Synthesis of Racemic Ethanolamine Plasmalogen

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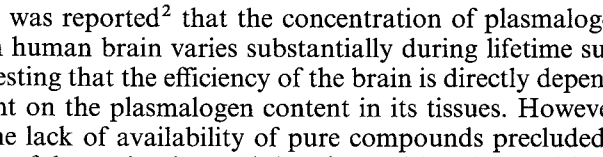
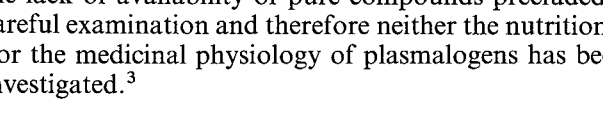
Racemic C_{12}-ethanolamine plasmalogen **5b** was prepared in high yield. The amino group was generated by selective reaction of azide **4b** with polymeric triphenylphosphine followed by mild hydrolysis of the intermediate phosphine imine. A novel universal phosphorylation reagent 2-azidoethyl dichlorophosphate (**7**) was used.

Plasmalogens are among the most widely distributed lipids occurring in nerve tissue and in cell membranes. As they are always associated with other lipids and as the individual side chains of plasmalogens, like in fat, show a great variety, pure compounds are rarely available from natural sources.[1]

It was reported[2] that the concentration of plasmalogen in human brain varies substantially during lifetime suggesting that the efficiency of the brain is directly dependent on the plasmalogen content in its tissues. However, the lack of availability of pure compounds precluded a careful examination and therefore neither the nutritional nor the medicinal physiology of plasmalogens has been investigated.[3]



5a



5b

C_{12} - ethanolamine plasmalogen

We wish to report the total synthesis of a racemic ethanolamine plasmalogen **5b**. The synthesis of a plasmalogen sodium salt has already been reported.[4] However, we found several inconsistencies[5] in this published report,[4] placing doubt on whether the reported method (using a phthalimido protection group) is useful in the preparation of the pure sodium salt of an ethanolamine plasmalogen and whether the material corresponded to the anticipated structural formula.

The enol ether moiety of plasmalogens is sensitive to acids, precluding the use of strongly acidic conditions during their preparation. Another obstacle appearing was transacylation during step **2** → **3**. We found that this rearrangement occurred during the purification by chromatography on silica gel. It can be avoided by chromatography at low temperature or, even better, by using the intermediate **3** without further purification.

The third and major difficulty in the preparation of ethanolamine plasmalogens lies in the suitable selection of an *N*-protection group. We could show that the phthalimido group, so far used for this purpose,[4,6] is less appropriate in the synthesis of plasmalogens since substantial deacylation occurs under the conditions required to cleave the *N*-substituted phthalimide.

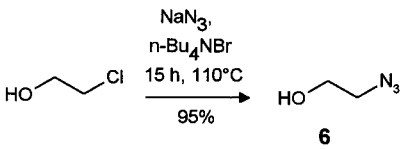
Consequently, a milder method was required to generate the amino function. This was achieved by using azides **4** as intermediates.

However, many experiments were necessary to find conditions for the conversion of **4** to **5**. Our initial idea was to hydrogenate **4b** with the Lindlar catalyst, but it turned out that this catalyst was not selective enough[7] and we observed the partial hydrogenation of the enol ether moiety. In addition, the impure product generated in this manner was shown to contain a *Z*/*E*-isomeric mixture of enol ethers **5b**.

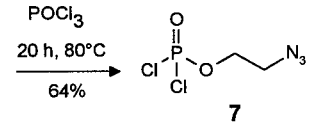
Most of the other known methods[8] for reduction of azides to amines also failed in our hands, e.g. those using hydrazine, diimine, $SnCl_2$, Bu_4NBH_4, $NaCNBH_3$, Pd/C with cyclohexene, triethyl phosphite, and hypophosphoric acid.

The best method was found in using triphenylphosphine with subsequent hydrolysis of the intermediate phosphine imine. This process, initiated by Staudinger,[9] was recently modified[10] by using polymer-bound triphenylphosphine, facilitating the separation from the byproduct triphenylphosphine oxide. In our hands, the polymer also provided most satisfactory results.

A key reagent in the preparation of ethanolamine plasmalogens was the novel reagent **7**, readily prepared from 2-azidoethanol (**6**) and phosphorus oxychloride. Reagent **7** can be stored at 0 °C without decomposition. The preparation[11] of **6** from 2-chloroethanol and sodium azide was improved by using tetrabutylammonium bromide as a catalyst, driving the reaction to completion.

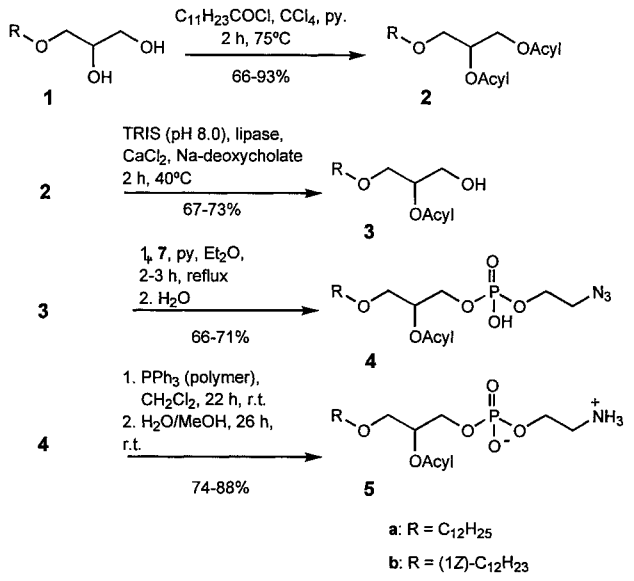


6



7

For a careful determination of the reaction conditions[12] leading to the final product **5b**, the saturated ether lipid **5a** was first prepared, as the starting material **1a** is easily available.[13]



After having performed these model reactions, the unsaturated sequence **1b** → **5b** was investigated.

Enol ether lipid **1b** was prepared according to a literature procedure,[14] and acylated as reported.[15,16] The enzymatic process **2** → **3** according to ref 16 is also well established. Inexpensive lipase from hog pancreas is very selective in generating regiospecifically the primary alcohol **3**. The optical rotations of **3a** and **4a** were found to be 0, indicating that there is no further preference of the enzyme for one individual enantiomer. Alcohol **3** was acylated according to ref 17 with the new reagent **7**, followed by hydrolysis of the intermediate phosphoric diester chloride and excess **7**. Finally, the azide function of **4** was efficiently converted to the free amino group, leading to **5**.

The advantage of the use of the polymeric triphenylphosphine is obvious, since pure **5** was obtained in high yield without chromatographic purification. In fact, in one preparation of **5b** only a single chromatography (of intermediate **4b**) was necessary.

It has been established[4] that aldehydogenic phospholipids are considerably more stable in the form of their sodium salts. However, the high mp of **5b** (179 °C, dec.) suggests that the zwitterion is also a preferred form of pure plasmalogens. Using the novel reagent **7** together with the above mentioned mild reactions, ethanolamine plasmalogens become accessible in pure crystalline form. We believe that the above mentioned method will also be selective in preparing other saturated or unsaturated lipids, such as the kephalins. As for the prepared ethanolamine plasmalogens, we hope that their investigation will reveal the role they play in nature.

All reagents and solvents were of commercial quality from freshly opened containers and purchased from Fluka Chemical Co. Et_2O was refluxed under N_2 with $LiAlH_4$ and distilled immediately before use. $CHCl_3$ and MeOH for column chromatography were distilled before use. Freshly distilled pyridine had a bp of 114–116 °C. Analytical silica gel TLC plates and silica gel were purchased from Merck Chemical Co. The TLC plates were developed by a phosphomolybdic acid spray reagent (10% in EtOH). Melting points were taken on a Büchi 535 apparatus and are uncorrected. Microanalyses were obtained using a Heraeus CHN Standard microanalyser, IR spectra were obtained using a Perkin-Elmer 1420 IR spectrophotometer. The NMR spectra (300 MHz for ^1H; 75 MHz for ^{13}C and 120 MHz for ^{31}P NMR) were obtained on a Bruker ARX 300. The 600 MHz spectrum was measured on a Bruker AMX 600. MS were obtained on Finnigan spectrometers (MAT 95 Q for the FAB spectra and MAT 90 for the EI spectrum).

2-Azidoethanol (6):
In a 50-mL flask, fitted with a reflux condenser, a mixture of 2-chloroethanol (9.80 g, 121 mmol), NaN_3 (10.25 g, 244 mmol) and n-Bu_4NBr (1.00 g, 3 mmol) was stirred magnetically for 15 h at 110 °C (safety shield). After cooling, the product was taken up with Et_2O (20 mL), the precipitate of NaCl, remaining NaN_3 and the phase transfer catalyst were removed by using a glass filter (G-3). The salts were washed with Et_2O (20 mL). The removal of the combined solvents in vacuo gave a yellow crude product which was purified by distillation in vacuo over a 10-cm Vigreux column (bp 35 °C/1 Torr) to yield 10.06 g (95%) of a colorless liquid. Organic azides of low molecular weight are **hazardous explosives!** In two runs, the distillation of **6** was performed at 13 Torr leading only to slightly reduced yields. However, for safety reasons the purification under high vacuum (1 Torr), using a safety shield, should be preferred.

IR (CH_2Cl_2): ν = 3610 vs (OH), 3450 s, 2940 s (CH), 2880 s (CH), 2140 vs (N_3), 1450 s, 1270 vs, 1070 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.94 (t, 1 H, $-OH$, J = 5.7 Hz), 3.42 (t, 2 H, $-CH_2-N_3$, J = 5.1 Hz), 3.75 (dt, 2 H, $-CH_2-OH$, J = 5.2 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 53.47 (1 C, $-CH_2-N_3$), 61.40 (1 C, $-CH_2-OH$).

2-Azidoethyl Dichlorophosphate (7):
Freshly distilled $POCl_3$ (26.58 g, 173 mmol) was placed under N_2 into a 50-mL flask, equipped with a dropping funnel and a magnetic stirrer. Over a period of 30 min, 2-azidoethanol (7.50 g, 86 mmol) was added dropwise at 0 °C to the stirred mixture. The funnel was replaced by a reflux condenser connected to a bubbler and the flask was heated to 70 °C until no further HCl gas formed (20 h). Excessive $POCl_3$ was evaporated in vacuo (10 mbar and at 0.001 mbar for 5 h). The crude product was distilled with a bath temperature not exceeding 80 °C using a short-path distillation apparatus (safety shield) in vacuo (bp 65–68 °C/0.001 mbar) to give 11.402 g (65%) of the colorless liquid product. In order to retain low pressure the forerun (bp 40–64 °C, ca. 3 g) was removed during the distillation (cooling of the distillation flask before augmenting pressure). To avoid hazards and decomposition an efficient vacuum pump should be used and the pressure watched during distillation.

$C_2H_4Cl_2N_3O_2P$	calc.	C 11.78	H 1.98
(203.95)	found	12.23	2.07

IR (CH_2Cl_2): ν = 3050 s, 2980 m (CH), 2120 vs (N_3), 1420 s (CH), 1350 s, 1280 vs (P=O), 1040 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 3.61 (dt, 2 H, $-CH_2-N_3$, J = 1.6, 4.9 Hz), 4.42 (m 2 H, $-CH_2-O-P$, J = 4.9 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 50.07 (d, 1 C, $-CH_2-O-$ or $-CH_2-N_3$), 69.49 (d, 1 C, $-CH_2-O-$ or CH_2-N_3).

^{31}P NMR (120 MHz, $CDCl_3$): δ = 8.56 (s, 1 P).

2,3-Di-*O*-dodecoyl-1-*O*-dodecylglycerol (2a) and 1-*O*-[(*Z*)-Dodecenyl]-2,3-di-*O*-dodecoylglycerol (2b); General Procedure:
The isomer-free diol **1** (2.30 mmol) was dissolved in dry CCl_4 (1.4 mL) and abs. pyridine (0.4 mL), and stirred magnetically at 0 °C in a dried 25-mL Schlenk flask, fitted with an N_2 filled balloon. For the following acylation a mixture of dodecoyl chloride (4.60 mmol) in dry CCl_4 (2.6 mL) was added slowly (3 h) at 0 °C, by dropping funnel. The milky white suspension was refluxed for 2 h and then diluted with CH_2Cl_2 (20 mL). After addition of 0.1 N HCl (20 mL), the organic layer was separated and washed once with sat. $NaHCO_3$ (20 mL) and water (3 × 5 mL). Organic traces were reextracted with CH_2Cl_2 (2 × 5 mL) and the collected organic

layers dried ($MgSO_4$). After evaporation of the solvent, the crude product was dried in vacuo (0.001 mbar) and used without further purification in the following reaction. The crude product could be purified by column chromatography on silica gel using hexane/Et_2O (6 : 1) to give a white solid.

2a: yield after chromatography 79%; mp 35.5°C (without recrystallization).

$C_{39}H_{76}O_5$ (625.03) calc. C 74.95 H 12.26
found 74.76 12.17

IR (CH_2Cl_2): ν = 2930 (vs (C–H), 2860 vs (C–H), 1740 vs (C=O), 1465 m, (C–H), 1380 m, 1160 s, 1120 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 0.85 (t, 9H, $-CH_3$, J = 6.6 Hz), 1.24 (s, 52H, aliphatic $-CH_2-$), 1.54 (m, 4H, 2 × $-CH_2CH_2-COO-$), 2.27 (t, 2H, $-CH_2-COO-$, J = 7.5 Hz), 2.29 (t, 2H, $-CH_2-COO-$, J = 7.5 Hz), 3.40 (m, 2H, $-CH_2CH_2-O-$), 3.51 (d, 2H, R–O–CH_2-, J = 5.3 Hz), 4.13 (dd, 1H, $-CH_2-O-$Acyl, J = 11.2 Hz), 4.31 (dd, 1H, $-CH_2-O-$Acyl, J = 11.9 Hz), 5.17 (m, 1H, $-O-CH(CH_2-O-)_2$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.01 (3C, $-CH_3$), 22.67–31.91 (25C, aliphatic $-CH_2-$), 24.91 (1C, $-CH_2CH_2-COO-$), 24.97 (1C, $-CH_2CH_2-COO-$), 26.04 (1C, $-CH_2CH_2-O-$), 34.15 (1C, $-CH_2-COO-$), 34.35 (1C, $-CH_2-COO-$), 62.78, 68.96, 70.10 and 71.75 (4C, 3 × $-O-CH_2-$ and $-O-CH(CH_2-O-)_2$), 173.09 (1C, $-C=O$), 173.40 (1C, $-C=O$).

MS (EI, M): m/z = 624.6 (calc. 624.583).

TLC: R_f 0.33 (hexane/Et_2O 6 : 1).

2b: yield after chromatography 66%; mp 28.5°C.

$C_{39}H_{74}O_5$ (623.02) calc. C 75.19 H 11.97
found 75.52 11.71

IR (CH_2Cl_2): ν = 2930 vs (CH), 2860 vs (CH), 1740 s (C=O), 1665 w (C=C enol ether), 1470 m (CH), 1190 vs, 1110 cm^{-1} s (CO).

^1H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, 9H, $-CH_3$, J = 6.7 Hz), 1.24 (s, 48H, aliphatic $-CH_2-$), 1.59 (m, 4H, 2 × $-CH_2CH_2-COO-$), 2.01 (m, 2H, $-CH_2-CH=$), 2.29 (t, 2H, $-CH_2-COO-$, J = 7.5 Hz), 2.30 (t, 2H, $-CH_2-COO-$, J = 7.5 Hz), 3.81 (d, 2H, R–O–CH_2-, J = 5.4 Hz), 4.14 (dd, 1H, $-CH_2-O$Acyl, J = 6.0, 11.9 Hz), 4.32 (dd, 1H, $-CH_2-O$Acyl, J = 4.0, 11.9 Hz), 4.36 (dt, 1H, $CH_2-CH=$, J = 6.3, 7.4 Hz), 5.18 (ddt, 1H, $-O-CH(CH_2-O-)_2$, J = 6.0, 4.0, 5.4 Hz), 5.86 (dt, 1H, $=CH-O-$, J = 1.3, 6.3 Hz).

^{13}C NMR (75 MHZ, $CDCl_3$): δ = 14.10 (3C, $-CH_3$), 22.69–31.92 (24C, aliphatic $-CH_2-$), 23.88 (1C, $-CH_2-CH=$), 24.93 (1C, $-CH_2CH_2-COO-$), 24.91 (1C, $-CH_2CH_2-COO-$), 34.12 (1C, $-CH_2-COO-$), 34.30 (1C, $-CH_2-COO-$), 62.27 (1C, $-O-CH(CH_2-O-)_2$), 69.94 (2C, R–O–CH_2- and $-CH_2-O$Acyl), 108.47 (1C, $CH_2-CH=$), 144.45 (1C, $=CH-O-$), 172.97 (1C, $-C=O$), 173.35 (1C, $-C=O$).

MS (+$FAB_{M=M+1}$): m/z = 623.6 (calc. 622.567).

TLC: R_f 0.17 (hexane/Et_2O 19 : 1).

2-*O*-Dodecoyl-1-*O*-dodecylglycerol (3a) and 1-*O*-[(*Z*)-1-Dodecenyl]-2-*O*-dodecoylglycerol (3b); General Procedure:

In a 100-mL Schlenk flask, fitted with an N_2 balloon and a magnetic stirrer, TRIS-buffer [tris-(hydroxymethyl)aminomethane, acidificated with conc. HCl to pH 8.0] (33 mL, 1.0 M) was heated to 40°C and stirred for at least 30 min to get a constant pH and a constant temperature. The diacyl compound **2** (0.96 mmol), 1% aq sodium deoxycholate solution (1.75 mL) and 45% aq $CaCl_2$ (3 mL) were added. A milky white suspension resulted. A mixture of hog pancreas lipase (375 mg, Fluka) in TRIS- buffer of pH 8.0 (7.5 mL) was added and the suspension was stirred vigorously for about 2 h at 40°C until the reaction was complete (TLC). To separate the lipase, the reaction mixture was first diluted with Et_2O (40 mL), filtered through a glass filter (G-3), which was filled with Celite (Celite was added up to a height of 5 cm in the glass filter), then washed with Et_2O (100 mL) and filtered again, using a G-4 glass filter and Et_2O (20 mL). After drying ($MgSO_4$) the reaction mixture was evaporated and the residue dried in vacuo (0.001 mbar) to give a colorless oil in 90% yield. The crude product could be used in the next reaction step without further purification. After column chromatography on silica gel, using toluene/EtOAc (9 : 1), the product remained as a white precipitate. It was very important to chromatograph quickly at −10°C, otherwise the product partly equilibrated to the 3-*O*-acyl isomer which was very difficult to separate from the desired 2-*O*-acyl product.

3a: yield after chromatography 73%.

$C_{27}H_{54}O_4$ (442.73) calc. C 73.25 H 12.30
found 73.49 12.32

IR (CH_2Cl_2): ν = 3600 w (OH), 2920 vs (CH), 2850 vs (CH), 1730 s (C=O), 1470 m (CH), 1170 s, 1120 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, 6H, $-CH_3$, J = 6.6 Hz), 1.24 (s, 34H, aliphatic $-CH_2-$), 1.53 (m, 2H, $-CH_2CH_2-O-$), 1.61 (m, 2H, $-CH_2-CH_2-COO-$), 2.21 (t, 1H, $-OH$, J = 6.2 Hz), 2.33 (t, 2H, $-CH_2-COO-$, J = 7.5 Hz), 3.41 (m, 2H, $-CH_2CH_2-O-$), 3.59 and 3.79 (m, 4H, R–O–CH_2- and $-CH_2-OH$), 4.97 (q_5, 1H, $-O-CH(CH_2-O-)_2$, J = 4.8 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.09 (2C, $-CH_3$), 22.68–31.91 (17C, aliphatic $-CH_2$), 22.50 (1C, $-CH_2CH_2-COO-$), 26.05 (1C, $-CH_2CH_2-O-$), 34.41 (1C, $-CH_2-COO-$), 63.04, 70.01, 71.92, and 72.87 (4C, 4 × $-O-CH_2$ and $-O-CH(CH_2-O-)_2$), 173.70 (1C, $-C=O$).

MS: (−$FAB_{M=M-1}$): m/z = 441.4 (calc. 441.412).

TLC: R_f 0.22 (toluene/EtOAc 9 : 1).

$[\alpha]_D^{20}$ 0.0 (racemic, $CHCl_3$, 1%).

3b: yield after chromatography 67%.

$C_{27}H_{52}O_4$ (440.71) calc. C 73.59 H 11.90
found 73.94 11.93

IR (CH_2Cl_2): ν = 3600 m (OH), 2930 vs (CH), 2860 vs (CH), 1740 vs (C=O), 1660 m (C=C enol ether), 1460 s (CH), 1380 m (OH), 1170 s, 1120 s (CO), 690 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, 6H, $-CH_3$, J = 6.7 Hz), 1.24 (s, 32H, aliphatic $-CH_2-$), 1.61 (m, 2H, $-CH_2CH_2-COO-$), 1.87 (t, 1H, $-OH$, J = 6.2 Hz), 2.01 (m, 2H, $-CH_2CH_2-CH=$), 2.33 (t, 2H, $-CH_2-COO-$, J = 7.6 Hz), 3.80 (m, 2H, $-CH_2-OH$, J = 3.9, 2.2, 2.9 Hz), 3.87 (d, 2H, R–O–CH_2-, J = 5.3 Hz), 4.37 (dt, 1H, $CH_2-CH=$, J = 7.8, 6.2 Hz), 5.01 (m, 1H, $-O-CH(CH_2-O-)_2$), 5.89 (dt, 1H, $=CH-O-$, J = 6.1, 1.4 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.10 (2C, $-CH_3$), 22.67–31.91 (16C, aliphatic $-CH_2$), 23.90 (1C, $-CH_2-CH=$), 24.94 (1C, $-CH_2CH_2-COO-$), 34.35 (1C, $-CH_2-COO-$), 62.13, 70.16, and 73.04 (3C, $-O-CH-(CH_2-O-)_2$, R–OCH_2- and $-CH_2-OH$), 108.28 (1C, $CH_2-CH=$), 114.53 (1C, $=CH-O-$), 173.59 (1C, $-C=O$).

MS (−$FAB_{M=M-1}$): m/z = 439.4 (calc. 440.396).

TLC: R_f 0.24 (toluene/EtOAc 9 : 1).

3-*O*-[2-Azidoethoxy(hydroxy)phosphoryl]-2-*O*-dodecoyl-1-*O*-dodecyl- (4a) and -1-*O*-[(*Z*)-1-dodecenyl]-2-*O*-dodecoyl-glycerol (4b); General Procedure:

In a well-dried 50-mL Schlenk flask with rubber septum and magnetic stirrer the phosphorylation reagent **7** (1.5 mmol) was dissolved in freshly distilled abs. Et_2O (15 mL). Abs. pyridine (720 µl, 9 mmol, bp 114–116°C) was added dropwise to give a white suspension. After 30 min of stirring, a mixture of the monoacyl compound **3** (0.5 mmol) in abs. Et_2O (15 mL) was added using a syringe. After 30 min the septum was replaced by a reflux condenser and the solution refluxed for about 150 min until the starting material (R_f 0.24) disappeared. Phosphoric acid chloride was hydrolyzed with water (1.5 mL) at 0°C to the corresponding acid. Stirring was continued for another 16 h. The mixture was reduced in vacuo to a volume of 5 mL and redissolved in a $CHCl_3$/MeOH mixture (2 : 1, 20 mL). The (lower) organic layer was washed twice with acidic water (acidified with 2N H_2SO_4 to pH 4). The acid water layers were reextracted with $CHCl_3$/MeOH (2 : 1, 3 × 10 mL), the residue was dried ($MgSO_4$) and finally dried in vacuo. The isolated white, noncrystalline solid could be purified by column chromatography using $CHCl_3$/MeOH (first 9 : 1 finally 9 : 2).

4a: yield after chromatography 71%.

IR (CH_2Cl_2): ν = 2950 vs (CH), 2860 vs (CH), 2120 s (N_3), 1740 s (C=O), 1470 s (CH), 1380 m, 1250 s (P=O), 1120 vs (CO), 1070 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, 6H, $-CH_3$, J = 6.7 Hz), 1.24 (s, 34H, aliphatic $-CH_2-$), 1.52 (m, 2H, $-CH_2-CH_2-O-$), 1.60 (m, 2H, $-CH_2CH_2-COO-$), 2.33 t, 2H, $-CH_2-COO-$, J = 7.6 Hz), 3.12 (broad, 2H, $-CH_2-N_3$), 3.38 (m, 4H, 2× $-CH_2-O-$), 3.55 (d, 2H, $R-O-CH_2-$, J = 5.1 Hz), 4.02 (m, 2H, $-CH_2-CH_2-N_3$), 5.13 (m, 1H, $-O-CH(CH_2-O-)_2$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.09 (2C, $-CH_3$), 22.68–31.91 (17C, aliphatic $-CH_2$), 24.92 (1C, $-CH_2CH_2-COO-$), 26.03 (1C, $-CH_2CH_2-O-$), 34.32 (1C, $-CH_2-COO-$), 51.05, 65.35, 68.35, 70.97, and 71.77 (6C, 5× $-C-O-$, and $-CH_2-N_3$), 173.31 (1C, $-C=O$).

^{31}P NMR (120 MHz, $CDCl_3$): δ = −3.55 (1P).

MS: ($-FAB_{M=M-1}$): m/z = 590.4 (calc. 591.412).

TLC: R_f 0.69 ($CHCl_3$/MeOH/NH_3 50 : 25 : 6).

$[\alpha]_D^{20}$ 0.0 (racemic, $CHCl_3$, 1%)

4b: yield after chromatography 66%.

IR (CH_2Cl_2): ν = 2940 vs (CH), 2860 vs (CH), 2120 s (N_3), 1740 s (C=O), 1660 m (C=C enol ether) 1590 m, 1465 m (CH), 1260 vs (P=O), 1120, 1080 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, 6H, $-CH_3$, J = 6.7 Hz), 1.24 (s, 32H, aliphatic $-CH_2-$), 1.59 (m, 2H, $-CH_2CH_2-COO-$), 2.00 (m, 2H, $-CH_2-CH=$), 2.33 (t, 2H, $-CH_2-COO-$, J = 7.6 Hz), 3.45 (m, 2H, $-CH_2-N_3$), 3.88 (m, 2H, $R-O-CH_2-$), 3.99 (broad signal, 4H, 2× $-CH_2-O-P-$), 4.32 (dt, 1H, $CH_2-HC=$, J = 6.3, 7.3 Hz), 5.25 (m, 1H, $-O-CH(CH_2-O-)_2$), 5.89 (d, 1H, $=CH-O-$, J = 6.1 Hz).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.09 (2C, $-CH_3$), 22.69–31.95 (16C, aliphatic $-CH_2$), 23.96 (1C, $-CH_2CH=$), 24.92 (1C, $-CH_2CH_2-COO-$), 34.46 (1C, $-CH_2-COO-$), 51.27 (1C, $-CH_2-N_3$), 64.05 (1C, $-CH_2-O-P-$), 64,63 (1C, $-CH_2-O-P-$), 70.32 (1C, $R-O-CH_2-$), 72.13 (1C, $-O-CH(CH_2-O-)_2$), 108.19 (1C, $-CH_2CH=$), 145.15 (1C, $=CH-O-$), 173.41 (1C, $-C=O$).

^{31}P NMR (120 MHz, $CDCl_3$): δ = −3.41 (s, 1P).

MS ($-FAB_{M=M-1}$): m/z = 588.4 (calc. 589.396).

TLC: R_f 0.65 ($CHCl_3$/MeOH/NH_3 50 : 25 : 6).

For further structure confirmation of **4a** and **4b** their *methyl esters* were prepared using excess of diazomethane.

Methyl ester of **4a**:

$C_{30}H_{60}N_3O_7P$	calc.	C 59.48	H 9.98	N 6.94
(605.80)	found	59.48	10.00	6.79

Methyl ester of **4b**:

$C_{30}H_{58}N_3O_7P$	calc.	C 59.68	H 9.68	N 6.96
(603.78)	found	59.53	10.03	6.91

3-*O*-[2-Aminoethoxy(hydroxy)phosphoryl]-2-*O*-dodecoyl-1-*O*-dodecyl- (5a) and -1-*O*-[(*Z*)-1-dodecenyl]-2-*O*-dodecoyl-glycerol (5b); General Procedure:

In a well-dried 10-mL Schlenk flask, fitted with a balloon, filled with Ar, and a magnetic stirrer, to a solution of the azide **4** (0.08 mmol) in abs CH_2Cl_2 (1.5 mL) polymer-bound triphenylphosphine (5 equiv, Fluka) was added. The reaction was stirred at r.t. for 22 h (the TLC spot of the starting material had to disappear). Then a MeOH/H_2O mixture (9 : 1, 1.5 mL) was added at 0°C in order to hydrolyse the resulting polymer-bound phosphine imine. The hydrolysis product could be detected by the resulting TLC spot. After 26 h stirring (Ar) at r.t., the polymer resin was removed by filtration, first through pressed cotton wool and then by using a G-4 glass filter. After washing the residue (CH_2Cl_2/MeOH/H_2O 19 : 9 : 1, 15 mL) the solvent was removed in vacuo, the residue dried under high vacuum to give approx. 75% of the white crude product of satisfactory purity.

5a: yield without purification 74%, after chromatography ($CHCl_3$/MeOH 2 : 1) 52%; mp 208°C ($CHCl_3$, dec.).

$C_{29}H_{60}NO_7P$	calc.	C 61.57	H 10.69	N 2.48
(565.77)	found	61.16	11.13	2.51

IR (CH_2Cl_2): ν = 2940 vs (CH), 2860 vs (CH), 1735 s (C=O), 1600 m, broad signal, 1465 m (CH), 1245 s (P=O), 1120, 1080 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, 6H, $-CH_3$, J = 6.7 Hz), 1.24 (s, 34H, aliphatic $-CH_2-$), 1.49 (m, 2H, $-CH_2CH_2-O-$), 1.57 (m, 2H, $-CH_2CH_2-COO-$), 2.28 (m, 2H, $-CH_2-COO-$, J = 7.4 Hz), 2.93 (broad signal, 2H, $-CH_2-NH_2$), 3.38 (m, 2H, $-CH_2-CH_2-O-$), 3.53 (broad signal, 2H, $R-O-CH_2-$), 3.97 (m, 4H, 2× $-CH_2-O-P$), 5.12 (m, 1H, $-O-CH(CH_2-O-)_2$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.07 (2C, $-CH_3$), 22.66–31.91 (17C, aliphatic $-CH_2$), 25.01 (1C, $-CH_2CH_2-COO-$), 26.08 (1C, $-CH_2CH_2-O-$), 34.39 (1C, $-CH_2-COO-$), 41.10 (1C, $-CH_2-NH_2$), 64.32 (2C, 2× $-CH_2-O-P$), 69.13 (1C, $R-O-CH_2$), 71.66 (2C, $-O-CH(CH_2-O-)_2$, $-CH_2-CH_2-O-$), 173.43 (1C, $-C=O$).

^{31}P NMR (120 MHz, $CDCl_3$): δ = −0.68 (s, 1P).

MS ($+FAB_{M=M+1}$): m/z = 566.4 (calc. 565.422).

TLC: R_f 0.47 (freshly prepared mixture of $CHCl_3$/MeOH/NH_3 50 : 25 : 6).

5b: yield without purification 88%, after chromatography 53%; mp 179°C ($CHCl_3$, dec.).

$C_{29}H_{58}NO_7P$	calc.	C 61.78	H 10.37	N 2.48
(563.76)	found	61.43	10.00	2.48

IR (CH_2Cl_2): ν = 2940 vs (CH), 2860 vs (CH), 1735 s (C=O), 1660 m (C=C enol ether), 1600 m, broad signal, 1465 m (CH), 1245 s (P=O), 1120, 1080 cm^{-1}.

^1H NMR (600 MHz, $CDCl_3$): δ = 0.86 (t, 6H, $-CH_3$, J = 6.7 Hz), 1.26 (s, 32H, aliphatic $-CH_2-$), 1.58 (m, 2H, $-CH_2CH_2-COO-$), 1.98 (m, 2H, $-CH_2-CH=$), 2.29 (t, 2H, $-CH_2-COO-$, J = 7.5 Hz), 3.10 (broad signal, 2H, $-CH_2-NH_2/CH_2-NH_3^+$), 3.90 (m, 4H, $-CH_2-O-P$ and $R-O-CH_2-$), 4.04 (broad signal, 2H, $-CH_2CH_2-NH_2$), 4.31 (dt, 1H, $CH_2-HC=$, J = 6.3, 7.1 Hz), 5.13 (m, 1H, $-O-CH(CH_2-O-)_2$), 5.88 (d, 1H, $=CH-O-$, J = 6.2 Hz), 8.48 (broad signal, 2H, $-NH_2$).

^{13}C NMR (150 MHz, $CDCl_3$): δ = 14.10 (2C, $-CH_3$), 22.69–32.76 (16C aliphatic $-CH_2-$), 23.94 (1C, $-CH_2-CH=$), 24.96 (1C, $-CH_2CH_2-COO-$), 34.36 (1C, $-CH_2-COO$), 40.47 (1C, $-CH_2-NH_2$), 62.41 (1C, $-CH_2CH_2-NH_2$), 63.83 (1C, $-CH_2-O-P$ at glycerol), 70.24 (1C, $R-O-CH_2-$), 71.45 (1C, $-O-CH(CH_2-O-)_2$), 107.81 (1C, $-CH_2CH=$), 144.75 (1C, $=CH-O-$), 173.43 (1C, $-C=O$).

^{31}P NMR (120 MHz, $CDCl_3$): δ = 15.18 (s, 1P).

MS ($+FAB_{M=M+1}$): m/z = 564.4 (calc. 563.406).

TLC: R_f 0.44 (freshly prepared mixture of $CHCl_3$/MeOH/NH_3 50 : 25 : 6), R_f 0.16 ($CHCl_3$/MeOH 2 : 1).

Deacylation of 5a:

The saturated compound **5a** (30 mg, 0.053 mmol), MeOH (1 mL), 0.1 N aq NaOH (530 µL, 0.053 mmol) and hydrazine hydrate (5.1 µL, 0.106 mmol) were placed in an ampoule containing a magnetic bar. The ampoule was sealed under Ar and stirred for 18 h at 70°C. After cooling to r.t. the ampoule was opened. The evaporated reaction mixture was neutralized with 0.1 N HCl (530 µL, 0.053 mmol) and evaporated again. The crude product was purified by column chromatography on silica gel, using $CHCl_3$/MeOH (first 9 : 1 then 2 : 1) as mobile phase. After evaporation and drying in vacuo (0.001 mbar) a white precipitate remained. Yield after chromatography ($CHCl_3$/MeOH 2 : 1) 52%.

IR (CH_2Cl_2): ν = 2930 vs (CH), 2880 vs (CH), 1600 m, broad signal, 1465 m (CH), 1240 s (P=O), 1080 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 0.86 (t, 3H, $-CH_3$, J = 6.5 Hz), 1.24 (s, 18H, aliphatic $-CH_2-$), 1.51 (m, 2H, $-CH_2CH_2-O-$), 3.23 (broad signal, 2H, $-CH_2-NH_2$), 3.39 (m, 4H, 2× $-CH_2-O-$), 3.87 (broad signal, 1H, $HO-CH(CH_2-O-)_2$, 3.94 (broad signal, 2H, $-O-CH_2-$), 4.15 (broad signal, 2H, $-CH_2-O-$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.09 (1C, $-CH_3$), 22.69–31.94

(9 C, aliphatic $-CH_2-$), 26.09 (1 C, $-CH_2CH_2-O-$), 41.17 (1 C, $-CH_2-NH_2$), 53.40, 67.96, 69.51, 71.37 and 71.80 (5 C, 4 × $-O-CH_2$ and $HO-CH-$).

TLC; R_f 0.32 (freshly prepared mixture of $CHCl_3$/MeOH/NH_3 50 : 25 : 6).

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