Antileishmanial Ring-Substituted Ether Phospholipids

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Three series of ring-substituted ether phospholipids were synthesized carrying *N*,*N*,*N*-trimethylammonium, *N*-methylpiperidino, or *N*-methylmorpholino headgroups. The first series is substituted by 2-cyclohexyloxyethyl or 2-(4-alkylidenecyclohexyloxy)ethyl groups, the second series by cyclohexylidenealkyl or adamantylidenealkyl moieties, and the third series by 2-aryloxyethyl or 6-aryloxyhexyl groups in the alkyl portion of the molecule. The antileishmanial activity of the new compounds was evaluated in vitro against the promastigote forms of *L. donovani* and *L. infantum* using an MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide)-based microassay as a marker of cell viability. Analogues **12**, **15**, **24**, **30**, **32**, **41**, **43**, and **45** were more potent than the control compound miltefosine (hexadecylphosphocholine) against both *L. donovani* and *L. infantum* while, derivatives **13** and **42** were equipotent to miltefosine. Analogues **16**, **17**, **19**, **20** were more potent than miltefosine against *L. infantum* and compounds **27**, **31**, **44** were more active than miltefosine against *L. donovani*. Differential scanning calorimetry (DSC) was used to probe the role of individual ether phospholipids on the physicochemical properties of model membranes. The DSC scans showed that the active compounds have a more profound effect on the thermotropic properties of model membrane bilayers than the less active ones.

Introduction

Leishmaniasis is a protozoan parasitic disease endemic in 88 countries, which causes considerable morbidity and mortality. At least 20 species of Leishmania can be transmitted by sandfly bites, originating cutaneous, diffuse cutaneous, mucocutaneous, and visceral leishmaniasis in humans, dogs, and various wild vertebrate hosts. The estimated yearly incidence is 1−1.5 million cases of cutaneous leishmaniasis and 500 000 cases of visceral leishmaniasis. The population at risk is estimated at 350 million people with an overall prevalence of 12 million.[1] Increasing risk factors are making leishmaniasis a growing public health concern for many countries around the world.[2]

The drugs most commonly used to treat leishmaniasis are the pentavalent antimonials sodium stibogluconate (Pentostam) and meglumine antimonate (Glucantime). Antimonial chemotherapy requires high dose regimens with long treatment courses using parenteral administration.[3] Second-line drugs, used in instances of antimonial-treatment failure, include amphotericin B (AMB), paromomycin (aminosidine), and pentamidine. However, all of these drugs are far from satisfactory due to unacceptable side effects at effective doses. The recently developed liposomal formulation of amphotericin B (AmBisome) showed good curative rates for antimony unresponsive cases of mucocutaneous leishmaniasis; however, drug administration is technically difficult and treatment costs are prohibitively expensive.[4]

The spreading resistance of the parasite toward the standby antimonial drugs, the high toxicity of most drugs in use, and the emergence of *Leishmania*/HIV coinfection as a new disease entity has triggered a continuous search for alternative therapies. Visceral leishmaniasis caused by *L. infantum* has emerged as an AIDS-associated opportunistic infection, particularly in southern Europe.[5] In recent years, alkyllysophospholipid analogues (ALPs) have received considerable interest due to their antineoplastic and immunomodulatory properties.[6] Their cytotoxic and cytostatic activity is remarkably selective for cancer cells, and they stimulate the host defense by activating macrophage cytotoxicity.[7] Whereas the majority of the conventional anticancer drugs may cause severe side effects due to bone marrow suppression, ALPs are known to exert minimal hematologic toxicity.[8] Also normal resting vascular endothelial cells are not affected by ALPs.[9] Edelfosine (ET-18-OCH_3, 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine) is the prototype of antineoplastic ether phospholipids and served as a lead drug for the synthesis of BM 41.440 (Ilmofosine), SRI 62-834 and others.

Extensive structure−activity relationship studies on a variety of ALPs showed that a long alkyl chain and a

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phosphocholine moiety may represent the minimal structural requirements for sufficient antineoplastic effects of ether lipid analogues.[10] This finding led to the synthesis of the alkylphosphocholines (APCs). Within the alkyl chain homologues, hexadecylphosphocholine (HePC, miltefosine) has therapeutically useful antitumor activity and was approved in 1992 as a drug in Germany for the topical treatment of metastasized mammary carcinoma.

Several in vitro and in vivo studies demonstrated that alkylphosphocholines including HePC, and alkylglycerophosphocholines such as edelfosine, ilmofosine, and SRI-62,834 possess antileishmanial activity.[11] Hexadecylphosphocholine was reported to be highly effective in treating mice infected with visceral leishmaniasis while oral treatment with miltefosine was 600-fold more effective than the subcutaneous administration *o*f pentostam. On the basis of these promising observations, miltefosine was evaluated in phase I and II clinical trials as oral therapy for Indian visceral leishmaniasis while phase III clinical trials are currently ongoing.[12] Cure rates of 88% to 100% were obtained using doses of 100−150 mg/day for 28 days. These results encouraged studies on the efficacy of miltefosine treatment for cutaneous leishmaniasis in the New World and currently phase II studies are being conducted. In a phase I study, the cure rate with miltefosine at doses of 100−150 mg for 3 weeks was 94%.[13] In the various clinical trials, the main side effects associated with miltefosine were gastrointestinal with the most common being moderate vomiting and diarrhea. Transient elevation of transaminases or urea/serum creatinine was noted in a number of patients and decreased under continued treatment. Although the toxicity associated with miltefosine sounds milder than that of some parenteral therapies, gastrointestinal symptoms could be of more consequence in severely ill patients, such as those who are malnourished or dehydrated. In addition, treatment of pregnant women is contraindicated because of miltefosine's teratogenic properties in animals.[14] Furthermore, miltefosine has a very long half-life and low therapeutic ratio and a course of treatment leaves a subtherapeutic level in the blood for several weeks. These drug characteristics might be expected to encourage development of resistance.[15] Additionally, miltefosine was shown to be only temporarily effective in HIV coinfected patients in Europe. Thus, more extensive SAR studies are needed to further explore the role of ether phospholipids as therapeutic agents for leishmanial infections.

Since the alkylphosphocholine analogues studied so far contain simple saturated or unsaturated alkyl chains in the lipid portion, we set out to probe the influence of cycloalkane rings on the antileishmanial activity. Specifically, we explored the effect of the presence of 4-alkylidenecyclohexyl and cyclohexylidene or adamantylidene groups in alkoxyethyl and alkoxy phosphodiester ether lipids, respectively. These can be envisaged as resulting from the deletion of the C2 or C1−C2 groups from the ALP structure. We also introduced aryl substituents in both categories in order to explore the role of an aromatic moiety on the antileishmanial activity. In addition, we evaluated how headgroup modifications affected in vitro antileishmanial activity.

Three series of analogues were synthesized carrying *N*,*N*,*N*-trimethylammonium, *N*-methylpiperidino, or *N*-methylmorpholino headgroups. The first series incorporates cyclohexyloxyethyl or 4-alkylidenecyclohexyloxyethyl groups, while, the second series has cyclohexylidene or adamantylidene moieties linked to the polar headgroup by an oligomethylene bridge of 5 or 11 carbons. The third series introduces 2-aryloxyethyl or 6-aryloxyhexyl groups in the alkyl portion of the molecule.

The antileishmanial activity of the new compounds was evaluated in vitro against the promastigote forms of *L. donovani* and *L. infantum* using an MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide-based enzymatic method as a marker of cell viability.

Chemistry

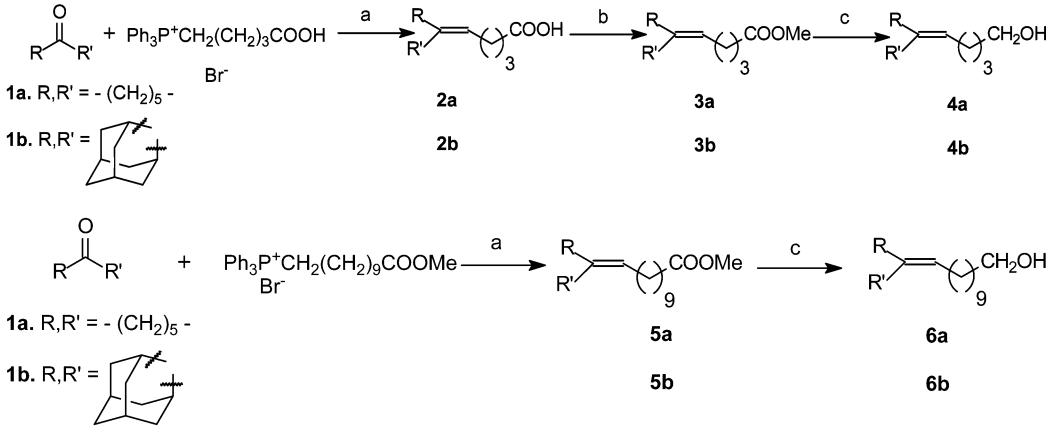
The synthetic strategy followed for the preparation of the ether phospholipids **9**−**40** is depicted in Scheme 3. The respective alcohols were treated with phosphorus oxychloride and triethylamine in THF to afford the corresponding pyridinium salts after hydrolysis and treatment with pyridine. These, in turn were coupled with the appropriate choline, *N*-methylpiperidino, or *N*-methylmorpholino headgroups in the presence of a condensing agent 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazolide (MSNT)[16] or triisopropylbenzenesulfonyl chloride (TIPS-Cl).[17] TIPS-Cl was employed for the synthesis of the 2-cyclohexyloxyethyl-containing ether phospholipids while MSNT was more suitable for the synthesis of cyclohexylidene- and adamantylidene-containing ether phospholipids.

Quaternization of *N*-methylpiperidine or *N*-methylmorpholine to *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide or *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide, respectively, was effected upon treatment with 1-bromoethanol.[18]

Alcohols **4a**,**b** were synthesized through a Wittig reaction between cyclohexanone (**1**) or adamantanone (**2**), respectively, and 4-carboxybutyl triphenylphosphonium bromide followed by esterification of the unsaturated acids **2a**,**b** with methanol, in the presence of a catalytic amount of H_2SO_4, and subsequent reduction using $LiAlH_4$ (Scheme 1). Initially, reduction of acids **2a**,**b** to the desired alcohols **4a**,**b** proceeded in low yields. However, the yields became quantitative when the respective methyl esters **3a**,**b** were used. The synthesis of alcohols **6a**,**b** is depicted in Scheme 1. Wittig reaction of cyclohexanone (**1**) or adamantanone (**2**) with 10-methoxycarbonyldecyl triphenylphosphonium bromide afforded the unsaturated esters **5a**,**b**, respectively, which were in turn reduced using $LiAlH_4$ to give the desired alcohols **6a**,**b**. The 10-methoxycarbonyldecyl triphenylphosphonium bromide was prepared via esterification of 11-bromoundecanoic acid in $MeOH/H^+$ and subsequent treatment of the corresponding methyl ester with triphenylphosphine in dry acetonitrile at 80 °C for 24 h. Crystallization using diethyl ether/acetone (1:2) afforded the pure phosphonium salt.

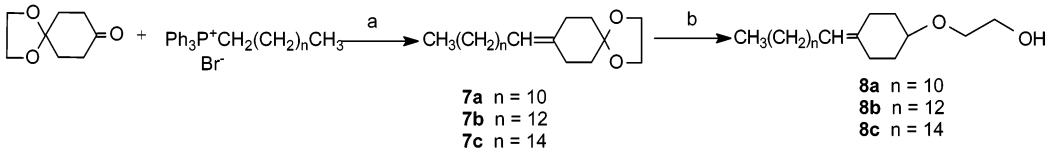
The 2-(4-alkylidenecyclohexyloxy)ethanols **8a**−**c** were synthesized as shown in Scheme 2. Wittig reaction of 1,4-cyclohexanedione monoethylene ketal with the ap-

Scheme 1[a]



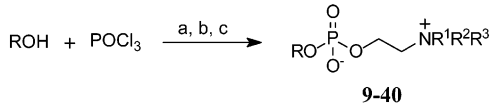
[a] Reagents and conditions: (a) $[(CH_3)_3Si]_2NK$, THF; (b) MeOH, H^+, 40 °C; (c) $LiAlH_4$, THF.

Scheme 2[a]



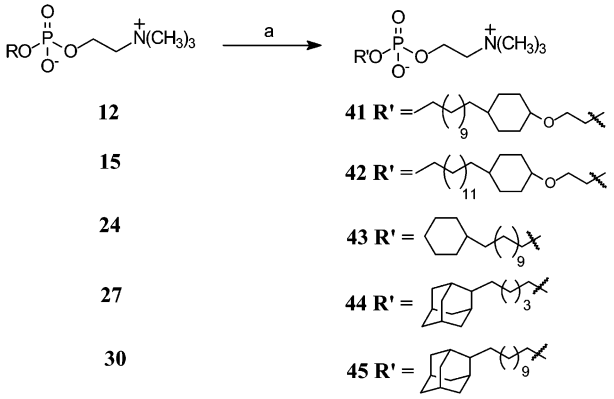
[a] Reagents and conditions: (a) $[(CH_3)_3Si]_2NK$, THF; (b) $NaBH_3CN$, $BF_3{\cdot}Et_2O$, THF.

Scheme 3[a]



[a] Reagents and conditions: (a) 1. $P(O)Cl_3$, Et_3N, THF; 2. H_2O, 2-propanol; (b) pyridine, 40 °C; (c) pyridine, MSNT or TIPS-Cl, $HOCH_2CH_2N^+R^1R^2R^3$, 40 °C.

Scheme 4[a]



[a] Reagents and conditions: (a) H_2, 10% Pd/C, EtOAc, 1 atm.

propriate alkyltriphenylphosphonium bromide led to the corresponding 4-alkylidenecyclohexyl-4-dioxolanes **7a**−**c.** Reductive opening of the ketals **7a**−**c** using $BF_3{\cdot}Et_2O$ and $NaBH_3CN$ in THF[19] gave the respective 2-(4-alkylidenecyclohexyloxy)ethanols **8a**−**c**. Synthesis of the aryloxyethanols or 6-aryloxyhexanols used for the preparation of ether phospholipids **35**−**40** was effected as previously described by *O*-alkylation of phenol or 2-naphthol with 2-bromoethanol or 6-bromohexanol in DMF in the presence of K_2CO_3.[18]

Catalytic hydrogenation of compounds **12**, **15**, **24**, **27**, **30** using 10% Pd/C in ethyl acetate afforded ether phospholipids **41**−**45**, respectively (Scheme 4).

Results and Discussion

The new analogues **9**−**40** (Figure 1) and **41**−**45** (Scheme 4) were evaluated in vitro for antileishmanial activity against promastigote cultures of *Leishmania donovani* MON 703 and *Leishmania infantum* MON 235 using MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide as a marker of cell viability (Table 1). In general 2-(4-alkylidenecyclohexyloxy)ethyl analogues **12**−**20**, 11-cyclohexylideneundecyl analogues **36**−**38** and 11-adamantylideneundecyl analogues **30**−**32** are active against both *Leishmania* strains and several of these are more potent than miltefosine. Conversely, the 2-cyclohexyloxyethyl derivatives **9**−**11** and those bearing aromatic rings in the lipid portion, i.e., compounds **34**−**40**, are inactive, exhibiting IC_{50} values higher than 100 μM. Generally, the trimethylammonium analogues were more potent than the respective *N*-methylpiperidino and *N*-methylmorpholino congeners.

A more detailed SAR is now described. In the 2-cyclohexyloxyethyl series introduction of an unsaturated long alkyl chain at the 4 position of the cyclohexane leads to ether phospholipids **12**−**20** that are active against both *Leishmania* strains. In particular, the tetradecylidene-substituted trimethylammonium compound **15** is the most potent of the series and also more potent than miltefosine with IC_{50} values 3.91 μM against *L. donovani* and 5.25 μM against *L. infantum*. The activity slightly decreases when the trimethylammonium group is substituted by *N*-methylpiperidino **16** (IC_{50} 29.7 μM against *L. donovani* and 11.4 μM against *L. infantum*). The decrease is more pronounced for the *N*-methylmorpholino substituted analogue **17** (IC_{50} 38.6 μM against *L. donovani* and 15.5 μM against *L. infantum*). Replacement of the tetradecylidene group by hexadecylidene results in reversal in order of activity within the headgroup analogues **18**−**20** with the *N*-methylmorpholino-bearing ether phospholipid, deriva-

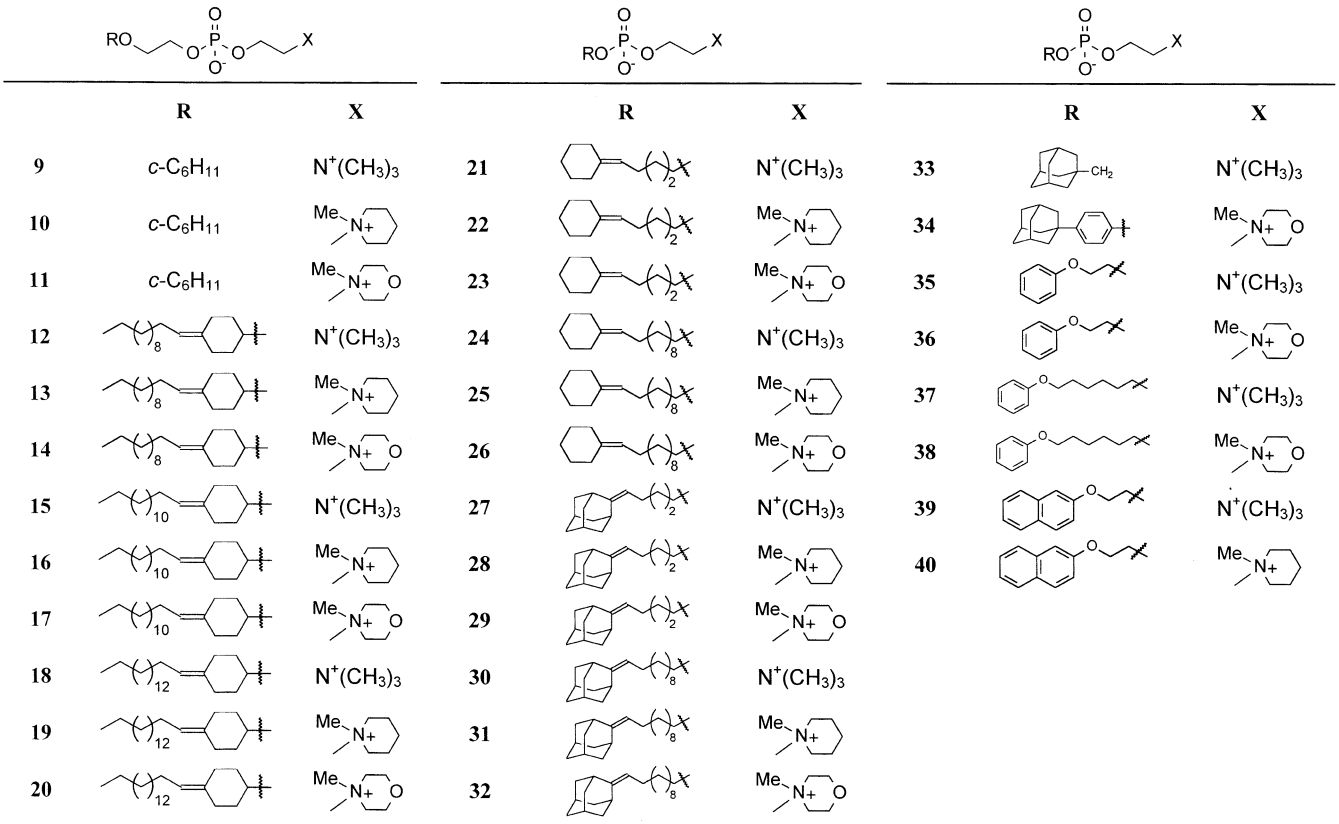


Figure 1. Structures of ether phospholipids **9**−**40**.

Table 1. In Vitro Antileishmanial Activity[a] against the Promastigote Forms of *L. infantum* and *L. donovani* of the New Ether Phospholipids

compd	IC50 (μM) *L. infantum* MON 235	IC50 (μM) *L. donovani* MON 703	compd	IC50 (μM) *L. infantum* MON 235	IC50 (μM) *L. donovani* MON 703
Miltefosine	22.56 ± 3.6	23.71 ± 4.07	**27**	>100	4.99 ± 1.50
9	>100	>100	**28**	>100	>100
10	>100	>100	**29**	>100	46.85 ± 8.7
11	>100	>100	**30**	6.75 ± 2.4	3.16 ± 0.63
12	3.25 ± 0.65	7.08 ± 1.2	**31**	22.58 ± 3.4	5.41 ± 1.14
13	23.07 ± 3.6	22 ± 3.25	**32**	6.64 ± 1.2	5.09 ± 1.86
14	16.46 ± 1.8	50.67 ± 3.6	**33**	>100	>100
15	5.25 ± 0.45	3.91 ± 0.21	**34**	>100	>100
16	11.4 ± 2.4	29.7 ± 3.6	**35**	>100	>100
17	15.5 ± 1.8	38.6 ± 3.2	**36**	>100	>100
18	21.19 ± 2.6	45.1 ± 7.2	**37**	>100	>100
19	6.5 ± 1.7	21.96 ± 1.99	**38**	>100	>100
20	3.7 ± 0.71	16.22 ± 2.29	**39**	>100	>100
21	>100	>100	**40**	>100	>100
22	>100	>100	**41**	5.65 ± 1.93	9.49 ± 1.4
23	>100	>100	**42**	23.3 ±3.5	23.65 ± 4.4
24	5.2 ± 1.5	2.4 ± 0.6	**43**	8.4 ± 0.8	10.3 ± 1.3
25	47.6 ± 7.33	8.7 ± 1	**44**	>100	4.02 ± 2.3
26	22.8 ± 1.7	8.25 ± 0.25	**45**	5.97 ± 1.06	2.88 ± 0.72

[a] Results are expressed as mean ± SEM, $n = 3-4$ (each run in duplicate).

tive **20**, being the most active of the three with IC_{50} 16.22 μM against *L. donovani* and 3.7 μM against *L. infantum* followed by the *N*-methylpiperidino-substituted analogue **19** to the trimethylammonium-substituted **18**. Both **19** and **20** are considerably more potent than miltefosine against *L. infantum* (IC_{50} 6.5 μM and 3.7 μM, respectively). Within the 2(4-dodecylidenecyclohexyloxy)ethyl series the choline derivative **12** is the most active and more potent than miltefosine against *L. infantum* ($IC_{50} = 3.25$ μM) and against *L. donovani* ($IC_{50} = 7.08$ μM). Thus, within this series **12**, **15**, **16**, **17**, **19**, and **20** are more potent than miltefosine against *L. infantum* while, ether phospholipids **12**, **15**, and **20** are more active than miltefosine against *L. donovani*.

In the second series the 5-cyclohexylidenepentyl derivatives **21**−**23** are inactive within the concentration range tested (IC_{50} values greater than 100 μM), while replacement of the cyclohexylidene portion in the above compounds by an adamantylidene group (**27**−**29**, respectively) establishes activity against *L. donovani* but not *L. infantum*. In particular, ether phospholipid **27** carrying a choline headgroup is more potent than miltefosine ($IC_{50} = 4.99$ μM), while *N*-methylmorpholino analogue **29** is less active ($IC_{50} = 46.85$ μM) and the

N-methylpiperidino compound **28** is inactive (IC_{50} > 100 μM).

The 11-cyclohexylideneundecyl analogues **24**−**26** are very potent. 11-Cyclohexylideneundecyloxy ether phospholipid **24** shows higher activity than miltefosine against both *L. donovani* and *L. infantum* (IC_{50} = 2.4 μM and IC_{50} = 5.2 μM, respectively), while the respective compounds **25**, **26** exhibit somewhat reduced activity. The decrease in activity of analogues **25**, **26** is more pronounced against *L. infantum* (IC_{50} = 47.6 μM and IC_{50} = 22.8 μM, respectively).

Ether phospholipid **30** bearing an 11-adamantylideneundecyl group is also more potent than miltefosine against both *L. donovani* and *L. infantum* (IC_{50} = 3.16 μM and IC_{50} = 6.75 μM, respectively). Within the headgroup analogues of **30** the *N*-methylmorpholino one is also more potent than miltefosine against both leishmania strains, while the *N*-methylpiperidino compound **32** exhibits decreased activity against *L. infantum* and similar activity to **31** against *L. donovani*.

Analogues **33**−**40** of the third series had IC_{50} values >100 μM regardless of the headgroup or the nature of the aryl substituent (phenyl or naphthyl). Elongation of the ethoxy group to hexyloxy (**37**, **38**) did not result in any increase in activity, while the 4-adamantylidenophenyl substituted derivative **34** was also inactive at the doses tested. On the basis of the above data, it cannot be deduced whether further elongation of the alkoxy group would improve the antileishmanial activity.

The presence of the double bond on antileishmanial activity was explored by comparing some of the most potent ether phospholipids **12**, **15**, **24**, **27**, **30** with their saturated congeners **41**−**45** obtained by catalytic hydrogenation. The absence of the double bond in the cyclohexylidene-substituted compounds results in decrease in activity, suggesting that the more rigid cycloalkylidene groups impart higher potencies. However, in the adamantylidene-substituted derivatives the absence or presence of the double bond does not seem to affect their potency.

The length of the alkyl chain of the most active compounds varies from 5 to 11 carbon atoms for the alkylphosphocholine analogues and from 12 to 14 for the alkoxyethylphosphocholine analogues. This could be advantageous for the solubility and/or the toxicity of the new compounds and also for their metabolic clearance.

Thus, we proceeded to assess the cytotoxicity of four of the most active ether phospholipids and miltefosine, in the human monocytic cell line THP1. THP1 monocytes infected with the appropriate *Leishmania* species are used for the evaluation of the leishmanicidal activity of compounds against the intracellular amastigote stages of the parasite. Our preliminary results (Figure 2) showed a strong cytotoxic effect of miltefosine on THP1 cells at concentrations as low as 12.5 μM, which was not observed with two of the most active analogues **24** and **30**. Conversely, compounds **12** and **15** are more cytotoxic than miltefosine. However, further experiments are required in order to evaluate the cytotoxicity of the other active new ether phospholipids and get a better understanding on the structural features that impart enhanced activity and minimal cytotoxicity.

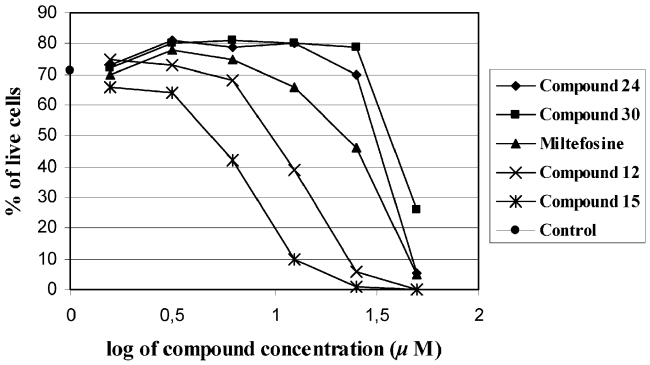


Figure 2. Percentage of live THP1 cells in the presence of different concentrations of ether phospholipids

Lipophilic alkylphospholipids influence membrane physiology in a pleiotrophic manner. In leishmania, this effect is believed to involve ether lipid metabolism, glycosylphosphatidylinositol (GPI) anchor biosynthesis, and signal transduction.[20] We used Differential scanning calorimetry (DSC) to probe the role of individual ether phospholipids on the physicochemical properties of model membranes.[21] DSC is a thermodynamic technique, which can be used to examine the effects of drug molecules on the thermotropic properties of membranes. For our experiments we used dipalmitoyl phosphatidyl choline (DPPC) bilayers as model membranes. When hydrated, phosphatidylcholines spontaneously form bilayers which are characterized by a mesomorphism. In particular, DPPC bilayers exist in the gel phase (L_{β}') at temperatures below 33 °C, and in the liquid crystalline phase (L_{α}') at temperatures above 42 °C. Between 33 and 42 °C the phospholipid bilayers exist in P_{β}' or ripple phase. DSC scans of DPPC bilayers show a small endothermic event (pretransition) around 35 °C and the main phase transition around 41 °C. The main phase transition is accompanied by several structural changes in the lipid molecules as well as systematic alterations in the bilayer geometry, but the most prominent feature is the trans−gauche isomerization taking place in the acyl chain conformation. The average number of gauche conformers indicates the effective fluidity, which depends not only on the temperature, but also on perturbations due to the presence of a drug molecule intercalating between the lipids. Three adamantyl-substituted ether phospholipids with different pharmacological profile were chosen for the DSC studies. In particular, compound **30** showed considerable activity against both *L. donovani* and *L. infantum*, analogue **27** was only active against *L. donovani*, and analogue **33** was inactive against both strains. The compounds were tested for these effects on bilayers at three concentrations (1%-mol (x = 0.01), 5%-mol (x = 0.05) and 10% (x = 0.10)) (Figure 3).

The two active compounds **30** and **27** had pronounced perturbing effects on DPPC bilayers causing broadening in the pre- and main transition peaks and lowering of the pre- and main phase transition temperatures (Table 2). The inactive ether phospholipid **33** caused only marginal effects on the phase properties of DPPC bilayers (Table 2). Interestingly, compound **30** affects the pretransition temperature to the same extent as **27** but causes more broadening of the main phase transition peak at the high concentrations of x = 0.05 and x = 0.10. These results may indicate similar perturbation

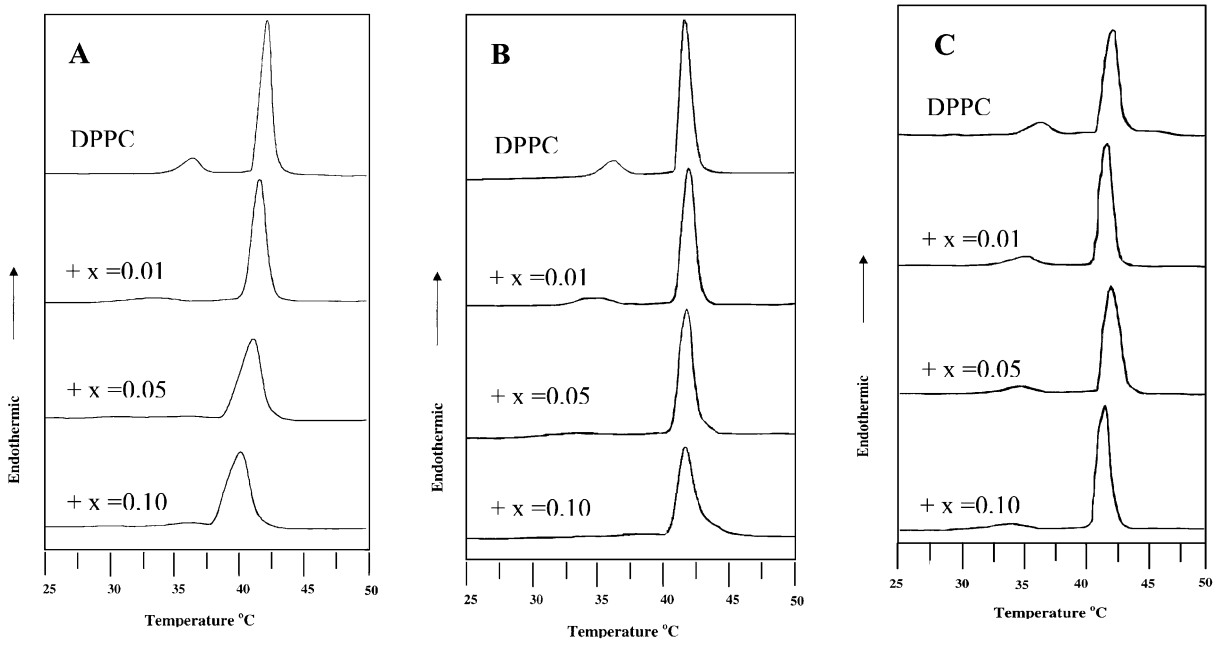


Figure 3. DSC scans of (A) DPPC with either phospholipid **30**, (B) DPPC with either phospholipid **27** and (C) DPPC with either phospholipid **33**.

Table 2. Values of Onset Temperature (T_{onset}), Half-Width Temperature ($T_m^{1/2}$), Peak Temperature T_m, and Enthalpy Change (ΔH) of DPPC/Ether Phospholipid preparations

sample	T_{onset}		T_m		$T_m^{1/2}$		ΔH (kcal/mol)	
DPPC alone								
DPPC+ **30** ($x = 0.01$)	29.0	39.6	32.0	40.8	-	1.9	4.0	43.3
DPPC + **27**($x = 0.01$)	31.5	40.2	33.9	41.2	3.1	1.1	4.8	42.9
DPPC + **33** ($x = 0.01$)	31.8	39.8	34.3	40.7	2.5	1.0	5.7	43.4
DPPC + **30** ($x = 0.05$)	-	38.1	-	40.2	-	1.9	-	40.9
DPPC + **27** ($x = 0.05$)	28.5	39.5	31.4	40.8	-	1.3	2.4	41.9
DPPC + **33** ($x = 0.05$)	31.1	40.1	33.9	41.0	3.1	1.2	5.0	43.8
DPPC + **30** ($x = 0.10$)	-	37.1	-	39.2	-	2.1	-	40.2
DPPC + **27** ($x = 0.10$)	-	39.1	-	40.2	-	1.5	-	37.7
DPPC + **33** ($x = 0.10$)	29.2	39.5	32.9	40.6	3.6	1.0	4.9	45.8

effects by both analogues on the polar region of the membrane bilayers, while compound **30** interacts effectively with the hydrophobic region as well. The above data indicate that the active ether phospholipids affect the physicochemical properties of DPPC bilayers to a greater extent than the inactive analogue.

To get some insight on the origin of the different biological activity and interaction with DPPC bilayers of the two adamantylidene derivatives **30** and **27** we studied their conformations.

Conformational Analysis. The conformational analysis for ether phospholipid derivatives **27** and **30** was carried out using a combination of NMR spectroscopy and computational analysis. Molecular dynamics using the most important ROE constraints and coupled with minimization algorithms were initially carried out for compound **27** and **30** to generate seven families of low energy conformers. From those conformers only three (Figure 4, A−C) for ether phospholipid **27** support the ROE data.

These conformers are characterized by a clustering of the adamantane ring with the phosphocholine moiety. The remaining four open forms are devoid of such clustering and express the flexibility of the alkyl chain.

Concerning ether phospholipid **30**, the resolution of the 2D-ROESY experiment did not allow to differentiate between the open and closed conformers since all methylene groups matched to one peak (Figure 5, D−J).

The obtained results from the conformational analysis suggest a similar conformation for both compounds. Thus, we can assume that the differential effects observed in the DSC by the two molecules result from their differences in the size of the alkyl chain. The analogue **30** with the longer alkyl chain, is embedded into the membrane bilayers causing significant perturbation. In contrast, compound **27** has access into the membrane core without disturbing the acyl chains of the bilayer.

Conclusions

This study evaluated how incorporation of cycloalkane or aromatic rings in the alkyl residues and changes in the choline based headgroup alters the antileishmanial activity of alkylphosphocholines. The most active analogues against both the promastigote forms *L. donovani* and *L. infantum* are 2-(4-dodecylidene cyclohexyloxy)-ethylphosphocholine (**12**), 2-(4-tetradecylidenecyclohexyloxy)ethylphosphocholine (**15**), 11-cyclohexylideneundecylphosphocholine (**24**), 11-adamantylideneundecylphosphocholine (**30**), and the hydrogenated derivatives **41**, **43**, **45** which are all more potent than miltefosine. Preliminary evaluation of the cytotoxicity in THP1 monocytes revealed that analogues **24** and **30** are less cytotoxic than miltefosine, while compounds **12** and **15** exhibit higher cytotoxicity. Several of the new compounds possess comparable activity to miltefosine against *L. donovani* but are more potent than miltefosine against *L. infantum*. With regard to headgroup SAR, choline is preferred with respect to activity against both leishmania strains. The introduction of cycloalkylidene groups in alkylphosphocholines results in enhanced activity and especially against the strain *L. infantum* that causes the AIDS associated coinfection in Europe. In particular, the presence of 2-(4-alkylidenecyclohexyloxy)ethyl moiety renders the resulting compounds more active against *L. infantum* while the presence of ω-cy-

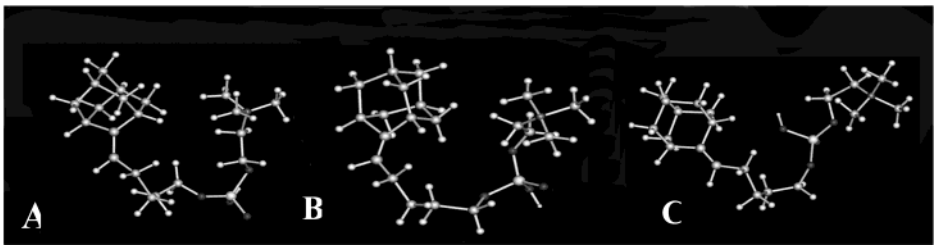


Figure 4. Lowest energy conformers of compound **27** (A−C) resulting from the combined use of NMR spectroscopy and computational chemistry. Hydrogens are not shown for clarity.

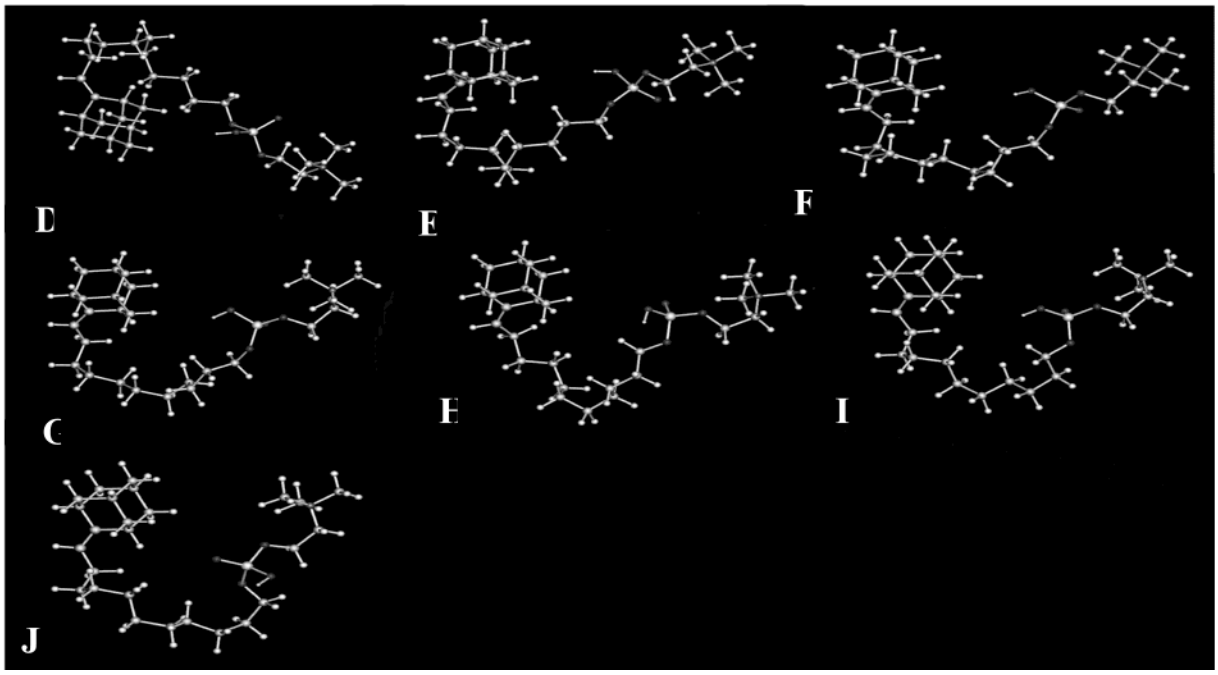


Figure 5. Lowest energy conformers of compound **30** (D−J) resulting from the combined use of NMR spectroscopy and computational chemistry. Hydrogens are not shown for clarity.

clohexylidenealkyl or ω-adamantylidenealkyl groups results in enhanced activity against *L. donovani*. Previous in vitro studies have shown variations in sensitivity of *Leishmania* species to different classes of drugs.[22] The difference in activity between the various ether phospholipids of this study may be due to dissimilarities in both membrane sterol[23,24] and lipid content[25] of the two *Leishmania* species studied. However, further investigations are required to verify this hypothesis.

Since alkylphosphocholines have been found active against other trypanosomatids[26] such as *Trypanosoma cruzi* and *T. brucei* as well as against the human intestinal parasite *Entamoeba histolytica*,[27] it will be of interest to examine the effect of the new ether phospholipids against these parasites.

Experimental Section

Chemistry. All reactions were carried out under scrupulously dry conditions. NMR spectra of all new compounds were recorded on a Bruker AC 300 spectrometer operating at 300 MHz for ^{1}H, 75.43 MHz for ^{13}C, and 121.44 MHz for ^{31}P. ^{1}H NMR spectra are reported in units δ with $CHCl_3$ resonance at 7.24 ppm used as the chemical shift resonance. ^{13}C NMR shifts are expressed in units relative to $CDCl_3$ at 77.00 ppm, while ^{31}P NMR spectra are reported in units of δ relative to 85% H_3PO_4 used as an external standard. Silica gel plates (Merck F_{254}) were used for thin-layer chromatography. Chromatographic purification was performed with silica gel (200−400 mesh). 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.

5-Cyclohexylidenepentanoic Acid (2a). A solution of 4-carboxybutyltriphenylphosphonium bromide (5.43 g, 12.24 mmol) in anhydrous THF (20 mL) was treated with potassium bis(trimethylsilyl)amide (4.88 g, 24.8 mmol), and the resulting orange mixture was stirred at room temperature for 15 min. A solution of cyclohexanone (**1**) (300 mg, 3.06 mmol) in anhydrous THF (10 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 3 h. The reaction was quenched with H_2O, and the water layer was extracted with ether to remove the unreacted cyclohexanone. The water layer was acidified with 10% HCl to pH 2, and it was extracted with ethyl acetate. The organic layer was washed with brine and was dried with Na_2SO_4, and the solvent was evaporated in vacuo to afford acid **2a** 450 mg (80%) as an oil which was used without further purification in the next step. ^{1}H NMR (δ): 5.02 (t, J = 6.7 Hz, 1H, C=C*H*), 2.21 (t, J = 7.9 Hz, 2H, CH_2CO), 1.94−1.84 (m, 6H), 1.53−1.32 (m, 8H).

5-Adamantylidenepentanoic Acid (2b). Acid **2b** was prepared according to the procedure described for acid **2a** in 75% yield, using 4-carboxybutyltriphenyl phosphonium bromide (3.55 g, 8 mmol) in anhydrous THF (20 mL), potassium bis(trimethylsilyl)amide (3.19 g, 16 mmol), and a solution of 2-adamantanone (**2**) (300 mg, 2 mmol) in anhydrous THF (10 mL) and refluxing the reaction mixture for 12 h. ^{1}H NMR (δ): 5.00 (t, J = 6.7 Hz, 1H, C*H*=C), 2.77 (s, 1H), 2.29 (t, J = 7.9 Hz, 2H, CH_2CO), 1.99−1.64 (m, 17H).

5-Cyclohexylidenepentanoic Acid Methyl Ester (3a). To a solution of acid **2a** in methanol (30 mL) was added 1 mL

concentrated H_2SO_4, and the mixture was stirred at 40 °C for 3 h. The solvent was evaporated in vacuo, and the residue was partitioned between water and ethyl acetate. The organic layer was washed with brine and dried over Na_2SO_4, and the solvent was evaporated in vacuo. The residue was purified by flash column chromatography (petroleum ether 40−60 °C/ethyl acetate (95:5)) to afford ester **3a** as an oil in 93% yield (450 mg); ^{1}H NMR (δ): 4.91 (t, J = 6.7 Hz, 1H, C=C*H*), 3.52 (s, 3H, COOCH_3), 2.16 (t, J = 7.9 Hz, 2H, CH_2CO), 1.98−1.91 (m, 6H), 1.53−1.40 (m, 8H).

5-Adamantylidenepentanoic Acid Methyl Ester (3b). Ester **3b** was prepared according to the procedure described for methyl ester **3a.** The crude reaction mixture was purified by flash column chromatography (petroleum ether 40−60 °C/ethyl acetate (9:1)) to afford ester **3b** in 84% yield; ^{1}H NMR (δ): 4.92 (t, J = 6.7 Hz, 1H, C*H*=C), 3.59 (s, 3H, COOCH_3), 2.70 (s, 1H, C*H*C=C), 2.27−2.21 (m, 3H, CH_2CO, C*H*C=), 1.96−1.57 (m, 16H).

5-Cyclohexylidenepentanol (4a). To a suspension of $LiAlH_4$ (139.6 mg, 3.68 mmol) in anhydrous THF (10 mL) was added dropwise at 0 °C a solution of ester **3a** (360 mg, 1.84 mmol) in anhydrous THF (10 mL), and the resulting mixture was stirred at room temperature for 2 h. Subsequently, the reaction was quenched at 0 °C by a mixture of H_2O/THF (1:1, 3 mL) followed by addition of ethyl acetate (30 mL). The resulting mixture was dried with anhydrous Na_2SO_4, the solid was filtered, and the filtrate was evaporated in vacuo to afford alcohol **4a** as a viscous oil in 96% yield (370 mg); ^{1}H NMR (δ): 4.99 (t, J = 6.7 Hz, 1H, C=C*H*), 3.54 (t, J = 6.7 Hz, 2H, CH_2-OH), 3.15 (broad s, 1H, O*H*), 2.06−1.93 (m, 6H), 1.51−1.28 (m, 10H).

5-Adamantylidenepentanol (4b). Alcohol **4b** was prepared according to the procedure described for alcohol **4a.** Yield: 80%; ^{1}H NMR (δ): 5.00 (t, J = 6.7 Hz, 1H, C*H*=C), 3.62 (t, J = 6.7 Hz, 2H, CH_2OH), 2.77 (s, 1H, C*H*C=C), 2.29 (s, 1H, C*H*C=C), 2.02−1.23 (m, 18H).

11-Cyclohexylideneundecanoic Acid Methyl Ester (5a). Ester (**5a**) was prepared according to the procedure described for acid **2a** using a solution of 10-methoxycarbonyldecyltriphenylphosphonium bromide (6.62 g, 12.24 mmol) in dry THF (20 mL), potassium bis(trimethylsilyl)amide (2.44 g, 12.24 mmol), and cyclohexanone (**1**) (300 mg, 3.06 mmol) in anhydrous THF (10 mL) and stirring at room temperature for 2 h. The reaction was quenched with H_2O, and the water layer was extracted with ethyl acetate. The organic layer was washed with brine and dried with Na_2SO_4, and the solvent was evaporated in vacuo. The crude residue was purified by flash column chromatography (petroleum ether 40−60 °C/diethyl ether (9:1)) to afford ester **5a** as viscous oil 680 mg (79% yield); ^{1}H NMR (δ): 5.07 (t, J = 6.7 Hz, 1H, C*H*=C), 3.65 (s, 3H, COOCH_3), 2.31 (t, J = 7.9 Hz, 2H, CH_2CO), 2.11−1.93 (m, 4H), 1.67−1.50 (m, 8H), 1.25 (broad s, 14H, (CH_2)$_7$).

11-Adamantylideneundecanoic Acid Methyl Ester (5b). Ester **5b** was prepared according to the procedure described for ester **5a** using 10-methoxycarbonyldecyltriphenylphosphonium bromide (4.33 g, 8 mmol), potassium bis(trimethylsilyl)amide (1.59 g, 8 mmol), and 2-adamantanone (**2**) (300 mg, 2 mmol) and heating at 80 °C. Purification of the ester was achieved by flash column chromatography (petroleum ether 40−60 °C/diethyl ether (9:1)) to afford ester **5b** as viscous oil 330 mg (50% yield). ^{1}H NMR (δ): 4.97 (t, J = 6.7 Hz, 1H, C*H*=C), 3.61 (s, 3H, COOCH_3), 2.75 (s, 1H, C*H*C=C), 2.28 (t, J = 7.3 Hz, 2H, CH_2CO), 2.22 (s, 1H, C*H*C=C), 1.92−1.55 (m, 16H), 1.24 (broad s, 12H).

11-Cyclohexylideneundecanol (6a). The procedure described for alcohol **4a** was followed using ester **5a** (680 mg, 2.43 mmol) to afford **6a** as a viscous oil (600 mg, 98% yield); ^{1}H NMR (δ): 5.01 (t, J = 6.7 Hz, 1H, C*H*=C), 3.55 (t, J = 6.7 Hz, 2H, CH_2OH), 3.12 (broad s, 1H, O*H*), 2.08−1.90 (m, 6H), 1.46 (broad s, 8H), 1.22 (broad s, 14H).

11-Adamantylideneundecanol (6b). The procedure described for alcohol **4a** was followed using ester **5b** (330 mg, 1 mmol) to afford **6b** as a viscous oil (290 mg, 95%); ^{1}H NMR (δ): 4.99 (t, J = 6.7 Hz, 1H, C*H*=C), 3.60 (t, J = 6.7 Hz, 2H, CH_2OH), 2.77 (s, 1H, C*H*C=C), 2.28 (s, 1H, C*H*C=C), 1.94−1.53 (m, 16H), 1.25 (broad s, 14H).

General Procedure for the Synthesis of 8-Alkylidene-1,4-dioxaspiro[4.5]decanes. To a solution of the corresponding alkylphosphonium salt (0.01mol) in dry THF (15 mL) was added potassium bis(trimethylsilyl) amide (2.19 g, 0.01 mmol), and the resulting orange solution was stirred at room temperature for 15 min. Subsequently, a solution of 1,4-dioxaspiro-[4.5]decan-8-one (1.5 g, 0.01 mmol) in dry THF (10 mL) was added dropwise, and the resulting mixture stirred at room temperature for 12 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution, and the aqueous layer was extracted with ethyl acetate. The organic layer was washed with brine and dried with Na_2SO_4, and the solvent evaporated in vacuo.

8-Dodecylidene-1,4-dioxaspiro[4.5]decane (7a). Purification of the crude residue by flash column chromatography (petroleum ether 40−60 °C/ethyl acetate (9:1)) afforded alkene **7a** as a viscous oil 2.8 g, (91% yield); ^{1}H NMR (δ): 5.10 (t, J = 6.7 Hz, 1H, C*H*=C), 3.91 (s, 4H, OCH_2CH_2O), 2.25−2.16 (m, 4H), 2.00−1.90 (m, 2H), 1.63−1.58 (m, 4H), 1.23 (s, 18H, (CH_2)$_9$), 0.83 (t, J = 7.0 Hz, 3H, CH_3).

8-Tetradecylidene-1,4-dioxaspiro[4.5]decane (7b). Purification of the crude residue by flash column chromatography (petroleum ether 40−60 °C/ethyl acetate (9:1)) afforded alkene **7b** as a viscous oil 1.6 g, (48% yield); ^{1}H NMR (δ): 5.12 (t, J = 6.7 Hz, 1H, C*H*=C), 3.93 (s, 4H, OCH_2CH_2O), 2.27−2.18 (m, 4H), 1.99−1.93 (m, 2H), 1.67−1.60 (m, 4H), 1.25 (bs, 22H, (CH_2)$_{11}$), 0.89 (t, J = 7.0 Hz, 3H, CH_3).

8-Hexadecylidene-1,4-dioxaspiro[4.5]decane (7c). Purification of the crude residue by flash column chromatography (petroleum ether 40−60 °C/ethyl acetate (9:1)) afforded alkene **7c** as a viscous oil 3.6 g (quantitative); ^{1}H NMR (δ): 5.05 (t, J = 6.7 Hz, 1H, C*H*=C), 3.85 (s, 4H, OCH_2CH_2O), 2.18−2.11 (m, 4H), 2.09 (t, J = 6.7 Hz, 2H, CH_2CH=C), 1.63−1.52 (m, 4H), 1.35 (broad s, 26H, (CH_2)$_{13}$), 0.95 (t, J = 7.0 Hz, 3H, CH_3).

General Procedure for the Synthesis of 2-(4-Alkylidenecyclohexyloxy)ethanols (8a−c). A solution of the corresponding ketal **7a−c** (5 mmol) in dry THF (5 mL) was sequentially treated with boron trifluoride etherate (0.76 mL, 6 mmol) and $NaBH_3CN$ (0.564 g, 9 mmol), and the resulting mixture was stirred at room temperature for 2 h. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$ solution, and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine and dried with anhydrous Na_2SO_4, and the solvent was evaporated in vacuo.

2-(4-Dodecylidenecyclohexyloxy)ethanol (8a). Purification of the crude residue by flash column chromatography (petroleum ether 40−60 °C/ethyl acetate (7:3)) afforded alcohol **8a** as a viscous oil (0.849 g, 55% yield); ^{1}H NMR (δ): 5.04 (t, J = 6.7 Hz, 1H, C*H*=C), 3.62 (broad s, 2H), 3.51−3.46 (m, 2H), 3.42−3.36 (m, 1H, CH$_2$C*H*OCH$_2$), 2.80 (broad s, 1H, O*H*), 2.41−2.36 (m, 1H), 2.19−2.15 (m, 1H), 1.95−1.77 (m, 6H), 1.41−1.33 (m, 2H, CH_2CH=C), 1.20 (broad s, 18H, (CH_2)$_9$), 0.87 (t, J = 7.0 Hz, 3H, CH_3).

2-(4-Tetradecylidenecyclohexyloxy)ethanol (8b). Purification of the crude residue by flash column chromatography (petroleum ether 40−60 °C/ethyl acetate (7:3)) afforded alcohol **8b** as a viscous oil (0.791 g, 47% yield); ^{1}H NMR (δ): 5.12 (t, J = 6.7 Hz, 1H, C*H*=C), 3.71−3.68 (m, 2H, OCH_2CH$_2$-OH), 3.58−3.55 (m, 2H, OCH$_2$CH_2OH), 3.48−3.44 (m, 1H, CH$_2$C*H*OCH$_2$), 2.47−2.41 (m, 1H), 2.26−2.21 (m, 1H), 2.19−1.86 (m, 6H), 1.50−1.41 (m, 2H, CH_2CH=C), 1.24 (broad s, 22H, (CH_2)$_{11}$), 0.89 (t, J = 7.0 Hz, 3H, CH_3).

2-(4-Hexadecylidenecyclohexyloxy)ethanol (8c). Purification of the crude residue by flash column chromatography (petroleum ether 40−60 °C/ethyl acetate (7:3)) afforded alcohol **8c** as a viscous oil (1.69 g, 93% yield); ^{1}H NMR (δ): 5.12 (t, J = 6.7 Hz, 1H, C*H*=C), 3.71−3.64 (m, 2H, OCH_2CH$_2$OH), 3.55−3.50 (m, 2H, OCH$_2$CH_2OH), 3.48−3.44 (m, 1H, CH$_2$C*H*OCH$_2$), 2.45−2.40 (m, 1H), 2.25−2.20 (m, 1H), 2.12−1.85 (m, 6H), 1.51−1.42 (m, 2H, CH_2CH=C), 1.27 (s, 26H, (CH_2)$_{13}$), 0.89 (t, J = 7.0 Hz, 3H, CH_3).

General Procedure for the Preparation of Ether Phospholipids. To a solution of phosphorus oxychloride (0.09 mL, 1 mmol) and triethylamine (0.25 mL, 1.8 mmol) in dry THF (5 mL) was added dropwise at 0 °C a solution of the corresponding alcohol (1 mmol) in dry THF (7 mL). The resulting mixture was stirred for 2 h at room temperature and subsequently hydrolyzed by the addition of water (3 mL). After 1 h of stirring at room temperature, the reaction mixture was diluted with water, and the aqueous layer was extracted with ethyl acetate and dichloromethane. The combined organic extracts were washed with brine and dried with anhydrous Na_2SO_4, and the solvent was evaporated in vacuo to afford the corresponding phosphoric acid derivative, which was transformed to the pyridinium salt by the addition of 7 mL of anhydrous pyridine and stirring for 2 h at 40 °C. After cooling, the solvent was evaporated in vacuo and pyridine (5 mL) was added to the residue. To the resulting solution was added dropwise, with cooling, a solution of 1-(mesitylen-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) (0.593 g, 2 mmol) or 2,4,6-triisopropylbenzenesulfonyl chloride (TIPS-Cl) (0.606 g, 2 mmol) in dry pyridine (2 mL) followed by the addition of choline chloride (0.210 g, 1.5 mmol) or *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide (0.448 g, 1.5 mmol) or *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide (0.452 g, 1.5 mmol). The reaction mixture was stirred at 40 °C for 48−56 h. After cooling, the mixture was hydrolyzed by the addition of H_2O (2 mL) and 2-propanol (7 mL) and stirred for 1 h at room temperature. The solvents were evaporated in vacuo, and the resulting crude solid was purified by gravity column chromatography using initially CH_2Cl_2/MeOH/25% NH_4OH (60/50/5) and subsequently MeOH/25% NH_4OH (95/5), and the solvents were evaporated in vacuo. The residue was diluted with $CHCl_3$ and filtered through a pore membrane (0.5 μM, FH Millipore). After evaporation of the solvent, the desired product was obtained.

1-{2-[(Cyclohexyloxyethyloxy)hydroxyphosphinyloxy]ethyl}-*N,N,N*-trimethylammonium Inner Salt (9). The general procedure described above using 2-(cyclohexyloxy)ethanol,[19] TIPS-Cl and choline chloride afforded compound **9** (80 mg, 26%). ^{1}H NMR (δ): 4.27 (broad s, 2H, POCH_2CH$_2$N), 3.90 (broad s, 2H, CH_2OP), 3.77 (broad s, 2H, OCH_2CH$_2$OP), 3.59 (broad s, 2H, CH_2N$^+$), 3.35 (s, 9H, N$^+$(CH_3)$_3$), 1.89 (broad s, 1H, C*H*O), 1.69−1.18 (m, 10H); ^{31}P NMR (δ): −2.17; ^{13}C NMR (δ): 67.7, 67.6, 66.1, 64.9, 59.2, 54.3, 32.4, 29.6, 25.7, 24.2; ESI-MS *m/z*: 332.1 (M$^+$ + Na), 310.1 (M$^+$ + 1); Anal. ($C_{13}H_{28}NO_5P\cdot H_2O$) C, H, N.

1-{2-[(Cyclohexyloxyethyloxy)hydroxyphosphinyloxy]ethyl}-1-methylpiperidinium Inner Salt (10). The general procedure described above using 2-(cyclohexyloxy)ethanol, TIPS-Cl, and *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide afforded compound **10** (70 mg, 20%).

^{1}H NMR (δ): 4.28 (s, 2H, POCH_2CH$_2$N), 3.90 (broad s, 2H, CH_2OP), 3.88 (broad s, 2H, OCH_2CH$_2$OP), 3.65−3.52 (m, 6H, CH_2N(CH_2)$_2$), 3.29 (s, 3H, N$^+$(CH_3)), 1.85−1.10 (m, 17 H); ^{31}P NMR (δ): −1.85; ^{13}C NMR (δ): 67.7, 64.7, 64.6, 63.5, 61.8, 58.5, 58.4, 48.5, 32.3, 25.7, 24.0, 20.8, 20.1; ESI-MS *m/z*: 372.1 (M$^+$ + Na$^+$), 350.1 (M$^+$ + 1); Anal. ($C_{16}H_{32}NO_5P\cdot 2H_2O$) C, H, N.

1-{2-[(Cyclohexyloxyethyloxy)hydroxyphosphinyloxy]ethyl}-1-methylmorpholinium Inner Salt (11). The general procedure described above using 2-(cyclohexyloxy)ethanol, TIPS-Cl and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide afforded compound **11** (0.122 g, 35%). ^{1}H NMR (δ): 4.29 (broad s, 2H, POCH_2CH$_2$N), 4.01−3.56 (m, 14H), 3.38 (s, 3H, N$^+$(CH_3)), 1.89−1.17 (m, 11H); ^{31}P NMR ($CDCl_3$) (δ): −2.32; ^{13}C NMR (δ): 67.6, 67.5, 64.8, 64.7, 64.4, 60.7, 58.5, 48.1, 32.4, 25.7, 24.2; ESI-MS *m/z*: 374.1 (M$^+$ + Na$^+$), 352.1 (M$^+$ + 1). Anal. ($C_{15}H_{30}NO_6P\cdot 3H_2O$) C, H, N

1-{2-{[(4-Dodecylidenecyclohexyloxy)ethyloxy]hydroxyphosphinyloxy}ethyl}-*N,N,N*-trimethylammonium Inner Salt (12). The general procedure described above using alcohol **8a**, TIPS-Cl and choline chloride afforded compound **12** (0.327 g, 69%). ^{1}H NMR (δ): 5.06 (t, J = 6.7 Hz, 1H, C=C*H*), 4.24 (broad s, 2H, POCH_2CH$_2$N), 3.89 (broad s, 2H), 3.76 (broad s, 2H), 3.57 (broad s, 2H), 3.40−3.35 (m, 1H, C*H*O), 3.30 (s, 9H, N$^+$(CH_3)$_3$), 2.40−1.72 (m, 8H), 1.42−1.33 (m, 2H), 1.24 (broad s, 18H, (CH_2)$_9$), 0.84 (t, J = 7.0 Hz, 3H, CH_3); ^{31}P NMR (δ): −2.16; Anal. ($C_{25}H_{50}NO_5P\cdot H_2O$) C, H, N

1-{2-{[(4-Dodecylidenecyclohexyloxy)ethyloxy]hydroxyphosphinyloxy}ethyl}-1-methylpiperidinium Inner Salt (13). The general procedure described above using alcohol **8a**, TIPS-Cl and *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide afforded compound **13** (0.350 g, 68%). ^{1}H NMR (δ): 5.16 (t, J = 6.70 Hz, 1H, C=C*H*), 4.24 (bs, 2H, POCH_2-CH$_2$N), 3.82−3.55 (m, 10H), 3.30 (broad s, 1H, C*H*O), 3.25 (s, 3H, N$^+$CH_3), 1.92−1.43 (m, 14H), 1.41−1.32 (m, 2H, CH_2CH=), 1.19 (broad s, 18H, (CH_2)$_9$), 0.83 (t, J = 7.0 Hz, 3H, CH_3); ^{31}P NMR (δ): −2.19; Anal. ($C_{28}H_{54}NO_5P\cdot 2H_2O$) C, H, N.

1-{2-{[(4-Dodecylidenecyclohexyloxy)ethyloxy]hydroxyphosphinyloxy}ethyl}-1-methylmorpholinium Inner Salt (14). The general procedure described above using alcohol **8a**, TIPS-Cl, and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide afforded compound **14** (0.330 g, 64%). ^{1}H NMR (δ): 5.11 (t, J = 6.7 Hz, 1H, C=C*H*), 4.13 (s, 2H, POCH_2-CH$_2$N), 3.82−3.32 (m, 15H), 3.16 (s, 3H, N$^+$CH_3), 1.92−1.47 (m, 8H), 1.42−1.34 (m, 2H, CH_2CH=C), 1.27 (broad s, 18H, (CH_2)$_9$), 0.83 (t, J = 7.0 Hz, 3H, CH_3); ^{31}P NMR (δ): −2.04; Anal. ($C_{27}H_{52}NO_6P\cdot 2H_2O$) C, H, N

1-{2-{[(4-Tetradecylidenecyclohexyloxy)ethyloxy]hydroxyphosphinyloxy}ethyl}-*N,N,N*-trimethylammonium Inner Salt (15). The general procedure described above using alcohol **8b**, TIPS-Cl, and choline chloride afforded compound **15** (0.166 g, 33%). ^{1}H NMR (δ): 5.05 (t, J = 6.7 Hz, 1H, C*H*=C), 4.23 (broad s, 2H, POCH_2CH$_2$N), 3.88 (broad s, 2H), 3.75 (broad s, 2H), 3.55 (broad s, 2H, CH_2N), 3.40−3.35 (m, 1H, C*H*O), 3.32 (s, 9H, N$^+$(CH_3)$_3$), 2.41−2.37 (m, 1H), 2.17−2.13 (m, 1H), 1.89−1.74 (m, 8H), 1.21 (broad s, 22H, (CH_2)$_{11}$), 0.89 (t, J = 7.0 Hz, 3H, CH_3); ^{31}P NMR (δ): −2.26; ^{13}C NMR: δ 136.9, 122.7, 74.3, 67.7, 64.7, 54.2, 33.4, 32.5, 31.9, 30.1, 29.6, 29.3, 27.4, 24.9, 22.6, 14.0; Anal. ($C_{27}H_{54}NO_5P$) C, H, N.

1-{2-{[(4-Tetradecylidenecyclohexyloxy)ethyloxy]hydroxyphosphinyloxy}ethyl}-1-methylpiperidinium Inner Salt (16). The general procedure described above using alcohol **8b**, TIPS-Cl, and *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide afforded compound **16** (0.201 g, 37%). ^{1}H NMR (δ): 5.21 (t, J = 6.7 Hz, 1H, C*H*=C), 4.31 (bs, 2H, POCH_2-CH$_2$N), 3.93−3.80 (m, 4H), 3.60−3.43 (m, 6H, CH_2N(CH_2)$_2$), 3.30 (broad s, 4H, NCH_3, C*H*O), 2.40−1.40 (m, 16H), 1.23 (broad s, 22H, (CH_2)$_{11}$), 0.88 (t, J = 7.0 Hz, 3H, CH_3); ^{31}P NMR (δ): −2.42; Anal. ($C_{30}H_{58}NO_5P$) C, H, N.

1-{2-{[(4-Tetradecylidenecyclohexyloxy)ethyloxy]hydroxyphosphinyloxy}ethyl}-1-methylmorpholinium Inner Salt (17). The general procedure described above using alcohol **8b**, TIPS-Cl and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide afforded compound **17** (0.218 g, 40%). ^{1}H NMR (δ): 5.06 (t, J = 6.7 Hz, 1H, C*H*=C), 4.07 (broad s, 2H, POCH_2-CH$_2$N), 3.49−3.17 (m, 5H), 3.11 (s, 3H, N$^+$CH_3), 1.99−1.34 (m, 10H), 1.08 (broad s, 22H, (CH_2)$_{11}$), 0.78 (t, J = 7.0 Hz, 3H, CH_3); ^{31}P NMR (δ): −1.99; Anal. ($C_{29}H_{56}NO_6P\cdot H_2O$) C, H, N.

1-{2-{[(4-Hexadecylidenecyclohexyloxy)ethyloxy]hydroxyphosphinyloxy}ethyl}- *N,N,N*-trimethylammonium Inner Salt (18). The general procedure described above using alcohol **8c**, TIPS-Cl, and choline chloride afforded compound **18** (0.196 g, 37%). ^{1}H NMR (δ): 5.08 (t, J = 6.7 Hz, 1H, C*H*=C), 4.09 (broad s, 2H, OP(O)CH_2CH$_2$N), 3.82 (broad s, 2H, OCH$_2$CH_2OP), 3.71 (broad s, 2H, OCH_2CH$_2$OP), 3.51−3.43 (m, 2H, CH_2N), 3.04 (s, 10H, C*H*O, N$^+$(CH_3)$_3$), 2.45−2.40 (m, 1H), 2.25−2.20 (m, 1H), 2.02−1.85 (m, 6H), 1.51−1.42 (m, 2H, CH_2CH=C), 1.09 (broad s, 26H, (CH_2)$_{13}$), 0.71 (t, J = 7.0 Hz, 3H, CH_3); ^{31}P NMR: δ −2.04; Anal. ($C_{29}H_{58}NO_5P\cdot H_2O$) C, H, N.

1-{2-{[(4-Hexadecylidenecyclohexyloxy)ethyloxy]hydroxyphosphinyloxy}ethyl}-1-methylpiperidinium Inner Salt (19). The general procedure described above using alcohol **8c**, TIPS-Cl, and *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide afforded compound **19** (0.211 g, 37%). ^{1}H NMR (δ): 5.13 (t, J = 6.7 Hz, 1H, C*H*=C), 4.35 (broad s, 2H, POCH_2), 3.87 (broad s, 2H), 3.78 (broad s, 2H), 3.62−3.45 (m, 6H), 3.26 (broad s, 4H), 2.27−1.63 (m, 14H), 1.52−1.41 (m, 2H, CH_2CH=

C), 1.24 (broad s, 26H, (CH_2)$_{13}$), 0.89 (t, J = 7.0 Hz, 3H, CH_3); ^{31}P NMR (δ): −2.01; ^{13}C NMR (δ): 138.0, 117.4, 75.1, 67.8, 67.7, 64.8, 63.3, 61.8, 58.7, 48.8, 37.2, 31.8, 30.1, 29.7, 29.6, 29.4, 29.3, 28.4, 27.7, 27.2, 22.5, 20.9, 20.1, 14.0; Anal. ($C_{32}H_{62}NO_5P\cdot 2H_2O$) C, H, N.

1-{2-[(4-Hexadecylidenecyclohexyloxy)ethyloxy]-hydroxyphosphinyloxy}ethyl}-1-methylmorpholinium Inner Salt (20). The general procedure described above alcohol **8c**, using TIPS-Cl and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide afforded compound **20** (0.206 g, 36%). ^{1}H NMR (δ): 5.06 (t, J = 6.70 Hz, 1H, CH=C), 4.41 (bs, 2H, POCH_2), 3.99−3.39 (m, 15H), 3.35 (s, 3H, N^+CH_3), 2.45−2.40 (m, 1H, CHCHOCH$_2$), 2.25−2.20 (m, 1H, CH$_2$CHOCH), 2.13−1.85 (m, 6H), 1.22 (broad s, 28H, (CH_2)$_{14}$), 0.89 (t, J = 7.0 Hz, 3H, CH_3); ^{31}P NMR (δ): −2.17; ^{13}C NMR (δ): 136.8, 122.8, 65.3, 60.7, 33.4, 33.3, 32.4, 31.8, 30.1, 29.6, 29.4, 29.3, 27.4, 24.9, 22.6, 14.0; Anal. ($C_{31}H_{60}NO_6P\cdot H_2O$) C, H, N.

1-{2-[(5-Cyclohexylidenepentyloxy)hydroxyphosphinyloxy]ethyl}- *N,N,N*-trimethylammonium Inner Salt (21). The general procedure described above using alcohol **4a**, MSNT, and choline chloride afforded compound **21** (0.219 g, 66%); ^{1}H NMR (δ): 4.99 (t, J = 6.7 Hz, 1H, C=CH), 4.21 (broad s, 2H, POCH_2CH$_2$N), 3.74 (broad s, 4H, CH_2OPOCH$_2$CH_2N), 3.34 (s, 9H, $N^+(CH_3)_3$), 2.09−1.84 (m, 6H), 1.55−1.28 (m, 10H); ^{31}P NMR (δ): −2.16; ^{13}C NMR (δ): 139.8, 120.8, 66.1, 65.4, 59.1, 54.2, 37.1, 30.6, 28.6, 27.8, 26.9, 26.4, 25.6; ESI-MS *m*/*z*: 356.2 ($M^+ + Na^+$), 334.2 ($M^+ + 1$); Anal. ($C_{16}H_{32}NO_4P$) C, H, N.

1-{2-[(5-Cyclohexylidenepentyloxy)hydroxyphosphinyloxy]ethyl}-1-methylpiperidinium Inner Salt (22). The general procedure described above using alcohol **4a**, MSNT, and *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide afforded compound **22** (0.153 g, 41%); ^{1}H NMR (δ): 5.02 (t, J = 6.7 Hz, 1H, C=CH), 4.28 (broad s, 2H, POCH_2CH$_2$N), 3.82−3.42 (m, 8H, CH_2OPOCH$_2$CH_2N(CH_2)$_2$), 3.31 (s, 3H, N^+CH_3), 2.08−1.48 (m, 16H), 1.23 (broad s, 6H, (CH_2)$_3$); ^{31}P NMR (δ): −2.04; ESI-MS *m*/*z*: 374.2 ($M^+ + 1$); Anal. ($C_{19}H_{36}NO_4P\cdot 2H_2O$) C, H, N.

1-{2-[(5-Cyclohexylidenepentyloxy)hydroxyphosphinyloxy]ethyl}-1-methylmorpholinium Inner Salt (23). The general procedure described above using alcohol **4a**, MSNT, and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide afforded compound **23** (0.153 g, 41%). ^{1}H NMR (δ): 5.01 (t, J = 6.7 Hz, 1H, C=CH), 4.29 (broad s, 2H, POCH_2CH$_2$N), 4.11−3.68 (m, 12H), 3.42 (s, 3H, N^+CH_3), 2.09−1.95 (m, 4H), 1.58−1.49 (m, 6H), 1.31 (broad s, 6H, (CH_2)$_3$); ^{31}P NMR (δ): −2.23; ESI-MS *m*/*z*: 376.2 ($M^+ + 1$); Anal. ($C_{18}H_{34}NO_5P\cdot 2\ H_2O$) C, H, N.

1-{2-[(11-Cyclohexylideneundecyloxy)hydroxyphosphinyloxy]ethyl}-*N,N,N*-trimethylammonium Inner Salt (24). The general procedure described above using alcohol **6a**, MSNT, and choline chloride afforded compound **24** (0.220 g, 52%). ^{1}H NMR (δ): 5.05 (t, J = 6.7 Hz, 1H, C=CH), 4.20 (broad s, 2H, POCH_2CH$_2$N), 3.75−3.68 (m, 4H, CH_2OPOCH$_2$CH_2N), 3.26 (s, 9H, $N^+(CH_3)_3$), 2.11−1.92 (m, 4H), 1.65−1.48 (m, 6H), 1.23 (broad s, 18H, (CH_2)$_9$); ^{31}P NMR (δ): −2.45; ^{13}C NMR (δ): 131.0, 124.8, 66.1, 66.0, 59.1, 54.1, 31.0, 30.2, 29.9, 29.7, 29.6, 29.5, 29.3, 28.6, 28.0, 27.8, 27.0, 26.9, 25.9, 25.7; ESI-MS *m*/*z*: 440.2 ($M^+ + Na^+$), 418.2 ($M^+ + 1$); Anal. ($C_{22}H_{44}NO_4P\cdot H_2O$) C, H, N.

1-{2-[(11-Cyclohexylideneundecyloxy)hydroxyphosphinyloxy]ethyl}-1-methylpiperidinium Inner Salt (25). The general procedure described above using alcohol **6a**, MSNT, and *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide afforded compound **25** (0.315 g, 69%). ^{1}H NMR (δ): 4.99 (t, J = 6.7 Hz, 1H, C=CH), 4.23 (bs, 2H, POCH_2CH$_2$N), 3.78−3.48 (m, 8H, CH_2OPOCH$_2$CH_2N(CH_2)$_2$), 3.27 (s, 3H, N^+CH_3), 2.04−1.45 (m, 16H), 1.18 (broad s, 18H, (CH_2)$_9$); ^{31}P NMR (δ): −2.04; ^{13}C NMR (δ): 130.9, 124.7, 65.3, 63.2, 58.4, 48.5, 37.0, 31.0, 30.9, 30.1, 29.8, 29.6, 29.5, 29.4, 29.2, 28.6, 27.9, 27.7, 26.8, 25.8, 25.6; ESI-MS *m*/*z*: 480.3 ($M^+ + Na^+$), 458.3 ($M^+ + 1$); Anal. ($C_{25}H_{48}NO_4P\cdot 2.5H_2O$) C, H, N.

1-{2-[(11-Cyclohexylideneundecyloxy)hydroxyphosphinyloxy]ethyl}-1-methylmorpholinium Inner Salt (26). The general procedure described above using alcohol **6a**, MSNT, and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide afforded compound **26** (0.117 g, 25%). ^{1}H NMR (δ): 5.05 (t, J = 6.7 Hz, 1H, C=CH), 4.29 (broad s, 2H, POCH_2CH$_2$N), 3.99−3.70 (m, 12H), 3.48 (s, 3H, N^+CH_3), 2.08−1.92 (m, 4H), 1.65−1.48 (m, 6H), 1.23 (s, 18H, (CH_2)$_9$); ^{31}P NMR (δ): −2.13; ^{13}C NMR (δ): 131.0, 124.8, 65.8, 64.3, 60.7, 58.5, 48.3, 37.1, 31.0, 30.9, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 28.6, 28.2, 28.0, 27.8, 27.0, 25.8, 25.7, 17.6; Anal. ($C_{24}H_{46}NO_5P\cdot 3H_2O$) C, H, N

1-{2-[(5-Adamantylidenepentyloxy)hydroxyphosphinyloxy]ethyl}-*N,N,N*-trimethylammonium Inner Salt (27). The general procedure described above using alcohol **4b**, MSNT, and choline chloride afforded compound **27** (0.223 g, 58%). ^{1}H NMR (δ): 4.96 (t, J = 6.7 Hz, 1H, C=CH), 4.22 (broad s, 2H, POCH_2CH$_2$N), 3.77−3.71 (m, 4H, CH_2OPOCH$_2$CH_2N), 3.29 (s, 9H, $N^+(CH_3)_3$), 2.75 (s, 1H, CHC=C), 2.27 (s, 1H, CHC=C), 1.95−1.53 (m, 16H), 1.34−1.29 (m, 2H); ^{31}P NMR (δ): −2.42; ^{13}C NMR (δ): 147.7, 115.9, 66.3, 65.5, 59.1, 54.3, 40.5, 39.8, 38.9, 37.2, 32.0, 30.6, 28.6, 26.6, 26.2; ESI-MS *m*/*z*: 408.1 ($M^+ + Na^+$), 386.1 ($M^+ + 1$); Anal. ($C_{20}H_{36}NO_4P\cdot 3H_2O$) C, H, N.

1-{2-[(5-Adamantylidenepentyloxy)hydroxyphosphinyloxy]ethyl}-1-methylpiperidinium Inner Salt (28). The general procedure described above using alcohol **4b**, MSNT, and *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide afforded compound **28** (0.272 g, 64%). ^{1}H NMR (δ): 4.93 (t, J = 6.7 Hz, 1H, C=CH), 4.25 (broad s, 2H, POCH_2CH$_2$N), 3.79−3.60 (m, 8H, CH_2OPOCH$_2$CH_2N(CH_2)$_2$), 3.32 (s, 3H, $N^+(CH_3)_3$), 2.72 (s, 1H, CHC=C), 2.24 (s, 1H, CHC=C), 1.92−1.50 (m, 22H), 1.31−1.26 (m, 2H); ^{31}P NMR (δ): −1.98; ^{13}C NMR (δ): 147.6, 115.9, 65.4, 65.3, 63.5, 58.6, 58.5, 48.6, 40.5, 39.8, 38.9, 37.2, 32.0, 30.7, 30.6, 28.6, 26.6, 26.2, 20.9, 20.2; ESI-MS *m*/*z*: 448.2 ($M^+ + Na^+$), 426.2 ($M^+ + 1$); Anal. ($C_{23}H_{40}NO_4P\cdot 2.5H_2O$) C, H, N.

1-{2-[(5-Adamantylidenepentyloxy)hydroxyphosphinyloxy]ethyl}-1-methylmorpholinium Inner Salt (29). The general procedure described above using alcohol **4b**, MSNT, and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide afforded compound **29** (0.239 g, 56%). ^{1}H NMR (δ): 4.94 (t, J = 6.7 Hz, 1H, C=CH), 4.27 (broad s, 2H, POCH_2CH$_2$N), 3.99−3.69 (m, 12H), 3.43 (s, 3H, N^+CH_3), 2.73 (s, 1H, CHC=C), 2.25 (s, 1H, CHC=C), 1.96−1.32 (m, 16H), 1.29−1.18 (m, 2H); ^{31}P NMR (δ): −2.16; ^{13}C NMR (δ): 147.8, 115.8, 65.6, 65.5, 64.3, 60.7, 58.5, 48.3, 40.5, 39.8, 38.9, 37.2, 32.0, 30.6, 30.5, 28.6, 26.6, 26.5; ESI-MS *m*/*z*: 450.2 ($M^+ + Na^+$), 428.2 ($M^+ + 1$); Anal. ($C_{22}H_{38}NO_5P\cdot 2.5H_2O$) C, H, N.

1-{2-[(11-Adamantylideneundecyloxy)hydroxyphosphinyloxy]ethyl}-*N,N,N*-trimethylammonium Inner Salt (30). The general procedure described above using alcohol **6b**, MSNT, and choline chloride afforded compound **30** (0.248 g, 53%). ^{1}H NMR (δ): 4.98 (t, J = 6.7 Hz, 1H, C=CH), 4.21 (broad s, 2H, POCH_2CH$_2$N), 3.75 (broad s, 4H, CH_2OPOCH$_2$CH_2N), 3.32 (s, 9H, $N^+(CH_3)_3$), 2.77 (s, 1H, CHC=C), 2.28 (s, 1H, CHC=C), 1.91−1.53 (m, 16H), 1.23 (broad s, 14H); ^{31}P NMR (δ): −2.16; ^{13}C NMR (δ): 147.2, 116.3, 66.1, 65.5, 59.2, 54.2, 40.5, 39.8, 38.9, 37.3, 32.0, 31.0, 30.9, 29.7, 29.6, 29.5, 29.2, 28.7, 26.5, 25.9; ESI-MS *m*/*z*: 492.2 ($M^+ + Na^+$), 470.2 ($M^+ + 1$); Anal. ($C_{26}H_{48}NO_4P\cdot H_2O$) C, H, N.

1-{2-[(11-Adamantylideneundecyloxy)hydroxyphosphinyloxy]ethyl}-1-methylpiperidinium Inner Salt (31). The general procedure described above using alcohol **6b**, MSNT, and *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide afforded compound **31** (0.168 g, 33%). ^{1}H NMR (δ): 4.98 (t, J = 6.7 Hz, 1H, C=CH), 4.27 (broad s, 2H, POCH_2CH$_2$N), 3.84−3.52 (m, 8H, CH_2OPOCH$_2$CH_2N(CH_2)$_2$), 3.32 (s, 3H, NCH_3), 2.76 (s, 1H, CHC=C), 2.27 (s, 1H, CHC=C), 1.92−1.53 (m, 22H), 1.23 (broad s, 14H); ^{31}P NMR (δ): −2.04; ^{31}C NMR (δ): 147.2, 116.3, 65.1, 62.1, 57.3, 47.4, 40.5, 39.9, 38.9, 37.5, 32.0, 30.3, 29.6, 29.5, 29.4, 29.2, 28.7, 26.4, 25.8, 20.9, 20.2; ESI-MS *m*/*z*: 532.3 ($M^+ + Na^+$), 510.3 ($M^+ + 1$); Anal. ($C_{29}H_{52}NO_4P\cdot 2H_2O$) C, H, N.

1-{2-[(11-Adamantylideneundecyloxy)hydroxyphosphinyloxy]ethyl}-1-methylmorpholinium Inner Salt (32).The general procedure described above using alcohol **6b**, MSNT, and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide af-

forded compound **32** (0.235 mg, 46%). ^{1}H NMR (δ): 4.99 (t, J = 6.7 Hz, 1H, C=C*H*), 4.29 (broad s, 2H, POCH_2CH$_2$N), 4.00−3.67 (m, 12H), 3.42 (s, 3H, N$^+$CH_3), 2.77 (s, 1H, C*H*C=C), 2.28 (s, 1H, C*H*C=C), 1.91−1.54 (m, 14H), 1.23 (s, 16H); ^{31}P NMR (δ): −2.29; ^{13}C NMR (δ): 147.2, 116.3, 65.7, 64.9, 60.7, 58.5, 48.3, 40.5, 39.8, 38.9, 37.3, 32.0, 30.9, 30.4, 29.7, 29.6, 29.5, 29.3, 28.6, 26.5, 25.8; ESI-MS *m*/*z*: 534.2 (M$^+$ + Na$^+$), 512.2 (M$^+$); Anal. ($C_{28}H_{50}NO_5P \cdot 3H_2O$) C, H, N.

1-{2-[(1-Adamantylmethyloxy)hydroxyphosphinyloxy]-ethyl}-*N,N,N*-trimethylammonium Inner Salt (33). The general procedure described above using 1-adamantylmethanol, TIPS-Cl, and choline chloride afforded compound **33** (0.060 g, 40%); ^{1}H NMR (CDCl$_3$/CD$_3$OD) (δ): 4.11 (broad s, 2H, POCH_2CH$_2$N), 3.62−3.25 (m, 4H, CH_2OPOCH$_2$CH_2N), 3.18 (s, 9H, N$^+$(CH_3)$_3$), 1.83−1.45 (m, 15H); ^{31}P NMR (CDCl$_3$/CD$_3$OD) (δ): −1.41; Anal. ($C_{16}H_{30}NO_4P$) C, H, N

1-{2-[4-(1-Adamantyl)phenyloxy]hydroxyphosphinyloxy]ethyl}-1-methylmorpholinium Inner Salt (34). The general procedure described above using 4-(1-adamantyl)-phenol, TIPS-Cl, and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide afforded compound **34** (0.131 g, 30%). ^{1}H NMR (δ): 7.15 (d, J = 7 Hz, 2H), 7.05 (d, J = 7 Hz, 2H), 3.89−3.62 (m, 4H), 3.45−3.15 (m, 8H), 2.90 (s, 3H), 2.00−1.50 (m, 15H); ^{31}P NMR (δ): −7.19. Anal. ($C_{23}H_{34}NO_5P$) C, H, N.

1-{2-[(2-(Phenyloxy)ethyloxy)]hydroxyphosphinyloxy}-ethyl}- *N,N,N*-trimethylammonium Inner Salt (35). The general procedure described above using 2-phenyloxyethanol, TIPS-Cl, and choline chloride afforded compound **35** (0.055 g, 18%). ^{1}H NMR: δ 7.25−7.18 (m, 2H), 6.90−6.81 (m, 3H), 4.30−4.08 (m, 6H), 3.67 (broad s, 2H), 3.21 (s, 9H); ^{31}P NMR (δ): −2.51. Anal. ($C_{13}H_{22}NO_5P$) C, H, N.

1-{2-{[2-(Phenyloxy)ethyloxy)]hydroxyphosphinyloxy}-ethyl}-1-methylmorpholinium Inner Salt (36). The general procedure described above using 2-phenyloxyethanol, TIPS-Cl, and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide afforded compound **36** (0.238 g, 69%). ^{1}H NMR (δ): 7.26−7.21 (m, 2H), 6.92−6.84 (m, 3H), 4.31−4.10 (m, 6H), 3.95−3.45 (m, 10H), 3.36 (s, 3H); ^{31}P NMR (δ): −2.32; ^{13}C NMR (δ): 158.6, 129.7, 121.1, 114.6, 67.9, 64.3, 63.9, 60.6, 58.6, 47.9. Anal. ($C_{15}H_{24}NO_6P \cdot 2H_2O$) C, H, N.

1-{2-{[6-(Phenyloxy)hexyloxy)]hydroxyphosphinyloxy}-ethyl}-*N,N,N*-trimethylammonium Inner Salt (37). The general procedure described above using 6-(phenyloxy)hexanol, TIPS-Cl, and choline chloride afforded compound **37** (0.190 g, 53%). ^{1}H NMR (CDCl$_3$/CD$_3$OD) (δ): 7.17−7.14 (m, 2H), 6.85−6.77 (m, 3H), 4.11 (broad s, 2H), 3.86−3.74 (m, 4H), 3.50−3.20 (m, 2H), 3.10 (s, 9H), 1.70−1.35 (m, 8H); ^{31}P NMR (δ): −2.18; ^{31}C NMR: δ 158.9, 129.3, 120.4, 114.3, 67.6, 66.4, 65.6, 58.7, 54.1, 30.5, 29.0, 25.6, 25.3; Anal. ($C_{17}H_{30}NO_5P \cdot 2H_2O$) C, H, N.

1-{2-{[6-(Phenyloxy)hexyloxy)]hydroxyphosphinyloxy}-ethyl}-1-methylmorpholinium Inner Salt (38). The general procedure described above using 6-(phenyloxy)hexanol, TIPS-Cl, and *N*-(2-hydroxyethyl)-*N*-methylmorpholinium bromide afforded compound **38** (0.197 g, 49%). ^{1}H NMR (δ): 7.25−7.20 (m, 2H), 6.91−6.82 (m, 3H), 4.26 (broad s, 2H), 3.96−3.65 (m, 14H), 3.42 (s, 3H), 1.76−1.35 (m, 8H); ^{31}P NMR (δ): −2.08; Anal. ($C_{19}H_{32}NO_6P \cdot 2H_2O$) C, H, N.

1-{2-{[2-(2-Naphthyloxy)ethyloxy)]hydroxyphosphinyloxy}ethyl} −*N,N,N*-trimethylammonium Inner Salt (39). The general procedure described above using 2-(2-naphthyloxy)ethanol, TIPS-Cl, and choline chloride afforded compound **39** (0.265 g, 75%). ^{1}H NMR (CDCl$_3$/CD$_3$OD) (δ): 7.67−7.62 (m, 3H), 7.37−7.01 (m, 4H), 4.19−4.18 (m, 6H), 3.45−3.42 (m, 2H), 3.03 (s, 9H); ^{31}P NMR (CDCl$_3$/CD$_3$OD) (δ): −2.20; ^{31}C NMR (CDCl$_3$/CD$_3$OD): δ 156.5, 134.4, 129.4, 128.9, 127.5, 126.6, 126.4, 123.7, 118.5, 106.9. 67.7, 67.6, 66.3, 63.9, 58.9; Anal. ($C_{17}H_{24}NO_5P \cdot 2H_2O$) C, H, N.

1-{2-{[2-(2-Naphthyloxy)ethyloxy)]hydroxyphosphinyloxy}ethyl}-1-methylpiperidinium Inner Salt (40). The general procedure described above using 2-(2-naphthyloxy)ethanol, TIPS-Cl, and *N*-(2-hydroxyethyl)-*N*-methylpiperidinium bromide afforded compound **40** (0.271 g, 69%). ^{1}H NMR (CDCl$_3$/CD$_3$OD) (δ): 7.66−7.61 (m, 3H), 7.36−7.02 (m, 4H), 4.19 (broad s, 6H), 3.37−3.15 (m, 6H), 2.95 (s, 3H), 1.67−1.65 (m, 4H), 1.49−1.46 (m, 2H); ^{31}P NMR (CDCl$_3$/CD$_3$OD) (δ): −2.13; Anal. ($C_{20}H_{28}NO_5P \cdot 4H_2O$) C, H, N.

General Procedure for the Hydrogenation of the Unsaturated Ether Phospholipids (41−45). To a solution of the desired ether phospholipid (1 mmol) in MeOH (10 mL) was added 10% Pd/C (10% w/w), and the resulting mixture was hydrogenated at 1 atm for 10 h. Filtration through Celite and evaporation of the filtrate in vacuo afforded the pure product.

1-{2-{[(4-Dodecylcyclohexyloxy)ethyloxy]hydroxyphosphinyloxy}ethyl}-*N,N,N*-trimethylammonium Inner Salt (41) Yield quantitative. ^{1}H NMR (δ): 4.31 (broad s, 2H, POCH_2CH$_2$N), 3.93 (broad s, 2H, CH_2OPOCH$_2$CH$_2$N), 3.82 (broad s, 2H), 3.59 (broad s, 2H, POCH$_2$CH_2N), 3.37 (broad s, 10H, C*H*O, N$^+$(CH_3)$_3$), 2.05−1.95 (m, 1H), 1.76−1.72 (m, 2H), 1.46−1.10 (m, 28H), 0.86 (t, J = 7.0 Hz, 3H, CH_3); Anal. ($C_{25}H_{52}NO_5P$) C, H, N.

1-{2-{[(4-Tetradecylcyclohexyloxy)ethyloxy]hydroxyphosphinyloxy}ethyl}-*N,N,N*-trimethylammonium Inner Salt (42). Yield quantitative. ^{1}H NMR (δ): 4.31 (broad s, 2H, POCH_2CH$_2$N), 3.93−3.82 (m, 4H), 3.59−3.15 (m, 12H), 1.96 (broad s, 1H), 1.76 (broad s, 2H), 1.42−1.09 (m, 32 H), 0.87 (t, J = 7.0 Hz, 3H, CH_3); Anal. ($C_{27}H_{56}NO_5P$) C, H, N.

1-{2-[(11-Cyclohexylundecyloxy)hydroxyphosphinyloxy]ethyl}-*N,N,N*-trimethylammonium Inner Salt (43). Yield quantitative. ^{1}H NMR (δ): 4.20 (broad s, 2H, POCH_2CH$_2$N), 3.75−3.68 (m, 4H, CH_2OPOCH$_2$CH_2N), 3.26 (s, 9H, N$^+$(CH_3)$_3$), 2.09−1.12 (m, 13H), 1.23 (s, 18H, (CH_2)$_9$); Anal. ($C_{22}H_{46}NO_4P$) C, H, N.

1-{2-[(5-Adamantylpentyloxy)hydroxyphosphinyloxy]-ethyl}-*N,N,N*-trimethylammonium Inner Salt (44).Yield quantitative ^{1}H NMR (δ): 4.27 (bs, 2H, POCH_2CH$_2$N), 3.79−3.09 (m, 13H), 2.02−1.25 (m, 23H); Anal. ($C_{20}H_{38}NO_4P$) C, H, N.

1-{2-[(11-Adamantylundecyloxy)hydroxyphosphinyloxy]ethyl}-*N,N,N*-trimethylammonium Inner Salt (45). Yield quantitative. ^{1}H NMR (δ): 4.27 (broad s, 2H, POCH_2CH$_2$N), 3.79−3.09 (m, 13H), 2.02−1.25 (m, 35H); Anal.-($C_{26}H_{50}NO_4P$) C, H, N.

NMR Spectroscopy. The high-resolution spectra were obtained using a Varian INOVA 600 MHz spectrometer. All data were collected using pulse sequences and phase-cycling routines provided in the Varian libraries of pulse programs. Data processing including sine-bell apodization, Fourier transformation, phasing, symmetrization, and plotting were performed using Varian software packages. The ROESY experiments were recorded using standard pulse sequence in the phase-sensitive mode and were measured at 150 ms mixing time. Typically, the homonuclear proton spectra were acquired with 4096 data points in t_2, 16−64 scans, 256−512 complex points in t_1, and a relaxation delay of 1−1.5 s.

Molecular Modeling. Computer calculations were performed on a Silicon Graphics O$_2$ workstation using Quanta 97 version of the Molecular Simulation Incorporated (MSI) program. The energy of the two molecules built were first minimized and then subjected to molecular dynamics experiments to explore their lower energy conformers, with the use of ROE constraints. The molecular dynamics calculations were run for the molecule using simulation time 6 ps at a temperature of 2000 K and a dielectric constant (ϵ = 1) that simulates the CDCl$_3$ environment. Family structures were generated using the dihedral angle criterion. The lowest energy conformers from each family were considered as the representatives ones.

The RMSD map generated the cluster threshold of 1.138 for compound **27** and 1.038 for compound **30** and included seven families for each molecule.

Differential Scanning Calorimetry. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC, purity >99%) was obtained from Avanti Polar Lipids (AL) and used without further purification. Appropriate amounts of phospholipid with or without compound under study were dissolved in spectroscopic chloroform. The solvent was then evaporated by passing a

stream of O_2-free nitrogen over the solution, and the residue was dried in vacuo (0.1 mmHg) for 12 h. The dry residue was dispersed in appropriate amounts of bidistilled water to a concentration of 50% (w/w) by vortexing at 45 °C, i.e., above the chain-melting transition of DPPC. Before temperature scan experiments, the samples were equilibrated at 4 °C for 4 h. After distilled water was added to the dry lipid/compound mixture (50% w/w), a portion of the sample (approximately 5 mg) was sealed in a stainless steel capsule. Thermograms were obtained on a Perkin-Elmer DSC-7 instrument. All samples were scanned at least twice until identical thermograms were obtained using a scanning rate of 2.5 °C/min. The temperature scale of the calorimeter was calibrated using fully hydrated DPPC and indium as standard samples. We found that thermograms of preparations containing up to 10% mol ($x = 0.10$) stored at freezer temperatures (−15 °C) for several days were identical to those run immediately after sample preparation.

Determination of in Vitro Antileishmanial Activity in Promastigote Cultures. Promastigotes of *Leishmania infantum* MHOM/TN/80/IPT1/LEM 235 and *Leishmania donovani* MHOM/IN/80/DD8/LEM 703 were grown in RPMI 1640 supplemented with 10% FCS, L-glutamine, and antibiotics, at 26 °C.

All compounds were dissolved in DMSO to a final concentration of 9.625 mM, and linear 3-fold dilutions were performed in the culture medium. A 25 μL amount of promastigote culture at 5×10^5 cells/mL was cultured in a 96-well flat-bottom plate (Costar 3696) and incubated with 25 μL of different drug concentrations at 26 °C. After 72 h, 10 μL of 5 mg/mL MTT in PBS (SIGMA M2128) was added, and incubation was continued for 3 h.[28] The reaction was stopped by the addition of 50 μL of 50% 2-propanol, 10% SDS under gentle shaking for 30 min. Absorbance was measured at 550 nm with reference at 620 nm in a TRITURUS microplate reader. Calculation of IC_{50} values was done according to Hills et al.[29,30]

Assessement of Cytotoxicity in THP1 Monocytic Cells. Staining with PI and SYBR-14. As a quantitative measurement of the cell damage after incubation with different concentrations of drugs dual staining with SYBR-14 and PI (Molecular Probes, The Neatherlands) was used. THP1 cell cultures were incubated at 1×10^6 cells/mL with different concentrations of the compounds ranging from 50 to 1.56 μM. After an incubation period of 72 h, approximately 4×10^6 cells were suspended in labeling buffer (10 mM HEPES, 150 mM NaCl, 10% BSA, pH 7.4) and 10 μg/mL PI and 0.1 mg/mL SYBR-14 were added. The cultures were incubated at 37 °C for 30 min before analysis by flow cytometry.

Flow Cytometric Analysis. Cell samples were analyzed on an Epics Elite model flow cytometer (Coulter, Miami, FL). The green fluorescence SYBR-14 and the red fluorescence of PI were excited at 488 nm. At least 10 000 cells were analyzed per sample, and each staining experiment was repeated twice. Data analysis was performed on fluorescence intensities that excluded cell autofluorescence and cell debris.

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Supporting Information Available: Analytical data for compounds **9**−**45** and assignment of ^{1}H NMR chemical shifts (ppm) of ether phospholipids **27** and **30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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