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Synthesis of Isosteric Phosphono Analogs of Biologically Active Alkylphosphocholines

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Summary. We describe a high yield reaction sequence for the conversion of long chain alcohols into 3-(trimethylammonio)propylphosphonates. The products are phospholipase D stable isosteric phosphono analogs of the respective alkylphosphocholines. The key step is the esterification of 3-chloropropylphosphonic acid with the respective long chain alcohols using *DCC/DMAP*. Deprotection and various acylations of 2-hydroxy and 2-amino groups following the introduction of the headgroup are described.

Keywords. Phosphonolipids, synthesis of; Alkylphosphocholines; Phospholipase D; *Michaelis-Becker* reaction.

Synthese isosterer Phosphonoanaloga biologisch aktiver Alkylphosphocholine

Zusammenfassung. Wir beschreiben eine in sehr guten Ausbeuten verlaufende Reaktionsfolge zur Überführung langkettiger Alkohole in 3-(Trimethylammonium)propanphosphonate. Die Produkte sind Phospholipase D-stabile isostere Phosphonoanaloga der entsprechenden Alkylphosphocholine. Schlüsselschritt ist die Veresterung von 3-Chlorpropanphosphonsäure mit den entsprechenden langkettigen Alkoholen unter Verwendung von *DCC/DMAP*. Entschützung und verschiedene Acylierungen von 2-Hydroxy- und 2-Amino-Gruppen nach Einführung der Kopfgruppe werden beschrieben.

Introduction

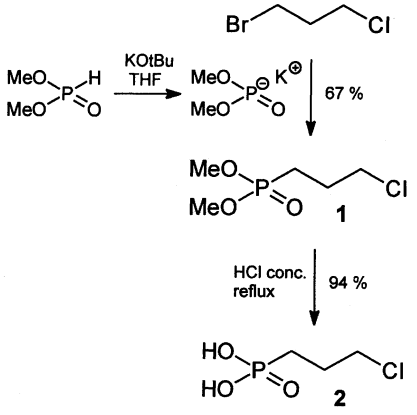
In most mammalian tissues, Phospholipase D (*PLD*) provides a major route for the hydrolysis of phosphatidylcholine [1]. Furthermore, *Ries* has demonstrated that the antineoplastic phospholipid hexadecylphosphocholine and the naturally occuring dipalmitoyl phosphatidylcholine are hydrolyzed by *PLD* at a similar rate [2]. Thus, it must be assumed that synthetic, pharmacologically interesting phospholipids bearing a phosphocholine headgroup will be biodegraded by *PLD* and therefore have a limited half-life, and toxic cleavage products may be formed. To avoid this biodegradation by *PLD* and hence increase the half-life of the pharmacologically interesting phospholipids in mammalian tissues, we prepared phosphono analogs of antineoplastic *n*-alkylphosphocholines, 1-O-phosphocholine-2-N-acyl-octadecanes (which are strong competitive inhibitors of 14 kDa phospholipases A_2), and 1-O-

phosphocholine-2-O-acyl-octadecanes bearing the isosteric 3-(trimethylammonio)-propylphosphonoyl headgroup without a cleavage point for *PLD*.

Results and Discussion

Synthesis of 3-chloropropylphosphonic acid (**2**)

The phosphonic acid **2** is usually prepared by acidic hydrolysis of diethyl-3-chloropropylphosphonate which can be obtained by *Michaelis-Arbuzov* reaction of 1-bromo-3-chloropropane with triethylphosphite [3]. We investigated an alternative route *via* dimethyl-3-chloropropylphosphonate (**1**) which was prepared by *Michaelis-Becker* reaction[1] from potassium dimethylphosphite and 1-bromo-3-chloropropane in 67% yield (Scheme 1). Applying the *Michaelis-Becker* reaction, neither high reaction temperatures nor distillation of the product were necessary. We found that potassium dimethylphosphite can be conveniently generated *in situ* by deprotonation of dimethylphosphite with the sterically hindered base potassium-*tert*-butylate in *THF*. To our knowledge, the use of potassium-*tert*-butylate in the *Michaelis-Becker* reaction has not yet been described previously. In the *Michaelis-Becker* reaction, dialkylphosphites usually are deprotonated with metallic sodium, sodium hydride, or sodium methoxide (or ethoxide), but it has been supposed that primary alcoholates compete in the nucleophilic displacement of the halogen, thus diminishing the yield [6]. 3-Chloropropylphosphonic acid (**2**) was obtained from **1** by acidic hydrolysis in fuming hydrochloric acid and subsequent crystallization from chloroform in 94% yield.



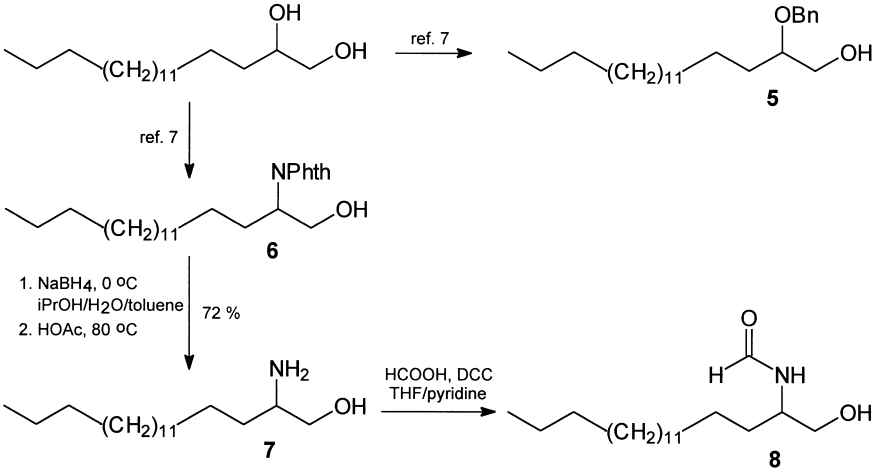
Scheme 1. Preparation of 3-chloropropylphosphonic acid (**2**) *via Michaelis-Becker*-reaction

[1] The *Michaelis-Becker* reaction, involving alkylation of (usually deprotonated) dialkyl phosphites, generally has been less used than the *Michaelis-Arbuzov* reaction, although it is convenient and dialkyl phosphites are commercially available [4, 5]. To our knowledge this is its first application to phosphonolipid synthesis.

Synthesis of 1-hydroxy-2-O-benzyl-octadecane (**5**), 1-*hydroxy-2-N-phthalimido-octadecane* (**6**) *and 1-hydroxy-2-N-formyl-octadecane* (**8**)

The alcohols **5**, **6**, and **8** needed for the esterification with 3-chloropropylphosphonic acid (**2**) to obtain the respective phosphonates were synthesized from racemic 1,2-octadecanediol (see Scheme 2). **5** and **6** were prepared as described elsewhere [7]. Briefly, the primary hydroxy group of 1,2-octadecanediol was selectively protected as a trityl ether. The secondary hydroxy group of the trityl ether was then either protected as benzyl ether or substituted with phthalimide under complete inversion of configuration by the *Mitsunobu* reaction. Acidic cleavage of the trityl ethers lead to **5** and **6**. Starting from enantiomerically pure 1,2-octadecanediol, this reaction sequence also allows the preparation of pure enantiomers of **5** and **6** [7].

Compound **8** was prepared from **6** *via* 1-hydroxy-2-amino-octadecene (**7**). Dephthalimidation of **6** was achieved in 72% yield according to *Osby*'s method by reduction with sodium borohydride and subsequent acidic cleavage of the resulting hydroxymethylbenzoylamide [8]. **7** was selectively N-formylated using formic acid and *DCC* as condensing agent [9]. Complete removal of the dicyclohexyl urea formed was not necessary for the following phosphonylation step.



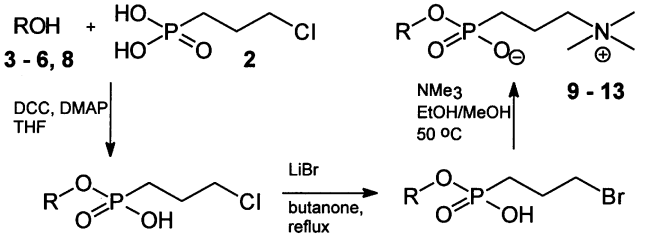
Scheme 2. Preparation of the alcohols **5**, **6**, and **8** from 1,2-octadecanediol

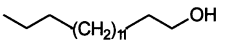
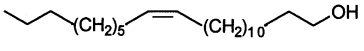
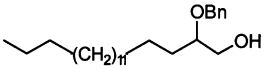
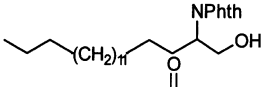
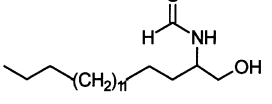
Formation of the 3-chloropropylphosphonoyl headgroup. Synthesis of the phosphonates **9**, **10**, **11**, **12**, *and* **13**

Phosphonolipids bearing the 2-(trimethylammonio)ethylphosphonoyl headgroup have been prepared since the sixties, whereas the synthesis of phosphonolipids with a 3-(trimethylammonio)propylphosphonoyl headgroup has not been described before 1991 (*Turcotte et al.* and *Ries et al.* [3, 10, 11]). The methods described by these authors include the esterification of the corresponding long chain alcohols with 3-chloro(bromo)-propylphosphonic acid monochloride which first has to be generated from the corresponding phosphonic acid. None of the methods described was satisfactory

for the phosphonylation of our alcohols, because the overall yield of these strategies was described to be lower than (partly far below) 50%.

During our search for a simple and generally applicable method to introduce the 3-(trimethylammonio)propylphosphonoyl headgroup in higher yields, we tested the widely used condensing agent *DCC* to carry out the esterification in one step from the alcohol (**5**, **6**, **8** or the commercially available hexadecanol or erucanol) and 3-chloro-propylphosphonic acid (**2**) (Scheme 3). Using *DCC* as condensing agent and *THF* as solvent, we observed quantitative turnover of the alcohols. Interestingly, in the case of the simple alcohols hexadecanol and erucanol (without side chain in the 2-position) the overall yields are the lowest (57% or 61%, respectively). Higher yields were achieved with alcohols **5**, **6**, or **8** bearing a formamido, phthalimido, or benzyloxy group in the 2-position. The crude phosphonic acid esters were treated with lithium bromide in butanone (*Finkelstein* reaction) to exchange the terminal chloride against bromide and then reacted with trimethylamine (without previous isolation of the intermediates) affording the respective 3-(trimethylammonio)-propylphosphonates. The complete reaction sequence (outlined in Scheme 3) gave overall yields ranging from 57% to 90%.



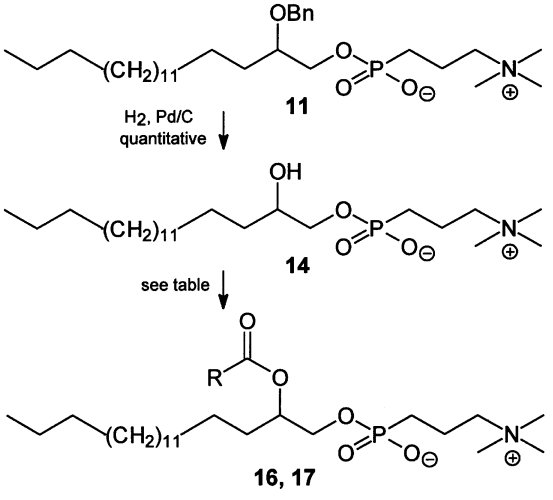
Alcohol ROH		respective phosphonate	overall yield
	**3**	**9**	57 %
	**4**	**10**	61 %
	**5**	**11**	90 % a)
	**6**	**12**	71 % a)
	**8**	**13**	60 % b)

a) yield includes the following deprotection step
b) yield includes the previous formylation step

Scheme 3. Preparation of the 3-(trimethylammonio)propylphosphonates **9–13**

1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-hydroxy-octadecane (**11**) *and 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-amino-octadecane* (**12**)

The O-benzyl-protected hydroxyl group in **11** was deprotected by hydrogenolysis in *THF*/1*N* HCl with palladium on activated charcoal as catalyst in quantitative yield. Deprotection of the N-phthalimide-protected amino group in **12** was achieved in high yields according to the method of *Osby* by reduction of the phthalimide with sodium borohydride in 2-propanol/water with the addition of toluene followed by acidic cleavage of the arising 2-hydroxymethylbenzoylamide which in this case was achieved by simply adding 6 *N* HCl to the solution and heating to 60°C [7, 8].

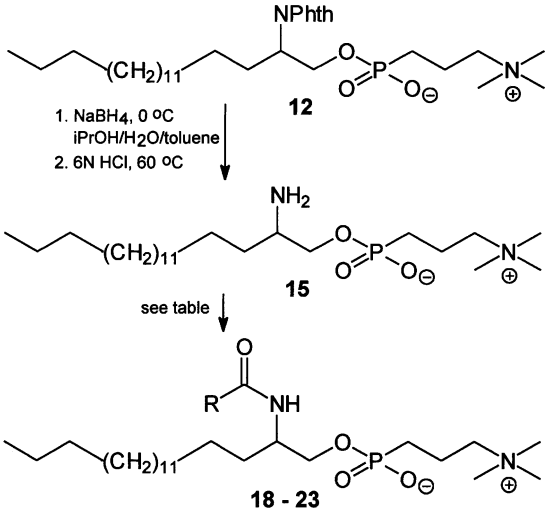


acylating agent	product	R	yield
acetyl chloride	**16**	H_3C-	48 %
palmitoyl chloride	**17**	H_3C-$(CH_2)_{14}$-	44 %

Scheme 4. Formation and acylation of 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-hydroxy-octadecane (**14**)

Acylation of 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-hydroxy-octadecane (**11**) *and 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-amino-octadecane* (**12**) *with various acid chlorides, imidazolides, and methyl chloroformate*

To minimize the total number of reaction steps, acylation of the 2-hydroxy or 2-amino group in **11** and **12** with different acyl groups was performed as the last reaction step (Schemes 4 and 5). Only in the case of formamide **13** the acylation was carried out before the headgroup was introduced. This procedure was necessary because the separation of the acylation product 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-N-formyl-octadecane (**13**) from the respective



acylating agent	product	R	yield
N-acetylimidazole	**18**	CH_3-	66 %
N-propionylimidazole	**19**	CH_3-CH_2-	74 %
N-palmitoylimidazole	**20**	CH_3-$(CH_2)_{14}$-	52 %
chloroacetyl chloride / DMAP	**21**	Cl-CH_2-	29 %
sodium fluoroacetate / perchloric acid / N,N'-carbonyldiimidazole	**22**	F-CH_2-	72 %
methyl chloroformate / DMAP / triethylamine	**23**	CH_3-O-	68 %

Scheme 5. Formation and acylation of 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-amino-octadecane (**15**)

hydroxy compound **12** is difficult due to very similar R_f values. All acylations were carried out in a 0.5 mmol scale by stirring the educts in chloroform at room temperature. The reaction times ranged from 1 h to 3 days depending on the type of acylation. According to earlier results, purification of phospholipids on silica gel causes a loss of substance which is not neglectible when small quantities are to be purified. In our case (0.5 mmol = about 300 mg phospholipid, 10 g silica gel) the recovery is about 75–80%.

Acylation of **11** and **12** with acid chlorides in the presence of triethylamine or *DMAP* gave good yields only in the case of chloroacetamide **21**[2] and carbamate **23**. In the case of the esters **16** and **17**, best yields were obtained with acid chlorides and without addition of any base. For the amides **18**, **19**, **20**, and **22**, the corresponding acylimidazoles proved to be the most suitable acylating agents.

[2] Although **21** was formed quantitatively (TLC control), it could be isolated in only 29% yield after purification on silica due to rapid decomposition, probably nucleophilic displacement of the chloride. The isolated product is not stable at room temperature over a longer period of time and soon becomes reddish.

To prepare the fluoroacetamide **22** we developed a new method that allows the use of the non-volatile sodium fluoroacetate as acylating agent. N-Fluoroacetylimidazole was formed *in situ* from N,N′-carbonyldiimidazole and fluoroacetic acid which was liberated from the sodium salt by perchloric acid (70%). Quantitative acylation was achieved when the surplus of carbonyldiimidazole added was at least equimolar to the amount of water contained in the perchloric acid.

Experimental

All chemicals were of reagent grade and used without further purification. THF and chloroform (ethanol free, stabilized with 60 ppm 2-methyl-2-butene) used for the acylations were dried over molecular sieve (0.4 nm). Removal of solvents was carried out in a rotatory evaporator under reduced pressure. Residual water, if not otherwise specified, was removed by azeotropic distillation with ethyl acetate. Solvent mixtures for extractions and chromatography are given in volume ratios, 'solvent *X*' thereby stands for chloroform/methanol/ammonia (25% aqueous solution) 60:40:*X*. All chromatographic separations were performed in glass columns (diameter 2 cm, fraction size 10–20 ml). Silica gel (Kieselgel 60, 0.063–0.100 mm) and TLC plates were obtained from Merck (Darmstadt, FRG). ^{1}H NMR spectra (δ in ppm) were recorded with a Bruker AC 250 spectrometer (250 MHz, *TMS* = 0 ppm) or a Bruker AM 400 spectrometer (400 MHz, *TMS* = 0 ppm). Infrared spectra were measured using a Perkin Elmer 16 PC FT-IR spectrometer. Peaks are given in cm^{-1} and labelled 'ss' (very strong) 's' (strong), and 'm' (medium). N-Acetylimidazole, N-propionylimidazole, and N-palmitoylimidazole were prepared according to the method described by *Staab* from imidazole and the acid chlorides and used without purification [12]. 1-Hydroxy-2-O-benzyl-octadecane (**5**) and 1-hydroxy-2-N-phthalimido-octadecane (**6**) were prepared from racemic 1,2-octadecanediol as described elsewhere [7]. Erucanol (**4**) was a gift from *H. Eibl*, Göttingen, FRG.

Dimethyl-3-chloropropylphosphonate (**1**)

Dimethylphosphite (50.5 ml, 60.6 g, 550 mmol) was added dropwise to a stirred solution of potassium-*tert*-butylate (56.1 g, 500 mmol) in 200 ml *THF* within 15 minutes. After cooling to room temperature, the resulting suspension was added dropwise to a stirred solution of 1-bromo-3-chloropropane (64.0 ml, 102 g, 650 mmol) in 100 ml *THF* within 40 minutes. The mixture was heated under reflux for 15 minutes. After cooling to room temperature, the precipitate (potassium bromide) was filtered off with suction and washed twice with 200 ml diisopropyl ether. Solvents, educts, and *tert*-butanol were evaporated (20 mbar, up to 170°C) to yield 62.4 g (334 mmol, 67%) pure **1**.

$R_f = 0.60$ (acetone); IR (Film): 2955 (s), 2850 (m), 1240 (s) P=O, 1055 (ss) P-O-C, 1030 (ss) P-O-C, 825 (s) C-Cl; ^{1}H NMR (400 MHz, $CDCl_3$): 1.86–1.98 (m, 2H, P-CH_2 CH_2), 2.01–2.13 (m, P-CH_2-), 3.61 (dt, $J_{P,H} = 1.0$ Hz, $^3J = 6.2$ Hz, 2H, -CH_2Cl), 3.76 (d, $J_{P,H} = 10.7$ Hz, 6H, O-CH_3).

3-Chloropropylphosphonic acid (**2**)

Compound **1** (15.1 g, 80.9 mmol) was dissolved in 150 ml (181 mmol) hydrochloric acid (37%) and refluxed for 9 h. The solvent was removed and the residue dried by azeotropic distillation with toluene. The jelly residue was crystallized from 200 ml chloroform to yield 12.1 g (76.3 mmol, 94%) **2** as colorless crystals.

$R_f = 0.63$ (methanol); IR (KBr): 3300–2400 (sb) OH, 2965 (m), 2310 (s), 1285 (m), 1235 (m) P=O, 1020 (s), 1000 (ss), 955 (s); ^{1}H NMR (250 MHz, D_2O): 1.41–1.52 (m, 2H, P-CH_2-), 1.52–1.70

(m, 2H, -CH_2CH$_2$-P), 3.26 (dt, $^3J = 6.0$ Hz, $J_{P,H} = 1.0$ Hz, 2H, -CH$_2$Cl), 4.40 (s, OH); ^{1}H NMR (250 MHz, CD$_3$CN): 1.81–2.15 (m, 4H, P-CH$_2$CH_2-), 3.68 (dt, $^3J = 6.3$ Hz, $J_{P,H} = 0.7$ Hz, 2H, -CH$_2$Cl), 9.46 (s, 2H, P-OH).

1-Hydroxy-2-amino-octadecane (**7**)

1-Hydroxy-2-N-phthalimido-octadecane (**6**, 4.16 g, 10.0 mmol) was dissolved in 90 ml 2-propanol and 15 ml water. Sodium borohydride (1.9 g, 50 mmol) was added stepwise within 2 h. The reaction mixture was stirred for further 12 h. Then, 30 ml glacial acetic acid were added, and the mixture was heated to 80°C for 4 h. After removal of the solvent the residue was dissolved in 90 ml dichloromethane/methanol 2:1 and extracted with 60 ml ammonia (10% acqueous solution). The aqueous phase was reextracted with 60 ml dichloromethane/methanol 5:1, and the solvents of the combined organic layers were removed. The residue was purified by recrystallization from 40 ml dichloromethan to yield 2.05 g (7.18 mmol, 72%) pure **7** as shimmering crystals.

R_f = 0.05 (ethyl acetate/methanol 2:1); IR (KBr): 3345 (s) NH$_2$, 3285 (s) NH$_2$, 2925 (ss) -CH$_2$-, 2850 (ss) -CH$_2$-, 1605 (s), NH, 1470 (s), 1070 (s); ^{1}H NMR (250 MHz, CDCl$_3$): 0.88 (t, $^3J = 6.6$ Hz, 3H, -CH$_3$), 1.62 (s, 30H, (3–17)-CH$_2$-), 2.01 (sb, 3H, OH and NH$_2$, H/D-exchangeable), 2.76–2.87 (m, 1H, 2-CH), 3.26 (dd, $^3J = 7.9$ Hz, $^2J = 10.6$ Hz, 1H, 1-CH), 3.58 (dd, $^3J = 3.8$ Hz, $^2J = 10.6$ Hz, 1H, 1-CH′).

1-Hydroxy-2-N-formyl-octadecane (**8**)

A 2 *M* solution of formic acid in *THF* (12.6 ml, 25.2 mmol) was added slowly to an ice-cold solution of *DCC* (2.60 g, 12.6 mmol) in 20 ml *THF*. After stirring the mixture for 20 min, a solution of 1-hydroxy-2-amino-octadecane (**7**, 1.80 g, 6.3 mmol) in 70 ml *THF*/pyridine 3:1 was added. After 1 day, 10 ml of water were added. The precipitate was filtered off with suction after another day, and the solvents were removed. Recrystallization from 35 ml cyclohexane afforded 2.25 g crude **8** which was phosphonylated according to the general procedure described below without further purification. For analytical purposes, a small amount of **8** was purified on silica gel (eluent: chloroform/methanol 95:5).

R_f = 0.63 (ethyl acetate/methanol 2:1); IR (KBr): 2915 (ss) -CH$_2$-, 2850 (s) -CH$_2$-, 1650 (ss) C=O, 1560 (m), 1465 (m), 1385 (m), 1070 (m), 725 (s); ^{1}H NMR (250 MHz, CDCl$_3$): 0.88 (t, $^3J = 6.4$ Hz, 3H, -CH$_3$), 1.25 (s, 28H, (4–17)-CH$_2$-), 1.41–1.64 (m, 2H, 3-CH$_2$-), 2.58 (t, $^3J = 4.9$ Hz, 1H, -OH, H/D-exchangeable), 3.60 (ddd, after H/D-exchange dd, $^2J = 11.1$ Hz, $^3J = 5.5$ Hz, $J_{H,OH} = 4.9$ Hz, 1H, 1-CH), 3.71 (ddd, after H/D-exchange dd, $^2J = 11.1$ Hz, $^3J = 3.5$ Hz, $J_{H,OH} = 4.9$ Hz, 1H, 1-CH′), 3.94–4.09 (m, 1H, 2-CH), 5.75 (d, $J = 7.0$ Hz, 1H, NH, H/D-exchangeable), 8.21 (s, 1H, formyl-H).

General procedure for the formation of the 3-(trimethylammonio)propylphosphonoyl headgroup

The alcohols **3**, **4**, **5**, **6**, or **8** (6.30 mmol), 3-chloropropylphosphonic acid (**2**) (1.10 g, 6.94 mmol), *DCC* (2.9 g, 14 mmol), and *DMAP* (70 mg, 0.6 mmol) were stirred in 60 ml *THF* for 24 h. To hydrolyze the surplus of *DCC*, 10 ml of water were added. After stirring the mixture for further 24 h, the precipitate was filtered off with suction, the solvent was removed, and lithium bromide (5.47 g, 63 mmol) was added. Butanone (50 ml) was added, and the mixture was refluxed for 4 h. After removal of the solvent, 30 ml methanol and 15 ml trimethylamine in ethanol (4.2 mol/l, 63 mmol) were added, and the sealed flask was kept at 50°C for 72 h with stirring. The volatile components were evaporated, and the residue was dissolved in 180 ml chloroform/methanol 1:1.2 and extracted with 80 ml water. The aqueous phase was extracted twice with 50 ml chloroform/methanol 4:1. The solvents of the combined organic layers were removed, and the crude phosphonates (**9**, **10**, **11**, **12**, or **13**) were purified or further reacted as described.

Hexadecyl-3-(trimethylammonio)propylphosphonate (**9**)

Hexadecanol (**3**, 1.53 g, 6.30 mmol) was converted into the corresponding phosphonate **9** according to the general procedure. Workup as described there and purification on silica gel (20 g) using first solvent 2 and then solvent 7 as eluents afforded 1.46 g (3.60 mmol, 57%) **9** as colorless solid.

$R_f = 0.21$ (solvent 7); IR (KBr): 2920 (ss) -CH_2-, 2850 (s) -CH_2, 1495 (m), 1470 (m), 1200 (s) P=O, 1075 (ss) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (2:1)): 0.89 (t, $^3J = 6.7$ Hz, 3H, -CH_3), 1.27 (s, 26H, (3–15)-CH_2-), 1.56 (dt, $J_{PH} = 17.0$ Hz, $^3J = 7.1$ Hz, 2H, P-CH_2), 1.62 (quint, $^3J = 6.7$ Hz, 2H, 2-CH_2-), 1.92–2.05 (m, 2H, P-CH_2-CH_2-), 3.12 (s, 9H, $N(CH_3)_3$), 3.41–3.46 (m, 2H, -CH_2-N), 3.84 (quart, $^3J = 6.7$ Hz, $J_{PH} = 6.7$ Hz, 2H, P-O-CH_2-).

Erucyl-3-(trimethylammonio)propylphosphonate (**10**)

13-*cis*-Docosenol (erucanol, **4**; 2.04 g, 6.30 mmol) was reacted according to the general procedure. Workup and purification as described for compound **9** afforded 1.87 g (3.83 mmol, 61%) **10** as colorless solid.

$R_f = 0.25$ (solvent 7); IR (KBr): 2925 (ss) -CH_2-, 2855 (s) -CH_2-, 1465 (m), 1200 (s) P=O, 1075 (ss) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$(2:1)): 0.89 (t, $^3J = 6.7$ Hz, 3H, -CH_3), 1.27 (s, 30H, (3–11, 16–21)-CH_2-), 1.58 (dt, $J_{PH} = 17.1$ Hz, $^3J = 7.2$ Hz, 2H, P-CH_2), 1.62 (quint, $^3J = 6.7$ Hz, 2H, 2-CH_2-), 1.93–2.05 (m, 6H, P-CH_2-CH_2- and allylic -CH_2-), 3.13 (s, 9H, $N(CH_3)_3$), 3.41–3.47 (m, 2H, -CH_2-N), 3.84 (quart, $^3J = 6.7$ Hz, $J_{PH} = 6.7$ Hz, 2H, P-O-CH_2-), 5.33–5.37 (m, 2H, vinylic CH).

1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-hydroxy-octadecane (**14**)

1-Hydroxy-2-O-benzyl-octadecane (**5**, 2.37 g, 6.30 mmol) was reacted according to the general procedure. The crude phosphonate **11** was dissolved in 15 ml *THF* and stirred with activated charcoal (100 mg) for 2 h. Palladium on activated charcoal (10% Pd, 75 mg) in 6 ml hydrochloric acid (1 mol/l) was added. Hydrogenolysis was peformed under intensive stirring at room temperature and atmospheric pressure until hydrogen uptake was completed (24 h). Charcoal and the catalyst were filtered off, and the solvents were removed. The crude product was purified on silica gel (30 g) using first solvent 2 and then solvent 7 as eluents to yield 2.55 g (5.67 mmol, 90%) **14** as colorless solid.

R_f(**11**) = 0.39, R_f(**14**) = 0.14 (solvent 7); IR (KBr): 3420 (s) OH, 2915 (ss) -CH_2-,2850 (s) -CH_2-, 1470 (s), 1200 (s) P=O, 1055 (s) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (2:1)): 0.88 (t, $^3J = 6.8$ Hz, 3H, -CH_3), 1.27 (s, 28H, (4–17)-CH_2-), 1.45 (s, 2H, 3-CH_2-), 1.66 (dt, $J_{P,H} = 17.2$ Hz, $^3J = 7.2$ Hz, 2H, P-CH_2-) 1.96–2.08 (m, 2H, P-CH_2CH$_2$-), 3.15 (s, 9H, $N(CH_3)_3$), 3.43–3.50 (m, 2H, -CH_2-N), 3.68–3.76 (m, 2H, 1-CH_2), 3.85–3.93 (m, 1H, 2-CH).

1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-amino-ctadecane (**15**)

1-Hydroxy-2-N-phthalimido-octadecane (**6**, 2.62 g, 6.30 mmol) was reacted according to the general procedure. The crude phosphonate **12** was dissolved in 30 ml 2-propanol/water/toluene (6:1:1.5). The solution was cooled to 0°C and sodium borohydride (1.13 g, 30 mmol) was added stepwise within 30 minutes. The mixture was stirred for 12 h; then, hydrochloric acid (10 ml, 6 mol/l) was added dropwise. The solution was stirred for 3.5 h at 60°C and cooled to room temperature. Then, 50 ml 2 *N* sodium hydroxide, 60 ml methanol, and 50 ml chloroform were added. The aqueous phase was reextracted with 50 ml chloroform/methanol 4:1, and the solvents of the combined organic layers were removed. Purification on silica gel (30 g) using first solvent 5 and then solvent 10 as eluents afforded 2.02 g (4.50 mmol, 71%) **15** as colorless solid.

R_f (**12**) = 0.37, R_f (**15**) = 0.11 (solvent 7); IR (KBr): 2915 (ss) -CH_2-, 2850 (s) -CH_2-, 1465 (s), 1200 (s) P=O, 1075 (s) P-O-C, 1050 (s) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD (2:1)): 0.88 (t, ^3J = 6.8 Hz, 3H, -CH_3), 1.27 (s, 28H, (4–17)-CH_2-), 1.45–1.56 (m, 2H, 3-CH_2-), 1.61 (dt, $J_{P,H}$ = 17.2 Hz, ^3J = 7.2 Hz, 2H, P-CH_2-), 1.93–2.07 (m, 2H, P-$CH_2C\mathit{H}_2$-), 3.05–3.13 (m, 1H, 2-CH), 3.14 (s, 9H, N(CH_3)$_3$), 3.41–3.47 (m, 2H, -CH_2-N), 3.67–3.75 (m, 1H, 1-CH), 3.93–4.00 (m, 1H, 1-CH′).

1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-N-formyl-octadecane **(13)**

Crude 1-hydroxy-2-N-formyl-octadecane (**8**, 2.25 g, prepared from 6.3 mmol **7** as described above) was reacted according to the general procedure (3.5). Workup as described there and purification on silica gel (30 g) using solvent 7 as eluent afforded 1.79 g (3.76 mmol, 60% with respect to **7**) **13** as slightly yellowish powder.

R_f = 0.14 (solvent 7); IR (KBr): 2920 (ss) -CH_2-, 2850 (s) -CH_2-, 1670 (ss) C=O, 1465 (m), 1195 (ss) P=O, 1065 (ss) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD (2:1)): 0.89 (t, ^3J = 6.7 Hz, 3H, -CH_3), 1.27 (s, 28H, alkyl-(4–17)-CH_2-), 1.43–1.62 (m, 4H, 3-CH_2- and P-CH_2-), 1.90–2.05 (m, 2H, P-CH_2-$C\mathit{H}_2$-), 3.12 (s, 9H, N(CH_3)$_3$), 3.38–3.44 (m, 2H, -CH_2N), 3.81 (ddd, ^2J = 10.5 Hz, ^3J = 6.4 Hz, J_{PH} = 6.4 Hz, 1H, 1-CH), 3.89 (ddd, ^2J = 10.5 Hz, ^3J = 3.8 Hz, J_{PH} = 6.1 Hz, 1H, 1-CH′), 4.02–4.10 (m, 1H, 2-CH), 8.11 (s, 1H, formyl-H).

1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-O-acetyl-octadecane (**16**)
and 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-O-palmitoyl-octadecane (**17**)

A solution of 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-hydroxy-octadecan (**14**, 225 mg, 0.500 mmol) and 5.00 mmol of the corresponding acid chloride (acetyl chloride: 0.355 ml, 393 mg; palmitoyl chloride: 3.00 ml, 2.73 g) in 10 ml chloroform was stirred in a stoppered flask at room temperature for 24 h. 12 ml methanol were added, and after 2 h of stirring the solution was extracted with 10 ml ammonia (10% aqueous solution). The aqueous phase was reextracted twice with 5 ml chloroform/methanol 4:1; then the solvents of the combined organic layers were removed. The crude products were purified on silica gel (10 g) using first solvent 2 and then solvent 7 as eluents to yield the respective esters as colorless solids.

a) 1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-O-acetyl-octadecane (**16**)

Yield: 117 mg (0.238 mmol, 48%); R_f = 0.26 (solvent 7); IR (KBr): 2920 (ss) -CH_2-, 2850 (s) -CH_2-, 1735 (s) C=O, 1245 (s), 1075 (s) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD (2:1)): 0.88 (t, ^3J = 6.8 Hz, 3H, alkyl-CH_3), 1.27 (s, 28H, alkyl-(4–17)-CH_2-), 1.52–1.63 (m, 4H, P-CH_2 and 3-CH_2-), 1.92–2.05 (m, 2H, P-$CH_2C\mathit{H}_2$-), 2.07 (s, 3H, acetyl-CH_3), 3.13 (s, 9H, N(CH_3)$_3$), 3.39–3.46 (m, 2H, -CH_2-N) 3.82–3.89 (m, 1H, 1-CH), 3.91–3.97 (m, 1H, 1-CH′), 3.97–4.05 (m, 1H, 2-CH).

b) 1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-O-palmitoyl-octadecane (**17**)

Yield: 152 mg (0.221 mmol, 44%); R_f = 0.53 (solvent 7); IR (KBr): 2915 (ss) -CH_2-, 2850 (s) -CH_2-, 1725 (s) C=O, 1465 (m), 1200 (s) P=O, 1075 (s) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD (2:1)): 0.89 (t, ^3J = 6.8 Hz, 6H, -CH_3), 1.27 (s, 52H, alkyl-(4–17)-CH_2- and acyl-(4–15)-CH_2-, 1.52–1.68 (m, 6H, P-CH_2-, alkyl-3-CH_2- and acyl-3-CH_2-), 1.92–2.04 (m, 2H, P-$CH_2C\mathit{H}_2$-), 2.25–2.38 (m, 2H, acyl-2-CH_2-), 3.12 (s, 9H, N(CH_3)$_3$), 3.40–3.46 (m, 2H, -CH_2-N), 3.82–3.96 (m, 2H, 1-CH_2), 3.99–4.06 (m, 1H, 2-CH).

1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-N-acetyl-octadecane (**18**),
1-O-(3-(trimethylammonio)propylphosphonoyl)-2-N-propionyl-octadecane (**19**),
and 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-N-palmitoyl-octadecane (**20**)

A solution of 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-amino-octadecane (**15**, 225 mg, 0.500 mmol) and 5.00 mmol of the corresponding N-acylimidazole (N-acetylimidazole: 551 mg; N-propionylimidazole: 621 mg; N-palmitoylimidazole: 1.53 g) in 15 ml chloroform was stirred in a stoppered flask at room temperature for 3 days. Methanol (18 ml) was added, and the solution was extracted with 15 ml water. The aqueous phase was extracted twice with 10 ml chloroform/methanol 4:1, and the solvents of the combined organic layers were removed. The crude amides were purified on silica gel (10 g) using first solvent 2 and then solvent 7 as eluents to yield the respective amides as colorless solids.

a) 1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-N-acetyl-octadecane (**18**)

Yield: 162 mg (0.330 mmol, 66%); $R_f = 0.17$ (solvent 7); IR (KBr): 2915 (ss) -CH_2-, 2850 (s) -CH_2-, 1650 (s) C=O, 1200 (s) P=O, 1065 (s) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD (2:1)): 0.89 (t, $^3J = 6.8$ Hz, 3H, alkyl-CH_3), 1.27 (s, 28H, alkyl-(4–17)-CH_2-), 1.42–1.49 (m, 2H, 3-CH_2-), 1.51–1.63 (m, 2H, P-CH_2-), 1.92–2.06 (m, 2H, P-$CH_2C\mathit{H}_2$-), 1.98 (s, 3H, acetyl-CH_3), 3.12 (s, 9H, $N(CH_3)_3$), 3.39–3.45 (m, 2H, -CH_2-N), 3.76–3.83 (m, 1H, 1-CH), 3.83–3.89 (m, 1H, 1-CH′), 3.92–3.99 (m, 1H, 2-CH).

b) 1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-N-propionyl-octadecane (**19**)

Yield: 187 mg (0.370 mmol, 74%); $R_f = 0.22$ (solvent 7); IR (KBr): 2915 (ss) -CH_2-, 2850 (s) -CH_2-, 1635 (ss) C=O, 1540 (s), 1465 (m), 1195 (ss) P=O, 1070 (ss) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD (2:1)): 0.89 (t, $^3J = 6.8$ Hz, 3H, Alkyl-CH_3), 1.15 (t, $^3J = 7.6$ Hz, 3H, propionyl-CH_3), 1.27 (s, 28H, alkyl-(4–17)-CH_2-), 1.41–1.61 (m, 4H, 3-CH_2- and P-CH_2-), 1.91–2.05 (m, 2H, P-$CH_2C\mathit{H}_2$-), 2.21 (dquart, $^3J = 7.6$ Hz, $^2J_{AB} = 14.5$ Hz, 1H, C(O)CH_AH$_B$CH$_3$), 2.24 (dquart, $^3J = 7.6$ Hz, $^2J_{AB} = 14.5$ Hz, 1H, C(O)CH$_A\mathit{H}_B$CH$_3$), 3.12 (s, 9H, $N(CH_3)_3$), 3.39–3.45 (m, 2H, -CH_2-N), 3.79 (ddd, $^2J = 10.5$ Hz, $^3J = 7.0$ Hz, $J_{PH} = 5.8$ Hz, 1H, 1-CH), 3.86 (ddd, $^2J = 10.5$ Hz, $^3J = 4.0$ Hz, $J_{PH} = 6.2$ Hz, 1H, 1-CH′), 3.93–4.00 (m, 1H, 2-CH).

c) 1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-N-palmitoyl-octadecane (**20**)

Yield: 178 mg (0.259 mmol, 52%); $R_f = 0.50$ (solvent 7); IR (KBr): 2920 (ss) -CH_2-, 2850 (s) -CH_2-, 1640 (s) C=O, 1200 (s) P=O, 1070 (s) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD (2:1)): 0.89 (t, $^3J = 6.8$ Hz, 6H, -CH_3), 1.27 (s, 52H, alkyl-(4–17)-CH_2- and acyl-(4–15)-CH_2-), 1.41–1.49 (m, 2H, alkyl-3-CH_2-), 1.51–1.67 (m, 4H, P-CH_2- and acyl-3-CH_2-), 1.91–2.04 (m, 2H, P-$CH_2C\mathit{H}_2$), 2.12–2.26 (m, 2H, acyl-2-CH_2-), 3.12 (s, 9H, $N(CH_3)_3$), 3.38–3.44 (m, 2H, -CH_2-N), 3.75–3.87 (m, 2H, 1-CH_2), 3.93–4.01 (m, 1H, 2-CH).

1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-N-chloroacetyl-octadecane (**21**)

A solution of 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-amino-octadecane (**15**, 225 mg, 0.500 mmol), *DMAP* (305 mg, 2.50 mmol), and chloroacetylchloride (0.159 ml, 226 mg, 2.00 mmol) in 15 ml chloroform was stirred in a stoppered flask at room temperature for 1 h. 0.4 ml Triethylamine and 20 ml methanol were added, and the solution was extracted with 15 ml water. After reextracting the aqueous phase twice with 15 ml chloroform/methanol 4:1, the solvents were removed. Purification on silica gel (10 g) using first solvent 2 and then solvent 7 as eluents afforded 75 mg (143 mmol, 29%) **21** as reddish solid.

$R_f = 0.25$ (solvent 7); IR (KBr): 2920 (ss) -CH_2-, 2850 (s) -CH_2-, 1650 (s) C=O, 1560 (m), 1470 (m), 1190 (s) P=O, 1070 (ss) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (2:1)): 0.89 (t, $^3J = 6.8$ Hz, 3H, -CH_3), 1.27 (s, 28H, alkyl-(4-17)-CH_2-), 1.47–1.63 (m, 4H, 3-CH_2- and P-CH-), 1.91–2.05 (m, 2H, P-CH_2-C*H*$_2$-), 3.12 (s, 9H, N$(CH_3)_3$), 3.39–3.44 (m, 2H, -CH_2N), 3,82 (ddd, $^2J = 10.7$ Hz, $^3J = 6.7$ Hz, $J_{PH} = 6.2$ Hz, 1H, 1-CH), 3.90 (ddd, $^2J = 10.7$ Hz, $^3J = 3.9$ Hz, $J_{PH} = 6.2$ Hz, 1H, 1-CH′), 3.97–4.05 (m, 1H, 2-CH), 4.02 and 4.04 (2d, $^2J_{AB} = 13.5$ Hz, 2H, -CH_AH_BCl).

1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-N-fluoroacetyl-octadecane (**22**)

Perchloric acid (70%, 0.171 ml, 2.00 mmol) was added to a suspension of sodium fluoracetate (0.20 g, 2.00 mmol) and carbonyldiimidazole (1.17 g, 7.20 mmol) in 8 ml *THF*. The mixture was stirred for 30 min, the precipitate was filtered off, and a solution of 1-O-(3-(trimethylammonio)-propylphosphonoyl)-2-amino-octadecane (**15**, 225 mg, 0.50 mmol) in 8 ml chloroform was added. The mixture was stirred for 1 day, then the solvents were removed. The residue was dissolved in 12 ml chloroform and 15 ml methanol, and the solution was extracted with 12 ml water. The aqueous phase was reextracted twice with 10 ml chloroform/methanol 5:1, and the solvents of the combined organic layers were removed. Purification on silica gel (10 g) using first solvent 2 and then solvent 7 as eluent afforded 184 mg (0.362 mmol, 72%) **22** as slightly yellowish solid.

$R_f = 0.19$ (solvent 7); IR (KBr): 2920 (ss) -CH_2-, 2850 (s) -CH_2-, 1660 (ss) C=O, 1560 (m), 1470 (m), 1190 (s) P=O, 1050 (ss) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (2:1)): 0.89 (t, $^3J = 6.8$ Hz, 3H, -CH_3), 1.27 (s, 28H, alkyl-(4–17)-CH_2-), 1.48–1.66 (m, 4H, 3-CH_2- and P-CH_2-), 1.92–2.05 (m, 2H, P-CH_2-C*H*$_2$-), 3.13 (s, 9H, N$(CH_3)_3$), 3.39–3.45 (m, 2H, -CH_2N), 3.84 (ddd, $^2J = 10.6$ Hz, $^3J = 6.4$ Hz, $^3J_{PH} = 6.4$ Hz,1H, 1-CH), 3.91 (ddd, $^2J = 10.6$ Hz, $^3J = 3.9$ Hz, $J_{PH} = 6.0$ Hz, 1H, 1-CH′), 4.04-4.12 (m, 1H, 2-CH), 4.74 and 4.86 (2s, 2H, -CH_AH_BF)

1-O-(3-(Trimethylammonio)propylphosphonoyl)-2-N-methoxycarbonyl-octadecane (**23**)

A solution of 1-O-(3-(trimethylammonio)propylphosphonoyl)-2-amino-octadecane (**15**, 225 mg, 0.500 mmol), *DMAP* (100 mg, 0.82 mmol), triethylamine (0.28 ml, 202 mg, 2.00 mmol), and methyl chloroformate (0.154 ml, 189 mg, 2.00 mmol) in 15 ml chloroform was stirred in a stoppered flask at room temperature for 24 h. Methanol (18 ml) was added, and the solution was extracted with 15 ml ammonia (5% aqueous solution). The aqueous phase was extracted with 10 ml chloroform/methanol 4:1, and the solvents of the combined organic layers were removed. Purification of the crude carbamate on silica gel (10 g) using first solvent 2 and then solvent 7 as eluents yielded 173 mg (341 mmol, 68%) **23** as slightly yellowish solid.

$R_f = 0.23$ (solvent 7): IR (KBr): 2920 (ss) -CH_2-, 2850 (s) -CH_2-, 1710 (ss) C=O, 1545 (m), 1470 (m), 1195 (s) P=O, 1075 (ss) P-O-C; ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (2:1)): 0.88 (t, $^3J = 6.8$ Hz, 3H, alkyl-CH_3), 1.27 (s, 28H, alkyl-(4–17)-CH_2-), 1.42–1.61 (m, 2H, 3-CH_2-), 1.57 (dt, $J_{PH} = 17.1$ Hz, $^3J = 7.2$ Hz, 2H, P-CH_2-), 1.91–2.04 (m, 2H, P-CH_2C*H*$_2$-), 3.12 (s, 9H, N$(CH_3)_3$), $_3$), 3.38–3.45 (m, 2H, -CH_2-N), 3.63 (s, 3H, -O-CH_3), 3.64–3.72 (m, 1H, 2-CH), 3.76–3.88 (m, 2H, 1-CH_2).

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