Journal of Medicinal Chemistry



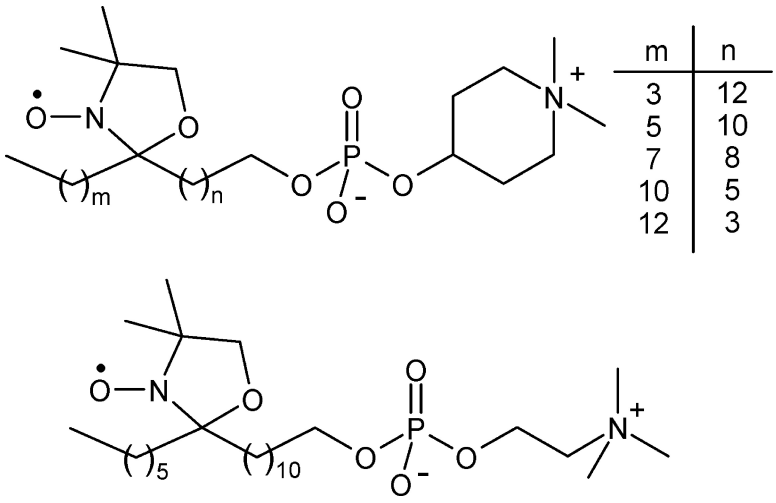
Article

# Synthesis and Biological Evaluation of Spin-Labeled Alkylphospholipid Analogs

Janez Mravljak, Reiner Zeisig, and Slavko Pec#ar

*J. Med. Chem.*, **2005**, 48 (20), 6393-6399• DOI: 10.1021/jm050189v • Publication Date (Web): 09 September 2005






## More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML



Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

# Synthesis and Biological Evaluation of Spin-Labeled Alkylphospholipid Analogs

Janez Mravljak,*,† Reiner Zeisig,‡ and Slavko Pečar†,§

*Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia, Max-Delbrück-Center for Molecular Medicine, Experimental Pharmacology, Robert-Rössle-Str. 10, 13092 Berlin-Buch, Germany, and Jožef Stefan Institute, Jamova 39, SI-1000 Ljubljana, Slovenia*

*Received March 1, 2005*

Alkylphospholipid analogues of perifosine and miltefosine bearing a nitroxide moiety at different positions on an alkyl chain were synthesized as electron paramagnetic resonance (EPR) probes. Their amphiphilic properties were characterized by determining their critical micelle concentration (cmc) and hemolytic activity on erythrocytes both in free and liposomal form. Spin-labeled analogues as membrane components of large unilamellar liposomes containing cholesterol and dicetyl phosphate or in free solution were evaluated using the MTT assay to determine growth inhibition on MT1, MT3, and MCF7 breast cancer cell lines. **4a** ($IC_{50}$ = 56.4 $\mu$M) was found to be significantly more active than the perifosine against the MCF-7 cell line. Its high cmc (194.03 $\mu$M) and low hemolytic activity shows that its cytotoxic activity might be more specific; therefore, **4a** can be an important molecular tool for further EPR investigations.

## Introduction

Alkylphospholipids (APLs) are interesting compounds with antineoplastic and immunomodulatory properties,[1] stimulating the host defense by inducing macrophage cytotoxicity.[2] Compared to most other antitumor drugs their cytotoxic and cytostatic activities are selective for tumor cells. Hexadecylphosphocholine (miltefosine, Figure 1) is the first APL anticancer agent to have been successfully introduced into therapy.[3] Because of its gastrointestinal (GI) toxicity and hemolytic activity, it is formulated for topical use in the clinical treatment of cutaneous breast cancer and other malignant lesions.[4] To overcome the GI toxicity, compounds obtained by modification of either the polar head or alkyl chains have been prepared and evaluated. Octadecyl(1,1-dimethyl-4-piperidiniumyl)phosphate (perifosine, OPP; Figure 1) belongs to the second generation and due to its significantly improved GI tolerance, especially in liposome preparations,[5] and may be the first therapeutically important heterocyclic APL drug.[6]

The primary target of APLs is thought to be the plasma membrane because of their similarity to membrane lipid components. Other possible mechanisms of action have also been discussed, such as interference with membrane signal transduction pathways[1,7] and induction of apoptosis.[8] Given the contradictory reports about their action, the exact mechanism on the molecular level is not known.[1,7,8] However, their selectivity and a molecular mechanism independently on DNA interaction strongly support further investigation.[1]

The purpose of this project was to synthesize new spin-labeled alkylphospholipid derivatives (SL-APL) and compare their amphiphilic, hemolytic, and cytotoxic properties with those of the parent compounds. These compounds bear a doxyl group on a single alkyl chain, unlike other described spin-labeled derivatives of ether-linked phospholipids.[9] They should be especially suitable as probes for investigating the interaction between selected APLs and the cellular membrane of tumor cells by electron paramagnetic resonance (EPR) spectroscopy.

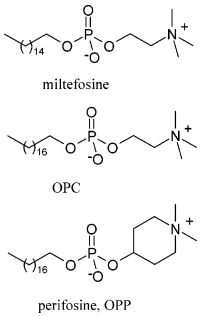


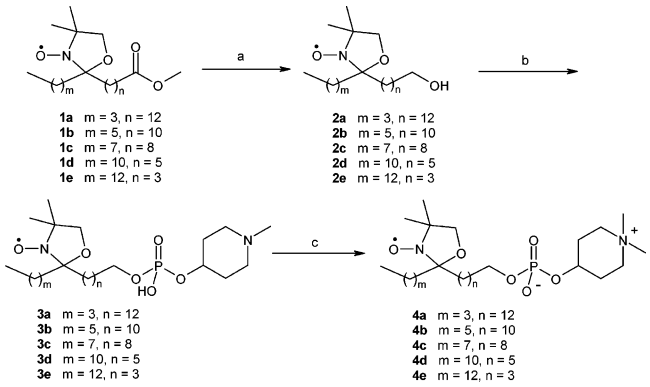

**Figure 1.** Structures of three alkylphospholipids: miltefosine, its octadecyl analogue OPC, and perifosine.

EPR is a powerful method for monitoring the biophysical characteristics of plasma membranes, their interactions with spin-labeled compounds, and the dynamics of the latter.[10] The fine structure of the EPR spectra provides information about the dynamic, structural, and redox properties of the spin-labeled molecule in the environment.[11] The successful application of spin-labeled compounds depends strongly on properly designed spin probes, which are mostly stable free radicals of the nitroxide type. To study the behavior of a compound/drug in a biological system, a spin-labeled analogue with similar biological activity has to be synthesized. Introduction of the five-membered heterocyclic oxazolidine ring containing a stable nitroxide group into the parent compound (e.g., APL molecule) slightly increases its molecular size. Consequently, the physicochemical properties and biological activity are changed also. Thus, the relevance of any further EPR studies using spin-labeled analogues and transfer of results obtained to the corresponding unlabeled derivatives should be supported by their biological evaluation.

* To whom correspondence should be addressed. Phone: +386-1-47-69-500. Fax: +386-1-42-58-031. E-mail: janez.mravljak@ffa.uni-lj.si.
† University of Ljubljana.
‡ Max-Delbrück-Center for Molecular Medicine.
§ Jožef Stefan Institute.

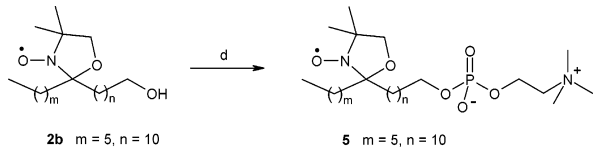
10.1021/jm050189v CCC: $30.25 
Published on Web 09/09/2005

**Scheme 1**[a]




[a] Reagents and conditions: (a) $LiAlH_4$, $Et_2O$; (b) (1) $POCl_3$, pyridine, $CH_2Cl_2$, 0 °C; (2) 4-hydroxy-*N*-methylpiperidine, pyridine, 10 °C; (3) $H_2O$; (c) *p*-TsOMe, $K_2CO_3$, ethanol, 65 °C.

**Scheme 2**[a]




[a] Reagents and conditions: (d) (1) $ClPO(OCH_2)_2$, $Et_3N$, TBME, 0 °C; (2) $Me_3N$, $CH_3CN$, 65 °C.

Here we present the synthesis of spin-labeled analogues of miltefosine and perifosine in which the doxyl group is placed at different positions on an octadecyl alkyl chain. Their cytotoxic activity was determined on three different cancer cell lines after applying SL-APL solutions or liposomes containing SL-APL. The results are discussed in terms of the influence on cytotoxic activity of the doxyl group and its position on the chain. The spin-labeled compounds are shown to be two to three times less active than the parent compounds, with the exception of **4a**, which is significantly more active against the MCF-7 cell line.

## Chemistry

Spin-labeled perifosine analogues **4a**−**e** were synthesized according to the general procedure depicted in Scheme 1. Spin-labeled fatty acid methyl esters **1a**−**e**[12] were reduced to the desired alcohols **2a**−**e** (**2b** and **2e** have already been described)[13] using $LiAlH_4$ by a modified procedure.[14] Phosphorylation of alcohols **2a**−**e** using phosphorus oxychloride in the presence of pyridine, followed by addition of 4-hydroxy-*N*-methylpiperidine, gave the desired alkylphospholipids **3a**−**e**. Quaternization of the tertiary amines to the inner salts **4a**−**e** was effected by treatment with 4-toluenesulfonic acid methyl ester (*p*-TsOMe) in anhydrous ethanol at 65 °C in the presence of $K_2CO_3$.[15]

Spin-labeled miltefosine analogue **5** was synthesized from the spin-labeled alcohol **2b** (Scheme 2). The alcohol **2b** was reacted with 2-chloro-2-oxo-1,3,2-dioxaphosphorane ($ClPO(OCH_2)_2$) in the presence of one equivalent of triethylamine in *tert*-butyl methyl ether (TBME) to produce the cyclic triester, which was immediately used in the next step. Ring opening with anhydrous trimethylamine in acetonitrile at 65 °C gave the target compound **5**.[16]

**Table 1.** Critical Micelle Concentration (cmc) Values and Diameter of Liposomes after Extrusion

| | | LUVET[c] | |
|---|---|---|---|
| SL-APL[a] (*m*,*n*) | cmc[b] [$\mu$M] | diameter ± SD [nm][d] | PI[e] |
| **4a** (3,12) | 194.03 | 194 ± 64 | 0.20 |
| **4b** (5,10) | 182.52 | 151 ± 55 | 0.28 |
| **4c** (7,8) | 116.46 | 156 ± 55 | 0.11 |
| **4d** (10.5) | 73.19 | 144 ± 57 | 0.43 |
| **4e** (12,3) | 4.22 | 151 ± 32 | 0.10 |
| **5** (5,10) | 99.45 | 112 ± 40 | 0.26 |

[a] Spin-labeled alkylphospholipid. For nomenclature, see Experimental Section. (*m*,*n*) indicate relative position of the doxyl group on the alkyl chain with *m* = chain between doxyl group and headgroup and *n* = chain between doxyl group and end of aliphatic chain. [b] Critical micellar concentration. [c] Large unilamellar vesicles were obtained by extrusion, liposome composition was APL/Ch/DCP in a molar ratio of 10:10:2, with a starting concentration of 5 $\mu$mol APL/mL. [d] Mean unimodal results of three measurements. [e] Polydispersity index.

## Results and Discussion

**Critical Micelle Concentration**. The cmc varies with the position of the doxyl group on the alkyl chain (Table 1). Among the piperidine SL-APL analogues the lowest value (4 $\mu$M) was found for **4e** (Schemes 1 and 2), in which the doxyl is closest to the polar headgroup. This value is also close to the cmc of miltefosine (2.5 $\mu$M).[17] **4e** has the longest effective alkyl chain residue (Scheme 1) and consequently the strongest amphiphilic nature. In all other compounds (**4a**−**d**) the effective alkyl chain is shorter, and consequently the cmc is higher. Compound **4a**, where doxyl is close to the end of the alkyl chain, has the highest cmc value (194 $\mu$M). Because all compounds (**4a**−**e**) have the same total length of alkyl chain the observed differences in cmc values result primarily from the position of the doxyl group. Choline derivative **5** has a considerably lower cmc than **4b** due to its different polar head. These results indicate that cmc values reflect the influence of the structure (presence and position of the polar doxyl group) of SL-APLs on micelle formation in water. Moreover, they suggest that the resulting perturbation of micelle formation by the doxyl group of SL-APLs is important for understanding their cytotoxic effects.

**Liposomes.** Multilamellar vesicles (MLV) containing SL-APL, cholesterol (Ch), and dicetyl phosphate (DCP) were obtained by the lipid film method and extruded through polycarbonate filters with defined pores of 200 nm to obtain a homogeneous population of small vesicles (LUVET). Dynamic light scattering measurements showed that the average diameter was around 150 nm, with a unimodal size distribution shown by the polydispersity index (PI, Table 1) and the graph of the size distribution. Exceptions were liposomes containing **4a** and the choline derivative **5**, which had average diameters of 200 and 110 nm, respectively. Attempts to prepare smaller vesicles of liposomes containing **4a**, **4c**, **4d**, and **4e** by an additional extrusion through membranes with 100 nm pores failed due to high loss of material and destruction of the filter membranes. The presence of the doxyl group on the alkyl chain of SL-APL incorporated into liposomes did not show a dependence of vesicle size on the doxyl group position as was seen for the cmc. Liposomes containing **4a** exhibited the largest diameter, the highest cmc value, and consequently the weakest amphiphilic character,

**Table 2.** Hemolytic Effect of Lipids and Corresponding Liposomes on Red Blood Cells

| | hemolysis[a] | | |
|---|---|---|---|
| | lipid | liposomes | |
| compound | 30 $\mu$M | 30 $\mu$M | 100 $\mu$M |
| **4a** | 16.1 ± 4.0 | 3.2 ± 2.5 | 5.3 ± 2.4 |
| **4b** | 18.3 ± 4.3 | 4.1 ± 0.3 | 5.7 ± 3.7 |
| **4c** | 34.6 ± 17.3 | 3.7 ± 1.5 | 5.9 ± 2.7 |
| **4d** | 95.2 ± 2.7 | 4.4 ± 3.3 | 6.7 ± 3.0 |
| **4e** | 99.0 ± 5.3 | 6.6 ± 1.7 | 17.9 ± 4.0 |
| **5** | 98.3 ± 3.5 | 4.9 ± 0.6 | 20.3 ± 3.7 |
| OPP | 98.1 ± 2.3 | 3.2 ± 2.5 | 93.5 ± 3.1 |
| PC | 18.1 ± 8.6 | n.d. | n.d. |

[a] Hemolysis was determined as described in the Experimental Section by incubating $8.6 \times 10^7$ erythrocytes with lipids or liposomes at the indicated concentration for 30 min. Results represent mean relative hemolysis (in percent) ± SD in comparison to control (100% hemolysis) for three samples. PC: phosphatidylcholin (Lipoid). n.d.: not done.

whereas liposomes with lipids **4b**−**d** were very similar in size. By comparing liposomes containing **5** or **4b**, which have the same position of the doxyl group but different polar headgroups, it appears that **5** occupies a smaller space in the lipid bilayer.

**Hemolytic Effect of SL-APL.** The hemolytic activities of dissolved SL-APL and liposomes containing SL-APL were determined for two concentrations of SL-APL (Table 2). OPP and noncytotoxic PC were used for control reasons. Compounds **4a**, **4b**, and **4c** at 30 $\mu$M expressed only a weak hemolytic effect, while **4d**, **4e,** and **5** caused practically total hemolysis like OPP. The obtained effect correlated well with the cmc values (Table 1). At 30 $\mu$M **4a** was practically dissolved (cmc = 194 $\mu$M) while OPP (cmc = 2,5 $\mu$M) was mostly in the form of micelles. It seems that the micellar form was responsible for the hemolytic effect.

Incorporation of tested compounds into liposomes at a 30 $\mu$M final concentration significantly reduced their hemolytic activity to a negligible level in all cases, probably due to reduction of the micellar portion. At higher concentrations (100 $\mu$M) only liposomes containing **4e** and **5** showed slightly increased hemolysis while OPP at this concentration produced total hemolysis; however, the lytic effect of OPP can be reduced in the presence of serum albumin.

**Cytotoxic Activity.** The spin-labeled analogues **4a**−**e** (Scheme 1) and **5** (Scheme 2) and the unlabeled parent compounds (OPP and OPC, Figure 1) were examined for antitumor activity against MT1, MT3, and MCF7 breast cancer cell lines using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide) as a marker of cell viability. The SL-APL compounds, applied in either solution or liposomes, inhibited cell growth less than OPP and OPC in all cell lines. Further, the small differences between the effects of SL-APL incorporated into liposomes and in micellar solutions were not significant (Figures 2 and 3). The only exception was compound **4a**, which had the highest cmc and gave the largest liposomes. It was more active when applied to MT1 cells in solution, whereas the opposite was observed for MT3 cells (Figure 4). In addition, the activity of **4a** in solution against MT1 was comparable to that of OPP in solution (Figure 2), while in liposomes it had the same activity as OPP in solution against MT3 (Figure 3). In the case of MCF7 (Figure 4) **4a** exerted

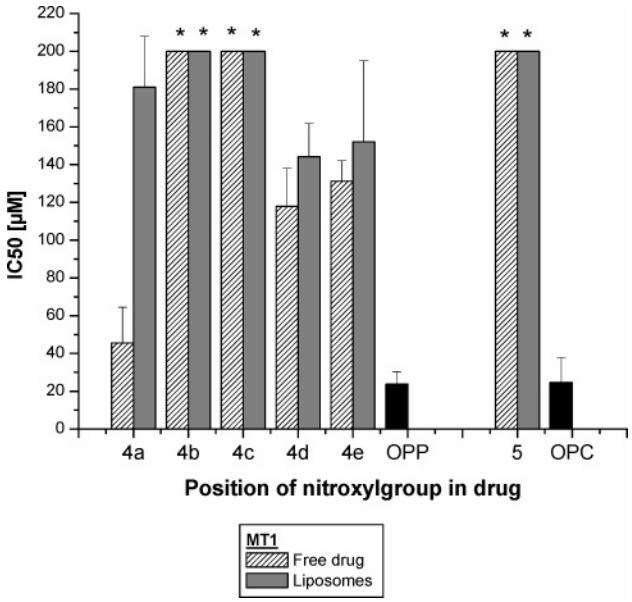



**Figure 2.** Growth inhibition of MT1 tumor cells by spin-labeled alkylphospholipids expressed as $IC_{50}$. The asterisk (*) indicates $IC_{50} > 200$ $\mu$M.

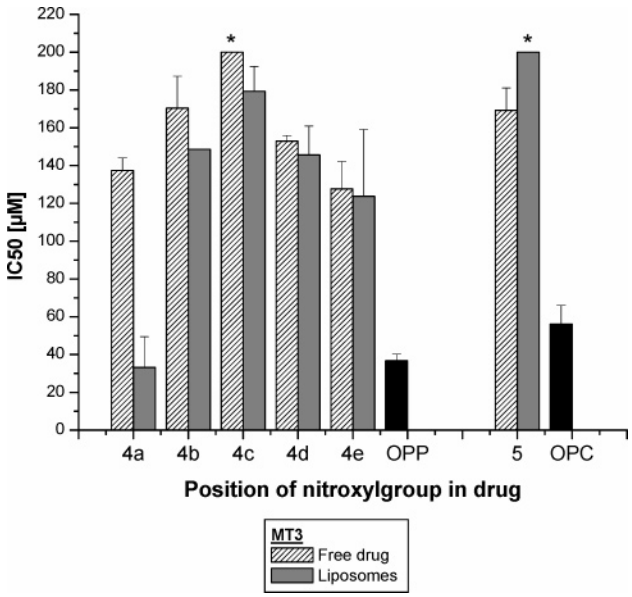



**Figure 3.** Growth inhibition of MT3 tumor cells by spin-labeled alkylphospholipids expressed as $IC_{50}$. The asterisk (*) indicates $IC_{50} > 200$ $\mu$M.

similar activity to OPP both in solution and in liposomes. The effects of **4a**, **4c**, and OPP, all in solution, on the growth of MCF7 cells are presented in Figure 5. Whereas **4a** showed a typical concentration-dependent inhibition, comparable to that of OPP, SL-APL **4c** was inactive in the concentration range between 3.15 and 200 $\mu$M. This indicates that two different modes of APL and probably SL-APL action exist, depending on the position of the doxyl group. The overall effect of APLs in micellar form was the result of a specific action at lower concentrations and a nonspecific lytic effect (detergent-like action) on membranes at elevated concentrations.[18] It can be assumed that due to the low cmc and considerable hemolytic activity of OPP this lipid causes a lytic effect over a broad concentration range while **4a** should provide a more specific action due to

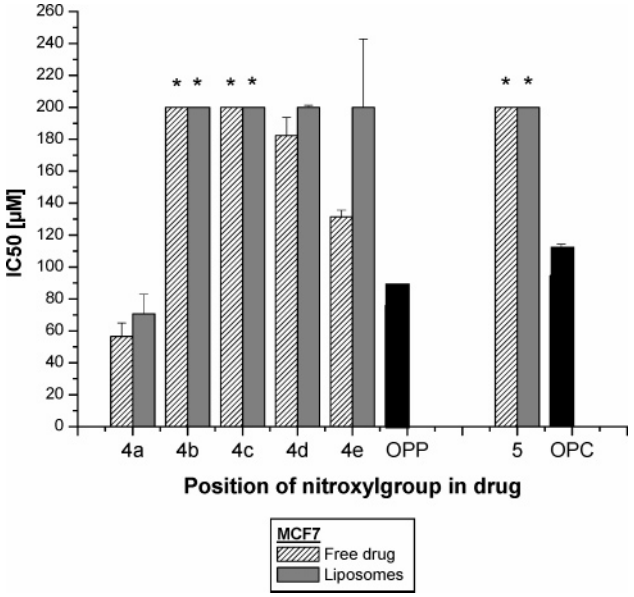



**Figure 4.** Growth inhibition of MCF7 tumor cells by spin-labeled alkylphospholipids expressed as $IC_{50}$. The asterisk (*) indicates $IC_{50} > 200$ $\mu$M.

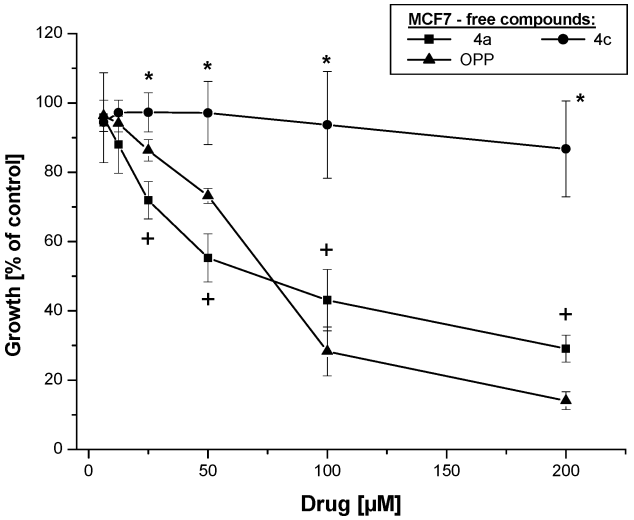



**Figure 5.** Inhibition curves (mean ± SD) of free solutions of **4a** and **4c** for MCF7 cell line compared to OPP: (+) significantly different from the corresponding result obtained with OPP ($p < 0.05$); (*) significantly different from the corresponding result obtained with OPP ($p < 0.05$).

its high cmc and negligible hemolytic activity. Under serum-free conditions the lytic effects for different APLs correspond generally well to their cmc's (all in the lower micromolar range) in water.[19] Whereas the hemolytic effect and cmc corresponds well, no clear correlation between the hemolytic effect and cytotoxic effect in vitro could be observed. This implicates that the detergent-like properties of APL only partly explains the cytotoxic effects of SL-APL and only for lipids where the doxyl group was close to the headgroup.

These results show that the position of the doxyl group has a significant influence on growth inhibition. Compounds in which the doxyl group was in the middle of the alkyl chain showed the lowest activity. Moving the doxyl group to the polar head increased the activity, but this was still smaller than that of OPP. On the other hand, compound **4a** was the most potent compound against all three cell lines. For example, it was significantly more potent against MCF7 ($IC_{50}$ 56.4 $\mu$M) than OPP ($IC_{50}$ 75.8 $\mu$M) (Figure 4). Choline derivative **5** was less active against all cell lines than the parent compound (OPC). In solution, **5** and **4b**, which differ only in the headgroup, had practically the same activity. Due to the cmc values, hemolytic data, and cytotoxic properties, **4a** had interesting properties for further investigation because its cytotoxic effect is practically free of lytic action (Table 2).

The chemical interactions of **4a**−**e** and **5** with MT1, MT3, and MCF7 cells are rather complex. It is known from published EPR investigations that the oxazolidine type of radical is reduced within living cells by different cellular reducents (ascorbate, etc.) and, furthermore, the corresponding hydroxylamine can be reoxidized in solution by dissolved oxygen.[9,20] The oxidation of **4a**−**e** to an oxoammonium product seems to be less important in cellular systems since it required a powerful oxidizer or strong acid. Also, the situation where the oxazolidine ring is opened up is less probable due to drastic reacting conditions.[20] At present we do not know whether the observed cytotoxic effect is the result of compounds **4a**−**e** or the corresponding hydroxylamines (reduced form). Also, the situation where the final effect is a contribution of both forms cannot be excluded. The radical and corresponding hydroxylamine forms differ only in their polarity, while the steric difference is negligible. Therefore, one cannot expect considerable differences in biological action.

## Conclusions

We have shown that it is possible to prepare spin-labeled perifosine analogues with the doxyl group at different positions in the alkyl chain. Introduction of the doxyl group into the perifosine and miltefosine molecules alters their physical properties and consequently the cytotoxic activity. cmc, hemolytic activity, as well as cytotoxicity were shown to depend on the position of the doxyl group in the alkyl chain. Cytotoxic activity was also dependent on the cell line. The most active analogue was **4a**, which, in liposomes or solution, was similar or even more potent than perifosine but had a negligible hemolytic activity. This finding makes compound **4a** an interesting and suitable molecular tool for further EPR investigations.

## Experimental Section

**Chemistry.** All reactions were carried out under scrupulously dry conditions and with magnetic stirring. Chemicals were from Acros, Aldrich, Fluka, Merck, Jannsen, and Sigma and used without further purification. Solvents were used without purification or drying, unless otherwise stated. Spin-labeled fatty acid methyl esters (**1a**−**e**) were prepared as described.[12] Reactions were monitored using analytical TLC plates (Merck, silica gel 60 $F_{254}$) with rhodamine G6 staining. Silica gel grade 60 (70−230 mesh, Merck) was used for column chromatography. Mass spectra were obtained with a VG-Analytical Autospec Q mass spectrometer with EI or FAB ionization (MS Centre, Jožef Stefan Institute). IR spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer. EPR spectra of nitroxide solutions were measured at room temperature in a glass capillary (1 mm i.d.) using a BRUKER X-band CW-ESR spectrometer ESP 300 (EPR Centre, Jožef Stefan Institute) at 10 mW microwave power. Elemental analyses were performed by the Department of Organic Chemistry, Faculty of Chemistry and Chemical Technology, Ljubljana, on

a Perkin-Elmer elemental analyzer 240 C. Melting points were determined using a Reichert hot-stage microscope and are uncorrected.

**General Procedure for Preparation of Spin-Labeled Fatty Alcohols.** A solution of the spin-labeled fatty acid methyl ester (3.00 g, 7.54 mmol) in dry diethyl ether (20 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (0.21 g, 5.51 mmol) in dry diethyl ether (50 mL). The resulting mixture was stirred for 3 h at room temperature and subsequently hydrolyzed by adding water (1.5 mL). After stirring at room temperature for 30 min, the solid residue was removed by filtration and washed with a small portion of ether, which was then added to the bulk solution. The ether solution was washed with brine and dried with anhydrous $Na_2SO_4$. The solvent was evaporated under reduced pressure to afford the corresponding spin-labeled fatty alcohol as an orange viscous oil. This was used in the next step without further purification.

**2-Butyl-2-(13-hydroxytridecyl)-3-oxyl-4,4-dimethyl-1,3-oxazolidine (2a)**. The general procedure described above, using methyl 13-(2-butyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)tridecanoate (**1a**), afforded compound **2a** (2.73 g, 98%). $R_f$ (ether:petroleum ether, 3:1) = 0.40. IR (NaCl) 3388, 2927, 2854, 1654, 1466, 1054 $cm^{-1}$. MS (FAB) *m/z*: 372 $(M + 2)^+$.

**2-Hexyl-2-(11-hydroxyundecyl)-3-oxyl-4,4-dimethyl-1,3-oxazolidine (2b)**. The general procedure described above, using methyl 11-(2-hexyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)undecanoate (**1b**), afforded compound **2b** (2.76 g, 99%). $R_f$ (ether:petroleum ether, 3:1) = 0.34. IR (NaCl) 3357, 2925, 2852, 1461, 1364, 1058 $cm^{-1}$. MS (FAB) *m/z*: 372 $(M + 2)^+$.

**2-(9-Hydroxynonyl)-2-octyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine (2c).** The general procedure described above, using methyl 9-(2-octyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)-nonanoate (**1c**), afforded compound **2c** (2.67 g, 96%). $R_f$ (ether:petroleum ether, 3:1) = 0.41. IR (NaCl) 3432, 2926, 2855, 1588, 1464, 1054 $cm^{-1}$. MS (FAB) *m/z*: 372 $(M + 2)^+$.

**2-(6-Hydroxyhexyl)-2-undecyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine (2d)**. The general procedure described above, using methyl 6-(3-oxyl-4,4-dimethyl-2-undecyl-1,3-oxazolidine-2-yl)hexanoate (**1d**), afforded compound **2d** (2.79 g, 100%). $R_f$ (ether:petroleum ether, 3:1) = 0.30. IR (NaCl) 3418, 2926, 2856, 1461, 1053 $cm^{-1}$. MS (FAB) *m/z*: 370 $M^+$.

**2-(4-Hydroxybutyl)-2-tridecyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine (2e)**. The general procedure described above, using methyl 4-(3-oxyl-4,4-dimethyl-2-tridecyl-1,3-oxazolidine-2-yl)butanoate (**1e**), afforded compound **2e** (2.76 g, 99%). $R_f$ (ether:petroleum ether, 3:1) = 0.29. IR (NaCl) 3405, 2924, 2854, 1460, 1055 $cm^{-1}$. MS (FAB) *m/z*: 370 $M^+$.

**General Procedure for Preparation of Ether Phospholipids.** A solution of the alcohol (**2a**−**e**) (1.00 g, 2.70 mmol) and pyridine (1.0 mL) in dry dichloromethane (6 mL) was added dropwise to a solution of phosphorus oxychloride (0.28 mL, 2.97 mmol) in dry dichloromethane (1.5 mL) at 0 °C. After stirring the mixture for 30 min at 5 °C, a solution of 4-hydroxy-*N*-methylpiperidine (0.40 g, 3.512 mmol) in pyridine (1.1 mL) was added dropwise with cooling. The resulting mixture was stirred for 3 h at 10 °C and then hydrolyzed by addition of water (0.5 mL). After stirring at room temperature for 1 h, the reaction mixture was washed successively with MeOH/$H_2O$ (1/1) (5 mL), 10% citric acid/MeOH (1/1) (5 mL), and MeOH/$H_2O$ (1/1) (5 mL) and dried with anhydrous $Na_2SO_4$. The solvent was evaporated under reduced pressure to afford a viscous orange oil. The resulting crude product was purified by gravity column chromatography using $CHCl_3$/MeOH/25% $NH_{3(aq)}$ (40/10/1). After evaporation of the solvent the desired product was obtained.

**13-(2-Butyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)-tridecyl 1-Methyl-4-piperidinyl Hydrogen Phosphate (3a)**. The general procedure described above, using 2-butyl-2-(13-hydroxytridecyl)-3-oxyl-4,4-dimethyl-1,3-oxazolidine (**2a**), afforded compound **3a** (0.59 g, 40%). $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.13. IR (NaCl) 3380, 2925, 2854, 1653, 1459, 1208, 1039 $cm^{-1}$. MS (FAB) *m/z*: 549 $(M + 2)^+$.

**11-(2-Hexyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)-undecyl 1-Methyl-4-piperidinyl Hydrogen Phosphate (3b)**. The general procedure described above, using 2-hexyl-2-(11-hydroxyundecyl)-3-oxyl-4,4-dimethyl-1,3-oxazolidine (**2b**), afforded compound **3b** (0.76 g, 52%). $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.15. IR (NaCl) 3386, 2924, 2853, 1659, 1466, 1214, 1038, 871 $cm^{-1}$. MS (FAB) *m/z*: 549 $(M + 2)^+$.

**9-(2-Octyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)-nonyl 1-Methyl-4-piperidinyl Hydrogen Phosphate (3c)**. The general procedure described above, using 2-octyl-2-(9-hydroxynonyl)-3-oxyl-4,4-dimethyl-1,3-oxazolidine (**2c**), afforded compound **3c** (0.59 g, 40%). $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.29. IR (NaCl) 3387, 2926, 2855, 1463, 1221, 1043, 845 $cm^{-1}$. MS (FAB) *m/z*: 548 $(M + 1)^+$.

**6-(2-Undecyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)-hexyl 1-Methyl-4-piperidinyl Hydrogen Phosphate (3d)**. The general procedure described above, using 2-undecyl-2-(6-hydroxyhexyl)-3-oxyl-4,4-dimethyl-1,3-oxazolidine (**2d**), afforded compound **3d** (0.59 g, 40%). $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.31. IR (NaCl) 3392, 2924, 2853, 1636, 1465, 1216, 1038, 867 $cm^{-1}$. MS (FAB) *m/z*: 548 $(M + 1)^+$.

**4-(2-Tridecyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)-butyl 1-Methyl-4-piperidinyl Hydrogen Phosphate (3e)**. The general procedure described above, using 2-tridecyl-2-(4-hydroxybutyl)-3-oxyl-4,4-dimethyl-1,3-oxazolidine (**2e**), afforded compound **3e** (0.65 g, 44%). $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.31. IR (NaCl) 3394, 2923, 2852, 1463, 1214, 1045 $cm^{-1}$. MS (FAB) *m/z*: 548 $(M + 1)^+$.

**General Procedure for the Alkylation of Tertiary Amine.** 4-Toluenesulfonic acid methyl ester (0.23 g, 1.25 mmol) and $K_2CO_3$ (0.11 g, 0.79 mmol) were added gradually over 1 h to a stirred solution of the ether phospholipids **3a**−**e** (0.50 g, 0.91 mmol) in anhydrous ethanol (10 mL) at 60 °C. The resulting mixture was stirred for an additional 30 min. After cooling the white precipitate was filtered off and the solvent evaporated under reduced pressure. The resulting crude product was purified by gravity column chromatography using initially $CHCl_3$/MeOH/25% $NH_{3(aq)}$ (85/15/1.5) and subsequently $CHCl_3$/MeOH/25% $NH_{3(aq)}$ (78/30/4.7). The solvents were evaporated under reduced pressure to yield the desired product.

**4-{[{[13-(2-Butyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)tridecyl]oxy}(oxy)phosphoryl]oxy}-1,1-dimethylpiperidinium Inner Salt (4a)**. The general procedure described above, using 13-(2-butyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)tridecyl 1-methyl-4-piperidinyl hydrogen phosphate (**3a**), afforded compound **4a** (0.40 g, 79%) as yellow semisolid. $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.12. IR (NaCl) 3375, 2925, 2860, 1649, 1461, 1226, 1064 $cm^{-1}$. MS (FAB) *m/z*: 562 $(M+1)^+$. $a_N$ (ethanol) = 1.487 mT. Anal. ($C_{29}H_{58}N_2O_6P \cdot 0.5H_2O \cdot 0.5NH_3$) C, H, N.

**4-{[{[11-(2-Hexyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)undecyl]oxy}(oxy)phosphoryl]oxy}-1,1-dimethylpiperidinium Inner Salt (4b)**. The general procedure described above, using 11-(2-hexyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)undecyl 1-methyl-4-piperidinyl hydrogen phosphate (**3b**), afforded compound **4b** (0.42 g, 82%) as yellow semisolid. $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.10. IR (NaCl) 3376, 2926, 2854, 1662, 1465, 1236, 1063, 837 $cm^{-1}$. MS (FAB) *m/z*: 562 $(M + 1)^+$. $a_N$ (ethanol) = 1.485 mT. Anal. ($C_{29}H_{58}N_2O_6P \cdot H_2O$) C, H, N.

**4-{[{[9-(2-Octyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)nonyl]oxy}(oxy)phosphoryl]oxy}-1,1-dimethylpiperidinium Inner Salt (4c)**. The general procedure described above, using 9-(2-octyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)nonyl 1-methyl-4-piperidinyl hydrogen phosphate (**3c**), afforded compound **4c** (0.38 g, 74%) as yellow semisolid. $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.09. IR (NaCl) 3394, 2925, 2854, 1654, 1464, 1231, 1069 $cm^{-1}$. MS (FAB) *m/z*: 562 $(M + 1)^+$. $a_N$ (ethanol) = 1.487 mT. Anal. ($C_{29}H_{58}N_2O_6P \cdot 0.5NH_3$) C, H, N.

**4-{[{[6-(2-Undecyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)hexyl]oxy}(oxy)phosphoryl]oxy}-1,1-dimethylpiperidinium Inner Salt (4d)**. The general procedure described above, using 6-(2-undecyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)hexyl 1-methyl-4-piperidinyl hydrogen phosphate (**3d**),

afforded compound **4d** (0.33 g, 65%) as yellow semisolid. $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.07. IR (NaCl) 3350, 2923, 2850, 1627, 1463, 1220, 1065 $cm^{-1}$. MS (FAB) *m/z*: 562 $(M + 1)^+$. $a_N$ (ethanol) = 1.485 mT. Anal. ($C_{29}H_{58}N_2O_6P\cdot 0.5H_2O$) C, H, N.

**4-{[{[4-(2-Tridecyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)butyl]oxy}(oxy)phosphoryl]oxy}-1,1-dimethylpiperidinium Inner Salt (4e)**. The general procedure described above, using 4-(2-tridecyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)butyl 1-methyl-4-piperidinyl hydrogen phosphate (**3e**), afforded compound **4e** (0.40 g, 78%) as yellow crystals: mp 215 °C. $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.08. IR (NaCl) 3383, 2920, 2850, 1652, 1466, 1246, 1063 $cm^{-1}$. MS (FAB) *m/z*: 562 $(M + 1)^+$. $a_N$ (ethanol) = 1.482 mT. Anal. ($C_{29}H_{58}N_2O_6P$) C, H, N.

**4-{[{[11-(2-Hexyl-3-oxyl-4,4-dimethyl-1,3-oxazolidine-2-yl)undecyl]oxy}(oxy)phosphoryl]oxy}-*N*,*N*,*N*-trimethyl-1-ethanaminium Inner Salt (5).** 2-Chloro-2-oxo-1,3,2-dioxaphosphorane (0.29 mL, 3.16 mmol) was added dropwise to a solution of 2-hexyl-2-(11-hydroxyundecyl)-3-oxyl-4,4-dimethyl-1,3-oxazolidine (**2b**) (0.50 g, 1.35 mmol) and triethylamine (0.44 mL, 3.16 mmol) in *tert*-butyl methyl ether (50 mL) at 0 °C. After stirring at room temperature for 3 h, the white precipitate was filtered off and the solvent evaporated under reduced pressure. The residue was dissolved in dry acetonitrile, and trimethylamine was added (1 mL). The flask was sealed and heated at 65 °C for 48 h. The resulting mixture was cooled and filtered, and the solvent was evaporated under reduced pressure. The resulting crude product was purified by gravity column chromatography using initially $CHCl_3$/MeOH/25% $NH_{3(aq)}$ (85/15/1.5) and subsequently $CHCl_3$/MeOH/25% $NH_{3(aq)}$ (78/30/4.7). The solvents were evaporated under reduced pressure to yield the desired product (0.61 g, 85%) as orange semisolid. $R_f$ ($CHCl_3$:MeOH:25% $NH_{3(aq)}$, 78:30:4.7) = 0.11. IR (NaCl) 3385, 2926, 2853, 1658, 1467, 1229, 1086 $cm^{-1}$. MS (FAB) *m/z*: 536 $(M + 1)^+$. $a_N$ (ethanol) = 1.489 mT. Anal. ($C_{27}H_{56}N_2O_6P\cdot H_2O$) C, H, N.

**Determination of Critical Micelle Concentrations.** cmc values were determined with a Krüss processor tensiometer K12. Starting solutions (1 M) of **4a**−**e** and **5** were progressively diluted with pure water. Surface tension was measured at 20.0 ± 0.2 °C using the plate method. cmc's were deduced by plotting the surface tension versus the logarithm of concentration.

**Preparation and Characterization of Liposomes.** Cholesterol and dicetyl phosphate were purchased from Sigma. MLV at a concentration of 11 mM total lipids were prepared by the lipid film/hydration method[5] by mixing stock solutions of spin-labeled alkylphospholipids (**4a**−**e**, **5**) (1.5 mL, 10 mM), cholesterol (1.5 mL, 10 mM), and dicetyl phosphate (1.5 mL, 2 mM) in $CH_2Cl_2$/MeOH (7:3, v/v). Lipid films were prepared in a round-bottomed flask by rotary evaporation, drying under high vacuum, and then hydrated with phosphate-buffered saline solution (PBS, pH 7.4, 3 mL) to a final concentration of 5 mM spin-labeled alkylphospholipid. MLV were obtained by shaking for 12 h. LUVET were prepared from these MLV by repeated extrusion through polycarbonate membranes of 200 nm pore size using a LiposoFast Basic system (Avestin, Ottawa, Canada). Vesicle size was determined by dynamic light scattering at 90° with a Coulter Counter N4 plus (Coulter Electronics, Hialeah). Size is expressed in nanometers as unimodal mean diameter ± SD and size distribution as polydispersity index (PI, varying from 0 for entirely monodisperse to 1 for completely polydisperse suspensions).

**Hemolytic Effect of SL-APL.** Hemolysis was performed according to Stensrud et al. with only minor adaptations as follows.[21] Blood from a healthy donor was collected in EDTA containing vacutainer tubes and centrifuged for 15 min at 470*g* to remove plasma. After washing with PBS (centrifugation at 840*g* for 10 min), cells were resuspended in PBS resulting in a suspension with $4.3 \times 10^6$ erythrocytes/$\mu$L. Erythrocytes were stored at 4 °C and used within 48 h.

One milliter of experimental sample was prepared containing lipids (30 nmol) or liposomes (30 or 100 nmol) in PBS (pH 7.5), 100 $\mu$L of 5.5 mM glucose solution, and 20 $\mu$L of erythrocyte-stock suspension (containing $8.59 \times 10^7$ erythrocytes). This sample was mixed and additionally shaken for 30 min at room temperature. Control samples without any lipid but with 10 $\mu$L of 10% Triton-X 100 to obtain the 100% values were treated similarly.

Samples were centrifuged again at 840*g* for 10 min, an aliquot of 400 $\mu$L was removed from the supernatant and transferred into a new tube, and 1600 $\mu$L of Drabkin's reagent was added. After vortexing, absorbance at 540 nm was measured. Each determination was performed in triplicate. Extinction was finally converted into percentage of hemolysis compared to control (complete hemolysis, obtained by addition of Triton-X 100).

**Cell Growth Inhibition Experiments in Vitro.** Human breast cancer cell lines MT1, MT3, and MCF7 were cultured in RPMI-1640 medium supplemented with L-glutamine (Gibco) and heat-inactivated fetal calf serum (10% FCS, Gibco). All reagents were free of endotoxin contamination. Cells in the exponential growth phase were seeded at a density of $2 \times 10^4$ per well in a 96-well microtiter plate on the day before the start of incubation with the experimental compound. Solution of compounds in liposomes and in free form were serially diluted with medium to concentrations between 200 and 6.25 $\mu$M and applied to the cells for 24 h in triplicate after removal of the old medium. MTT (0.5 mg/mL) was added to the cells, which were incubated for another 4 h. Finally, 180 $\mu$L of the supernatant was carefully removed and the formazan formed was completely dissolved in DMSO with shaking for 15 min. The formazan was quantified at 540 nm (Rainbow, Tecan SLT, Crailshaim, Germany). The percentage inhibition was calculated relative to the growth of control cells handled similarly but without drug exposure. $IC_{50}$ was determined from growth inhibition curves and expressed as mean ± SD of at least three independent experiments.

**Acknowledgment.** This research was supported by the Ministry of Education, Science and Sports of the Republic of Slovenia and by a bilateral agreement between Germany and Slovenia (BI-DE/03-04-002). The position of R.Z. was financed in part by a fund of the European Community. The authors thank the EPR Centre, Jožef Stefan Institute, for EPR spectra, the MS Centre, Jožef Stefan Institute, for mass spectra, the Faculty of Chemistry and Chemical Technology, for elemental analyses, and Prof. Roger Pain for his critical reading of the manuscript.

**Supporting Information Available:** Results from combustion analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) (a) Houlihan, W. J.; Lohmeyer, M.; Workman, P.; Cheon, S. H. Phospholipid Antitumor Agents. *Med. Res. Rev.* **1995**, *15*, 157−223. (b) Brachwitz, H.; Vollgraf, C. Analogs of Alkyllysophospholipids: Chemistry, Effects on the Molecular Level and Their Consequences for Normal and Malignant Cells. *Pharmacol. Ther.* **1995**, *66*, 39−82. (c) Principe, P.; Braquet, P. Advances in ether phospholipids treatment of cancer. *Crit. Rev. Oncol. Hematol.* **1995**, *18*, 155−178.

(2) Grosman, N. Effect of anti-neoplastic agents edelfosine (ET-18-$OCH_3$), ilmofosine (BM 41.440) and the hexadecylphosphocholines D-20133 and D-21266 on histamine release from isolated rat mast cells. *Immunopharmacology* **1999**, *44*, 211−221.

(3) (a) Unger, C.; Eibel, H.; Breiser, A.; Von Heyden, H. W.; Engel, J.; Hilgard, P.; Sindermann, H.; Peukert, M.; Nagel, G. A. Hexadecylphosphocholine (D-18506) in the topical treatment of skin metastases − a phase-I trial. *Onkologie.* **1988**, *11*, 295−296. (b) Unger, C.; Herrmann, R.; Berdel, W. E.; Kleeberg, U. R.; Gatzemeier, U.; Illiger, H. J.; Wander, H. E.; Westerhausen, M.; Becher, R.; Bremer, K.; Rieche, K.; Essers, U.; Queisser, W.; Heidemann, E.; Fiebif, H. H.; Possinger, K.; Jourdainmandl, B.; Heim, M. E.; Edler, L. Topically applied miltefosine (hexadecylphosphocholine) in patients with skin-metastasized breast-cancer − a phase II study. *Onkologie* **1993**, *16*, 170−173.

(4) Unger, C.; Peukert, M.; Sindermann, H.; Hilgard, P.; Nagel, G.; Eibl, H. Hexadecylphosphocholine in the topical treatment of skin metastases in breast cancer patients. *Prog. Exp. Tumor Res.* **1992**, *34*, 153−159.
(5) Zeisig, R.; Arndt, D.; Stahn, R.; Fichtner, I. Physical properties and pharmacological activity in vitro and in vivo of optimised liposomes prepared from a new cancerostatic alkylphospholipid. *BBA-Biomembranes* **1998**, *1414*, 238−248.
(6) (a) Hilgard, P.; Klenner, T.; Stekar, J.; Nossner, G.; Kutscher, B.; Engel, J. D-21266, a new heterocyclic alkylphospholipid with antitumour activity. *Eur. J. Cancer.* **1997**, *33*, 442−446. (b) Engel, J.; Hilgard, P.; Klenner, T.; Kutscher, B.; Nossner, G.; Traiser, M.; Voss, V. Perifosine−Oncolytic−Ether phospholipid. *Drugs Future* **2000**, *25*, 1257−1260.
(7) Grunicke, H. H.; Maly, K.; Uberall, F.; Schubert, C.; Kindler, E.; Stekar, J.; Brachwitz, H. Cellular signaling as a target in cancer chemotherapy. Phospholipid analogues as inhibitors of mitogenic signal transduction. *Adv. Enzyme Regul.* **1996**, *36*, 385−407.
(8) (a) Mollinedo, F.; Gajate, C.; Martin-Santamaria, S.; Gago, F. ET-18-OCH3 (Edelfosine): A selective antitumour lipid targeting apoptosis through intracellular activation of Fas/CD95 death receptor. *Curr. Med. Chem.* **2004**, *11*, 3163−3184. (b) Ogretmen, B.; Hannun, Y. A. Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat. Rev. Cancer* **2004**, *4*, 604−616. (c) Wieder, T.; Orfans, C. E.; Geilen, C. C. Induction of Ceramide-mediated Apoptosis by the Anticancer Phospholipid Analog, Hexadecylphosphocholine. *J. Biol. Chem.* **1998**, *273*, 11025−11031. (d) Ramos, B.; Moueddem, M.; Carlo, E.; Jackowski, S. Inhibition of CTP:Phosphocholine Citidylyltransferase by $C_2$−Ceramide and Its Relationship to Apoptosis. *Mol. Pharmacol.* **2002**, *62*, 1068−1075. (e) Jiménez-López, J. M.; Carrasco, M. P.; Segovia J. L.; Marco, C. Hexadecylphosphocholine inhibits phosphatidylcholine biosynthesis and the proliferation of HepG2 cells. *Eur. J. Biochem.* **2002**, *269*, 4649−4655. (f) Rahmani, M.; Reese, E.; Dai, Y.; Bauer, C.; Payne, S. G.; Dent, P.; Spiegel, S.; Grant, S. Coadministration of Histone Deacetylase Inhibitors and Perifosine Synergistically Induces Apoptosis in Human Leukemia Cells through Akt and ERK1/2 Inactivation and the Generation of Ceramide and Reactive Oxygen Species. *Cancer Res.* **2005**, *65*, 2422−2431. (g) Ruiter, G. A.; Zerp, S. F.; Bartelnik, H.; Blitterswijk, W. J.; Verheij, M. Alkyl-Lysophospholipids Activate the SAPK/JNK Pathway and Enhance Radiation-induced Apoptosis. *Cancer Res.* **1999**, *59*, 2457−2463.
(9) (a) Joseph, J.; Shih, C. C. Y.; Lai, C. S. Synthesis of the spin-labeled derivative of an ether-linked phospholipid possessing high antineoplastic activity. *Chem. Phys. Lipids* **1991**, *58*, 19−26. (b) Sosnovsky, G.; Lukszo, J.; Brasch, R. C. Preparation of an Aminoxyl Analog of the Anticancer Agent Miltefosine. *Z. Naturforsch. B* **1996**, *51*, 888−890.
(10) (a) Griffith, O. H.; Jost, P. C. Lipid Spin Labeles in Biological Membranes. In *Spin Labeling I, Theory and Applications;* Berliner, L. J., Ed.; Academic Press Inc.: New York, 1976; pp 453−523. (b) McConnell, H. M. Molecular Motion in Biological Membranes. In *Spin Labeling I, Theory and Applications*; Berliner, L. J., Ed.; Academic Press Inc.: New York, 1976; pp 525−560. (c) Štrancar, J.; Koklič, T.; Arsov, Z. Soft Picture of Latheral Heterogeneity in Biomembranes. *J. Membr. Biol.* **2003**, *196*, 135−146.
(11) (a) Smith, I. C. P.; Butler, K. W. Oriented Lipid Systems as Model Membranes. In *Spin Labeling I, Theory and Applications*; Berliner, L. J., Ed.; Academic Press Inc.: New York, 1976; pp 411−451. (b) Marsh, D.; Páli, T.; Horváth, L. I. Progressive Saturation and Saturation Transfer EPR for Measuring Exchange Processes and Proximity Relations in Membranes. In *Spin Labeling, The Next Millenium*, *Biological Magnetic Resonance*; Berliner, L. J., Ed.; Plenum Press: New York, 1998; Vol. 14, pp 23−82.
(12) (a) Hubbell, W. L.; McConnell, H. M. Molecular Motion in Spin-Labeled Phospholipids and Membranes. *J. Am. Chem. Soc.* **1971**, *93*, 314. (b) Hubbell, W. L.; McConnell, H. M. Orientation and motion of amphiphilic spin labels in membranes. *Proc. Nat. Acad. Sci. U.S.A.* **1969**, *64*, 20. (c) Keana, J. F. W. Synthesis and Chemistry of Nitroxide Spin Labels. *In Spin Labeling in Pharmacology*; Holtzman, J. L., Ed.; Academic Press: New York, 1984; pp 1−85. (d) Gaffney, B. J. The Chemistry of Spin Labels. In *Spin Labeling I, Theory and Applications;* Berliner, L. J., Ed.; Academic Press Inc.: New York, 1976; pp 184−238.
(13) (a) Keana, J. F. W.; Bernard, E. M.; Roman, R. B. Selective Reduction of Doxyl and Proxyl Nitroxide Carboxylic Acids to the Corresponding Alcohols with Borane Methyl Sulfide. *Synth. Commun.* **1978**, *8*, 169−173. (b) Sanson, A.; Ptak, M.; Rigaud, J. L.; Gary-Bobo, C. M. An ESR study of the anchoring of spin-labeled stearic acid in lecithin multilayers. *Chem. Phys. Lipids* **1976**, *17*, 435−444.
(14) Becker, H. G. O.; Berger, W.; Domschke, G.; Fanghänel, E.; Faust, J.; Fischer, M.; Gentz, F.; Gewald, K.; Gluch, R.; Mayer, R.; Müller, K.; Pavel, D.; Schmidt, H.; Schollberg, K.; Schwetlick, K.; Seiler, E.; Zeppenfeld, G. Reduction von Carbonylverbindungen durch Aluminium- und Borhydride. In *Organikum*; Wiley-VCH Verlag GmbH: Weinheim, 2001; pp 568−572.
(15) Noessner, G.; Kutscher, B.; Engel, J.; Schumacher, W.; Stekar, J.; Hilgard, P. Novi alkilni ali alkenski fosfati, postopek za njihovo pripravo in njihova uporaba kot zdravilnega sredstva. SI Patent 9 300 365 A, 1993.
(16) (a) Chandrakumar, N. S.; Hajdu, J. Stereospecific Synthesis of Ether Phospholipids. Preparation of 1-Alkyl-2-(acylamino)-2-deoxyglycerophosphorylcholines. *J. Org. Chem.* **1983**, *48*, 1197−1202. (b) Bhatia, S. K.; Hajdu, J. Stereospecific Synthesis of Ether and Thioether Phospholipids. The Use of L-Glyceric Acid as a Chiral Phospholipid Precursor. *J. Org. Chem.* **1988**, *53*, 5034−5039. (c) Menger, F. M.; Chen, X. Y.; Brocchini, S.; Hopkins, H. P.; Hamilton, D. Synthesis and Thermotropic Properties of Macrocyclic Lipids Related to Archebacterial Membranes. *J. Am. Chem. Soc.* **1993**, *115*, 6600−6608.
(17) Rakotomanga, M.; Loiseau, P. M.; Saint-Pierre-Chazalet, M. Hexadecylphosphocholine interaction with lipid monolayers. *Biochim. Biophys. Acta.* **2004**, *1661*, 212−218.
(18) (a) Bratton, D. L.; Harris, R. A.; Clay, K. L.; Henson, P. M. Effects of platelet activating factor and related lipids on phase transition of dipalmitoylphosphatidylcholine. *Biochim. Biophys. Acta.* **1988**, *941*, 76−82. (b) Noseda, A.; Godwin, P. L.; Modest, E. J. Effects of antineoplastic ether lipids on model and biological membranes. *Biochim. Biophys. Acta.* **1988**, *945*, 92−100.
(19) (a) Fleer, E. A. M.; Berkovic, D.; Unger, C.; Eibl, H. Cellular uptake and metabolic-fate of hexadecylphosphocholine. *Prog. Exp. Tumor Res.* **1992**, *34*, 33−46. (b) Kötting, J.; Marschner, N. W.; Neumüller, W.; Unger, C.; Eibl, H. Hexadecylphosphocholine and octadecyl-methyl-glycero-3-phosphocholine − a comparison of hemolytic-activity, serum binding and tissue distribution. *Prog. Exp. Tumor Res.* **1992**, *34*, 131−142.
(20) (a) Kocherginsky, N.; Swartz, H. M. Chemical Reactivity of Nitroxides. In *Nitroxide Spin Labels*; Kocherginsky, N., Swartz, H. M., Eds.; CRC Press: Boca Raton, FL, 1995; pp 27−66. (b) Swartz, H. M.; Šentjurc, M.; Kocherginsky, N. Metabolism of Nitroxides and Their Products in Cells. In *Nitroxide Spin Labels;* Kocherginsky, N., Swartz, H. M., Eds.; CRC Press: Boca Raton, FL, 1995; pp 27−66. (c) Prelesnik, T.; Nemec, M.; Pečar, S.; Demšar, F.; Kveder, M.; Schara, M. Oxygen in free radical reactions. *Vestn. Slov. Kem. Drus.* **1986**, *33*, 153−155.
(21) Stensrud, G.; Passi, S.; Larsen, T.; Sandset, P. M.; Smistad, G.; Mönkkönen, J.; Karlsen, J. Toxicity of gamma irradiated liposomes. 1. In vitro interactions with blood components. *Int. J. Pharm.* **1999**, *178*, 33−46.

JM050189V