Synthesis and Properties of Photoactivatable Phospholipid Derivatives Designed To Probe the Membrane-Associate Domains of Proteins

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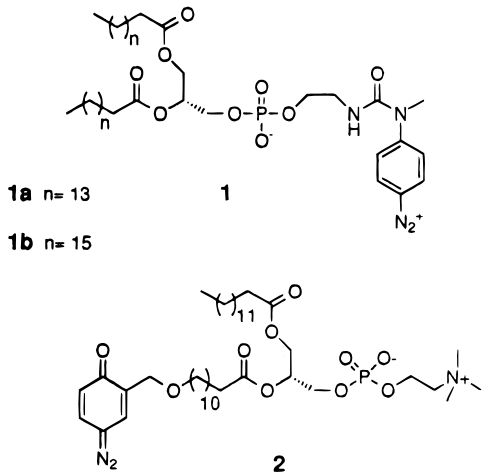
The total syntheses of photoactivatable phospholipidic probes **1** and **2** are described. These probes contain either an aryldiazonium function at their polar head (probes **1a** and **1b**) or an diazocyclohexadienonyl group attached to the end of one fatty acid side chain (probe **2**) and have been designed to probe the lipid/water interface and the hydrophobic core of the membrane, respectively. The synthetic schemes include the possibility of incorporating a radio-labeled atom (tritium) for further labeling investigations. Both probes were stable in the dark under physiological conditions and could be efficiently photodecomposed at wavelengths above 300 nm, leading to the generation of highly reactive species, aryl cations and cyclohexadienonyl carbene, respectively. In addition, these probes displayed UV-absorption spectra which are compatible with tryptophan-mediated energy transfer photoactivation, which can lead potentially to an efficient mapping of the membrane-associate protein domains.

Introduction

Site-directed labeling experiments are complementary approaches to site-directed mutagenesis for the investigation of ligand−receptor interactions.[1] Both use functional properties of a protein (i.e. ligand-binding measurements) to evaluate the perturbation caused by either mutation or chemical modification. However, other protein domains such as the membrane-associate domains which are not directly subjected to specific ligand−receptor interactions often condition and modulate the protein functioning.[2] In order to enhance the structural information on membrane-associate domains of proteins, the hydrophobic photolabeling approach[3] has been developed to replace site-directed labeling studies. Different types of hydrophobic labeling reagents have been used to achieve such labeling, including the photoactivatable phospholipids,[4] which present the advantage of resembling closely the natural membrane components. Among the difficulties encountered using such probes are the low yields of coupling the probes to the target proteins as opposed to surrounding lipids and the necessity of photochemically generating highly reactive species to ensure efficient chemical coupling with nonpolar amino acids.

A protein-mediated energy transfer photoactivation method[5] should allow efficient labeling of the protein. In such an energy transfer process, the excited tryptophan residues (donors) transfer their energy to the photoactivatable probes (acceptors) which possess a suitable chromophore. Consequently, the phospholipid probes should be efficiently photoactivated only when in close vicinity to tryptophan-containing protein domains, which would restrict the mapping area to the membrane-associated domains in close proximity to the proteins.[6] Among the photosensitive chromophores which act as acceptors in tryptophan-mediated energy transfer reactions,[6] we selected two species: *N*-ureyl-*p*-phenyldiazonium and 1,4-diazocyclohexadienone, whose absorption spectra showed good overlap with the emission spectrum of tryptophan. Furthermore, both probes generate by photoactivation highly reactive aryl cations and cyclohexadienonyl carbenes, respectively. In the present work, two complementary types of photoactivatable phospholipids (Scheme 1) were designed for mapping membrane-associate protein domains. Probes **1a** and **1b** contain an aryldiazonium moiety in their polar head and are proposed for probing the lipid/water interface of the membranes, while probe **2** has a 1,4-diazocyclohexadienonyl moiety in one fatty acid side chain of the phospholipid and is therefore designed to probe the hydrophobic membrane core. We describe here the synthesis

Scheme 1



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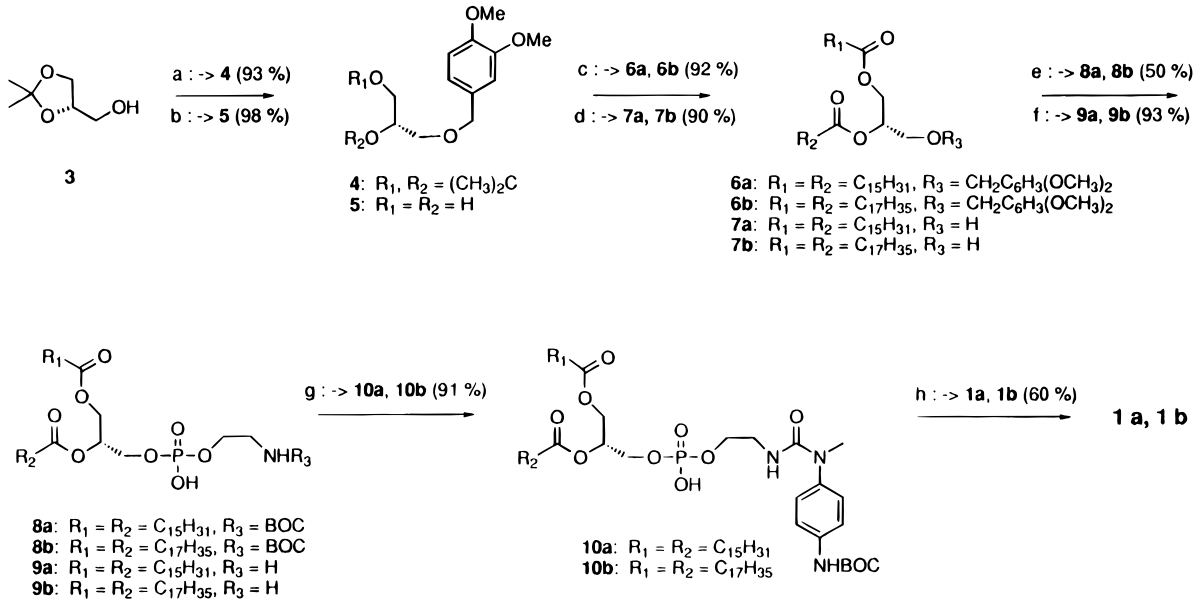
Scheme 2[a]



[a] Reagents and conditions: (a) NaH, $(MeO)_2C_6H_3CH_2Cl$, $(Bu)_4N^+I^-$, THF, rt; (b) 10% AcOH, 65 °C; (c) $C_{15}H_{31}COCl$ or $C_{17}H_{35}COCl$, pyridine, CH_2Cl_2, rt; (d) DDQ, H_2O/CH_2Cl_2; (e) 1. $POCl_3$, NEt_3, rt, 2. $HOCH_2CH_2NHBoc$, NEt_3, rt, 3. H_2O, rt; (f) TFA, CH_2Cl_2, rt; (g) $BocNHC_6H_4N(Me)COCl$, NEt_3, 65 °C; (h) TFA, rt; (i) $NaNO_2$, −10 °C.

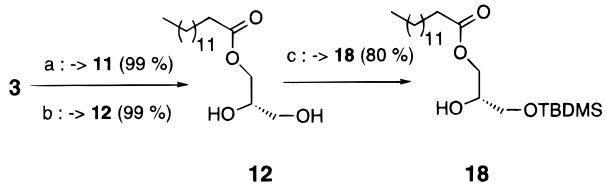
and characterization of these two types of probes. The proposed synthetic schemes allow the introduction of radioactive isotopes (^3H) to ensure their potential use as markers for membrane-associate protein domains.

Results and Discussion

The synthetic strategies developed for probes **1a**, **1b**, and **2** (Schemes 2 and 4) propose a potential incorporation of tritium at a step preceeding the final diazotization procedures used for the synthesis of the photosensitive moieties.

Synthesis of Probes 1a and 1b. The synthesis of probes **1a** and **1b** used the optically active glycerol acetonide **3** ((*S*)-enantiomer) as the starting material. The synthesis of dipalmitoyl and distearyl glycerides **7a** and **7b** (Scheme 2) used successive protection of **3** with the 3,4-dimethoxybenzyl group (compound **4**), removal of the acetonide group (compound **5**), subsequent acylations with palmitoyl chloride or stearyl chloride (compounds **6a** or **6b**), and a final deprotection of the 3,4-dimethoxybenzyl group (compounds **7a** or **7b**). The 3,4-dimethoxybenzyl group was used rather than the benzyl group to avoid hydrogenation during the deprotection reaction.[7] The mild deprotection conditions (DDQ)[8] allow the potential synthesis of unsaturated photoactivatable phospholipid probes. During the purification of compounds **7a** or **7b** on silica gel, very small amounts of side product resulting from transacylation reaction were observed. Fortunately, both compounds could be obtained in pure form by recrystallization from ether/hexane. Their conversion to phosphatidylethanolamine derivatives **9a** and **9b** used known procedures.[7] Coupling of **9a** and **9b** to the bifunctional reagent[9] led to the formation of **10a** and **10b** (Scheme 2), stable precursors of the final probes. This reagent has been synthesized in a tritiated form[10] and has been used in several instances for the synthesis of radio-labeled photoaffinity probes.[11−13] The deprotection of **10a** and **10b** with trifluoroacetic acid to the corresponding amines, followed by direct treatment with $NaNO_2$, gave the final probes **1a** and **1b** with almost quantitative yield. Both probes **1a** and **1b** were characterized spectroscopically and could be purified by HPLC.

Scheme 3[a]



[a] Reagents and conditions: (a) $CH_3(CH_2)_{12}COOH$, DCC, DMAP, CH_2Cl_2, rt (**11**); (b) AcOH 80%, 80 °C (**12**); (c) TBDMSCl, iPr_2EtN, DMF, rt (**18**).

Synthesis of Probe 2. Glycerol acetonide **3** was used again, but now as an (*R*)-enantiomer, to have access to the TBDMS, myristoyl glyceride derivative **18**, after a series of usual transformations[14−16] allowing gram quantities of preparations of this intermediate (Scheme 3). The chosen TBDMS protecting group gave excellent results during protection and deprotection reactions, in terms both of high yields and of nonracemization. The expected compound **12** possessed an α_D in agreement with the described α_D value for the (*S*)-enantiomer.[17]

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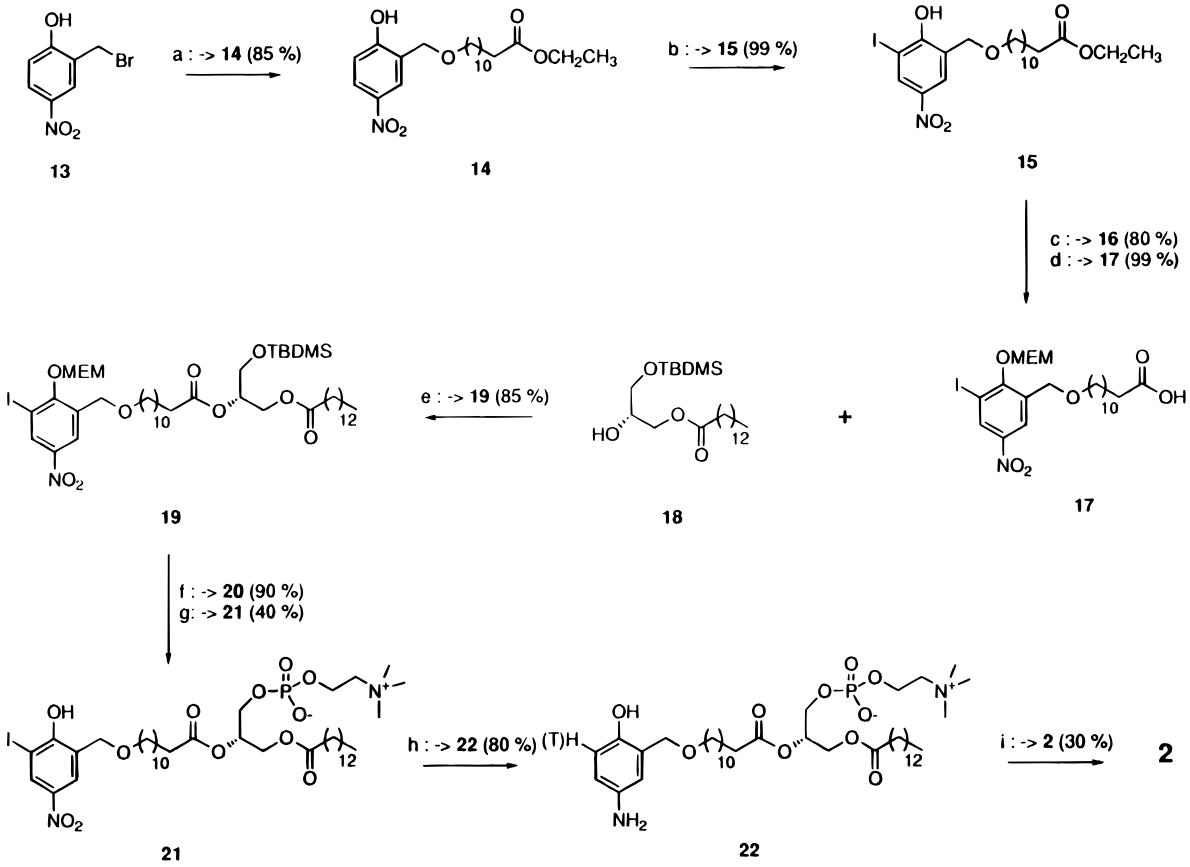
Scheme 4[a]



[a] Reagents and conditions: (a) Ag_2O, $HO(CH_2)_{11}COOEt$, CH_2Cl_2, rt; (b) BTMA· ICl_2, $NaHCO_3$, CH_2Cl_2/MeOH, rt; (c) iPr_2EtN, MEMCl, CH_2Cl_2, rt (**16**: 80%); (d) NaOH 4M, DME/H_2O, rt (**17**: 100%); (e) DCC, DMAP, CH_2Cl_2, rt; (f) HF−pyridine, THF, rt (**20**: 90%); (g) $POCl_3$, NEt_3, CH_2Cl_2, $HO(CH_2)_2N(CH_3)_3OTs$, pyridine, rt (**21**: 40%); (h) H_2, Pd/C, NEt_3, MeOH, rt; (i) $ONO(CH_2)_2CH(CH_3)_2$, AcOH, 10 °C.

The overall synthesis of probe **2** is outlined in Scheme 4 and describes the esterification of the secondary alcohol of glyceride **18** by a lauric acid derivative **17** functionalized at the ω-carbon by a substituted benzyloxy moiety, which allows the potential incorporation of both the radioactive isotope and the photosensitive chromophore. The acid **17** was initially synthesized from 2-hydroxy-5-nitrobenzyl bromide (**13**) which allowed the introduction of an iodine atom in an ortho position to the hydroxyl group as a potential exchangeable position by tritium. The iodination reaction (step b, Scheme 4) gave quantitative yields using benzyltrimethylammonium iododichloride in basic conditions,[18] while other classical iodination conditons gave less satisfactory results. The removal of the TBDMS protecting group of **19** (step f, Scheme 4) was best achieved using an HF-pyridine complex in THF.[19] The alcohol **20** obtained was not further purified to avoid transacylation of the diglyceride, but was transformed directly to the phosphatidylcholine derivative **21**.[20] Finally, a single catalytic hydrogenation reaction (step h, Scheme 4) permitted a simultaneous reduction of the aromatic nitro group to amine and substitution of the iodine atom by a hydrogen atom. This reaction combined the generation of the final precursor of the photosensitive function with the possible incorporation of a tritium atom at a stable position. Compound **22** was then converted to the probe **2** by mild diazotization using isoamyl nitrite. The final probe was purified by HPLC and characterized spectroscopically. The overall yield for the synthesis of probe **2** from the starting benzyl bromide derivative **13** was about 5%.

Stereochemical Inferences for Probes 1 and 2. The retention of the natural glyceride configuration in probes **1a** and **1b** was ascertained by comparing the α_D values of compounds **7a** and **7b** with the ones described in the literature.[21]

The *o*-iodo-*p*-nitrophenol derivative **21** is a stable precursor of probe **2** which can be conveniently stored and transformed to the final molecule without isolation and purification of the intermediates. It was important to demonstrate that this key precursor **21** was enantiomerically pure and retained the original configuration, knowing in particular the possibility of racemization during acidic deprotection conditions (i.e. step b, Scheme 3) or transacylation reactions at the level of compounds **12** and **20**. Consequently, we developed an alternative synthesis of **21** which used a natural phospholipid derivative to assess the optical activity of this chemical (Scheme 5). Dimyristoylphosphatidylcholine (DMPC) was first converted to the corresponding lysomyristoylphosphatidylcholine (LMPC) by selective hydrolysis using phospholipase A_2.[22] The coupling[23] of an LMPC−$CdCl_2$ complex[24] to an activated acid **17** as an 2-mercaptothiazoline deriv-

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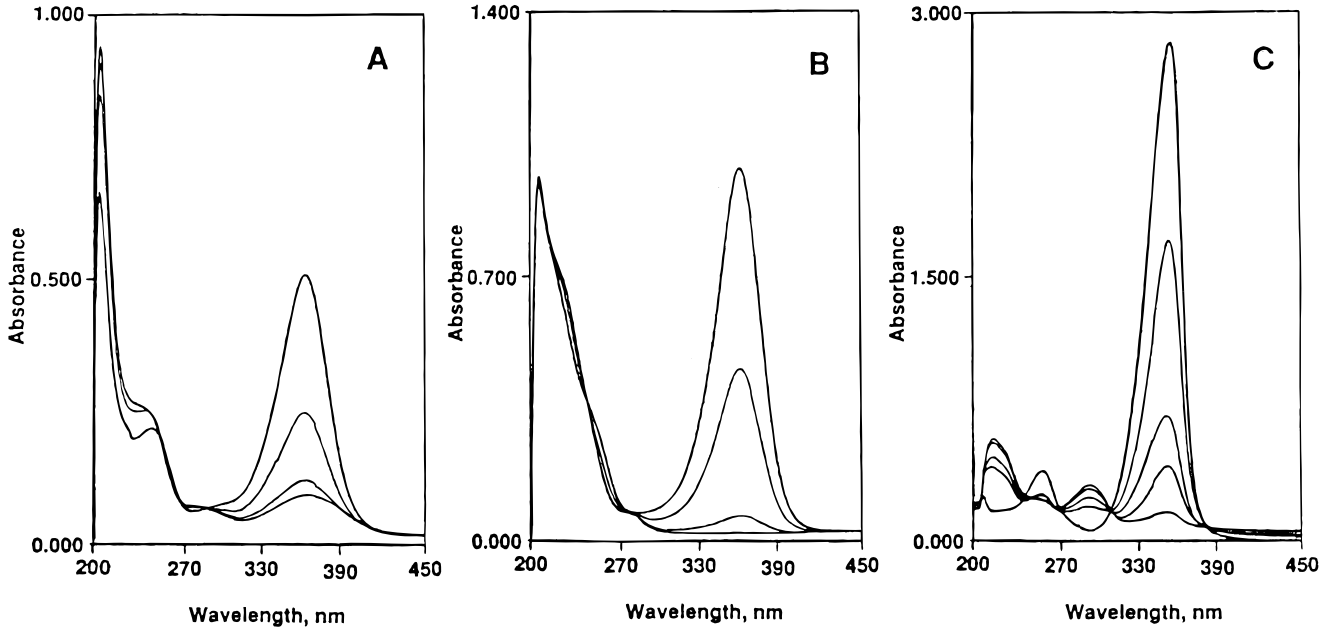
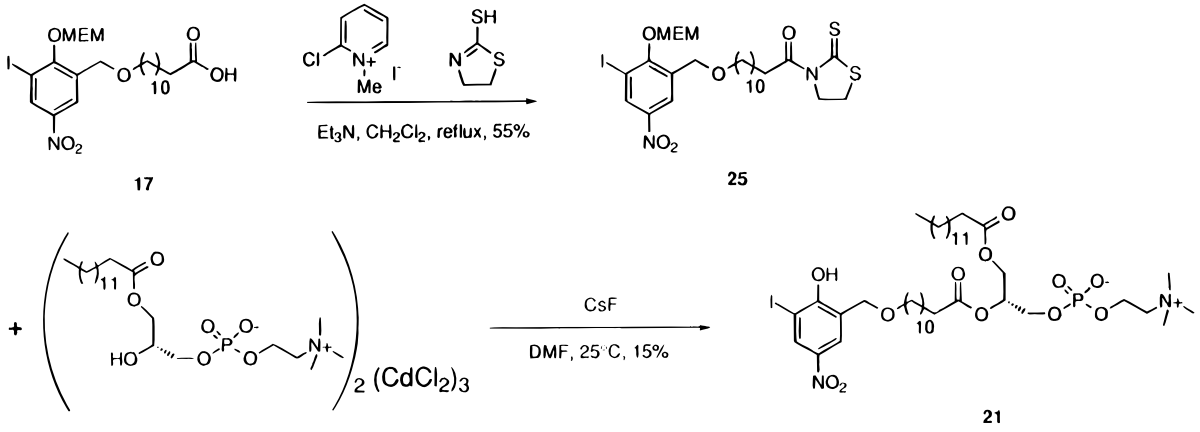


Figure 1. UV-spectral recording of the photoirradiation of **1a** or **2** at 364 nm, 4 °C: (A) 3×10^{-5} M **1a** in 50 mM phosphate buffer, pH = 7.2 with 0.07% SDS and (B) 3×10^{-5} M **1a** in MeOH; (C) 18×10^{-5} M **2** in 500 mM phosphate buffer (+ 4 μL MeOH), pH = 7.2. The time interval between two spectra was 20 s in **A**, 10 s in **B**, and 30 s in **C**.

Scheme 5



ative, **25**,[25] gave the expected **21**, which possessed an identical α_D to that of the previously synthesized derivative. This demonstrated that no racemization or transacylation occurred during the synthesis of probe **2**. Although this alternative synthetic route is slightly shorter, it presents two major drawbacks, which are the high cost of the natural phospholipid DMPC and poor reaction yields, especially for the coupling reaction leading to **21**.

Physicochemical Properties of Probes 1 and 2. (a) Chemical Stability. In order to be used as irreversible photochemical markers, these probes should present sufficient chemical stability in the absence of light. This property is best evaluated by measuring half-lives in buffered medium. The half-lives of probes **1a** and **1b** are 24 h at pH 7.4 and 25 °C, which can be increased to 6 days at pH 6.5 and 4 °C. Probe **2** has a half-life of 3 days at pH 7.2 and 4 °C. Thus, both probes **1** and **2** show satisfactory stability for further use in photolabeling experiments.

(b) Photochemical Properties. Figure 1 shows the absorption spectra of probes **1a** and **1b** and **2** as well as their photodecomposition by irradiation at 364 nm in buffered medium or in methanol. Both compounds show strong absorptions at 367 nm (ϵ = 25 000) and 357 nm (ϵ = 15 000), respectively. Irradiation of each compound led to complete disappearance of these bands, and the observed isobestics are illustrative of a unique photodecomposition process. As indicated earlier, absorption spectra of both chromophores are well adapted to overlap the tryptophan emission spectra (λ_{max} = 320−340 nm) and therefore constitute good candidates for Trp-mediated energy transfer photoactivation.[5]

The use of aryldiazonium derivatives as topographical markers[1] of protein binding sites in photoaffinity labeling experiments has demonstrated the extreme reactivity of aryl cations,[26,27] which renders these probes appropriate for nondiscriminative labeling of a binding site by react-

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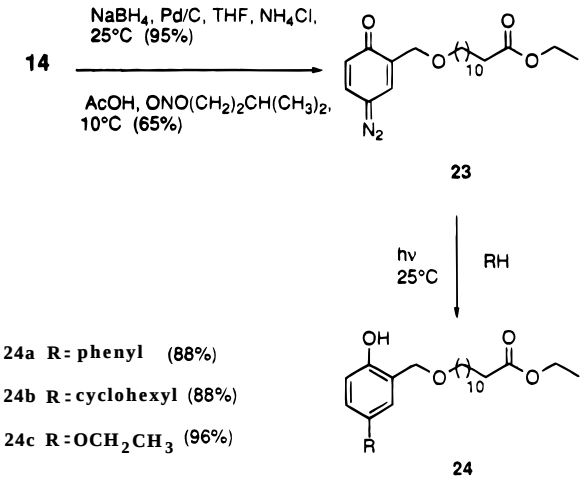
Scheme 6



ing with any amino acid residue. Probes **1a** and **1b**, designed for the mapping of the lipid/protein interface, might however be partially quenched by water molecules after photoactivation. Although they are charged species, aryldiazonium salts constitute well-adapted reagents to probe hydrophobic quaternary ammonium binding sites on nicotinic acetylcholine receptor[28,29] and acetylcholinesterase.[30]

Diazocyclohexadienonyl phospholipid derivative **2** was designed to probe the core of the membrane, including the hydrophobic membrane-embedded domains. To evaluate the photochemical reactivity of this probe, we used compound **23**, derived from the ω-substituted lauric ester **14**, as a model probe. The photodecomposition experiments in different solvents (Scheme 6) show efficient insertion reaction of the derived carbene in O—H bonds (ethanol), C(sp^3)—H bonds (cyclohexane), and C(sp^2)—H bonds (benzene). The 1:1 product ratio obtained from irradiation of **23** in an equimolar ethanol/cyclohexane solution reflects a preferential carbene insertion reaction with OH over CH bonds. This property has been described in the literature for similar species and demonstrated to occur through singlet carbene reactivity.[31] Laser flash photolysis studies on diazocyclohexadienonyl derivatives showed singlet insertion reactions in both methanol and cyclohexane with a diffusion-controlled insertion rate in methanol where 100−500 ps lifetimes for cyclohexadienoyl carbenes have been estimated.[32] Clearly, probe **2** should present the expected reactivity for efficient labeling of chemically unreactive species within the membrane core.

Conclusion

Two types of photoactivatable phospholipidic probes were proposed to label either the water/lipid membrane interface (probes **1a** and **1b**) or the membrane core (probe **2**). The total synthesis of these probes was described using synthetic strategies to permit incorporation of tritium isotopes. These probes photochemically generate highly reactive species, aryl cations and carbene, able to react covalently with any type of amino acid residue including the nonactivated hydrophobic residues. In addition, both probes present characteristic absorptions permitting a tryptophan-mediated energy transfer photoactivation intended to efficiently label the membrane-associate domains of proteins. These probes constitute, therefore, new tools for structural investigation of membrane constituents.

Experimental Section[33]

Thin-layer chromatography was performed on Merck 60 silica gel plates with a fluorescence indicator. The plates were visualized with UV light and/or by spraying with molybdenum blue (stain for phospholipids; Sigma). Phospholipid derivatives were purified on BIO-RAD silica acid Bio-Sil A, 100−200 mesh, by column chromatography. All other compounds were purified on Merck silica gel grade 60, 230−400 mesh. HPLC was carried out with a Zorbax SB C18 column (4.6 × 250 mm) and Zorbax 5μ Sil column (4.6 × 250 mm).

Chemicals were obtained from Aldrich Chemical Corporation and were used without prior purification. Choline tosylate[34] was prepared as decribed previously.

4-(*S*)-(((3,4-Dimethoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolane (4). To a suspension of (*S*)-isopropylidene glycerol (**3**) (380 mg, 2.62 mmol) and NaH (120 mg, 2.94 mmol) in THF (8 mL) were added tetrabutylammonium iodide (105 mg, 0.26 mmol) and 3,4-dimethoxybenzyl chloride (490 mg, 2.94 mmol) at 25 °C under argon. After being refluxed at 65 °C for 14 h, the reaction was quenched with methanol (1 mL) and neutralized with NH_4Cl (300 mg, 5.24 mmol). The reaction mixture was extracted with ether (100 mL), and the extract was washed with saturated NaCl solution (100 mL), dried over $MgSO_4$, and reduced *in vacuo*. The residue was purified by flash chromatography on silica gel with ether/hexane (1:1) to give **4** (690 mg, 93%) as a colorless oil: TLC (hexane/ether (1:1)) R_f 0.30; $[\alpha]^{25}_D$ +16.1 (c = 0.067, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 3H), 1.42 (s, 3H), 3.40−3.57 (m, 2H), 3.72 (dd, 1H, J = 6.34, 8.24 Hz), 3.87 (s, 3H), 3.88 (s, 3H), 4.04 (dd, 1H, J = 6.48, 8.12 Hz), 4.23−4.32 (m, 1H), 4.40−4.60 (m, 2H), 6.79−6.89 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 25.29, 26.70, 55.73, 66.74, 70.68, 73.31, 74.67, 109.33, 110.71, 110.92, 120.27, 130.38, 148.60, 148.94; IR (film) 2986, 2932, 2890, 1592, 1514, 1458, 1420, 1370, 1328, 1262, 1238, 1156, 1137, 1086, 1044, 1030 cm^{-1}. Anal. Calcd for $C_{15}H_{22}O_5$: C, 63.81; H, 7.85. Found: C, 63.57; H, 7.66.

2(*R*)-3-((3,4-Dimethoxybenzyloxy)propane-1,2-diol (5). A solution of **4** (130 mg, 0.47 mmol) in 10% acetic acid (in H_2O, 1 mL) was heated at 65 °C for 1 h. After lyophilization, the obtained residue was purified by flash chromatography on silica gel with EtOAc and yielded **5** (112 mg, 98%) as a colorless oil: TLC (EtOAc) R_f 0.20; $[\alpha]^{25}_D$ +2.50 (c = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 2.40 (s, br, 1H), 2.75 (s, br, 1H), 3.47−3.76 (m, 4H), 3.88 (s, 3H), 3.89 (s, 3H), 3.81−3.96 (m, 1H), 4.49 (s, 2H), 6.81−6.90 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 55.71, 63.84, 70.64, 71.22, 73.25, 77.20, 110.75, 110.99, 120.31, 130.07, 148.55, 148.82; IR (film) 3414 br, 2935, 2868, 1608, 1593, 1516, 1464, 1420, 1363, 1328, 1265, 1238, 1158, 1138, 1086, 1027 cm^{-1}. Anal. Calcd for $C_{12}H_{18}O_5$: C, 59.49; H, 7.49. Found: C, 59.65; H, 7.72.

Hexadecanoic Acid 3-((3,4-Dimethoxylbenzyl)oxy)-2-(hexadecanoyloxy)propyl Ester (6a). To a solution of **5** (100 mg, 0.41 mmol) in pyridine (1 mL) and CH_2Cl_2 (5 mL) was added dropwise at 0 °C palmitoyl chloride (0.28 mL, 0.86 mmol). The reaction mixture was stirred for 50 h at rt before being quenched with methanol (2 mL). Then the reaction mixture was filtered through Celite, and the filtrate was

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reduced *in vacuo*. The residue was dissolved in ether (50 mL). This solution was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, and reduced. The obtained residue was purified by chromatography on silica gel with ether/hexane (7:3) and gave **6a** (273 mg, 92.3%) as a white crystalline compound: mp 62−64 °C; TLC (hexane/ether (4:1)) R_f 0.25; $[\alpha]^{25}_D$ +6.50 (c = 0.080, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 6.30 Hz), 1.25 (s, 48H), 1.52−1.68 (m, 4H), 2.23−2.35 (m, 4H), 3.56 (d, 2H, J = 5.20 Hz), 3.87 (s, 3H), 3.89 (s, 3H), 4.17 (dd, 1H, J = 6.44, 11.86 Hz), 4.34 (dd, 1H, J = 3.72, 11.86 Hz), 4.47 (AB system, 2H, ν_A = 4.45 ppm, ν_B = 4.49 ppm, J_{AB} = 14.1 Hz), 5.18−5.25 (m, 1H), 6.80−6.88 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 14.06, 22.63, 24.87, 29.05, 29.26, 29.62, 31.87, 34.04, 34.26, 55.77, 62.63, 67.86, 69.90, 73.15, 110.72, 110.86, 120.22, 130.10, 148.64, 173.05, 173.34; IR (film) 2954, 2916, 2848, 1732, 1514, 1453, 1444, 1420, 1258, 1246, 1142, 1084, 1020 cm^{-1}. Anal. Calcd for $C_{44}H_{78}O_7$: C, 73.49; H, 10.93. Found: C, 73.44; H, 11.08.

Octadecanoic Acid 3-((3,4-Dimethoxylbenzyl)oxy)-2-(octadecanoyloxy)propyl Ester (6b). Using a similar procedure to that of **6a**, **6b** was obtained as a white crystalline compound: mp 70−72 °C; TLC (hexane/ether (4:1)) R_f 0.25; $[\alpha]^{25}_D$ +6.20 (c = 0.080, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, J = 6.14 Hz), 1.25 (s, 56H), 1.52−1.64 (m, 4H), 2.24−2.36 (m, 4H), 5.57 (d, 2H, J = 5.12 Hz), 3.87 (s, 3H), 3.89 (s, 3H), 4.18 (dd, 1H, J = 6.38, 11.74 Hz), 4.34 (dd, 1H, J = 3.48, 11.84 Hz), 4.47 (AB system, 2H, ν_A = 4.45 ppm, ν_B = 4.49 ppm, J_{AB} = 14.1 Hz), 5.18−5.25 (m, 1H), 6.78−6.88 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 14.09, 22.67, 24.90, 28.60, 29.09, 29.32, 31.90, 34.08, 34.30, 55.81, 62.66, 67.90, 69.93, 73.18, 110.75, 110.89, 120.25, 130.14, 148.66, 149.02, 173.07, 173.37; IR (film) 2954, 2917, 2850, 1738, 1592, 1516, 1466, 1264, 1159, 1098, 1028 cm^{-1}. Anal. Calcd for $C_{48}H_{68}O_7$: C, 74.37; H, 11.18. Found: C, 74.19; H, 11.43.

Hexadecanoic Acid 2-(Hexadecanoyloxy)-3-hydroxypropyl Ester (7a). To a mixture of **6a** (3.00 g, 4.17 mmol) in CH_2Cl_2 (18 mL) and H_2O (1 mL) was added dichlorodicyanobenzoquinone (1.01 g, 4.38 mmol). The colored reaction mixture turned progressively black. After 10 min at rt, the reaction mixture was filtered through Celite, and the filtrate was reduced *in vacuo*. The residue was purified by flash chromatography on silica gel with ether/hexane (1:3), followed by recrystallization with ether and hexane to give **7a** (2.07 g, 90%) as white crystals: mp 67−69 °C; TLC (hexane/ether (3:1)) R_f 0.25; $[\alpha]^{25}_D$ −2.60 (c = 0.080, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H, J = 6.40 Hz), 1.26 (s, 48H), 1.56−1.72 (m, 4H), 2.05 (t, 1H, J = 6.57 Hz), 2.30−2.39 (m, 4H), 3.74 (dd, 2H, J = 4.30, 5.59 Hz), 4.24 (dd, 2H, J = 5.61, 11.93 Hz), 4.33 (dd, 1H, J = 4.53, 11.89 Hz), 5.07−5.11 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.09, 22.67, 24.90, 29.10, 29.35, 29.67, 31.90, 34.08, 34.26, 61.49, 61.98, 72.05, 173.42, 173.79; IR (film) 3505, 2922, 2852, 1731, 1702, 1464, 1380, 1288, 1215, 1179, 1091, 1065 cm^{-1}. Anal. Calcd for $C_{35}H_{68}O_5$: C, 73.89; H, 12.05. Found: C, 74.14; H, 12.13.

Octadecanoic Acid 2-(Octadecanoyloxy)-3-hydroxypropyl Ester (7b). Using a similar procedure to that of **7a**, **7b** was obtained as white crystals: mp 75−77 °C; TLC (hexane/ether (4:1)) R_f 0.18; $[\alpha]^{25}_D$ −2.50 (c = 0.060, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H, J = 6.39 Hz), 1.26 (s, 56H), 1.56−1.70 (m, 4H), 2.05 (t, 1H, J = 6.48 Hz), 2.29−2.39 (m, 4H), 3.74 (dd, 2H, J = 4.53, 5,70 Hz), 4.24 (dd, 1H, J = 5.62, 12.0 Hz), 4.33 (dd, 1H, J = 4.63, 12.0 Hz), 5.07−5.11 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.09, 22.67, 24.89, 29.10, 29.35, 29.67, 31.91, 34.08, 34.26, 61.39, 62.08, 72.06, 173.44, 173.76; IR (film) 3503, 2955, 2916, 2849, 1732, 1709, 1465, 1380, 1287, 1255, 1177, 1091 cm^{-1}. Anal. Calcd for $C_{39}H_{76}O_5$: C, 74.94; H, 12.26. Found: C, 74.68; H, 12.42.

Hexadecanoic Acid 3-[((2-((*tert*-Butoxycarbonyl)amino)ethoxy)hydroxyphosphoryl)oxy]-2-(hexadecanoyloxy)propyl Ester (8a). To a solution of triethylamine (0.146 mL, 1.05 mmol) and phosphorus oxychloride (0.096 mL, 1.05 mmol) in THF (5 mL) at 0 °C was added dropwise a solution of **7a** (580 mg, 1.05 mmol) in THF (5 mL) over 30 min. After the reaction mixture was stirred at 0 °C for 1 h and at rt for 4 h, a solution of *N*-(*tert*-butoxycarbonyl)ethanolamine (190 mg, 1.47 mmol) and triethylamine (0.351 mL, 2.52 mmol) in THF (5 mL) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and at rt for 12 h before the addition of H_2O (2 mL), and the mixture was stirred again at rt for an additional 4 h. Then the organic solvent was removed, and 1% HCl (10 mL) was added to acidify the solution to pH = 1. After extraction of the product with $CHCl_3$ (15 mL), the extract was dried over $MgSO_4$ and reduced *in vacuo*. The obtained residue was purified by chromatography on Bio-Sil A with MeOH/CH_2Cl_2 (1:9) to give **8b** (410 mg, 50%) as a white solid: mp 41−43 °C; TLC (MeOH/CH_2Cl_2 (1:9)) R_f 0.15; $[\alpha]^{25}_D$ +2.50 (c = 0.020, $CHCl_3$);^{1}H NMR ($CD_3OD/CDCl_3$ (1:1)) δ 0.85 (t, 6H, J = 6.30 Hz), 1.24 (s, 48H), 1.41 (s, 9H), 1.52−1.64 (m, 4H), 2.26−2.32 (m, 4H), 3.16 (t, 1H, J = 5.54 Hz), 3.56 (t, 1H, J = 5.53 Hz), 3.80−4.00 (m, 4H), 4.13 (dd, 1H, J = 7.09, 12.0 Hz), 4.42 (dd, 1H, J = 2.32, 12.0 Hz), 5.25−5.55 (m, 1H); ^{13}C NMR ($CD_3OD/CDCl_3$ (1:1)) δ 14.40, 23.25, 25.49, 28.70, 29.75, 29.97, 30.29, 32.53, 34.62, 34.76, 41.58, 43.24, 61.68, 63.30, 64.24, 65.20, 71.03, 71.21, 79.99, 157.58, 174.12, 174.49; IR (film) 3300−3400 (br), 2954, 2917, 2850, 1740, 1516, 1467, 1244, 1170, 1053 cm^{-1}. Anal. Calcd for $C_{42}H_{82}NPO_{10}$: C, 63.77; H, 10.32; N, 1.77. Found: C, 63.8; H, 10.39; N, 1.92.

Octadecanoic Acid 3-[((2-((*tert*-Butoxycarbonyl)amino)ethoxy)hydroxyphosphoryl)oxy]-2-(octadecanoyloxy)propyl Ester (8b). Using a similar procedure to that of **8a**, **8b** was obtained as a white solid: mp 51−53 °C; TLC (MeOH/CH_2Cl_2 (1:9)) R_f 0.15; $[\alpha]^{25}_D$ +2.80 (c = 0.022, $CHCl_3$); ^{1}H NMR ($CD_3OD/CDCl_3$ (1:1)) δ 0.85 (t, 6H, J = 6.29 Hz), 1.24 (s, 56H), 1.41 (s, 9H), 1.52−1.64 (m, 4H), 2.28−2.36 (m, 4H), 3.22−3.35 (m, 2H), 3.83−3.98 (m, 4H), 4.13 (dd, 1H, J = 7.00, 12.0 Hz), 4.39 (dd, 1H, J = 2.62, 12.0 Hz), 5.12−5.27 (m, 1H); ^{13}C NMR ($CD_3OD/CDCl_3$ (1:1)) δ 14.42, 23.28, 25.51, 28.72, 29.79, 30.00, 30.33, 32.56, 34.64, 34.77, 41.63, 63.33, 64.22, 65.18, 71.05, 71.21, 79.98, 157.58, 174.11, 174.46; IR (film) 3350, 2917, 2850, 1740, 1708, 1698, 1652, 1558, 1540, 1467, 1367, 1255, 1174, 1098 cm^{-1}. Anal. Calcd for $C_{46}H_{90}NPO_{10}$: C, 65.21; H, 10.58; N, 1.65. Found: C, 65.06; H, 10.66; N, 1.58.

Hexadecanoic Acid 3-[((2-Aminoethoxy)hydroxyphosphoryloxy)-2-(hexadecanoyloxy)propyl Ester (9a). A solution of **8a** (177 mg, 0.22 mmol) in CH_2Cl_2 (9 mL) and TFA (1 mL) was stirred at rt for 45 min. After removal of the solvent, the residue was purified by chromatography on Bio-Sil A with MeOH/CH_2Cl_2 (1:9) to give **9a** (167 mg, 93%) as a white solid: mp 103−107 °C; TLC (MeOH/CH_2Cl_2 (1:9)) R_f 0.12; $[\alpha]^{25}_D$ +3.48 (c = 0.022, $CHCl_3$ + 0.05% TFA); ^{1}H NMR ($CD_3OD/CDCl_3$ (1:1)) δ 0.84 (t, 6H, J = 6.28 Hz), 1.23 (s, 48H), 1.48−1.65 (m, 4H), 2.15−2.31 (m, 4H), 3.10 (t, 2H, J = 4.28 Hz), 3.94−4.23 (m, 5H), 4.36 (dd, 1H, J = 2.89, 12.0 Hz), 5.12−5.24 (m, 1H); ^{13}C NMR ($CD_3OD/CDCl_3$ (1:1)) δ 14.31, 23.17, 25.40, 29.64, 29.87, 30.20, 32.46, 34.55, 34.67, 39.70, 62.24, 63.10, 64.32, 71.39, 108.40, 114.18, 119.97, 124.75, 162.04, 162.75, 163.46, 164.17, 174.30, 174.60; ^{31}P NMR (CD_3OD/$CDCl_3$ (9:1) + 0.05% TFA) δ 0.23; IR (film) 3300−3000 (br), 2954, 2918, 2850, 1740, 1467, 1205, 1165, 1080, 1025 cm^{-1}; FABMS$^+$ (*m*-nitrobenzyl alcohol) *m*/*z* 692 (M + H$^+$, 65), 551 (M$^+$ − $HOPO_3CH_2CH_2NH_2$, 100).

Octadecanoic Acid 3-[((2-Aminoethoxy)hydroxyphosphoryl)oxy]-2-(octadecanoyloxy)propyl Ester (9b). Using a similar procedure to that of **9a**, **9b** was obtained as a white solid: mp 124−127 °C; TLC (MeOH/CH_2Cl_2 1:9) R_f 0.12; $[\alpha]^{25}_D$ +3.59 (c = 0.034, $CHCl_3$ + 0.05% TFA); ^{1}H NMR (CD_3OD/$CDCl_3$ (1:1)) δ 0.84 (t, 6H, J = 6.40 Hz), 1.22 (s, 56H), 1.50−1.95 (m, 4H), 2.24−2.31 (m, 4H), 3.08−3.16 (m, 2H), 3.91−4.15 (m, 5H), 4.36 (dd, 1H, J = 2.89, 12.0 Hz), 5.12−5.24 (m, 1H); ^{13}C NMR ($CD_3OD/CDCl_3$ (1:1)) δ 14.33, 23.16, 25.39, 29.63, 29.87, 30.20, 32.45, 34.54, 34.67, 40.92, 62.24, 63.10, 64.32, 70.96, 108.20, 114.14, 119.93, 125.90, 162.66, 163.37, 174.30, 174.60; IR (film) 3300−3000 (br), 2916, 2850, 1742, 1683, 1467, 1175, 1162, 1096, 1065, 1026 cm^{-1}; FABMS$^+$ (*m*-nitrobenzyl alcohol) *m*/*z* 748 (M + H$^+$, 40), 607 (M$^+$ − $HOPO_3CH_2CH_2NH_2$, 100).

Hexadecanoic Acid 3-(({2-[3-(4-((*tert*-Butoxycarbonyl)amino)phenyl)-3-methylureido]ethoxy}hydroxyphosphoryl)oxy)-2-(hexadecanoyloxy)propyl Ester (10a). A solution of **9a** (100 mg, 0.115 mmol) and *N*-[4-((*tert*-butoxycarbonyl)amino)phenyl]-*N*-methylcarbamic chloride (33 mg, 0.115 mmol) in dry triethylamine (1 mL) and THF (1 mL)

was refluxed at 80 °C for 2 h. Then MeOH/$CHCl_3$ (1:1) (5 mL) was added to the reaction mixture, followed by addition of 5 mL of 10% HCl. The aqueous phase was extracted with MeOH/$CHCl_3$ (1:1) (3 × 5 mL). The combined organic phase was washed with MeOH/H_2O (1:1) (5 mL), dried over $MgSO_4$, and reduced *in vacuo*. The residue was purified by chromatography on Bio-Sil A with MeOH/$CHCl_3$ (1:9) to give **10a** (105 mg, 0.105 mmol, 91%) as a white solid: mp 43−45 °C; TLC (MeOH/CH_2Cl_2 (1:9)) R_f 0.15; $[\alpha]^{25}_D$ +3.03 (c = 0.0217, $CHCl_3$); ^{1}H NMR (CD_3OD/$CDCl_3$ (1:1)) δ 0.82 (t, 6H, J = 6,47 Hz), 1.20 (s, 48H), 1.47 (s, 9H), 1.38−1.55 (m, 4H), 2.24−2.31 (m, 4H), 3.17 (s, 3H), 3.29−3.31 (m, 2H), 3.51−3.90 (m, 4H), 4.00−4.35 (m, 2H); 5.10−5.24 (m, 1H), 7.11 (d, 2H, J = 8.74 Hz), 7.44 (d, 2H, J = 8.74 Hz); ^{13}C NMR (CD_3OD/$CDCl_3$ (1:1)) δ 14.21, 22.90, 25.11, 28.42, 29.39, 29.60, 29.92, 32.17, 34.31, 34.44, 37.59, 41.27, 62.90, 63.79, 64.89, 70.67, 80.74, 120.31, 127.97, 136.99, 138.83, 153.82, 158.44, 173.81, 174.20; IR (film) 3400−3200 (br), 2918, 2850, 1741, 1641, 1527, 1467, 1235, 1162, 1050 cm^{-1}. Anal. Calcd for $C_{50}H_{90}N_3PO_{11}$: C, 63.87; H, 9.65; N, 4.47. Found: C, 63.93; H, 9.71; N, 4.41.

Octadecanoic Acid 3-(({2-[3-(4-((*tert*-Butoxycarbonyl)amino)phenyl)-3-methylureido]ethoxy}hydroxyphosphoryl)oxy)-2-(octadecanoyloxy)propyl Ester (10b). Using a similar procedure to that of **10a**, **10b** was obtained as a white solid: mp 52−54 °C; TLC (MeOH/CH_2Cl_2 1:9) R_f 0.15; $[\alpha]^{25}_D$ +2.82 (c = 0.020, $CHCl_3$); ^{1}H NMR (CD_3OD/$CDCl_3$ (1:9)) δ 0.84 (t, 6H, J = 6,46 Hz), 1.22 (s, 56H), 1.49 (s, 9H), 1.40−1.60 (m, 4H), 2.24−2.34 (m, 4H), 3.17 (s, 3H), 3.29−3.35 (m, 2H), 3.90−4.15 (m, 5H), 4.36 (dd, 1H, J = 2.74, 12.0 Hz), 5.08−5.20 (m, 1H), 7.08 (d, 2H, J = 8.72 Hz), 7.40 (d, 2H, J = 8.70 Hz); ^{13}C NMR (CD_3OD/$CDCl_3$ (1:9)) δ 14.37, 23.20, 25.45, 28.63, 29.71, 29.92, 30.25, 34.60, 34.73, 37.89, 41.66, 63.25, 64.12, 65.12, 70.97, 80.81, 120.57, 128.23, 137.40, 139.31, 155.90, 158.88, 174.11, 174.50; IR (film) 3400−3100 (br), 2923, 2853, 1728, 1641, 1522, 1457, 1235, 1162, 1050 cm^{-1}. Anal. Calcd for $C_{54}H_{98}N_3PO_{11}$: C, 65.09; H, 9.91; N, 4.22. Found: C, 65.31; H, 10.15; N, 3.99.

4-(3-{2-[(2,3)-Bis(hexadecanoyloxy)propoxy)hydroxyphosphoryl)oxy]ethyl}-1-methylureido)benzenediazonium Trifluoroacetate (1a). A solution of **10a** (15 mg, 0.015 mmol) in TFA (0.4 mL) and MeOH/$CHCl_3$ (1:1) (0.6 mL) was stirred at rt for 1 h. After cooling at −10 °C an aqueous solution (15 μL) of 1 M $NaNO_2$ was added in 5 portions (each 3 μL). The reaction was followed by UV spectroscopy (at 379 nm, in $CHCl_3$). Then the solution was dried *in vacuo*, and the residue was purified by HPLC to give **1a** (10 mg, 60%) as a yellow solid: UV (MeOH): λ_{max} = 367 nm, ϵ_{367} = 26 000; HPLC: t_R = 8.2 min (Zorbax SB C18, isocratic: 70% CH_3CN and 30% MeOH with 0.1% TFA); ^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$ (1:1)) δ 0.85 (t, 6H, J = 6.78 Hz), 1.23 (s, 48H), 1.50−1.65 (m, 4H), 2.25−2.31 (m, 4H), 3.47 (s, 3H), 3.49−3.55 (m, 2H), 3.63−3.72 (m, 1H), 3.98−4.02 (m, 3H), 4.20 (dd, 1H, J = 6.78, 12.0 Hz), 4.41 (dd, 1H, J = 3.21, 11.51 Hz), 5.18−5.26 (m, 1H), 7.60 (d, 2H, J = 9.42 Hz), 8.36 (d, 2H, J = 9.42 Hz); ^{13}C NMR (75 MHz, CD_3OD/$CDCl_3$ (1:1)) δ 13.76, 22.64, 24.89, 29.11, 29.34, 29.51, 29.67, 31.92, 34.05, 34.18, 35.99, 41.87, 62.67, 63.72, 70.42, 70.50, 98.05, 120.32, 134.15, 155.99, 156.07, 161.50, 161.99, 173.81, 174.20; FABMS$^+$ (*m*-nitrobenzyl alcohol) *m*/*z* 847 (MH − N_2 + Na^+, 25), 740 (22), 551 (45). $t_{1/2}$ = 1 day (pH = 7.4, phosphate buffer, 25 °C); $t_{1/2}$ = 3.5 days (pH = 7.4, phosphate buffer, 4 °C); $t_{1/2}$ = 6 days (pH = 6.5, phosphate buffer, 4 °C).

4-(3-{2-[(2,3-Bis(octadecanoyloxy)propoxy)hydroxyphosphoryl)oxy]ethyl}-1-methylureido)benzenediazonium Trifloroacetate (1b). Using a similar procedure to that of **1a**, **1b** was obtained as a yellow solid: UV (MeOH): λ_{max} = 367 nm, ϵ_{367} = 25 000; HPLC: t_R = 15 min, (Zorbax SB C18 isocratic: 70% CH_3CN and 30% MeOH with 0.2% TFA); ^{1}H NMR (CD_3OD/$CDCl_3$ (1:1)) δ 0.84 (t, 6H, J = 6.37 Hz), 1.22 (s, 56H), 1.48−1.66 (m, 4H), 2.24−2.33 (m, 4H), 3.41 (s, 3H), 3.51−3.61 (m, 2H); 3.95−4.18 (m, 5H), 4.32−4.42 (m, 1H), 5.15−5.26 (m, 1H), 7.55 (d, 2H, J = 9.30 Hz), 8.32 (d, 2H, J = 9.30 Hz); ^{13}C NMR (75 MHz, CD_3OD/$CDCl_3$ (1:1)) δ 13.74, 22.64, 24.89, 29.11, 29.34, 29.67, 31.92, 34.05, 34.18, 35.99, 41.87, 62.67, 63.72, 70.42, 70.50, 98.05, 114.70, 118.40, 120.32, 134.15, 155.99, 156.07, 161.50, 161.99, 173.81, 174.20; FABMS$^+$ (*m*-nitrobenzyl alcohol) *m*/*z* 903 (MH − N_2 + Na^+, 12), 796 (8), 607 (85). $t_{1/2}$ = 1 day (pH = 7.4, phosphate buffer, 25 °C); $t_{1/2}$ = 6 days (pH = 6.5, phosphate buffer, 4 °C); $t_{1/2}$ = 5 days (MeOH/$CHCl_3$ 1:1, 25 °C).

12-Hydroxydodecanoic Acid Ethyl Ester. A solution of 12-hydroxydodecanoic acid (2 g, 9.24 mmol) in dry EtOH (150 mL) and sulfuric acid (95%, 10 mL) was refluxed for 4 h. After addition of a saturated solution of Na_2CO_3, the mixture was extracted with EtOAc. The combined organic layers were washed with water and saturated NaCl solution, dried over $MgSO_4$, and evaporated *in vacuo* to give the ester (2.24 g, 99%) as a white solid: mp 24−25 °C; TLC (hexane/EtOAc (5:5)) R_f 0.63; ^{1}H NMR ($CDCl_3$) δ 1.10−1.26 (m, 17H), 1.40−1.60 (m, 4H), 2.22 (t, 2H, J = 7.46 Hz), 3.48 (t, 2H, J = 6.58 Hz), 4.03 (q, 2H, J = 7.14 Hz); ^{13}C NMR ($CDCl_3$) δ 14.04, 24.89, 25.69, 29.07, 29.48, 32.75, 34.05, 60.09, 62.90, 174.22; IR (film) 3427, 2920, 2851, 1737, 1466, 1254, 1200 cm^{-1}.

12-((2-Hydroxy-5-nitrobenzyl)oxy)dodecanoic Acid Ethyl Ester (14). To a solution of 2-hydroxy-5-nitrobenzyl bromide, **13** (0.10 g, 0.43 mmol), in dry CH_2Cl_2 (1 mL) was added silver oxide (0.10 g, 0.43 mmol), followed by a dropwise addition of the above-synthesized ester alcohol (0.105 g, 0.43 mmol) at rt under nitrogen. The dark mixture was stirred for 2 days at rt in the dark. The solid was removed by filtration through Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel with EtOAc/hexane (3:7) to give **14** (0.142 g, 85%) as a yellow solid: mp 46−47 °C; TLC (hexane/EtOAc (5:5)) R_f 0.70; ^{1}H NMR ($CDCl_3$) δ 1.20−1.39 (m, 17H), 1.55−1.67 (m, 4H), 2.27 (t, 2H, J = 7.34 Hz), 3.57 (t, 2H, J = 6.52 Hz), 4.10 (q, 2H, J = 7.12 Hz), 4.74 (s, 2H), 6.92 (d, 1H, J = 8.92 Hz), 7.97 (d, 1H, J = 2.74 Hz), 8.08 (dd, 1H, J = 2.74, 8.92 Hz); ^{13}C NMR ($CDCl_3$) δ 14.04, 24.76, 25.77, 29.20, 34.19, 60.09, 71.04, 71.44, 116.54, 122.91, 123.83, 125.18, 140.48, 161.87, 174.01; IR (film) 3325, 2930, 2856, 1736, 1548, 1342, 1251, 1092, 1005, 978 cm^{-1}. Anal. Calcd for $C_{21}H_{33}NO_6$: C, 63.77; H, 8.40; N, 3.54. Found: C, 63.96; H, 8.56; N, 3.38.

Benzyltrimethylammonium Iododichloride (BTMA·ICl_2). To a black solution of ICl (10 g, 61.60 mmol) in CH_2Cl_2 (123 mL) was added dropwise, under stirring at rt, a solution of benzyltrimethylammonium chloride (11.44 g, 61.60 mmol) in water (100 mL). After 30 min of stirring, the CH_2Cl_2 layer was separated, dried over $MgSO_4$, and then evaporated *in vacuo* to give the residue, which was recrystallized from CH_2Cl_2/ether (3:1) to afford BTMA·ICl_2 (21.1 g, 90%) as brilliant yellow needles: mp 125−126 °C; ^{1}H NMR (CD_3COCD_3) δ 3.42 (s, 9H), 4.86 (s, 2H), 7.61−7.69 (m, 3H), 7.73−7.80 (m, 2H); ^{13}C NMR (CD_3COCD_3) δ 53.10, 69.95, 129.90, 131.42, 133.68, 206.19. Anal. Calcd for $C_{10}H_{16}ICl_2N$: C, 34.50; H, 4.63; N, 4.03. Found: C, 34.51; H, 4.67; N, 4.11.

12-((2-Hydroxy-3-iodo-5-nitrobenzyl)oxy)dodecanoic Acid Ethyl Ester (15). To a solution of nitrophenol **14** (1.55 g, 3.92 mmol) in CH_2Cl_2 (38 mL) and MeOH (16 mL) were added BTMA·ICl_2 (1.80 g, 4.70 mmol) and $NaHCO_3$ (0.857 g, 10.20 mmol). The mixture was stirred for 19 h at rt. The yellow solution turned gradually to light brown. The excess $NaHCO_3$ was filtered off, the filtrate was concentrated, and 5% aqueous $NaHCO_3$ (16 mL) was added to the obtained residue. After extraction with EtOAc, the organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to give **15** (2.03 g, 99%) as a yellow solid: mp 38−39 °C; TLC (hexane/EtOAc 7:3) R_f 0.58; ^{1}H NMR ($CDCl_3$) δ 1.20−1.45 (m, 17H), 1.50−1.75 (m, 4H), 2.28 (t, 2H, J = 7.30 Hz), 3.63 (t, 2H, J = 6.50 Hz), 4.12 (q, 2H, J = 7.12 Hz), 4.75 (s, 2H), 7.97 (d, 1H, J = 2.66 Hz), 8.58 (d, 1H, J = 2.66 Hz); ^{13}C NMR ($CDCl_3$) δ 14.16, 24.88, 25.64, 25.82, 29.04, 29.16, 29.32, 34.29, 60.08, 71.37, 71.86, 84.06, 122.37, 123.56, 134.32, 141.05, 160.79, 173.71; IR (film) 3186, 2927, 2854, 1732, 1524, 1338, 1091 cm^{-1}.

12-[(3-Iodo-2-((2-methoxyethoxy)methoxy)-5-nitrobenzyl)oxy]dodecanoic Acid Ethyl Ester (16). To a solution of nitrophenol **15** (1.80 g, 3.45 mmol) in diisopropylethylamine (3.70 mL) was added dropwise at 0 °C (methoxyethoxy)methyl chloride (0.51 mL, 4.49 mmol). The reaction mixture was warmed up to rt before dry CH_2Cl_2 (3.70 mL) was added. The mixture was stirred for 6 h at rt before being quenched with

a saturated aqueous $NaHCO_3$ solution (16 mL). The mixture was extracted with EtOAc. The combined organic layers were washed with water and saturated NaCl solution, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel with EtOAc/hexane (3:7) to give **16** (1.68 g, 80%) as a yellow solid: mp 32−34 °C; TLC (hexane/EtOAc (7:3)) R_f 0.59; ^{1}H NMR ($CDCl_3$) δ 1.20−1.40 (m, 17H), 1.55−1.67 (m, 4H), 2.27 (t, 2H, J = 7.34 Hz), 3.38 (s, 3H), 3.52 (t, 2H, J = 6.52 Hz), 3.55−3.62 (m, 2H), 3.94−4.01 (m, 2H), 4.10 (q, 2H, J = 7.10 Hz), 4.63 (s, 2H), 5.23 (s, 2H), 8.33 (d, 1H, J = 2.70 Hz), 8.56 (d, 1H, J = 2.70 Hz); ^{13}C NMR ($CDCl_3$) δ 14.16, 24.87, 26.09, 29.04, 29.14, 29.34, 29.57, 34.27, 59.01, 60.03, 67.28, 69.93, 71.29, 71.47, 91.42, 99.59, 124.37, 133.52, 135.01, 144.75, 160.06, 173.79; IR (film) 3019, 2931, 2856, 1725, 1525, 1437, 1343, 1216, 1158, 1107 cm^{-1}. Anal. Calcd for $C_{25}H_{40}INO_8$: C, 49.26; H, 6.61; N, 2.29. Found: C, 49.56; H, 6.95; N, 2.38.

12-[(3-Iodo-2-((2-methoxyethoxy)methoxy)-5-nitrobenzyl)oxy]dodecanoic Acid (17). To a solution of **16** (1.30 g, 2.13 mmol) in a 1:1 mixture of DME/H_2O (17 mL) was added at rt an aqueous solution of 4 M NaOH (3.73 mL). After the solution was stirred at rt for 6 h, an aqueous solution of 6 M HCl was added to neutralize the solution. The mixture was extracted with EtOAc. The combined organic layers were washed with water and saturated NaCl solution, dried over $MgSO_4$, and evaporated *in vacuo* to give **17** (1.23 g, 99%) as a light brown solid: mp 53−55 °C; TLC (hexane/EtOAc (1:9)) R_f 0.46; ^{1}H NMR ($CDCl_3$) δ 1.22−1.40 (m, 14H), 1.55−1.70 (m, 4H), 2.34 (t, 2H, J = 7.38 Hz), 3.39 (s, 3H), 3.53 (t, 2H, J = 6.56 Hz), 3.56−3.67 (m, 2H), 3.96−4.01 (m, 2H), 4.64 (s, 2H), 5.24 (s, 2H), 8.34 (d, 1H, J = 2.60 Hz), 8.57 (d, 1H, J = 2.60 Hz); ^{13}C NMR ($CDCl_3$) δ 24.61, 26.10, 28.97, 29.14, 29.41, 29.59, 33.96, 59.06, 66.56, 69.95, 71.33, 71.73, 91.48, 99.60, 124.42, 133.57, 134.98, 144.76, 160.10, 179.65; IR (film) 3433, 2927, 2854, 1708, 1525, 1341, 1109, 927 cm^{-1}. Anal. Calcd for $C_{23}H_{36}INO_8$: C, 47,51; H, 6,24; N, 2,40. Found: C, 47.67; H, 6.36; N, 2.30.

12-[(3-Iodo-2-((2-methoxyethoxy)methoxy)-5-nitrobenzyl)oxy]dodecanoylthiazolidine-2-thione (25). To a suspension of 2-chloro-1-methylpyridinium iodide (0.04 g, 0.156 mmol) in dry CH_2Cl_2 (1.30 mL) were added, at rt under argon, **17** (0.070 g, 0.120 mmol) and 2-mercaptothiazoline (0.016 g, 0.132 mmol), followed by dropwise addition of triethylamine (0.040 mL, 0.288 mmol). After the solution was stirred under reflux for 6 h, water (0.360 mL) was added before the mixture was extracted with CH_2Cl_2. The combined organic layers were washed with water and saturated NaCl solution, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel with EtOAc/hexane (5:5) to give **25** (0.045 g, 55%) as a yellow oil: TLC (hexane/EtOAc (5:5)) R_f 0.62; ^{1}H NMR ($CDCl_3$) δ 1.20−1.50 (m, 14H), 1.50−1.70 (m, 4H), 3.19−3.32 (m, 4H), 3.39 (s, 3H), 3.53 (t, 2H, J = 6.56 Hz), 3.56−3.64 (m, 2H), 3.97−4.02 (m, 2H), 4.58 (t, 2H, J = 7.52 Hz), 4.65 (s, 2H), 5.25 (s, 2H), 8.35 (d, 1H, J = 2.67 Hz), 8.58 (d, 1H, J = 2.67 Hz); ^{13}C NMR ($CDCl_3$) δ 24.51, 25.99, 28.11, 29.25, 34.02, 38.27, 55.88, 58.83, 66.44, 67.75, 71.20, 71.63, 91.61, 99.50, 123.74, 133.03, 134.39, 144.61, 169.77, 174.68, 201.33; IR (film) 3433, 2927, 2854, 1708, 1690, 1525, 1370, 1280, 1150, 1055 cm^{-1}.

Tetradecanoic Acid 2,2-Dimethyl-1,3-dioxolan-4-yl Methyl Ester (11). To a solution of alcohol **3** (0.200 g, 1.510 mmol), myristic acid (0.415 g, 1.81 mmol), and 4-pyrrolidinopyridine (0.022 g, 0.150 mmol) in dry CH_2Cl_2 (43 mL) was added a solution of DCC (0.344 g, 1.660 mmol) in dry CH_2Cl_2 (2 mL) at rt. The reaction mixture was stirred for 16 h at rt, and the white solid was removed by filtration through Celite. After evaporation, the residue was purified by flash chromatography on silica gel with hexane/ether (5:5) to give **11** (0.515 g, 99%) as a white solid: mp 23−24 °C; TLC (hexane/ether (8:2)) R_f 0.40; $[\alpha]^{25}_D$ +0.52 (c = 0.054, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 6.10 Hz), 1.24 (s, 20H), 1.35 (s, 3H), 1.42 (s, 3H), 1.55−1.67 (m, 2H), 2.32 (t, 2H, J = 7.34 Hz), 3.72 (dd, 1H, J = 6.20, 8.38 Hz), 4.02−4.19 (m, 3H), 4.24−4.35 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 15.04, 23.61, 25.81, 26.31, 27.60, 30.04, 30.17, 30.28, 30.38, 32.84, 35.02, 65.42, 67.27, 74.59, 110.71, 174.51; IR (film) 4214, 3683, 3620, 3015, 2927, 2855, 2400, 2253, 1733, 1522, 1475, 1423, 1383, 1231, 1047 cm^{-1}. Anal. Calcd for $C_{20}H_{38}O_4$: C, 70.13; H, 11.18. Found: C, 70.24; H, 11.21.

Tetradecanoic Acid 2,3-Hydroxypropyl Ester (12). A solution of **11** (4.50 g, 13.14 mmol) in 80% acetic acid (13.50 mL) was heated at 80 °C for 4 h. The solution was cooled to rt, and the solvent was removed by azeotropic evaporation with toluene (3 × 10 mL) to remove the last traces of water before being dried under high vacuum to afford **12** (3.90 g, 99%) as a white solid: mp 70−71 °C; TLC (ether/hexane (5:95)) R_f 0.60; $[\alpha]^{25}_D$ +4.05 (c = 0.057, pyridine);^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.80 Hz), 1.20−1.35 (m, 20H), 1.55−1.68 (m, 2H), 2.36 (t, 2H, J = 7.36 Hz), 3.56 (part B of the ABX system, 1H, J_{BX} = 6.00 Hz, J_{AB} = 11.69 Hz), 3.67 (part A of the ABX system, 1H, J_{AX} = 3.57 Hz, J_{AB} = 11.69 Hz), 3.85−3.96 (part X of the ABX system, m, 1H), 4.13 (d, 2H, J = 5.44 Hz); ^{13}C NMR ($CDCl_3$) δ 14.03, 22.60, 24.81, 29.06, 29.19, 29.27, 29.39, 31.83, 34.07, 63.29, 64.97, 70.16, 174.33; IR (film) 4214, 3683, 3620, 3028, 2927, 2855, 2400, 2253, 1733, 1522, 1423, 1231, 1046 cm^{-1}. Anal. Calcd for $C_{17}H_{34}O_4$: C, 67.51; H, 11.33. Found: C, 67.61; H, 11.57.

Tetradecanoic Acid 3-((*tert*-Butyldimethylsilyl)oxy)-2-hydroxypropyl Ester (18). To a solution of **12** (0.127 g, 0.419 mmol) in dry DMF (1.20 mL) at 0 °C under nitrogen were added diisopropylethylamine (0.220 mL, 1.26 mmol) and *tert*-butyldimethylsilyl chloride (0.076 g, 0.50 mmol). The mixture was stirred for 5 min at 0 °C and then for 12 h at rt before being quenched by slow addition of saturated aqueous NH_4Cl solution (1.50 mL). The mixture was extracted with EtOAc, and the combined organic layers were washed with water and saturated NaCl solution, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel with EtOAc/hexane (2:8) to give **18** (0.137 g, 80%) as a clear oil: TLC (hexane/EtOAc (9:1)) R_f 0.50; $[\alpha]^{25}_D$ +1.91 (c = 0.100, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.07 (s, 6H), 0.87 (t, 3H, J = 7.10 Hz), 0.90 (s, 9H), 1.20−1.35 (m, 20H), 1.55−1.65 (m, 2H), 2.33 (t, 2H, J = 7.36 Hz), 3.60 (part B of the ABX system, 1H, J_{BX} = 6.18 Hz, J_{AB} = 10.26 Hz), 3.66 (part A of the ABX system, 1H, J_{AX} = 4.75 Hz, J_{AB} = 10.26 Hz), 3.79−3.92 (part X of the ABX system, m, 1H), 4.10 (part B of the ABX system, 1H, J_{BX} = 2.05 Hz, J_{AB} = 9.68 Hz), 4.15 (part A of the ABX system, 1H, J_{AX} = 1.58 Hz, J_{AB} = 9.68 Hz); ^{13}C NMR ($CDCl_3$) δ −5.25, 14.06, 18.22, 22.63, 24.89, 25.78, 29.30, 29.59, 31.87, 34.15, 63.67, 64.94, 69.96, 173.92; IR (film) 3461, 2954, 2920, 2855, 1741, 1464, 1389, 1255, 1177, 1113 cm^{-1}.

Tetradecanoic Acid 3-((*tert*-Butyldimethylsilyl)oxy)-2-[(12-((3-iodo-2-(2-methoxyethoxy)methoxy)-5-nitrobenzyl)oxy)dodecanoyl)oxy]propyl Ester (19). To a solution of alcohol **18** (0.527 g, 1.26 mmol), acid **17** (0.809 g, 1.39 mmol), and DMAP (0.016 g, 0.126 mmol) in dry CH_2Cl_2 (3 mL) was added a solution of DCC (0.287 g, 1.39 mmol) in dry CH_2Cl_2 (1 mL) at rt. The reaction mixture was stirred at rt overnight, and the white solid was removed by filtration through Celite. After evaporation, the oily residue was purified by flash chromatography on silica gel with hexane/EtOAc (8:2) to give **19** (1.16 g, 85%) as a yellow oil: TLC (hexane/EtOAc (8:2)) R_f 0.52; $[\alpha]^{25}_D$ +4.12 (c = 0.008, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 6H), 0.80−0.90 (m, 12H), 1.20−1.45 (m, 34H), 1.55−1.64 (m, 6H), 2.30 (t, 4H, J = 7.40 Hz), 3.40 (s, 3H), 3.53 (t, 2H, J = 6.52 Hz), 3.59−3.64 (m, 2H), 3.72 (d, 2H, J = 5.36 Hz), 3.97−4.01 (m, 2H), 4.16 (part B of the ABX system, 1H, J_{BX} = 6.42 Hz, J_{AB} = 11.93 Hz), 4.34 (part A of the ABX system, 1H, J_{AX} = 3.59 Hz, J_{AB} = 11.93 Hz), 4.65 (s, 2H), 5.05−5.24 (part X of the ABX system, m, 1H), 5.25 (s, 2H), 8.35 (d, 1H, J = 2.70 Hz), 8.58 (d, 1H, J = 2.70 Hz); ^{13}C NMR ($CDCl_3$) δ −5.21, 14.04, 18.11, 22.60, 24.83, 25.67, 26.11, 29.03, 29.21, 29.39, 31.83, 34.07, 34.25, 59.03, 61.34, 62.33, 67.28, 69.94, 71.30, 71.45, 71.57, 91.44, 99.59, 124.36, 133.53, 135.00, 144.74, 160.07, 173.03, 173.38; IR (film) 2926, 2855, 2358, 1742, 1528, 1462, 1342, 1252, 1163, 1111, 839 cm^{-1}. Anal. Calcd for $C_{46}H_{82}INO_{11}Si$: C, 56.36; H, 8.43; N, 1.43. Found: C, 56.76; H, 8.80; N, 1.41.

Tetradecanoic Acid 3-Hydroxy-2-[(12-((3-iodo-2-((2-methoxyethoxy)methoxy)-5-nitrobenzyl)oxy)dodecanoyl)oxy]propyl Ester (20). Silylated compound **19**

(0.130 g, 0.132 mmol) was azeotropically dried with benzene, dissolved in dry THF (2.90 mL), and transferred to a dry polyethylene vial. The cooled (0 °C) solution was treated under argon with HF−pyridine complex (0.20 mL), and after 30 min the ice bath was removed. After 4 h at rt, additional amounts of HF−pyridine (0.40 mL) were added until the reaction was completed. At 0 °C, the cooled solution was diluted with ether (15 mL) and neutralized with saturated $NaHCO_3$ solution until no more carbon dioxide was liberated. The combined organic layers were washed with water and saturated NaCl solution, dried over $MgSO_4$, and coevaporated with toluene *in vacuo*. The ester **20** was obtained (0.103 g, 90%) as a yellow solid: mp 34−35 °C; TLC (hexane/EtOAc 75:25) R_f 0.27; $[\alpha]^{25}_D$ −1.70 (c = 0.024, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.12 Hz), 1.20−1.45 (m, 34H), 1.55−1.75 (m, 6H), 2.36 (t, 4H, J = 6.20 Hz), 3.40 (s, 3H), 3.53 (t, 2H, J = 6.56 Hz), 3.59−3.64 (m, 2H), 3.73 (d, 2H, J = 4.58 Hz), 3.97−4.02 (m, 2H), 4.23 (part B of ABX system, 1H, J_{BX} = 5.81 Hz, J_{AB} = 11.96 Hz), 4.31 (part A of the ABX system, 1H, J_{AX} = 4.44 Hz, J_{AB} = 11.96 Hz), 4.65 (s, 2H), 5.05−5.24 (part X of the ABX system, m, 1H), 5.25 (s, 2H), 8.35 (d, 1H, J = 2.84 Hz), 8.58 (d, 1H, J = 2.84 Hz); ^{13}C NMR ($CDCl_3$) δ 14.06, 22.63, 24.85, 26.15, 29.22, 29.41, 29.59, 31.87, 34.06, 34.24, 59.08, 61.50, 61.97, 67.35, 70.00, 71.37, 71.55, 72.07, 91.46, 99.63, 124.45, 133.61, 135.06, 144.83, 160.08, 173.38, 173.74; IR (film) 3460, 2925, 2853, 2358, 1738, 1526, 1462, 1341, 1239, 1164, 1109 cm^{-1}. Anal. Calcd for $C_{40}H_{68}INO_{11}$: C, 55.48; H, 7.91; N, 1.61. Found: C, 55.64; H, 8.17; N, 1.64.

1-Tetradecanoic Acid 3-((((*N*,*N*,*N*-Trimethylammonio)-ethoxy)hydroxyphosphoryl)oxy)-2-[(12-((2-hydroxy-3-iodo-5-nitrobenzyloxy)dodecanoyl)oxy]propyl Ester (21). To a mixture of phosphorus oxychloride (11.65 μL, 0.126 mmol) and freshly distilled triethylamine (305 μL, 2.18 mmol) was added dropwise at 0 °C under nitrogen a solution of **20** (0.10 g, 0.115 mmol) in dry CH_2Cl_2 (0.778 mL). After 15 min at 0 °C, the mixture was allowed to warm up to rt and was stirred for 30 min. Then a solution of choline tosylate (0.095 g, 0.346 mmol) in freshly distilled pyridine (1.15 mL) was added, and the mixture was stirred under nitrogen for 63 h. Water (0.030 mL) was added, and the obtained solution continued to stir for 2 h. After removal of the solvents *in vacuo*, the residue was taken up in 2 mL of CH_2Cl_2/toluene (1:1), and the mixture was filtered. The filtrate was evaporated, and the obtained residue was purified by flash chromatography on Bio-Sil A with chloroform/methanol/H_2O (65:35:4) to give **21** (0.043 g, 40%) as a yellow oil: TLC ($CHCl_3$/MeOH/H_2O (65:35:4)) R_f 0.30; $[\alpha]^{25}_D$ +4.75 (c = 0.013, CH_3OH); ^{1}H NMR (CD_3OH) δ 0.86 (t, 3H, J = 6.56 Hz), 1.20−1.45 (m, 34H), 1.55−1.64 (m, 6H), 2.30 (q, 4H, J = 7.16 Hz), 3.20 (s, 9H), 3.54 (t, 2H, J = 6.34 Hz), 3.59−3.66 (m, 2H), 3.97 (t, 2H, J = 6.34 Hz), 4.14 (dd, 1H, J = 6.94, 12.08 Hz), 4.21−4.32 (m, 2H), 4.40 (dd, 1H, J = 3.18, 12.08 Hz), 4.57 (s, 2H), 5.15−5.27 (m, 1H), 8.13 (d, 1H, J = 2.66 Hz), 8.48 (d, 1H, J = 2.66 Hz); ^{13}C NMR (CD_3-OH) δ 14.46, 23.73, 26.01, 27.25, 30.19, 30.46, 30.66, 30.76, 33.06, 34.92, 35.09, 54.71, 60.51, 63.66, 64.86, 67.47, 71.71, 71.86, 72.06, 100.06, 125.09, 127.28, 135.04, 140.65, 164.48, 174.62, 174.94; IR (film) 3368, 2923, 2852, 2359, 1730, 1518, 1467, 1337, 1233, 1086, 968 cm^{-1}; UV (CH_3OH) λ_{max} = 408 nm, ϵ = 4350; FABMS$^+$ (*m*-nitrobenzyl alcohol) *m*/*z* 943 (M + H$^+$, 60), 776 (47); HPLC t_R 23.5 min, (Zorbax 5μ Sil column, from 0 to 60 min, iPrOH/H_2O (7:3) from 60% to 100%, 40% hexane from 40% to 0%).

Alternative procedure: To a solution of **25** (0.070 g, 0.102 mmol) in dry DMF (4.56 mL) were added the LMPC·$CdCl_2$ complex (0.138 g, 0.0931 mmol) and activated CsF (0.141 g, 0.931 mmol), and the mixture was stirred at rt under argon for 6 days in the dark. Dry MeOH (0.330 mL) was then added, and the mixture was stirred overnight. After centrifugation (3000 rpm × 5 min), the supernatant liquid was diluted with H_2O at 0 °C and extracted with $CHCl_3$ containing small amounts of MeOH (6 × 1.5 mL). After removal of solvents *in vacuo*, the residue was purified by flash chromatography on Bio-Sil A with chloroform/methanol/water (65:35:4) to give **21** (0.010 g, 15%) as a yellow oil: $[\alpha]^{25}_D$ +4.76 (c = 0.0065, CH_3-OH).

1-Tetradecanoic Acid 3-((((*N*,*N*,*N*-Trimethylammonio)-ethoxy)hydroxyphosphoryl)oxy)-2-[(12-((2-hydroxy-5-aminobenzyl)oxy)dodecanoyl)oxy]propyl Ester (22). A mixture of nitrophenol **21** (0.010 g, 0.0106 mmol), triethylamine (22 μL, 0.0159 mmol), and Pd/C (10%) (0.010 g) in dry MeOH (2.50 mL) was stirred under hydrogen atmosphere for 2 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated *in vacuo* to give **22** (0.0066 g, 80%) as a yellow solid: TLC ($CHCl_3$/MeOH/H_2O (65:35:4)) R_f 0.15; ^{1}H NMR (CD_3OH) δ 0.87 (t, 3H, J = 6.58 Hz), 1.20−1.38 (m, 34H), 1.49−1.65 (m, 6H), 2.30 (q, 4H, J = 7.16 Hz), 3.21 (s, 9H), 3.50 (t, 2H, J = 6.64 Hz), 3.60−3.68 (m, 2H), 3.97 (t, 2H, J = 6.42 Hz), 4.15 (dd, 1H, J = 6.84, 12.22 Hz), 4.21−4.32 (m, 2H), 4.38 (d, 1H, J = 3.20 Hz), 4.44 (s, 2H), 5.15−5.27 (m, 1H), 6.62 (s, 2H), 6.78 (s, 1H); ^{13}C NMR (CD_3OH) δ 14.42, 23.72, 26.00, 27.27, 30.18, 30.34, 30.44, 30.54, 30.64, 30.73, 33.05, 34.90, 35.08, 54.70, 60.47, 63.66, 64.91, 67.45, 71.45, 71.73, 72.92, 116.90, 117.79, 118.43, 126.27, 140.19, 149.49, 174.61, 174.95.

This product was stored under inert atmosphere because of its extreme sensitivity to oxygen.

1-Tetradecanoic Acid 3-((((*N*,*N*,*N*-Trimethylammonio)-ethoxy)hydroxyphosphoryl)oxy)-2-[(12-(2-(4-diazocyclohexa-2,5-dienonyl)methylenoxy)dodecanoyl)oxy]-propyl Ester (2). To a solution of aminophenol **22** (0.010 g, 0.0127 mmol) in glacial acetic acid (0.060 mL) was added isoamyl nitrite (2 μL, 0.0139 mmol) at 10 °C in the dark. The mixture was stirred for 1 h. (The evolution of the reaction was followed by UV spectroscopy at 357 nm, in MeOH.) After neutralization to pH = 7 using a 0.5 M phosphate buffer, the mixture was extracted with $CHCl_3$ (3 × 1 mL). The organic solution was reduced *in vacuo*, and the residue was purified by HPLC to give **2** (0.003 g, 30%) as a yellow solid: TLC ($CHCl_3$/MeOH/H_2O (65:35:4)) R_f 0.15; ^{1}H NMR (CD_3OH) δ 0.87 (t, 3H, J = 6.58 Hz), 1.20−1.45 (m, 34H), 1.49−1.65 (m, 6H), 2.30 (q, 4H, J = 7.16 Hz), 3.21 (s, 9H), 3.54 (t, 2H, J = 6.64 Hz), 3.59−3.66 (m, 2H), 3.97 (t, 2H, J = 6.42 Hz), 4.14 (dd, 1H, J = 6.84, 12.22 Hz), 4.21−4.32 (m, 2H), 4.33 (s, 2H), 4.38 (d, 1H, J = 3.18 Hz), 5.15−5.27 (m, 1H), 6.40 (d, 1H, J = 9.16 Hz), 7.81 (dd, 1H, J = 2.92, 9.16 Hz), 7.84 (d, 1H, J = 2.92 Hz); ^{13}C NMR (CD_3OH) δ 14.44, 23.71, 26.04, 27.26, 30.14, 30.18, 30.35, 30.45, 30.59, 30.63, 30.73, 33.06, 34.91, 35.06, 54.70, 60.49, 63.66, 64.89, 67.46, 71.45, 71.73, 72.26, 124.94, 129.96, 133.64, 140.01, 174.61, 174.95, 175.61, 182.07; UV (EtOH) λ_{max} = 357 nm, ϵ = 15 000; HPLC t_R = 41 min, (Zorbax 5μ Sil column, from 0 to 20 min, isocratic 60% (iPrOH/H_2O 7:3 = A), 40% hexane and from 20 to 50 min, 60% of A to 100%); FABMS$^+$ (*m*-nitrobenzyl alcohol) *m*/*z* 772 (M + H$^+$ − N_2, 50), 666 (14), 184 ($HOPO_3(CH_2)_2N(CH_3)_3$, 100).

12-((2-Hydroxy-5-aminobenzyl)oxy)dodecanoic Acid Ethyl Ester. To a solution of nitrophenol **14** (0.162 g, 0.409 mmol) in THF (2.30 mL) were added $NaBH_4$ (0.155 g, 4.09 mmol) and Pd/C (20%, 0.032 g). The reaction mixture was stirred for 2 h at rt, and a saturated NH_4Cl solution (5.10 mL) was added slowly until destruction of excess reagent. The catalyst was removed by filtration through Celite, and the filtrate was concentrated *in vacuo*. After extraction with EtOAc (120 mL), the combined organic layers were washed with water and saturated NaCl solution, dried over $MgSO_4$, and evaporated *in vacuo* to give the ester (0.142 g, 95%) as a yellow solid; the product was stored under nitrogen: TLC (hexane/EtOAc (5:5)) R_f 0.37; ^{1}H NMR ($CDCl_3$) δ 1.20−1.45 (m, 17H), 1.50−1.70 (m, 4H), 2.28 (t, 2H, J = 7.32 Hz), 3.50 (t, 2H, J = 6.52 Hz), 4.12 (q, 2H, J = 7.16 Hz), 4.59 (s, 2H), 6.40 (d, 1H, J = 2.66 Hz), 6.57 (dd, 1H, J = 2.66, 8.42 Hz), 6.71 (d, 1H, J = 8.42 Hz); ^{13}C NMR ($CDCl_3$) δ 14.08, 24.79, 25.87, 28.95, 29.06, 34.19, 59.99, 70.66, 71.85, 115.20, 116.25, 116.77, 123.02, 138.61, 148.75, 173.80; IR (film) 3363, 2926, 2854, 1733, 1627, 1502, 1458, 1372, 1208, 1090 cm^{-1}.

Alternative procedure: A mixture of nitrophenol **14** (0.030 g, 0.0575 mmol) and 20% palladium on carbon (6 mg) in MeOH (4.11 mL) was stirred under hydrogen atmosphere for 2 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated *in vacuo* to give the aminophenol derivative (0.019 g, 90%) as a yellow solid.

12-[2-(4-Diazo-1-oxocyclohexa-2,5-dienyl)methylenoxy]-dodecanoic Acid Ethyl Ester (23). To a solution of the previously synthesized aminophenol (0.140 g, 0.383 mmol) in glacial acetic acid (1.70 mL) was added isoamyl nitrite (57 μL, 0.421 mmol) at 10 °C in the dark. The mixture was stirred for 1 h. (The progress of the reaction was followed by UV spectroscopy at 358 nm, in MeOH.) Then a saturated $NaHCO_3$ solution was added dropwise to neutralize (pH = 7). The mixture was extracted with $CHCl_3$ (3 × 2 mL). The extract was reduced *in vacuo*, and the residue was purified by flash chromatography on silica gel with CH_2Cl_2/MeOH (9:1) to give **23** (0.094 g, 65%) as a red solid: TLC (CH_2Cl_2/MeOH (9:1)) R_f 0.50; ^{1}H NMR ($CDCl_3$) δ 1.20−1.45 (m, 17H), 1.55−1.75 (m, 4H), 2.28 (t, 2H, J = 7.38 Hz), 3.56 (t, 2H, J = 6.56 Hz), 4.12 (q, 2H, J = 7.18 Hz), 4.42 (s, 2H), 6.43 (d, 1H, J = 9.52 Hz), 7.42 (dd, 1H, J = 3.04, 9.52 Hz), 7.52 (d, 1H, J = 3.04 Hz); ^{13}C NMR ($CDCl_3$) δ 14.23, 26.16, 24.94, 28.33, 29.23, 29.50, 29.68, 34.36, 60.14, 67.60, 71.43, 124.64, 126.26, 129.46, 136.69, 173.92, 180.57; UV (EtOH) λ_{max} = 358 nm, ϵ = 15 145; FABMS$^+$ (*m*-nitrobenzyl alcohol) *m*/*z* 377 (M + H$^+$, 100), 349 (M + H$^+$ − N_2, 28), 258 (M$^+$ − diazocyclohexadienyl, 41).

General Photolysis Procedure. Solutions of **23** (0.02 g, 0.053 mmol) in benzene, cyclohexane, and ethanol, respectively (53 mL), were photolyzed under stirring using a 125 W Philips lamp for 40−45 min. The reaction was followed spectroscopically by the disappearance of the absorption band at 357 nm.

After concentration under reduced pressure, the residues were purified by flash chromatography on silica gel with hexane/EtOAc (8:2) to give **24a**, **24b**, and **24c**, respectively.

12-((2-Hydroxy-5-benzylbenzyl)oxy)dodecanoic Acid Ethyl Ester (24a). By following the general photolysis procedure, **24a** was obtained as a red solid (0.020 g, 88%): mp 43−45 °C; TLC (hexane/EtOAc (8:2)) R_f 0.40; ^{1}H NMR ($CDCl_3$) δ 1.20−1.45 (m, 17H), 1.50−1.75 (m, 4H), 2.29 (t, 2H, J = 7.30 Hz), 3.58 (t, 2H, J = 6.52 Hz), 4.13 (q, 2H, J = 7.14 Hz), 4.77 (s, 2H), 6.97 (d, 1H, J = 8.38 Hz), 7.21−7.56 (m, 7H), 7.83 (s, 1H, OH); ^{13}C NMR ($CDCl_3$) δ 14.21, 24.93, 26.00, 29.10, 29.19, 34.35, 60.10, 71.10, 72.64, 116.84, 122.54, 126.60, 127.95, 128.66, 132.96, 140.74, 155.92, 173.90; IR (film) 3355, 2926, 2853, 1735, 1613, 1513, 1486, 1455, 1372, 1263, 1176, 1093 cm^{-1}. Anal. Calcd for $C_{27}H_{38}O_4$: C, 76.02; H, 8.97. Found: C, 76.09; H, 9.21. CI-MS (NH_3) *m*/*z* 444 (M + NH_4, 32), 427 (M + H$^+$, 29).

12-((2-Hydroxy-5-cyclohexylbenzyl)oxy)dodecanoic Acid Ethyl Ester (24b). Following the general photolysis procedure, **24b** was obtained as a red oil (0.020 g, 88%): TLC (hexane/EtOAc (8:2)) R_f 0.61; ^{1}H NMR ($CDCl_3$) δ 1.20−1.45 (m, 23H), 1.55−1.75 (m, 4H), 1.75−1.90 (m, 4H), 2.29 (t, 2H, J = 7.36 Hz), 2.40−2.50 (m, 1H), 3.54 (t, 2H, J = 6.52 Hz), 4.12 (q, 2H, J = 7.10 Hz), 4.67 (s, 2H), 6.81 (d, 1H, J = 8.24 Hz), 6.82 (d, 1H, J = 2.34 Hz), 7.04 (dd, 1H, J = 2.34, 8.24 Hz), 7.53 (s, 1H, OH); ^{13}C NMR ($CDCl_3$) δ 14.24, 24.96, 26.04, 26.92, 29.22, 29.44, 34.37, 34.72, 43.65, 60.13, 71.00, 72.76, 116.15, 121.92, 126.13, 127.48, 139.48, 154.23, 173.95; IR (film) 3389, 2919, 2848, 2343, 1736, 1597, 1501, 1449, 1370, 1246, 1180, 1091 cm^{-1}. Anal. Calcd for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.79; H, 10.41.

12-((2-Hydroxy-5-ethoxybenzyl)oxy)dodecanoic Acid Ethyl Ester (24c). Following the general photolysis procedure, **24c** was obtained as a red oil (0.021 g, 96%): TLC (hexane/EtOAc (8:2)) R_f 0.50; ^{1}H NMR ($CDCl_3$) δ 1.20−1.50 (m, 20H), 1.55−1.75 (m, 4H), 2.28 (t, 2H, J = 7.34 Hz), 3.52 (t, 2H, J = 6.52 Hz), 3.95 (q, 2H, J = 6.98 Hz), 4.12 (q, 2H, J = 7.14 Hz), 4.64 (s, 2H), 6.58 (d, 1H, J = 2.50 Hz), 6.76 (d, 1H, J = 2.50 Hz), 6.78 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.22, 14.92, 24.96, 26.03, 29.23, 34.38, 60.14, 64.05, 70.96, 72.44, 114.38, 115.01, 116.93, 122.99, 150.06, 152.18, 173.92; IR (film) 3389, 2919, 2848, 2355, 1731, 1496, 1460, 1437, 1372, 1237, 1190, 1096, 1043 cm^{-1}. Anal. Calcd for $C_{23}H_{38}O_4$: C, 72.97; H, 10.11. Found: C, 72.71; H, 10.39.

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