Notes

Alkyl and Alkoxyethyl Antineoplastic Phospholipids

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Two series of phosphodiester ether lipid analogs with (*N*-methylmorpholino)ethyl or (*N*-methylpiperidino)ethyl polar head groups and long aliphatic or alkoxyethyl chains in the nonpolar portion of the molecule were synthesized as potential antineoplastic agents. The cytotoxic activity of these compounds (**9**–**19**) was evaluated *in vitro* against a panel of six human tumor xenografts and in two biochemical, mechanism-based screens (cdc2 kinase and cdc25 phosphatase). Analogs **13**, **14**, **17**, and **19** showed activity in the *in vitro* tests. Specifically, **14** and **17** were more active than the reference compound hexadecylphosphocholine (Miltefosine, He-PC) while **13** and **19** possessed activity similar to that of the control. Of the analogs tested the one with the highest potency and least toxicity (**17**) has an *N*-methylpiperidino head group and a C_{16} alkyl chain. In the mechanism-based tests **11** showed weak inhibitory activity in the cdc25 phosphatase screen.

Introduction

Ether lipid (EL) and alkyllysophospholipid (ALP) analogs have received much interest in recent years because of their cytotoxic effects on malignant cells *in vitro* and *in vivo*.[1] A number of these ether lipids including *rac*-2-*O*-methyl-1-*O*-octadecylglycero-3-phosphocholine (*rac*-ET-18-OCH_3, *rac*-Edelfosine),[2] hexadecylphosphocholine (Miltefosine, He-PC),[3] and others[4] have been studied in some detail. ELs structurally resemble the natural membrane components and are completely different from the classical antitumor drugs as their primary target appears to be the plasma membrane. Several mechanisms of action have been postulated to account for these biological effects, including enhancement of the cytotoxic properties of macrophages, alteration of phospholipid metabolism, and inhibition of PKC.[4d,5]

With the first-generation ALPs, and in particular ET-18-OCH_3 as a reference structure, many laboratories have embarked on the chemical synthesis of a variety of structurally related compounds and screening for antineoplastic activity. In addition, the use of synthetic ELs in the autologous bone marrow transplant technique is of considerable interest due to their nonhematotoxic effects.[6]

Structure–activity relationship (SAR) studies on a variety of synthetic alkyllysophosphocholines showed that a long alkyl chain and a phosphocholine moiety may represent the minimal structural requirements for sufficient antineoplastic effects of ether lipid analogs.[7,8] This finding led to the synthesis of a new group of analogs, the alkylphosphocholines.

Within the alkyl chain homologs, He-PC has therapeutically useful antitumor activity.[9] Alkylphosphocholines with longer alkyl chains such as octadecyl are antitumor effective but at the same time far too toxic to be used as potential drugs.[9a] *In vitro* studies on He-PC revealed antineoplastic activity on HL60, U937, Raji, and K562 leukemia cell lines. In addition, He-PC, administered orally, was superior for the treatment of dimethylbenzanthracene-induced rat mammary carcinomas when compared to intravenously administered cyclophosphamide.[7] In a clinical pilot study on breast cancer patients with widespread skin involvement, topically applied He-PC showed skin tumor regressions without local or systemic side effects.[7] On the basis of these promising observations, regular phase I and II trial studies (oral and topical applications) were performed.[10,11] In the phase II trials, He-PC was administered orally to patients with advanced non small cell lung carcinoma. The treatment lasted an average of 8 weeks, but the results, as with ET-18-OCH_3, were unsatisfactory. Furthermore, there were gastrointestinal side effects (WHO > 2) such as abdominal pain, anorexia, nausea, vomiting, and diarrhea.[1h] Since there seems to be a discrepancy between the biological activity of ALPs *in vitro* and in clinical trials, more extensive SAR studies are needed to further explore the role of these molecules as therapeutic agents.

As an ongoing effort aimed at exploring the structural and stereochemical requirements of amphipathic ether lipids for optimal cytotoxicity and/or immunomodulation,[12] we studied the effect of different head groups and

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Table 1. Antimitotic and Cytotoxic Activity of Alkylphospholipids

	inhibitory activity on cell cycle control enzymes, (IC_{50} μM)		*in vitro* cytotoxicity in six human tumor xenografts	
alkylphospholipid	Cdc2 kinase	Cdc25 phosphatase	xenografts[a] active/total	mean IC_{70} μg/mL
9	>1000	500	0/6	>10
10	>1000	290	1/6 (RXF)	>10
11	>1000	40	1/6 (RXF)	>10
12	>1000	610	1/6 (LXFS)	>10
13	>1000	380	2/6 (LXFS, RXF)	8.6
14	>1000	1000	3/6 (LXFS, MEXF, RXF)	7.8
15	>1000	>1000	1/6 (LXFS)	>10
16	>1000	490	0/6	>10
17	>1000	460	4/6 (LXFL, LXFS, MEXF, OVXF)	5.6
18	>1000	300	1/6 (LXFS)	>10
19	>1000	100	2/6 (LXFL, LXFS)	7.9
He-PC	>1000	25	2/6 (LXFL, RXF)	8.0

[a] Nonsmall cell lung carcinoma line LXFL 529, small cell lung carcinoma cell line LXFS 538, ovarian adenocarcinoma OVXF 1023, melanoma MEXF 989, colorectal adenocarcinoma CXF 1103, and renal cancer RXF 423. All compounds were tested at the 10 μg/mL dose level.

9. R = $C_{12}H_{25}$
10. R = $C_{14}H_{29}$
11. R = $C_{16}H_{33}$
12. R = $C_{12}H_{25}O(CH_2)_2$
13. R = $C_{14}H_{29}O(CH_2)_2$
14. R = $C_{16}H_{33}O(CH_2)_2$
15. R = $C_{12}H_{25}$
16. R = $C_{14}H_{29}$
17. R = $C_{16}H_{33}$
18. R = $C_{14}H_{29}O(CH_2)_2$
19. R = $C_{16}H_{33}O(CH_2)_2$

Figure 1. Structures of alkylphospholipids.

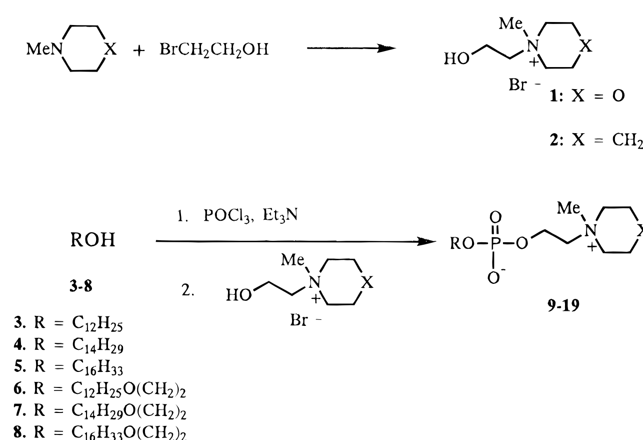
alkyl chains on the *in vitro* antineoplastic activity of ALPs. Specifically, we explored the effect of deleting the C2 or C1–C2 groups from the ALP structure to obtain the corresponding alkoxyethyl and the alkoxy phosphodiester ether lipids, respectively. We also evaluated how head group modifications affected *in vitro* cytotoxicity.

Two series of analogs were synthesized carrying *N*-methylmorpholino or *N*-methylpiperidino head groups and long aliphatic or alkoxyethyl chains in the alkyl portion of the molecule (Figure 1). The cytotoxic activity of these new compounds was then evaluated *in vitro* against a panel of human tumor xenografts[13] and in two biochemical, mechanism-based screens (cdc2 kinase and cdc25 phosphatase).[14,15] The tests showed that **13**, **14**, **17**, and **19** were active in the *in vitro* tests while **11** had a weak inhibitory activity in the cdc25 phosphatase screen.

Chemistry

Alkylphospholipids **9–19** were synthesized according to the general procedure depicted in Scheme 1. Quaternization of *N*-methylmorpholine or *N*-methylpiperidine to *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide (**1**) or *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide (**2**), respectively, was effected upon treatment with 1-bromoethanol. Phosphorylation of alcohols **3–8** using

Scheme 1. Synthetic Scheme for Alkylphospholipids **9–19**



phosphorus oxychloride in the presence of triethylamine followed by the addition of salts **1** and **2** gave the desired alkylphospholipids **9–19**. Alkoxyethanols **6–8** were prepared according to our previously reported method.[16]

Results and Discussion

The antimitotic activity of alkylphospholipids **9–19** (Table 1) was evaluated using purified cell cycle regulators as molecular targets. The first screening test uses the p34[cdc2]/cyclin B[cdc13] protein-kinase, affinity-purified on p9[CKShs] sepharose beads. The enzyme activity was assayed in the presence of potential inihibitors using Histone H1 and ^{32}P-labeled ATP.[15] The second screening test uses a highly purified human recombinant glutathione-S-transferase/cdc25 fusion protein assayed colorimetrically for *p*-nitrophenyl phosphate phosphatase activity in microtitration plates.[14] The use of these major cell cycle control proteins provides highly specific mechanism-based screens for antimitotic drug discovery. The level of activity for the cdc2 kinase and cdc25 phosphatase screens is defined at an IC_{50} value

of <50 μM. The calculated IC_{50} values for **9**−**19** reported in Table 1 show that only **11** exhibits weak inhibitory activity in cdc25 (IC_{50} = 40 μM). However, this analog was inactive in cdc2 (IC_{50} >100 μM). All other compounds were inactive in both screens. Similarly, hexadecylphosphocholine is active in cdc25 with an IC_{50} value of 25 μM but lacks inhibitory activity in cdc2. The cytotoxicity of alkylphospholipids **9**−**19** was tested in a panel of six human tumor xenografts (HTXs)[13] (Table 1). The panel consists of two sensitive lines, nonsmall cell lung carcinoma LXFL 529 and small cell lung carcinoma LXFS 538; two intermediately sensitive lines, ovarian adenocarcinoma OVXF 1023 and melanoma MEXF 989; and two resistant lines, colorectal adenocarcinoma CXF 1103 and renal cancer RXF 423.

In this *in vitro* assay, tumor cells taken from human tumor xenografts which were serially transplanted in nude mice are incubated continuously (for at least 1 week) with three concentrations of the drug, 0.1, 1, and 10 μg/mL, in cell culture medium. This is done for six HTXs, and colony formation is scored for each tumor. Drug effects are expressed as percentage survival obtained by comparing the mean number of colonies in treated plates with the mean number of colonies of control plates (test/control, % = T/C, %). A compound is considered to be cytotoxic if it reduces colony formation to less than 30% of the control value (T/C < 30%). The new analogs **9**−**19** were compared with He-PC which was active in two out of six human tumor xenografts at 10 μg/mL.

Our data show that the compounds tested were inactive at the dose levels of 0.1 and 1 μg/mL. As shown in Table 1, at the dose level of 10 μg/mL compounds **13**, **14**, **17**, and **19** were active with alkylphospholipids **14** and **17** being cytotoxic in a larger number of HTXs than He-PC. Ether lipids **10**, **11**, **12**, **15**, and **18** were less active, all having mean IC_{70} values greater than 10 μg/mL while **9** and **16** showed no activity. The most active analog **17**, which contains a C_{16} aliphatic chain in the alkyl portion of the molecule and a *N*-methylpiperidino component in the polar head group, had a mean IC_{70} value equal to 5.6 μg/mL and showed overall cytotoxicity 1.5-fold higher than that of the control He-PC (IC_{70} = 8 μg/mL).

In more detail, **10**, **11**, **13**, and **14** bearing the *N*-methylmorpholino head group showed activity against the resistant RXF HTX. In contrast, analogs with a *N*-methylpiperidino head group (**15**−**19**) are inactive in the RXF HTX but show cytotoxicity in the sensitive lines LXFL or LXFS.

Among the alkoxyethyl phospholipids, **13**, **14**, and **19** were found to be cytotoxic (Table 2). Of these the *N*-methylmorpholino analogs were less active in the RXF HTX than He-PC. In the LXFL HTX these analogs showed comparable activity to that of He-PC while in the LXFS HTX they were more potent. The *N*-methylpiperidino alkoxyethyl phospholipid **19** was substantially less active than He-PC in the RXF HTX, while it showed increased activity in the LXFL and LXFS HTXs compared to the control.

The most potent analog **17** showed significantly higher activity than He-PC in all HTXs with the exception of RXF. In particular, analog **17** was exceptionally potent in the LXFS HTX with an IC_{70} value of 1.9 μg/mL (Table 2).

Table 2. *In Vitro* Cytotoxicity in HTXs (IC_{70}, μg/mL) of Alkylphospholipids

tumor	13	14	17	19	He-PC (control)
CXF 1103	>10.000	>10.000	>10.000	>10.000	>10.000
LXFL 529	10.000	10.378	4.328	5.379	9.574
LXFS 538	5.849	5.115	1.895	4.540	>10.000
MEXF 989	>10.000	8.756	4.641	>10.000	>10.000
OVXF 1023	>10.000	>10.000	7.796	>10.000	>10.000
RXF 423	6.738	4.757	10.000	10.000	2.754
mean	8.562	7.776	5.564	7.906	8.007

The above data indicate that, in general, analogs with C_{16} alkyl residues are more cytotoxic than those with shorter aliphatic chains. Also, replacement of the long aliphatic chains of analogs **9**−**11** with alkoxyethyl groups leads to more potent compounds. With regard to head group SAR, the *N*-methylpiperidino analogs were more potent than the respective *N*-methylmorpholino and trimethylammonium congeners.

Conclusion

This study evaluated how changes in the choline head group and alkyl residues of alkylphosphocholines altered the *in vitro* cytotoxic efficacy of this class of compounds in six human tumor xenografts. The most active ether lipid and least toxic of all the compounds tested was **17** which has a *N*-methylpiperidino head group and a C_{16} alkyl chain. Thus, *N*-methylpiperidino may be an attractive head group modification when designing novel alkoxyphospholipids. In contrast, in the alkoxyethyl series the *N*-methylmorpholino head group is preferred not only with respect to activity but also in that the most promising analogs **13** and **14** are cytotoxic against resistant tumor cell line RXF.

Earlier studies[12b] have shown that ether lipid analogs exhibit selective toxicities toward cancer cells when compared to normal cells. Even though there is still no clearly accepted mechanism of action which could explain this selectivity, an attractive explanation may be based on the ability of ELs to preferentially accumulate at higher concentrations in cancer cell membranes, thus resulting in the destruction of these cells. This selective accumulation may, in turn, be due to differences in membrane organization and/or composition between cancer and normal cells.

Experimental Section

Antimitotic Activity. The antimitotic activity of the new compounds was evaluated according to the procedures by Rialet and Meijer[14] and Baratte *et al.*[15]

(a) $P34^{cdc2}$/Cyclin B Assay. Starfish oocytes were induced to enter into M phase with 10 μM 1MeAde, frozen in liquid nitrogen, and stored at −80 °C. When required, the oocytes were homogenized in homogenization buffer (1 g of oocytes/2 mL of buffer) and centrifuged for 45 min at 24000*g*.[15,17] The supernatant was loaded on $p9^{CKShs}$-sepharose beads at a ratio of 40 mL of supernatant/mL $p9^{CKShs}$-sepharose beads. The beads were prepared from recombinant human $p9^{CKShs}$ as described in Azzi *et al.*[18] After 30 min at 4 °C, under constant rotation, the beads were washed extensively with bead buffer. Active $p34^{cdc2}$/cyclin B kinase was eluted with free $p9^{CKShs}$ (3 mg/mL). The eluted kinase was assayed using Histone H1 as a substrate according to Arion *et al.*[17] and Meijer *et al.*[19] with

a few minor modifications. Briefly, 10 μL of p34^{cdc2}/cyclin B kinase, 5 μL of Histone H1 (5 mg/mL), 7 μL of buffer C, and 3 μL of inhibitor were mixed in a Rhesus tube. The kinase assay was started by addition of 5 μL of [^{32}P]ATP (90 μM) and run for 10 min at 30 °C. The assay was terminated by transferring 25 μL on 2.5 × 3 cm pieces of P81-phosphocellulose paper. After 20 s, these papers were washed six times with 10 mL of phosphoric acid/L for at least 5 min/wash. The filters were then dried and counted in a scintillation counter. Background activity (the kinase being replaced by buffer) was substracted from all data.

(b) P80^{cdc25} Phosphatase Assay. An *Escherichia coli* strain containing a plasmid encoding the genes fusion construct of glutathione-S-transferase (GST) and human cdc25A under the control of IPTG was used. *E. coli* were first grown in 100 μg of ampicillin/mL of LB medium. When the optical density at 600 nm had reached a value between 0.8 and 1.00, IPTG was added and the culture incubated for 7 h. Cells were then harvested by a 3000*g* centrifugation for l5 min at 4 °C. Pellets were kept frozen at −80 °C until extraction. The bacterial pellet was disrupted by sonication in lysis buffer at 4 °C. The homogenate was centrifuged for 30 min at 4 °C at 100000*g*. The supernatant was recentrifuged under similar conditions; the final supernatant was then immediately mixed and rotated with glutathione−agarose beads (equilibrated with lysis buffer) for 30 min at 4 °C. The glutathione−agarose beads were washed three times with 10 volumes of lysis buffer, followed by four washes with 10 volumes of Tris buffer A. Elution of the fusion protein was induced by three or four successive washes with 10 mM glutathione in Tris buffer A. The efficiency of the elution was monitored by the following phosphatase assay. Twenty microliters of GST-cdc25A protein (diluted to an activity of ∂ OD 405 nm = 0.2−0.3/60 min) was mixed with 20 μL of 100 mM DTT (in Tris buffer A) and 140 μL of Tris buffer A, in 96-wells microplates. The plates were preincubated at 37 °C for 15 min in a Denley Wellwarm 1 microplate incubator. The assays were initiated by addition of 20 μL of 500 mM p-NPP (in Tris buffer A). After a 60 min incubation at 37 °C, absorbance at 405 nm was measured in a BioRad microplate reader. Blank values (no GST-cdc25A protein added) were automatically substracted.

***In Vitro* Antitumor Activity.** A modification of the double-layer soft-agar assay as described by Hamburger and Salmon[20] was used as described recently.[13] Briefly, solid human tumor xenografts were mechanically disaggregated and subsequently incubated with an enzyme cocktail consisting of collagenase 0.05%, DNAse 0.07%, and hyaluronidase 0.1% in RPMI 1640 at 37 °C for 30 min. The cells were washed twice and passed through sieves of 200 and 50 μm each size. The percentage viable cells was determined in a hemocytometer using trypan blue exclusion. The tumor cell suspension was plated into 24-multiwell plates over a bottom layer consisting of 0.2 mL of Iscoves' modified Dulbecco's medium with 20% fetal calf serum and 0.7% agar. Next, 20 000 to 200 000 cells were added to 0.2 mL of the same culture medium and 0.4% agar and plated onto the base layer. Cytostatic drugs were applied by continuous exposure (drug overlay) in 0.2 mL of medium. In each assay, six control plates received the vehicle only; drug-treated groups were plated in triplicate in three concentrations: 0.1, 1, and 10 μg/mL. Drug effects are expressed as percentage survival obtained by comparing the mean number of colonies in treated plates with the mean number of colonies of control plates (test/control, %, T/C, %). Cytotoxicity is considered to be significant if the compound reduced colony formation to less than 30% of the control value (T/C < 30%).

The composition of the panel is based more on the sensitivity of the lines to standard anticancer agents than on ensuring that frequently occurring human tumor types are represented. Currently, the panel consists of two sensitive lines, nonsmall cell lung carcinoma line LXFL 529 and small cell lung carcinoma LXFS 538; two intermediately sensitive lines, ovarian adenocarcinoma OVXF 1023 and melanoma MEXF 989; and two resistant lines, colorectal adenocarcinoma CXF 1103 and renal cancer RXF 423. Most of the standard anticancer agents are active at a dose of ≤1 μg/mL.

A compound is considered to be active in the panel if it shows cytotoxic activity in ≥20% of the number of tumors tested at the dose of ≤10 μg/mL.

Materials and Experimental Procedures

All reactions were carried out under scrupulously dry conditions with magnetic stirring. Organic phases were dried over Na_2SO_4, and the solvent was evaporated under vacuum. Silica gel grade 60 (200−400 mesh, E. Merck, Germany) and ASTM (150−230 mesh, E. Merck, Germany) was used for flash and gravity column chromatography, respectively. All compounds were demonstrated to be homogeneous by analytical TLC on precoated silica gel TLC plates (grade 60, F254, E. Merck, Germany), and chromatograms were visualized by phosphomolybdic acid staining. All NMR spectra were recorded on a Bruker AC300 spectrometer operating at 300 MHz for ^{1}H and 121.44 MHz for ^{31}P. ^{1}H NMR spectra are reported in units of δ relative to internal $CHCl_3$ at 7.24 ppm. ^{31}P NMR spectra were proton noise decoupled and are reported in units of δ relative to 85% phosphoric acid as external standard; positive shifts are downfield. Analyses indicated by the symbols of the elements were carried out by the microanalytical section of the Institute of Organic and Pharmaceutical Chemistry of the National Hellenic Research Foundation and the microanalytical section of the Chemistry Department of the University College London.

General Procedure for the Quaternization of *N*-Methylmorpholine and *N*-Methylpiperidine. To a stirred solution of amine (49.5 mmol) in EtOH (10 mL) was added dropwise at room temperature 2-bromoethanol (9.2 g, 74 mmol). The resulting mixture was refluxed for 2 h. After cooling, the excess 2-bromoethanol and EtOH were evaporated *in vacuo*. Trituration of the crude mixture with ether afforded the desired quaternary salts.

***N*-(2-Hydroxyethyl)-*N*-methylmorpholinium bromide (1):** yield 99%; TLC (CH_2Cl_2/MeOH (85:15)) R_f 0.20; ^{1}H NMR (DMSO-d_6) δ 5.40 (t, 1H, J = 4.8 Hz), 4.10−3.92 (m, 6H), 3.72−3.52 (m, 6H), 3.32 (s, 3H).

***N*-(2-Hydroxyethyl)-*N*-methylpiperidinium bromide (2):** yield 99%; TLC (CH_2Cl_2/MeOH (85:15)) R_f 0.20; ^{1}H NMR (DMSO-d_6) δ 3.92−3.81 (m, 2H), 3.78−3.48 (m, 6H), 3.30 (s, 3H), 1.95−1.78 (m, 4H), 1.72−1.61 (m, 2H).

General Procedure for the Preparation of Alkylphospholipids and (Alkoxyethyl)phospholipids. To a stirred solution of phosphorus oxychloride (0.307 g, 2 mmol) and triethylamine (0.36 g, 3.6 mmol) in tetrahydrofuran (2.5 mL) was added dropwise at 0 °C a solution of the corresponding alcohol or alkoxyethanol (2 mmol) in tetrahydrofuran (2.5 mL). The resulting mixture was stirred for 10 min at 0 °C and subsequently hydrolyzed by the addition of H_2O (0.5 mL). After 1 h of stirring at room temperature, the reaction mixture was diluted with ether and the organic layer was washed with H_2O, brine and dried (Na_2SO_4). The solvent was evaporated *in vacuo* to afford the crude phosphoric acid derivative which was transformed to the pyridinium salt by the addition of pyridine (14 mL) and stirring for 2 h at 50 °C. After cooling the solvent was evaporated at high vacuum and pyridine (6 mL) was added to the residue. To the resulting solution was added dropwise at 0 °C a solution of triisopropylbenzenesulfonyl chloride (1.2 g, 3.96 mmol) in pyridine (23 mL) followed by the addition of *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide or *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide (3 mmol). The resulting mixture was stirred at 35−40 °C for 5 h. After cooling, the mixture was hydrolyzed by the addition of H_2O (1.5 mL) and 2-propanol (6 mL) and stirred for 10 min at room temperature, and the solvents were evaporated *in vacuo.* The resulting crude solid was purified by gravity column chromatography (CH_2Cl_2/MeOH/NH_4OH (60:50:5) followed by MeOH/NH_4OH (95:5)), and the solvents were evaporated *in vacuo*. The residue was diluted with $CHCl_3$ (5 mL) and filtered through a pore membrane (0.5 μm, FH Millipore) and the solvent removed under vacuum to give the desired product.

(*N*-Methylmorpholino)ethanol dodecyl phosphonate (9): yield 25%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.25;

^{1}H NMR δ 4.38−4.15 (m, 2H), 4.05−3.85 (m, 6H), 3.70−3.58 (m, 6H), 3.44 (bs, 3H), 1.55−1.42 (m, 2H), 1.32−1.06 (m, 18H), 0.82 (t, 3H, J = 6.3 Hz); ^{31}P NMR δ −2.0. Anal. ($C_{19}H_{40}NO_5P\cdot 2H_2O$) C, H, N.

(*N*-Methylmorpholino)ethanol tetradecyl phosphonate (10): yield 21%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.25; ^{1}H NMR δ 4.32−4.20 (m, 2H), 3.95−3.85 (m, 6H), 3.75−3.58 (m, 6H), 3.45 (s, 3H), 1.55−1.42 (m, 2H), 1.32−1.12 (m, 22H), 0.82 (t, 3H, J = 6.7 Hz); ^{31}P NMR δ −2.0. Anal. ($C_{21}H_{44}NO_5P\cdot 1.45H_2O$) C, H, N.

(*N*-Methylmorpholino)ethanol hexadecyl phosphonate (11): yield 22%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.25; ^{1}H NMR δ 4.32−4.25 (m, 2H), 4.05 −3.90 (m, 6H), 3.80−3.55 (m, 6 H), 3.47 (s, 3H), 1.60−1.48 (m, 2H), 1.35−1.15 (m, 26H), 0.86 (t, 3H, J = 6.5 Hz); ^{31}P NMR δ −2.0. Anal. ($C_{23}H_{48}NO_5P\cdot 0.5H_2O$) C, H, N.

(*N*-Methylmorpholino)ethanol 2-(dodecyloxy)ethyl phosphonate (12): yield 40%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.20; ^{1}H NMR δ 4.40−4.22 (m, 4H), 4.05−3.82 (m, 6H), 3.80−3.58 (m, 4H), 3.52−3.48 (m, 2H), 3.42 (s, 3H), 3.35 (t, 2H, J = 6.9 Hz), 1.55−1.38 (m, 2H), 1.30−1.11 (m, 18H), 0.82 (t, 3H, J = 6.6 Hz); ^{31}P NMR δ −2.0. Anal. ($C_{21}H_{44}NO_6P\cdot 1.5H_2O$) C, H, N.

(*N*-Methylmorpholino)ethanol 2-(tetradecyloxy)ethyl phosphonate (13): yield 31%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.20; ^{1}H NMR δ 4.35−4.18 (m, 4H), 4.00−3.85 (m, 6H), 3.74−3.59 (m, 4H), 3.52−3.49 (m, 2H), 3.41 (s, 3H), 3.36 (t, 2H, J = 6.9 Hz), 1.58−1.42 (m, 2H), 1.30−1.18 (m, 22H), 0.83 (t, 3H, J = 6.6 Hz); ^{31}P NMR δ −2.0. Anal. ($C_{23}H_{48}NO_6P\cdot 2H_2O$) C, H, N.

(*N*-Methylmorpholino)ethanol 2-(hexadecyloxy)ethyl phosphonate (14): yield 48.6%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.20; ^{1}H NMR δ 4.35−4.22 (m, 4H), 4.01−3.80 (m, 6H), 3.72−3.56 (m, 4H), 3.55−3.48 (m, 2H), 3.40 (s, 3H), 3.33 (t, 2H, J = 6.9 Hz), 1.50−1.38 (m, 2H), 1.30−1.12 (m, 26H), 0.80 (t, 3H, J = 6.6 Hz); ^{31}P NMR δ −2.0. Anal. ($C_{25}H_{52}NO_6P\cdot 0.5H_2O$) C, H, N.

(*N*-Methylpiperidino)ethanol dodecyl phosphonate (15): yield 16%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.24; ^{1}H NMR δ 4.32−4.18 (m, 2H), 3.92−3.81 (m, 2H), 3.80−3.48 (m, 6H), 3.32 (s, 3H), 1.95−1.78 (m, 4H), 1.72−1.61 (m, 2H), 1.60−1.48 (m, 2H), 1.35−1.15 (m, 18H), 0.83 (t, 3H, J = 6.3 Hz); ^{31}P NMR δ −1.5. Anal. ($C_{20}H_{42}NO_4P\cdot 2H_2O$) C, H, N.

(*N*-Methylpiperidino)ethanol tetradecyl phosphonate (16): yield 14%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.27; ^{1}H NMR δ 4.30−4.18 (m, 2H), 3.90−3.42 (m, 8H), 3.23 (s, 3H), 1.92−1.85 (m, 4H), 1.70−1.58 (m, 2H), 1.57−1.42 (m, 2H), 1.32−1.10 (m, 22H), 0.81 (t, 3H, J = 6.5 Hz); ^{31}P NMR δ −1.5. Anal. ($C_{22}H_{46}NO_4P\cdot H_2O$) C, H, N.

(*N*-Methylpiperidino)ethanol hexadecyl phosphonate (17): yield 16%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.25; ^{1}H NMR δ 4.15−4.06 (m, 2H), 3.70−3.32 (m, 8H), 3.15 (s, 3H), 1.78−1.62 (m, 4H), 1.58−1.30 (m, 4H), 1.22 −0.95 (m, 26H), 0.66 (t, 3H, J = 6.5 Hz); ^{31}P NMR δ −1.5. Anal. ($C_{24}H_{50}NO_4P\cdot 1.5H_2O$) C, H, N.

(*N*-Methylpiperidino)ethanol 2-(tetradecyloxy)ethyl phosphonate (18): yield 37.6%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.32; ^{1}H NMR δ 4.32−4.20 (m, 2H), 3.98−3.78 (m, 4H), 3.70−3.48 (m, 6H), 3.37 (t, 2H, J = 6.8 Hz), 3.29 (s, 3H), 1.90−1.80 (m, 4H), 1.70−1.60 (m, 2H), 1.54−1.42 (m, 2H), 1.32−1.14 (m, 22H), 0.84 (t, 3H, J = 6.5 Hz); ^{31}P NMR δ −2.2. Anal. ($C_{24}H_{50}NO_5P\cdot 2H_2O$) C, H, N.

(*N*-Methylpiperidino)ethanol 2-(hexadecyloxy)ethyl phosphonate (19): yield 30%; TLC (CH_2Cl_2/MeOH/NH_4OH (60:50:5)) R_f 0.32; ^{1}H NMR δ 4.40−4.25 (m, 2H), 4.07−3.73 (m, 4H), 3.70−3.48 (m, 6H), 3.37 (t, 2H, J = 6.8 Hz), 3.29 (s, 3H), 1.95−1.75 (m, 4H), 1.75−1.60 (m, 2H), 1.55−1.45 (m, 2H), 1.35−1.15 (m, 28H), 0.84 (t, 3H, J = 6.5 Hz); ^{31}P NMR δ −2.1. Anal. ($C_{26}H_{54}NO_5P\cdot 1.5H_2O$) C, H, N.

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