$, $CHCl_3$); ESI-HRMS $[M+H]^+$ *m/z* calcd for $C_{26}H_{54}N_2O_7P$: 537.3663, found: 537.3659.

2′-(Trimethylammonio)ethyl 4-hexadecyloxy-3(*S*)-*N*-methylcarbamoyl-1-butanephosphonate (1). Me_3SiBr (500 μL, 3.79 mmol) was added to a solution of carbamoyl phosphonate **11** (512 mg, 1.07 mmol) in 25 mL CH_2Cl_2. After the mixture was allowed to stand overnight at room temperature, volatile materials were removed under reduced pressure to give a residue. Choline toluenesulfonate (1.45 g, 3.01 mmol) was added to the residue, and the mixture was dried overnight under high vacuum. After the dry mixture was dissolved in pyridine (50 mL), Cl_3CCN (1.5 mL, 15.0 mmol) was added, and the reaction mixture was heated for 48 h at 50 °C (oil bath temperature). Upon removal of most of the pyridine by rotary evaporation, a brown residue was formed, which was dissolved in THF/H_2O (10 mL, 9:1 *v/v*) and passed through a column of TMD-8 resin (previously equilibrated with the same solvent mixture). The product was purified by silica gel chromatography (elution with $CHCl_3$/MeOH/H_2O 65:25:4). The fractions containing the product were pooled and concentrated under reduced pressure. The residue was dissolved in $CHCl_3$ (15–25 mL) and passed through an Osmonics filter three times to remove the suspended silica gel. The filtrate was concentrated to give a residue, which was lyophilized from benzene to afford phosphonate **1** (395 mg, 69%) as a white powder; $[\alpha]_D^{25} = -2.53$ ($c = 0.21$, $CHCl_3$/MeOH 1:1); ^1H NMR ($CDCl_3/CD_3OD$): $\delta = 0.89$ (t, 3H, $J = 6.4$ Hz), 1.26 (br s, 26H), 1.32–1.35 (m, 2H), 1.44–1.62 (m, 4H), 1.86–1.95 (m, 2H), 2.86 (d, 3H, $J =$ 4.8 Hz), 3.24 (s, 9H), 3.30–3.70 (m, 8H), 4.90–5.00 ppm (m, 1H);

^{13}C NMR ($CDCl_3$): δ = 14.1, 20.7 (d, J = 143 Hz), 22.6, 25.1 (d, J = 4.1 Hz), 26.0, 26.1, 26.4, 29.3, 29.4, 29.5, 29.6, 31.9, 56.5 (d, J = 4.7 Hz), 65.8, 70.6 (d, J = 5.8 Hz), 75.4, 75.5, 74.9, 153.7 ppm; ESI-HRMS $[M+H]^+$ m/z calcd for $C_{27}H_{58}N_2O_6P$: 537.4027, found: 537.4037.

2′-(Trimethylammonio)ethyl 4-hexadecyloxy-3(*R*)-*N*-methylcarbamoyl-1-butanephosphonate (2). The enantiomer of **1** was prepared in 66% yield from dimethyl 4-hexadecyloxy-3(*R*)-*N*-methylcarbamoyl-1-butanephosphonate by the procedure described above; $[\alpha]_D^{25} = +2.44$ (c = 0.21, $CHCl_3$/MeOH 1:1); ESI-HRMS $[M+H]^+$ m/z calcd for $C_{27}H_{58}N_2O_6P$: 537.4027, found: 537.4029.

2′-(Trimethylammonio)ethyl 4-hexadecyloxy-3(*S*)-[*N*-(*N*′-methylcarbamoyl)-*N*-methylcarbamoyl]-1-butanephosphonate (3). Compound **3** was prepared in 71% yield from dicarbamoyl phosphonate **12** by the procedure described above; $[\alpha]_D^{25} = -2.07$ (c = 0.23, $CHCl_3$/MeOH 1:1); ^{1}H NMR ($CDCl_3$/CD_3OD): δ = 0.89 (t, 3H, J = 6.4 Hz), 1.26 (br s, 26H), 1.44–1.62 (m, 4H), 1.86–1.95 (m, 2H), 2.87 (d, 3H, J = 4.0 Hz), 3.24 (s, 3H), 3.26 (s, 9H), 3.35–3.70 (m, 8H), 4.95–5.05 ppm (m, 1H); ^{13}C NMR ($CDCl_3$): δ = 14.1, 20.7 (d, J = 143 Hz), 22.6, 25.1 (d, J = 4.1 Hz), 26.0, 26.1, 26.4, 29.3, 29.4, 29.5, 29.6, 31.9, 56.5 (d, J = 4.7 Hz), 65.8, 70.6 (d, J = 5.8 Hz), 75.4, 75.5, 74.8, 155.4, 156.1 ppm; ESI-HRMS $[M+H]^+$ m/z calcd for $C_{29}H_{61}N_3O_7P$: 594.4242, found: 594.4246.

2′-(Trimethylammonio)ethyl 4-hexadecyloxy-3(*R*)-[*N*-(*N*′-methylcarbamoyl)-*N*-methylcarbamoyl]-1-butanephosphonate (4). The enantiomer of **3** was prepared in 68% yield from dimethyl 4-hexadecyloxy-3(*R*)-[*N*-(*N*′-methylcarbamoyl)-*N*-methylcarbamoyl]-1-butanephosphonate by the procedure described above; $[\alpha]_D^{25} = +1.98$ (c = 0.21, $CHCl_3$/MeOH 1:1); ESI-HRMS $[M+H]^+$ m/z calcd for $C_{29}H_{61}N_3O_7P$: 594.4242, found: 594.4250.

2(*S*)-Azido-2-deoxy-3-*O*-hexadecyl-1,3-propanediol (14). Diisopropyl azodicarboxylate (DIAD; 3.2 mL, 15 mmol) was added to a solution of 3-*O*-hexadecyl-*sn*-glycerol (**13**)[33] (3.17 g, 10.0 mmol) and Ph_3P (3.42 g, 13.0 mmol) in 180 mL CH_2Cl_2 at 0 °C. After the mixture was stirred for 3 h under N_2 gas, Me_3SiN_3 was added. The mixture was stirred at the same temperature for 3 h, and then at room temperature until glycerol **13** had reacted completely. The reaction mixture was concentrated to give a yellow residue, which was dissolved in a minimal volume of CH_2Cl_2 and passed through a pad of silica gel in a sintered glass funnel. The pad was rinsed with hexane/EtOAc (50:1) until the excess yellow DIAD began to elute. After concentration of the eluted silyloxy azide, the residue was dissolved in 30 mL THF and treated with a solution of $(n\text{Bu})_4$NF (1 M, 25 mL) in THF. The mixture was stirred at room temperature until all of the silyloxy azides were consumed completely, and then was diluted with 250 mL Et_2O and washed with H_2O and brine. The organic layer was separated, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography on silica gel (elution first 150 mL of 50:1 hexane/EtOAc and then with 6:1 hexane/EtOAc) to give azido alcohol **14**[34] (2.60 g, 76%) as a white solid; mp: 37–39 °C; $[\alpha]_D^{25} = +14.5$ (c = 1.0, $CHCl_3$), ref. [34]: $[\alpha]_D^{27} = +14.1$ (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, 3H, J = 6.4 Hz), 1.26 (s, 26H), 1.52–1.62 (m, 2H), 2.19 (br s, 1H), 3.47 (t, 2H, J = 6.8 Hz), 3.55–3.63 (m, 4H), 3.65–3.80 ppm (m, 1H); ^{13}C NMR ($CDCl_3$): δ = 14.1, 22.7, 26.0, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 62.3, 63.0, 70.9, 71.9 ppm; ESI-HRMS $[M+Na]^+$ m/z calcd for $C_{19}H_{39}N_3NaO_2$: 364.2935, found: 364.2941.

2′-(Trimethylammonio)ethyl 2(*S*)-azido-3-hexadecyloxypropanephosphate (16). 2-Chloro-1,3,2-dioxaphospholane-2-oxide (250 μL, 2.72 mmol) was added to a solution of **14** (342 mg, 1.00 mmol) and $(i\text{Pr})_2$NEt (390 mg, 3.02 mmol) in 20 mL CH_2Cl_2 at 0 °C. After azido alcohol **14** was consumed completely to afford intermediate **15**, Me_3SiBr (1.0 mL, 7.6 mmol) was added at 0 °C to carry out the opening of the phospholane ring. After the mixture was stirred for 2 h at room temperature, the volatile material was removed under reduced pressure to give a residue, which was dissolved in 40 mL $CHCl_3$/MeCN/*i*PrOH (0.9:1.5:1.5) and treated with an aqueous solution of Me_3N (40 mL, 40%) for two days at room temperature. After concentration under reduced pressure, the residue was purified by column chromatography on silica gel (elution with $CHCl_3$/MeOH/H_2O 65:25:4) to give azido phosphocholine **16** (350 mg, 69%) as a white solid; $[\alpha]_D^{25} = -4.3$ (c = 0.11, $CHCl_3$), $[\alpha]_D^{25} = -4.5$ (c = 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$/CD_3OD): δ = 0.88 (t, 3H, J = 6.4 Hz), 1.26 (s, 26H), 1.50–1.60 (m, 2H), 3.30 (s, 9H), 3–43–3.65 (m, 4H), 3,25–3.50 (m, 3H), 3.90–4.10 (m, 2H), 4.35–4.45 ppm (m, 2H); ^{13}C NMR ($CDCl_3$/CD_3OD): δ = 13.6, 22.3, 25.6, 29.0, 29.1, 29.2, 29.3, 31.6, 44.4, 53.9, 59.5 (d, J = 5.0 Hz), 60.7 (d, J = 7.0 Hz), 65.7 (d, J = 5.0 Hz), 69.6, 71.7 ppm; ^{31}P NMR ($CDCl_3$/CD_3OD): δ = −2.03 ppm.

2′-(Trimethylammonio)ethyl 2(*S*)-(*N*-methoxycarbonylamido)-3-hexadecyloxypropanephosphate (5). A mixture of **16** (102 mg, 0.20 mmol) and Pd/C (30 mg) in EtOH was stirred overnight under H_2 atmosphere. After the catalyst was removed by filtration, the filtrate was concentrated under reduced pressure to give crude amine **17**. Et_3N (60 μL, 0.44 mmol) and $ClCO_2Me$ (30 μL, 0.39 mmol) were added to a solution of vacuum-dried amine **17** in 10 mL alcohol-free $CHCl_3$ at 0 °C. The mixture was stirred overnight at room temperature and then concentrated under reduced pressure to give a residue, which was purified by column chromatography on silica gel (elution with $CHCl_3$/MeOH/H_2O 65:25:4) to give carbamate phosphocholine **5** (93 mg, 85%) as a white solid after filtration of a $CHCl_3$ solution of **5** through an Osmonics filter to remove suspended silica gel: $[\alpha]_D^{25} = -3.9$ (c = 0.10, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, 3H, J = 6.4 Hz), 1.26 (s, 26H), 1.50–1.60 (m, 2H), 3.43 (s, 9H), 3.40–3.75 (m, 4H), 3.67 (s, 3H), 3.90–4.30 (m, 5H), 4.60–4.80 (m, 2H), 6.51 (br s, 1H); ^{31}P NMR ($CDCl_3$): δ = −2.61; ESI-HRMS $[M+H]^+$ m/z calcd for $C_{26}H_{56}N_2O_7P$: 593.3820, found: 593.3830.

Biological methods

Cell culture. Cell lines were obtained from frozen stocks of cell lines originally obtained from ATTC. The cells were grown in basal medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin. PC3 cells were grown in F12K medium; BT549 in RPMI; MDA-MB-231 in MEM; A549 in F12; and DU145, MCF-7, HepG2, and HeLa cells were grown in DMEM.

Antiproliferation assay. Cell proliferation was monitored by the CyQuant assay according to the instructions of the manufacturer (Invitrogen, Mississauga, ON, Canada) and as previously described.[21] Cells were dispersed 96-well plates and incubated until they were in log phase. The medium was subsequently replaced with one containing varying concentrations of compounds **1**–**5** (0–20 μM dissolved in EtOH) for 48 h. The concentration of EtOH in all wells was 0.1%. Each drug concentration was replicated eight times.

Toxicity assay. The toxicity of **1**, **2**, and **3** was measured by using the ToxiLight assay kit according to the instructions of the manufacturer (Lonza, Rockland, ME, USA) as previously described[21] in clear-bottom 96-well black plates.

Assay of mitochondrial membrane potential. The effect of compound **2** on the mitochondrial membrane potential was determined with the JC-10 reagent (ABD Bioquest, Sunnyvale, CA, USA) by using the assay previously described for JC-1.[21]

Caspase activation. The activation of caspases in cells was monitored by using the CaspGlow fluorescein caspase staining kit (Biovision Inc., Mountain View, CA, USA) as previously described.[21,35]

Animal studies. Rag2M mice (8–10 weeks old) from the Animal Resource Centre of the British Columbia Cancer Research Centre (Vancouver, BC, Canada) were used for the studies. The methodology used for the studies was approved by the Institutional Animal Care Committee at the University of British Columbia prior to conducting the studies (protocol #A05-1582). The toxicity of the selected doses of compound **2** to be used in the efficacy studies was established as follows: The mice were randomly distributed into groups of four and individually weighed. Compound **2** was dissolved in saline solution for intravenous injections or in double-distilled water for oral gavage. The compound was introduced according to body weight. The animals were monitored for acute signs of toxicity for the first 2 h; if this was observed, they were sacrificed by CO_2 asphyxiation followed by necropsies. All mice were monitored daily for 14 days for signs of toxicity, and body weight was monitored.

Xenograft studies were conducted as follows: Rag2M mice were injected subcutaneously with 10×10^6 PC3 cells in a volume of 50 μL. Tumors were allowed to grow for 29–35 days. Animals were selected and randomized into groups comprising: 1) vehicle-treated control, 2) oral **2**, and 3) intravenous **2**. Compound **2** was delivered once per week by oral gavage (200 μL per 20 g mouse) or intravenously (200 μL per 20 g mouse) for 3 weeks. Tumor growth was monitored with digital calipers beginning on the day of administering the compounds and then every 2–3 days thereafter. Tumor volume was calculated according to the formula: volume = (length) × (width)^2/2. The body weight of the animals was determined at the time of tumor measurement.

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[1] A. Jemal, T. Murray, A. Samuels, A. Ghafoor, E. Ward, M. J. Thun, *CA Cancer J. Clin.* **2003**, *53*, 5 – 26.
[2] H. Saitoh, M. Hida, T. Shimbo, K. Nakamura, J. Yamagata, T. Satoh, *Cancer* **1984**, *54*, 3078 – 3084.
[3] S. C. Jacobs, *Urology* **1983**, *21*, 337 – 344.
[4] M. Koutsilieris, G. Tolis, *Prostate* **1985**, *7*, 31 – 39.
[5] S. Chowdhury, S. Burbridge, P. G. Harper, *Int. J. Clin. Pract.* **2007**, *61*, 2064 – 2070.
[6] A. M. Lin, C. J. Ryan, E. J. Small, *Crit. Rev. Oncol. Hematol.* **2007**, *61*, 243 – 254.
[7] B. Pandit, A. L. Gartel, *Prostate* **2010**, *70*, 825 – 833.
[8] G. Arthur, R. Bittman, *Biochim. Biophys. Acta Lipids Lipid Metab.* **1998**, *1390*, 85 – 102.
[9] C. Gajate, A. M. Santos-Beneit, M. Modolell, F. Mollinedo, *Mol. Pharmacol.* **1998**, *53*, 602 – 612.
[10] P. Samadder, R. Bittman, H.-S. Byun, G. Arthur, *J. Med. Chem.* **2004**, *47*, 2710 – 2713.
[11] T. Nieto-Miguel, C. Gajate, F. Gonzalez-Camacho, F. Mollinedo, *Oncogene* **2008**, *27*, 1779 – 1787.
[12] T. Nieto-Miguel, R. I. Fonteriz, L. Vay, C. Gajate, S. Lopez-Hernandez, F. Mollinedo, *Cancer Res.* **2007**, *67*, 10368 – 10378.
[13] C. Gajate, F. Mollinedo, *Blood* **2007**, *109*, 711 – 719.
[14] F. Mollinedo, C. Gajate, S. Martin-Santamaria, F. Gago, *Curr. Med. Chem.* **2004**, *11*, 3163 – 3184.
[15] W. E. Berdel, *Br. J. Cancer* **1991**, *64*, 208 – 211.
[16] P. Samadder, G. Arthur, *Cancer Res.* **1999**, *59*, 4808 – 4815.
[17] W. J. van Blitterswijk, M. Verheij, *Curr. Pharm. Des.* **2008**, *14*, 2061 – 2074.
[18] W. J. Houlihan, M. Lohmeyer, P. Workman, S. H. Cheon, *Med. Res. Rev.* **1995**, *15*, 157 – 223.
[19] A. Ashagbley, P. Samadder, R. Bittman, R. V. Erukulla, H.-S. Byun, G. Arthur, *Anticancer Res.* **1996**, *16*, 1813 – 1818.
[20] W. E. Berdel, R. Andreesen, P. G. Munder in *Phospholipids and Cellular Regulation, Vol. 2* (Ed.: J. F. Kuo), CRC Press, Boca Raton, **1985**, pp. 41 – 73.
[21] P. Samadder, R. Bittman, H.-S. Byun, G. Arthur, *Biochem. Cell Biol.* **2009**, *87*, 401 – 414.
[22] L. Jahreiss, M. Renna, R. Bittman, G. Arthur, D. C. Rubinsztein, *Autophagy* **2009**, *5*, 835 – 846.
[23] R. K. Erukulla, X. Zhou, P. Samadder, G. Arthur, R. Bittman, *J. Med. Chem.* **1996**, *39*, 1545 – 1548.
[24] G. Yang, R. W. Franck, H.-S. Byun, R. Bittman, P. Samadder, G. Arthur, *Org. Lett.* **1999**, *1*, 2149 – 2151.
[25] R. Bittman, H.-S. Byun, B. Mercier, H. Salari, *J. Med. Chem.* **1994**, *37*, 425 – 430.
[26] B. Herradon, *Tetrahedron: Asymmetry* **1991**, *2*, 191 – 194.
[27] K. Yokoyama, D. L. Baker, T. Virag, K. Liliom, H.-S. Byun, G. Tigyi, R. Bittman, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2002**, *1582*, 295 – 308.
[28] L. He, M. Wanunu, H.-S. Byun, R. Bittman, *J. Org. Chem.* **1999**, *64*, 6049 – 6055.
[29] N. M. Navone, C. J. Logothetis, A. C. von Eschenbach, P. Troncoso, *Cancer Metastasis Rev.* **1998**–**1999**, *17*, 361 – 371.
[30] R. E. Sobel, M. D. Sadar, *J. Urol.* **2005**, *173*, 342 – 359.
[31] S. T. Smiley, M. Reers, C. Mottola-Hartshorn, M. Lin, A. Chen, T. W. Smith, G. D. Steele, Jr., L. B. Chen, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 3671 – 3675.
[32] A. E-H. De Mendoza, M. A. Campanero, J. de La Iglesia-Vincente, C. Gajate, F. Mollinedo, M. J. Blanco-Prieto, *Clin. Cancer Res.* **2009**, *15*, 858 – 864.
[33] P. N. Guivisdalsky, R. Bittman, *J. Org. Chem.* **1989**, *54*, 4637 – 4642.
[34] M. M. Ponpipom, R. L. Bugianesi, *Chem. Phys. Lipids* **1984**, *35*, 29 – 37.
[35] R. Bittman, Z. Li, P. Samadder, G. Arthur, *Cancer Lett.* **2007**, *251*, 53 – 58.

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