Synthesis and Biophysical Characterization of Chlorambucil Anticancer Ether Lipid Prodrugs

Palle J. Pedersen, Mikkel S. Christensen, Tristan Ruysschaert, Lars Linderoth, Thomas L. Andresen, Fredrik Melander, Ole G. Mouritsen, Robert Madsen and Mads H. Clausen*

Supporting Information

Experimental procedures for the synthesis of 5a, 5b, 6a, 6b, 7a, 7b, 9 and 10.

(*R*)-1-*O*-Hexadecyl-3-(*p*-toluenesulfonyl)-glycerol (5a)[1]

Epoxide **4** (2.0 g, 8.76 mmol) and hexadecanol (3.0 g, 12.37 mmol) were dissolved in anhydrous CH_2Cl_2 (40 mL) under N_2 and $BF_3{\cdot}OEt_2$ (0.5 mL) was added. The solution was stirred for 24 h at 20 °C and concentrated *in vacuo*. A white solid was formed and recrystallized from hexane (35 mL) to give 2.75 g of **5a**. The filtrate, which contain some product, was concentrated *in vacuo* and purified by column chromatography (CH_2Cl_2:Et_2O 10:1) to give 0.98 g of **5a** (89% overall). R_f = 0.58 (CH_2Cl_2:Et_2O 10:1); mp = 53-55 °C (lit.[2] 53-54 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.81 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 8.4 Hz, 2H), 4.12-3.93 (m, 3H), 3.48-3.27 (m, 4H), 2.46 (s, 3H), 2.18 (s, 1H), 1.51 (t, *J* = 6.4 Hz, 2H), 1.32-1.26 (m, 26H), 0.88 (t, *J* = 6.7 Hz, 3H).

(*R*)-1-*O*-Octadecyl-3-(*p*-toluenesulfonyl)-glycerol (5b)[1]

Performed as for **5a** using **4** (5.25 g, 23.00 mmol) and octadecanol (8.7 g, 32.20 mmol) to afford 11.16 g (97 %) of **5b** as white crystals. R_f = 0.65 (CH_2Cl_2:Et_2O 10:1); mp = 66-68 °C (lit.[3] 67-68 °C). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.1 Hz, 2H), 7.38 (d, *J* = 8.1 Hz, 2H), 4.05-3.95 (m, 3H), 3.45-3.35 (m, 4H), 2.43 (s, 3H), 2.10 (s, 1H), 1.51 (m, 2H), 1.35-1.23 (m, 30H), 0.90 (t, *J* = 6.7 Hz, 3H).

(*S*)-1-*O*-Hexadecyl-2-*O*-(4-methoxybenzyl)-glycerol (6a)

Alcohol **5a** (1.192 g, 2.532 mmol) and 4-methoxybenzyl trichloroacetimidate (1.098 g, 3.885 mmol) were dissolved in anhydrous toluene (21 mL) under N_2 and $La(OTf)_3$ (70 mg, 0.119 mmol) was added. The mixture was stirred at 20 °C for 2.5 h and concentrated *in vacuo* to give ((*R*)-1-*O*-hexadecyl-2-*O*-(*p*-methoxybenzyl)-3-(*p*-toluenesulfonyl)-glycerol) as a white solid. The crude product was dissolved in DMSO (20 mL) and DMF (5 mL) under N_2 and CsOAc (1.491 g, 7.379 mmol) was added. The mixture was heated to 60 °C and stirred 14 h, after which the reaction mixture was washed with H_2O (30 mL) and the organic layer was isolated via extraction with Et_2O (3×50 mL). The combined organic phases were dried over $MgSO_4$ and filtration followed by

[1] Andresen, T. L.; Jensen, S. S.; Madsen, R.; Jørgensen, K. Synthesis and Biological Activity of Anticancer Ether Lipids That Are Specifically Released by Phospholipase A_2 in Tumor Tissue. *J. Med. Chem.* **2005**, *48*, 7305-7314.
[2] Baylis, R. L.; Bevan, T. H.; Malkin, T. The synthesis of cephalin (phosphatidylethanolamine) and batyl, chimyl, glycol and alkyl analogues. *J. Chem. Soc.* **1958**, 2962-2966.
[3] Hirth, G.; Barner, R. Synthesis of glyceryl etherphosphatides. 1. Preparation of 1-O-octadecyl-2-O-acetyl-*sn*-glyceryl-3-phosphorylcholine (Platelet Activating Factor), of its enantiomer and of some analogous compounds. *Helv. Chim. Acta* **1982**, *65*, 1059-1084.

concentration *in vacuo* gave ((*R*)-1-*O*-hexadecyl-2-*O*-(*p*-methoxybenzyl)-3-acetyl-glycerol). The crude product was dissolved in MeOH (40 mL) under N_2, NaOMe (140 mg, 2.592 mmol) was added and the mixture was heated to 40 °C and stirred 14 h. The mixture was neutralized by addition of conc. HCl, washed with H_2O (20 mL) and extracted with CH_2Cl_2 (3×20 mL). The combined organic phases were dried over $MgSO_4$ and after concentration *in vacuo* the residue was purified by column chromatography (1:1 heptane:EtOAc) yielding 772 mg (70% over 3 steps) of the desired product **6a** as a greasy solid. R_f = 0.46 (heptane:EtOAc 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (d, *J* = 8.6 Hz, 2H), 6.89 (d, *J* = 8.6 Hz, 2H), 4.66 (d, *J* = 11.4 Hz, 1H), 4.56 (d, *J* = 11.4 Hz, 1H), 3.81 (s, 3H), 3.78-3.50 (m, 5H), 3.44 (t, *J* = 6.7 Hz, 2H), 1.61-1.53 (m, 2H), 1.32-1.26 (m, 26H), 0.88 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.4, 130.5, 129.6 (2C), 114.0 (2C), 77.5, 72.0, 71.9, 71.3, 63.2, 55.4, 32.1, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 26.3, 22.9, 14.3. Anal ($C_{27}H_{48}O_4$): C, H. IR (KBr) 3374, 2923, 1695 1613, 1513, 1466, 1248, 1110, 824 cm^{-1}.

(*S*)-1-*O*-Octadecyl-2-*O*-(4-methoxybenzyl)-glycerol (6b)

Performed as for **6a** using **5b** (2.211 g, 4.433 mmol) to afford 1.600 g (78%) of **6b** as a greasy solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.29 (d, *J* = 8.6 Hz, 2H), 6.89 (d, *J* = 8.6 Hz, 2H), 4.66 (d, *J* = 11.4 Hz, 1H), 4.56 (d, *J* = 11.4 Hz, 1H), 3.81 (s, 3H), 3.78-3.50 (m, 5H), 3.44 (t, *J* = 6.7 Hz, 2H), 1.62-1.53 (m, 2H), 1.32-1.26 (m, 30H), 0.88 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.4, 130.5, 129.6 (2C), 114.0 (2C), 77.5, 72.0, 71.9, 71.3, 63.2, 55.4, 32.1, 29.9, 29.8, 29.8, 29.6, 29.5, 26.3, 22.8, 14.3. Anal ($C_{29}H_{52}O_4$): C, H. IR (KBr) 3524, 2916, 1709 1612, 1511, 1472, 1253, 1108, 1032, 824 cm^{-1}.

1-*O*-Hexadecyl-2-lyso-*sn*-glycero-3-phosphocholine (7a)

To a solution of $POCl_3$ (240 µL, 2.58 mmol) in anhydrous CH_2Cl_2 (10 mL) at 0 °C was added a solution of **6a** (900 mg, 2.06 mmol) and anhydrous Et_3N (375 µL, 2.68 mmol) in anhydrous CH_2Cl_2 (15 mL) dropwise over 20 min. The reaction was stirred 30 min under N_2 at 20 °C, after which anhydrous pyridine (1.30 mL, 16.5 mmol) and choline tosylate (1139 mg, 4.12 mmol) were added. The reaction was stirred for 19 h at 20 °C, then H_2O (1.3 mL) was added and stirring was continued for 40 min. Continuous concentration with ethanol:toluene 1:1 (50 mL) gave the crude product as a white foam. The residue was dissolved in THF:H_2O 9:1, slowly passed through an MB-3 column, and the solvent was removed by continuous concentration with ethanol:toluene 1:1 (50 mL). The crude product was purified by column chromatography (CH_2Cl_2:MeOH; then CH_2Cl_2:MeOH:H_2O

65:25:4) giving 860 mg (69%) of 1-*O*-hexadecyl-2-*O*-(4-methoxybenzyl)-*sn*-glycero-3-phosphocholine as an oil. R_f = 0.19 (CH_2Cl_2:MeOH:H_2O 30:15:1). ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD 4:1): δ 7.31 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 8.7 Hz, 2H), 4.65 (d, *J* = 11.5 Hz, 1H), 4.60 (d, *J* = 11.5 Hz, 1H), 4.17 (s, 2H), 4.02-3.88 (m, 2H), 3.81 (s, 3H), 3.58-3.41 (m, 7H), 3.11 (s, 9H), 1.58-1.54 (m, 2H), 1.32-1.26 (m, 26H), 0.88 (t, *J* = 6.6 Hz, 3H). 1-*O*-Hexadecyl-2-*O*-(4-methoxybenzyl)-*sn*-glycero-3-phosphocholine (50 mg, 0.083 mmol) was dissolved in CH_2Cl_2:H_2O 18:1 (5 mL) under N_2 and DDQ (38 mg, 0.166 mmol) was added. The mixture was stirred for 2 h after which it was concentrated *in vacuo* and the residue was purified by column chromatography (MeOH until the eluent was colorless, then CH_2Cl_2:MeOH:H_2O 65:25:4) to give 40 mg (99%) of **7a** as an oil. R_f = 0.10 (CH_2Cl_2:MeOH:H_2O 65:25:4). ^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD 4:1): δ 4.26 (s, 2H), 3.99-3.82 (m, 3H), 3.61-3.60 (m, 2H), 3.48-3.44 (m, 4H), 3.21 (s, 9H), 1.59-1.54 (m, 2H), 1.32-1.26 (m, 26H), 0.88 (t, *J* = 6.9 Hz, 3H).[4]

1-*O*-Octadecyl-2-lyso-*sn*-glycero-3-phosphocholine (7b)

Performed as for **7a** starting from **6b** (199 mg, 0.43 mmol) affording 124 mg (46%) of 1-*O*-octadecyl-2-*O*-(4-methoxybenzyl)-*sn*-glycero-3-phosphocholine as an oil. R_f = 0.14 (CH_2Cl_2:MeOH:H_2O 30:20:1). ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD 4:1): δ 7.31 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 8.7 Hz, 2H), 4.65 (d, *J* = 11.2 Hz, 1H), 4.60 (d, *J* = 11.2 Hz, 1H), 4.19 (s, 2H), 4.02-3.88 (m, 2H), 3.81 (s, 3H), 3.62-3.41 (m, 7H), 3.11 (s, 9H), 1.60-1-51 (m, 2H), 1.32-1.26 (m, 30H), 0.88 (t, *J* = 6.6 Hz, 3H). 1-*O*-Octadecyl-2-*O*-(4-methoxybenzyl)-*sn*-glycero-3-phosphocholine (79 mg, 0.346 mmol) was converted into 70 mg (79%) of **7b** using DDQ (79 mg, 0.35 mmol) via the same procedure as for **7a**. R_f = 0.10 (CH_2Cl_2:MeOH:H_2O 30:10:2). ^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD 4:1): δ 4.26 (s, 2H), 3.99-3.82 (m, 3H), 3.61-3.60 (m, 2H), 3.48-3.44 (m, 4H), 3.21 (s, 9H), 1.59-1.54 (m, 2H), 1.32-1.26 (m, 30H), 0.88 (t, *J* = 6.9 Hz, 3H).[4]

(*S*)-1-*O*-(4-Methoxybenzoyl)-glycerol (9)

$K_2OsO_4 \cdot 2H_2O$ (271 mg, 0.74 mmol), $(DHQD)_2PHAL$ (687 mg, 0.88 mmol), $K_3Fe(CN)_6$ (72.6 g, 220.6 mmol) and K_2CO_3 (30.5 g, 220.6 mmol) were dissolved in a mixture of ^{t}BuOH (370 mL) and H_2O (370 mL) under an atmosphere of N_2 and stirred for 15 min at 20 °C, after which **8** (14.12 g, 73.5 mmol) was added. After 2 h excess reagent was quenched by addition of Na_2SO_3 (111 g, 0.88 mol) and the organic layer was isolated by extraction with EtOAc (3×500 mL). Toluene (200 mL)

[4] Andresen, T. L.; Jensen, S. S.; Madsen, R.; Jørgensen, K. Synthesis and Biological Activity of Anticancer Ether Lipids That Are Specifically Released by Phospholipase A_2 in Tumor Tissue. *J. Med. Chem.* **2005**, *48*, 7305-7314.

was added to the combined organic phases and concentration *in vacuo* afforded **9** (12.97 g, 57.3 mmol, 78%) as a white solid. The enantiomeric excess (97%) was determined from chiral HPLC. HPLC (chiral) Chiralpak AS-H at 20 °C, λ= 254 nm, hexane:2-propanol 75:25, retention times 17.8 min (*S*), 23.4 min *(R)* at 0.4 mL/min flow rate. R_f = 0.49 (EtOAc). ^{1}H NMR (300 MHz, d_6-acetone): δ 8.00 (d, *J* = 9.0 Hz, 2H), 7.02 (d, *J* = 9.0 Hz, 2H), 4.36 (dd, *J* = 11.2, 4.5 Hz, 1H), 4.27 (dd, *J* = 11.2, 6.2 Hz, 1H), 4.01-3.92 (m, 1H), 3.88 (s, 3H), 3.65-3.63 (m, 2H).[5]

(*R*)-1,2-Di-*O*-*tert*-butyldimethylsilyl-glycerol (10)

The diol (**9**) (7.35g, 32.5 mmol) was dissolved in CH_2Cl_2 (100 mL), cooled to 0 °C and diisopropylethylamine (23 mL, 130 mmol) was added. Then, *tert*-butyldimethylsilyl triflate (20 mL, 87 mmol) was added dropwise and the mixture was stirred for 2 h before another portion *tert*-butyldimethylsilyl triflate was added (6.0 mL, 26 mmol). After stirring for 48 h at 4 °C excess reagent was quenched with MeOH (10 mL), the mixture was stirred for 1 h, washed with water (100 mL) and sat. aq. $NaHCO_3$ (100 mL), dried over $MgSO_4$ and concentrated. The residue was filtered through a plug of silica (CH_2Cl_2:heptane 1:1) and concentrated, then taken up in CH_2Cl_2 (125 mL) and cooled to -78 °C before diisobutylaluminum hydride in hexane (65 mL, 1 M, 65 mmol) was added dropwise during 15 min. The mixture was stirred for 75 min before MeOH (4 mL) was added and stirring was continued for 1 h. Sat. aq. Rochelle´s salt (50 mL) was added and the mixture was allowed to reach 20 °C, diluted with water (200 mL) and extracted with EtOAc (4×100 mL). The combined organic extracts were dried over Na_2SO_4, concentrated and the residue was purified by column chromatography (CH_2Cl_2) affording the title compound as a clear oil (3.30 g, 63%).

$[\alpha]^D$: +18.5° (*c* 0.6, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.80-3.73 (m, 1H), 3.69-3.52 (m, 4H), 2.12 (dd, *J* = 7.6, 5.1 Hz, 1H), 0.89 (s, 18H), 0.09 (s, 3H), 0.08 (s, 3H), 0.06 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 72.5, 64.9, 64.8, 25.9 (3C), 25.8 (3C), 18.3, 18.1, -4.6, -4.9, -5.4, -5.5. IR (neat): 3442, 2929, 1472, 1257, 1095, 1005, 836 cm^{-1}. m/z ($M+Na^+$) 343.21.

[5] Corey, E. J.; Guzman-Perez, A.; Noe, M. C. The Application of a Mechanistic Model Leads to the Extension of the Sharpless Asymmetric Dihydroxylation to Allylic 4-Methoxybenzoates and Conformationally Related Amine and Homoallylic Alcohol Derivatives. *J. Am. Chem. Soc.* **1995**, *117*, 10805-10816.

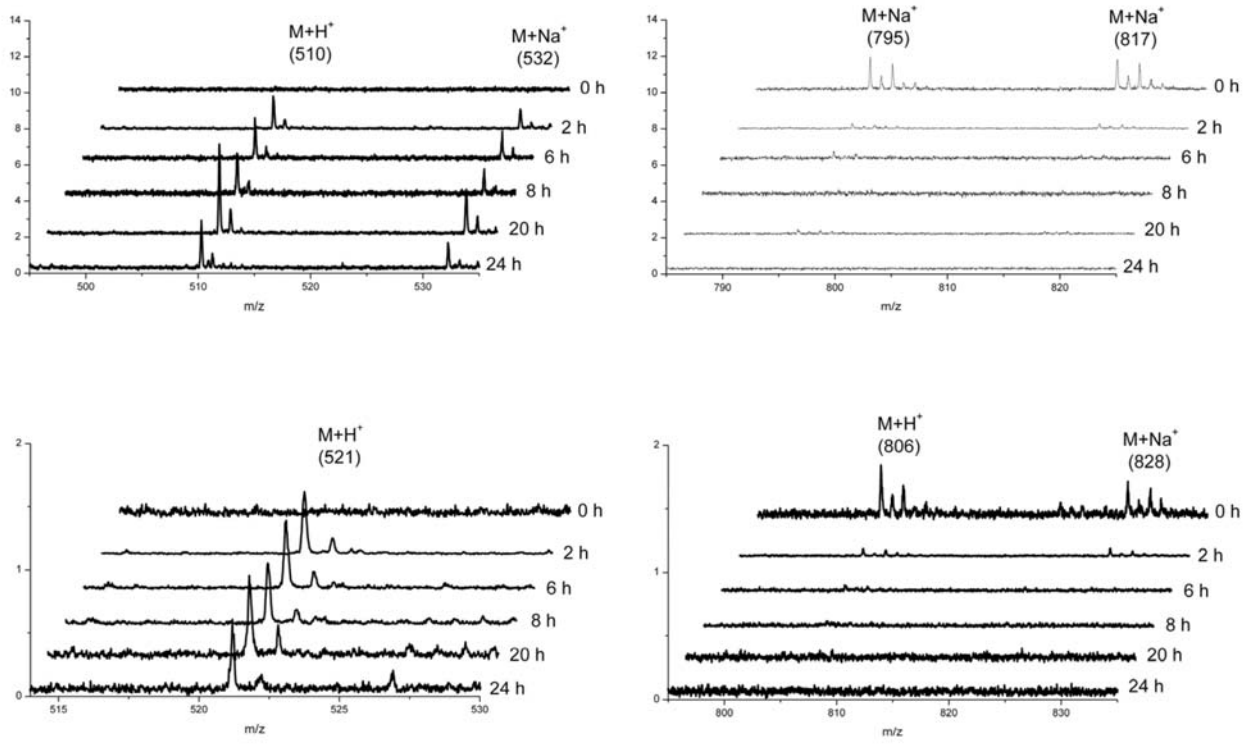


Figure S1. MALDI-TOF MS monitoring of $sPLA_2$ activity on chlorambucil AEL prodrug **1b** (top) and **2b** (bottom). The spectra demonstrates that the prodrugs (right) are consumed and the AELs (left) are released.

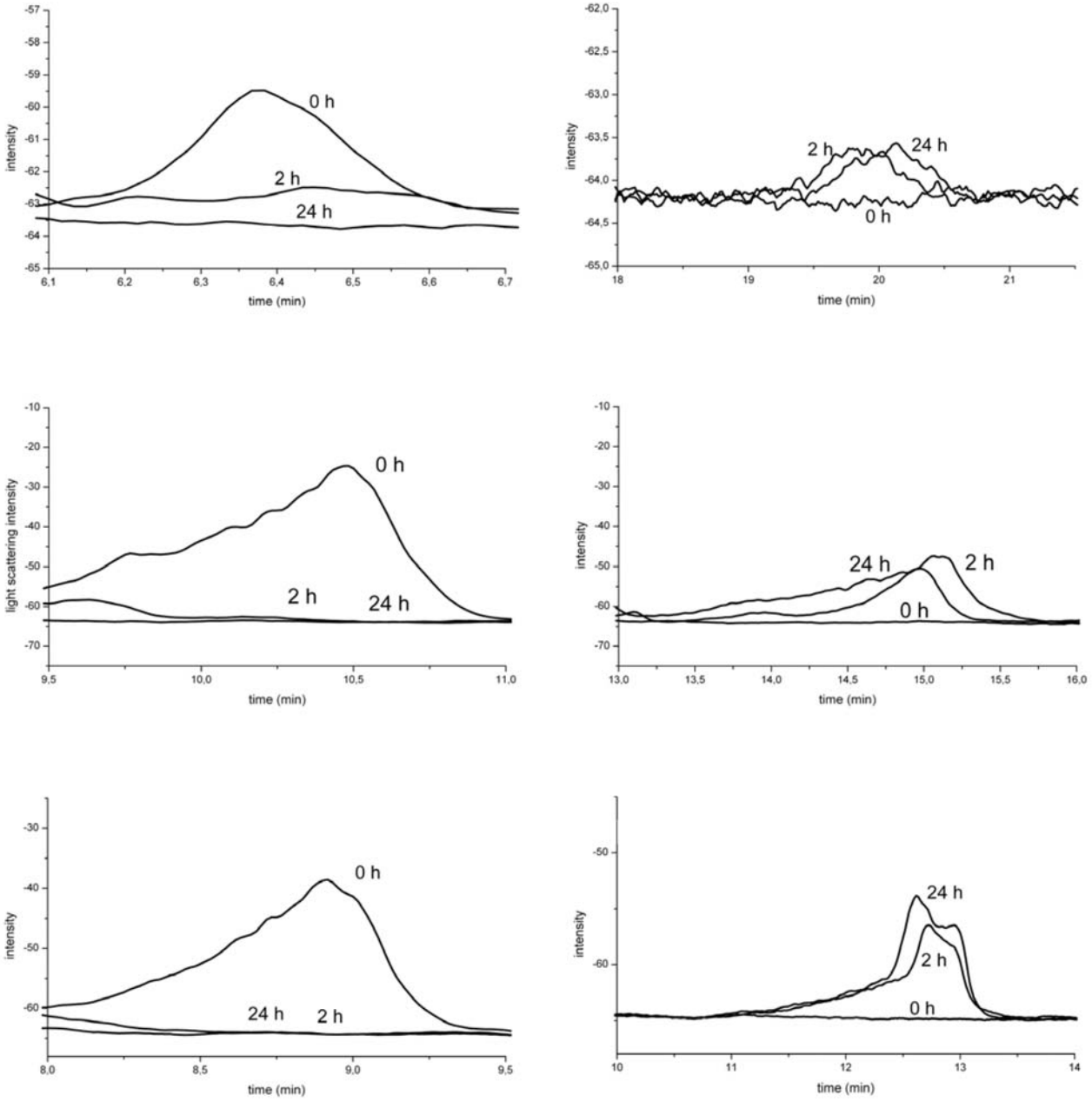


Figure S2. HPLC chromatograms for the $sPLA_2$ experiment on chlorambucil AEL prodrugs **1b**, **2a** and **2b** showing the amount of prodrug (left) and AEL (right) before the addition of the enzyme and after 2 and 24 h.

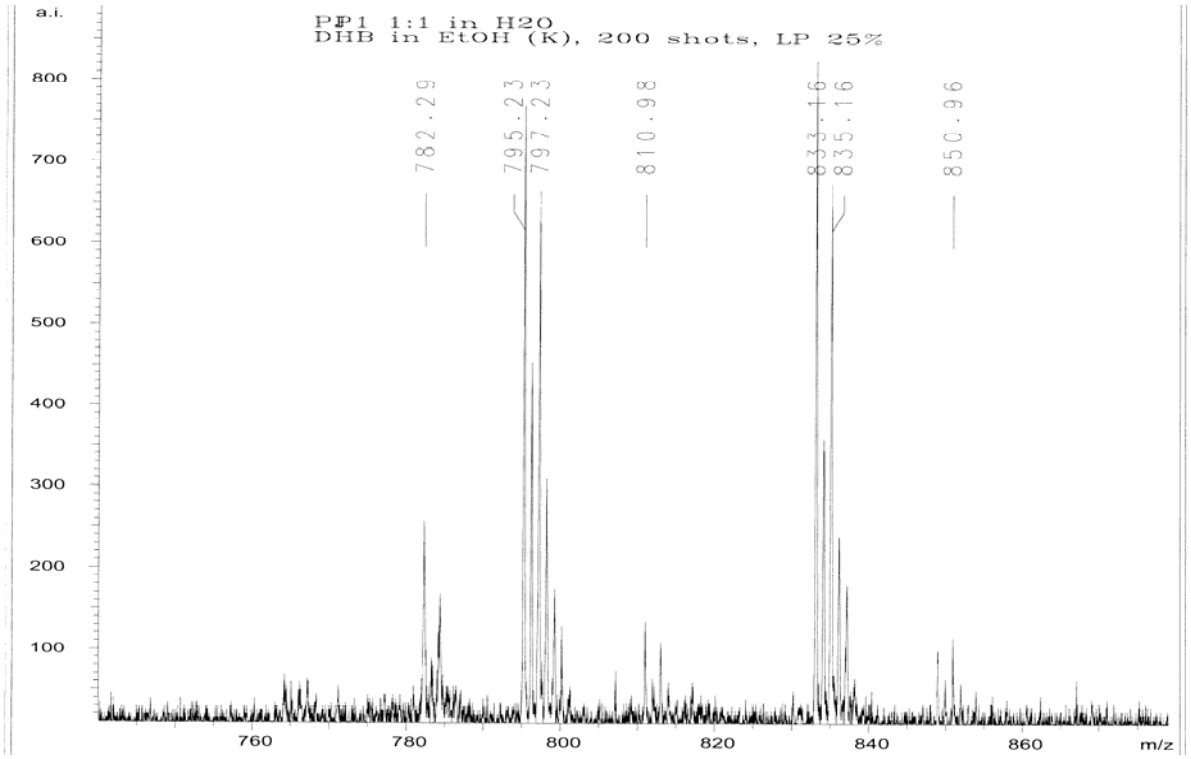


Figure S3. MALDI-TOF MS of prodrug **1b** after 42 days at 20 °C showing the major peaks at 795/797 [M+H] and 833/835 [M+K].

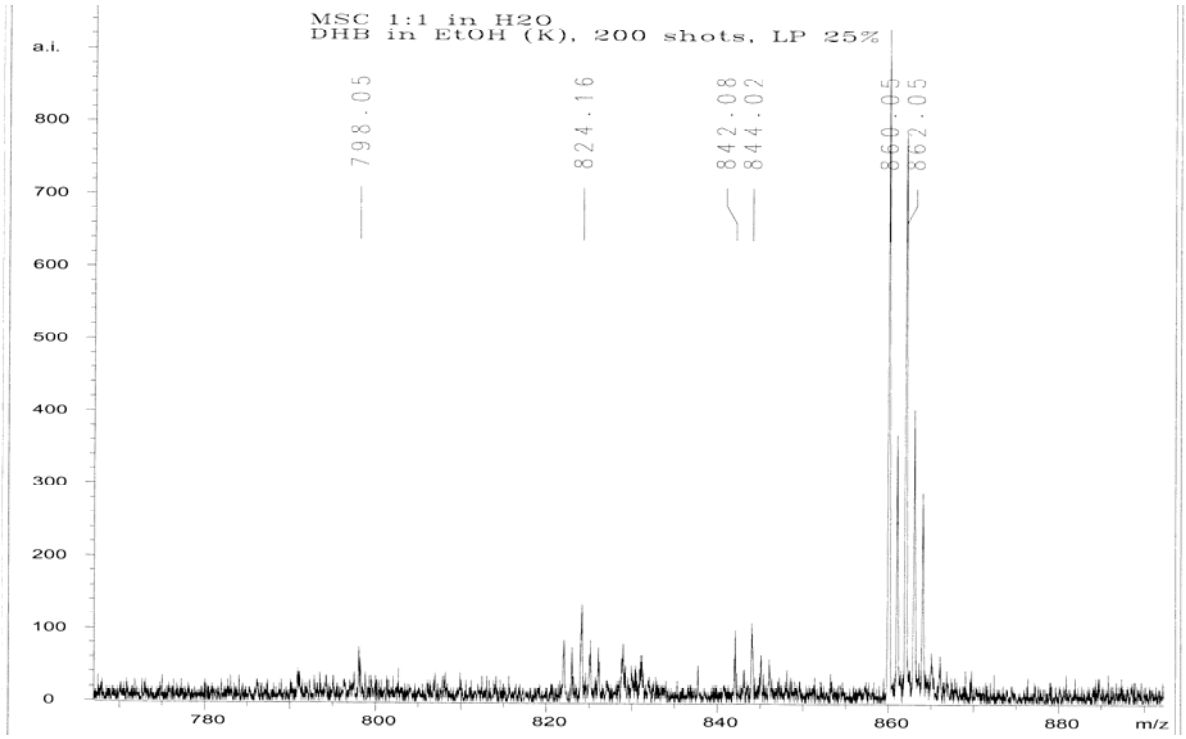


Figure S4. MALDI-TOF MS of prodrug **2b** after 42 days at 20 °C showing the major peaks at 822/824 [M+H+K] and 860/862 [M+2K].

(*R*)-1-[(*S*)-Methoxytrifluoromethylphenylacetyl]-2,3-bis(*tert*-butyldimethylsilyl)glycerol (Mosher (*S*)-ester)

The alcohol (**10**) (80 mg, 0.25 mmol) was dissolved in pyridine (0.5 mL) and added to the cold commercial ampule with (*R*)-methoxytrifluoromethylphenylacetyl chloride (100 mg, 0.40 mmol) and shaken. After standing for 1 h the mixture was poured into sat. aq. $NaHCO_3$ (5 mL), which was subsequently extracted with CH_2Cl_2 (3×5 mL) and the organic phase dried over Na_2SO_4. After drying *in vacuo* the crude ester (116 mg, 87%) was subjected to NMR analysis.

IR (neat): 2954, 2930, 2886, 2858, 1753, 1253, 1169, 1103, 1023, 991 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ 7.55-7.37 (m, 5 H), 4.58 (dd, *J* = 3.8, 11.2 Hz, 1H), 4.17 (dd, *J* = 4.6, 11.2 Hz, 1H), 3.89-3.82 (m, 1H), 3.54-3.48 (m, 5H), 0.87 (s, 9H), 0.85 (s, 9H), 0.02-0.01 (m, 12H).

^{19}F NMR (282 MHz, $CDCl_3$): δ -72.21 (s).

(*R*)-1-[(*R*)-Methoxytrifluoromethylphenylacetyl]-2,3-bis(*tert*-butyldimethylsilyl)glycerol (Mosher (*R*)-ester)

The alcohol (**10**) (80 mg, 0.25 mmol) was dissolved in pyridine (0.5 mL) and added to the cold commercial ampule with (*S*)-methoxytrifluoromethylphenylacetyl chloride (100 mg, 0.40 mmol) and shaken. After standing for 1 h the mixture was poured into sat. aq. $NaHCO_3$ (5 mL), which was subsequently extracted with CH_2Cl_2 (3×5 mL) and the organic phase dried over Na_2SO_4. After drying *in vacuo* the crude ester (152 mg, quantitative) was subjected to NMR analysis.

IR (neat): 2954, 2930, 2886, 2858, 1752, 1253, 1168, 1103, 1023, 989 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ 7.43-7.23 (m, 5H), 4.43 (dd, *J* = 3.8, 11.2 Hz, 1H), 4.03 (dd, *J* = 3.8, 11.2 Hz, 1H), 3.80-3.73 (m, 1H), 3.49-3.32 (m, 5H), 0.72 (s, 9H), 0.71 (s, 9H), -0.06 (s, 6H), -0.14 (s, 6H).

^{19}F NMR (282 MHz, $CDCl_3$): δ -71.99 (s).

Table S1. Combustion analysis and HRMS values.

Compound	Formula	Anal. Calc'd	Anal. found	HRMS calc'd	HRMS found
6a	$C_{27}H_{48}O_4$	C 74.26, H 11.08	74.26, 11.03		
6b	$C_{29}H_{52}O_4$	C 74.95, H 11.28	75.08, 11.24		
1a	$C_{38}H_{69}Cl_2N_2O_7P$			$[M+H^+]$ 767.4298	767.4312
1b	$C_{40}H_{73}Cl_2N_2O_7P$			$[M+Na^+]$ 817.4425	817.4430
10	$C_{15}H_{36}O_3Si_2$			$[M+Na^+]$ 343.2096	343.2110
11	$C_{24}H_{53}N_2O_4PSi_2$			$[M+Na^+]$ 543.3174	543.3186
12a	$C_{48}H_{92}Cl_2NO_9PSi_2$			$[M+Na^+]$ 1006.5317	1006.5348
12b	$C_{51}H_{96}Cl_2NO_9PSi_2$			$[M+Na^+]$ 1034.5631	1034.5665
2a	$C_{36}H_{63}Cl_2NNaO_9P$			$[M+H^+]$ 778.3588	778.3612
2b	$C_{38}H_{67}Cl_2NNaO_9P$			$[M+Na^+]$ 828.3721	828.3681

Alkylating assay[6]

The chlorambucil AEL prodrugs (2 mM) were hydrated in an aqueous buffer (0.15 M KCl, 30 μM $CaCl_2$, 10 μM EDTA, 10 mM HEPES, pH = 7.5) for 1 h at 60 °C, and then sonicated for 1 h at 60 °C providing a clear solution. The formulated chlorambucil AEL prodrugs (0.40 mL, 2 mM) were diluted in an aqueous buffer (2.1 mL, 0.15 M KCl, 30 μM $CaCl_2$, 10 μM EDTA, 10 μM HEPES, pH = 7.5) and 4-nitrobenzyl pyridine (63 mg, 0.29 mmol) was added and the mixture was stirred at 37 °C. When $sPLA_2$ was used to degrade the liposomes, the catalytic reaction was initiated by addition of snake (*Agkistrodon piscivorus piscivorus*) venom $sPLA_2$ (20 μL, 42 μM). The purified snake venom $sPLA_2$ was donated by Dr. R. L. Biltonen (University of Virginia, VA, USA). After 1.5 h incubation 2-amino-1-propanol in *tert*-butyl alcohol (25% v/v, 3.0 mL) was added and the coloration of the reaction mixture was measured using a visible spectrophotometer (PerkinElmer, Lambda 25 UV/VIS Spectrophotometer) at 450-700 nm with a maximum absorption at 560 nm for the colored pigment (Figures S6 and S7). Measurements of pure chlorambucil samples in the aqueous buffer (HEPES buffer) with and without 4-nitrobenzyl pyridine, of HEPES buffer with and without $sPLA_2$ and of pure 4-nitrobenzyl pyridine in HEPES buffer were obtained as controls (Figure S5).

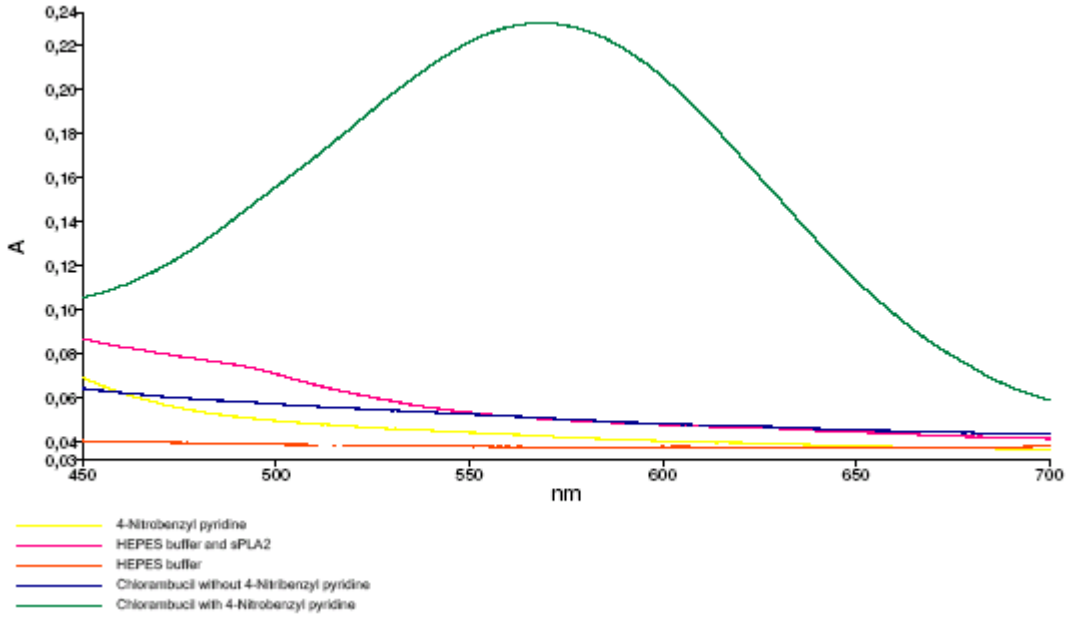


Figure S5: Alkylating assay control experiments, which illustrate that only the combination of chlorambucil and 4-nitrobenzyl pyridine gives absorption at 560 nm.

[6] a) Epstein, J.; Rosenthal, R. W., Ess, R. J. Use of p-(4-nitrobenzyl)pyridine as analytical reagent for ethylenimines and alkylating agents. *Anal. Chem.* **1955**, *27*, 1435-1439. b) Friedman, O. M.; Boger, E.; Chlorimetric estimation of nitrogen mustard in aqueous media. *Anal. Chem.* **1961**, *33*, 906-910. c) Genka, S.; Deutsch, J.; Shetty, U. H.; Stahle, P. L.; John, V.; Lieberburg, I. M.; Ali-Osmant, F.; Rapoport, S. I.; Greig, N. H. Development of lipophilic anticancer agents for the treatment of brain tumors by the esterification of water-soluble chlorambucil. *Clin. Exp. Metastasis,* **1993**, *11*, 131-140.

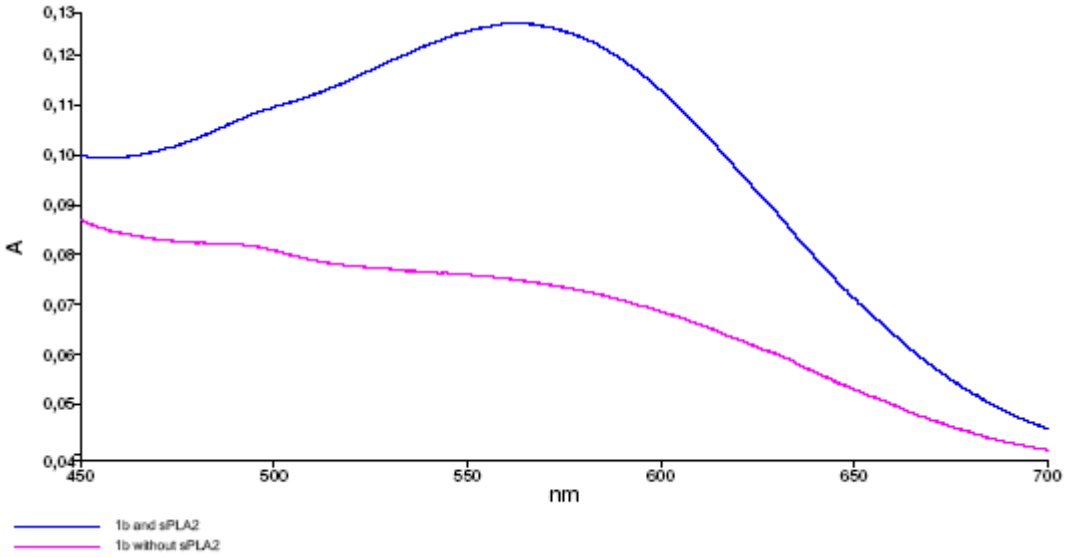


Figure S6: Alkylating activity of AEL prodrug **1b** with and without $sPLA_2$. When AEL prodrug **1b** was subjected to $sPLA_2$ it provided a colored solution, with a strong absorption at 560 nm, illustrating alkylating activity, whereas AEL prodrug **1b** without subjection of $sPLA_2$ provided a colorless solution, with a weak absorption at 560 nm, illustrating that the liposome formulation shield the chloroethyl moiety of **1b**.

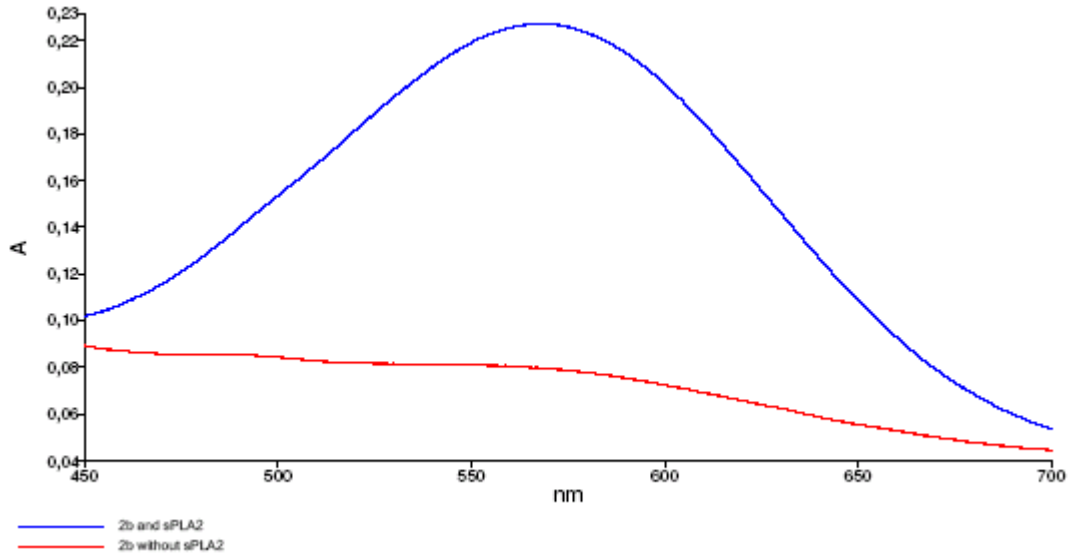
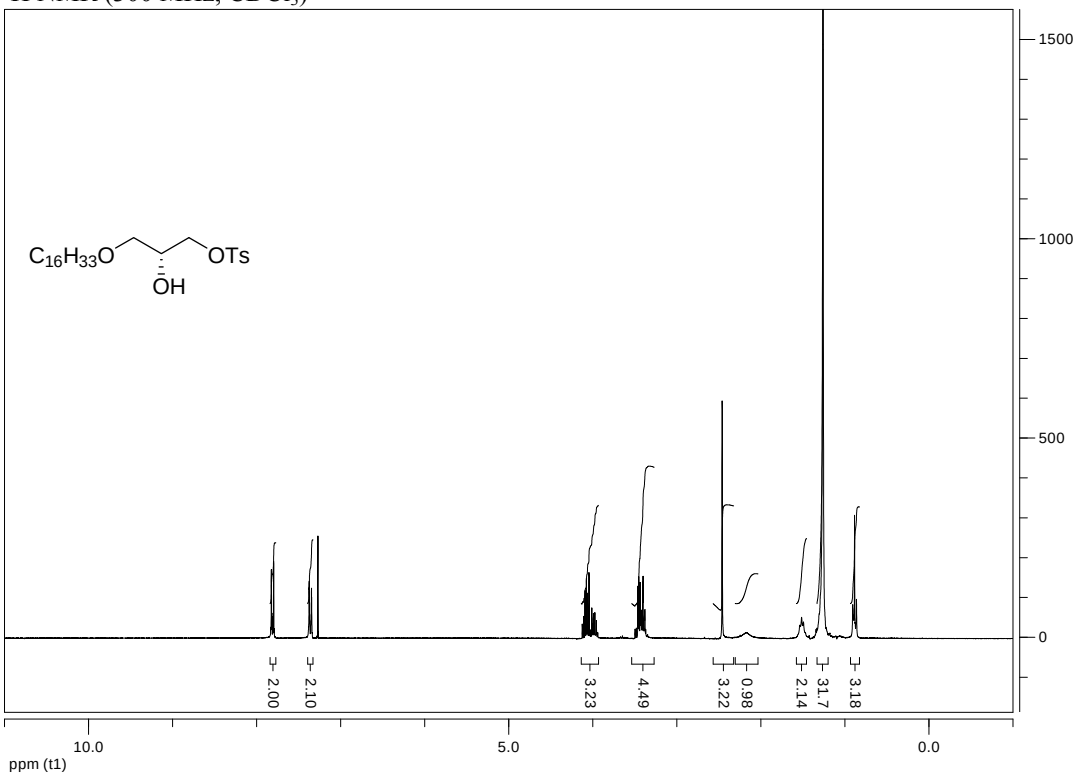


Figure S7: Alkylating activity of AEL prodrug **2b** with and without subjection of $sPLA_2$. When AEL prodrug **1b** was subjected to $sPLA_2$ it provided a colored solution, with a strong absorption at 560 nm, illustrating alkylating activity, whereas AEL prodrug **2b** without subjection of $sPLA_2$ provided a colorless solution, with a weak absorption at 560 nm, illustrating that the liposome formulation shield the chloroethyl moiety of **2b**.

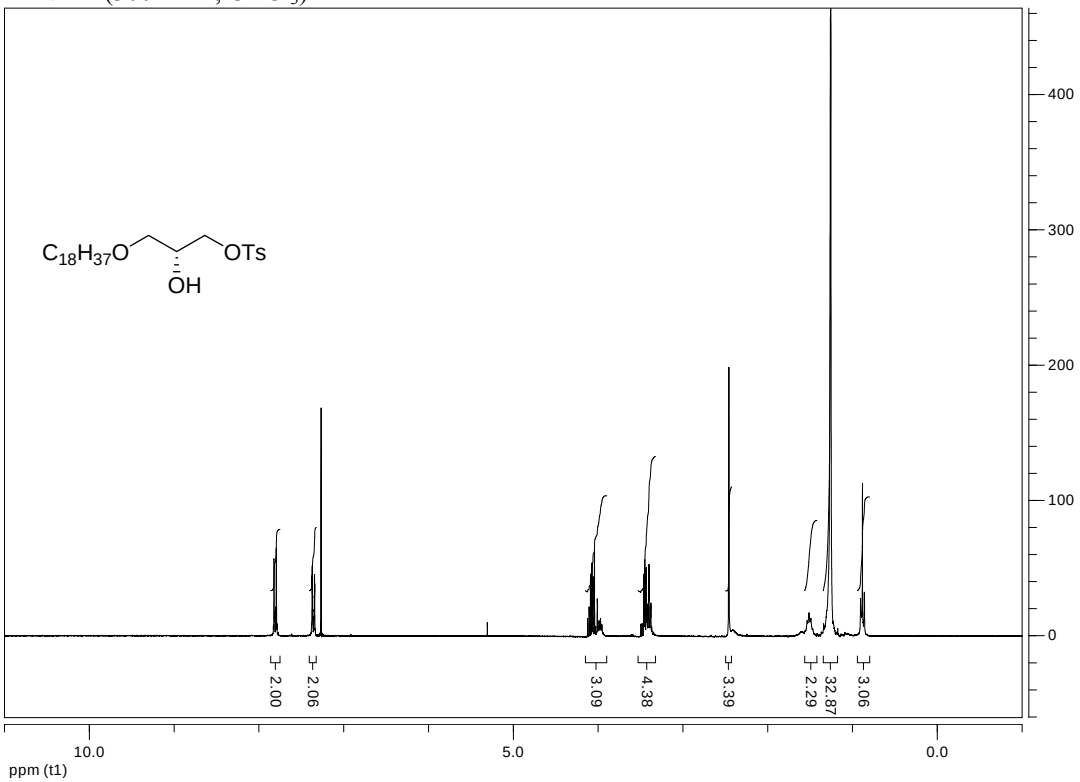
(*R*)-1-*O*-Hexadecyl-3-(*p*-toluenesulfonyl)-glycerol (5a)

^{1}H NMR (300 MHz, $CDCl_3$)



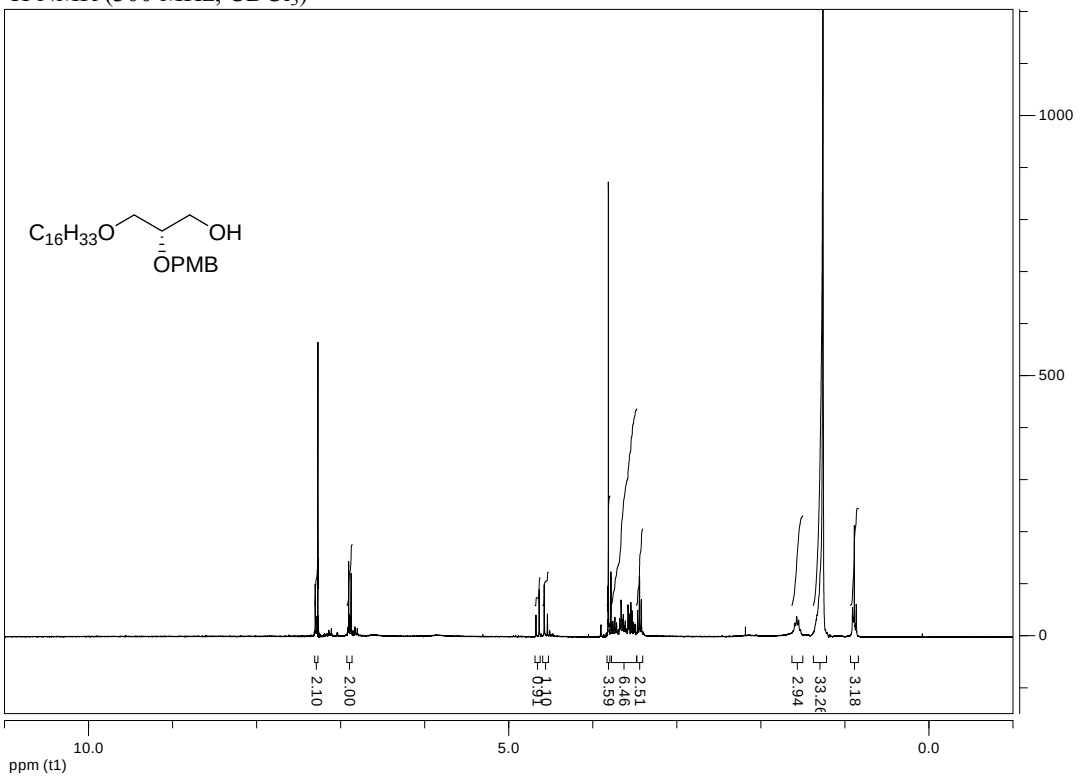
(*R*)-1-*O*-Octadecyl-3-(*p*-toluenesulfonyl)-glycerol (5b)

^{1}H NMR (300 MHz, $CDCl_3$)

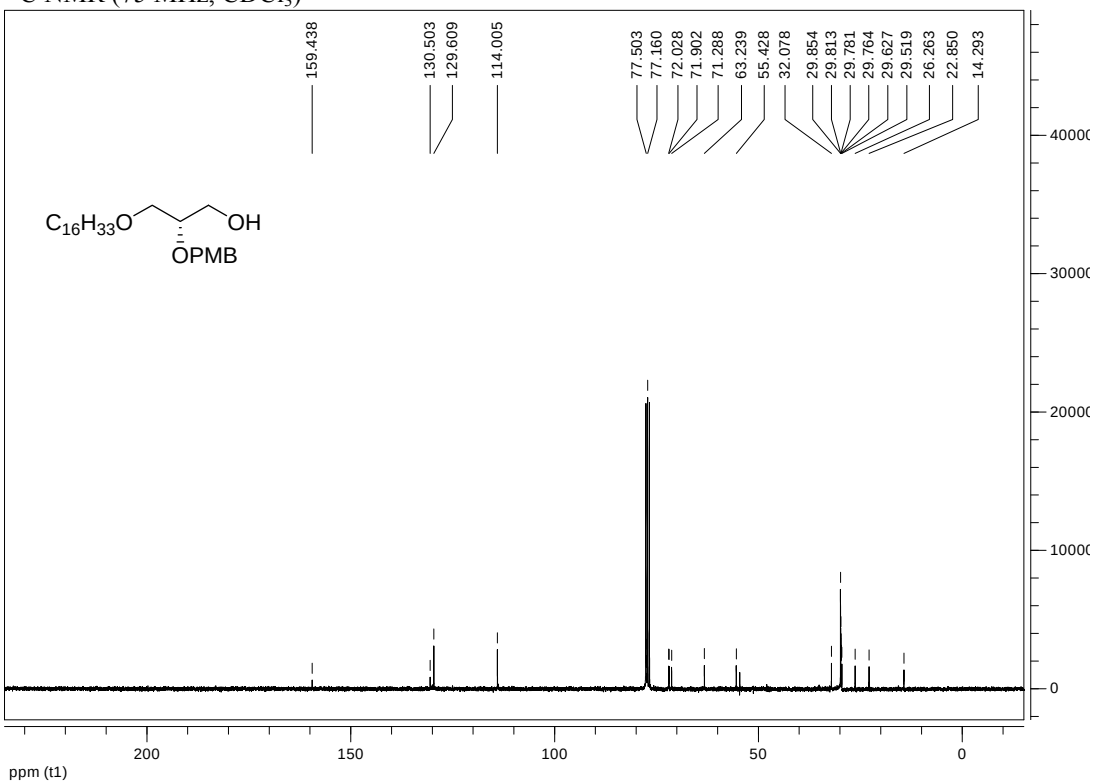


(*S*)-1-*O*-Hexadecyl-2-*O*-(4-methoxybenzyl)-glycerol (6a)

^{1}H NMR (300 MHz, $CDCl_3$)

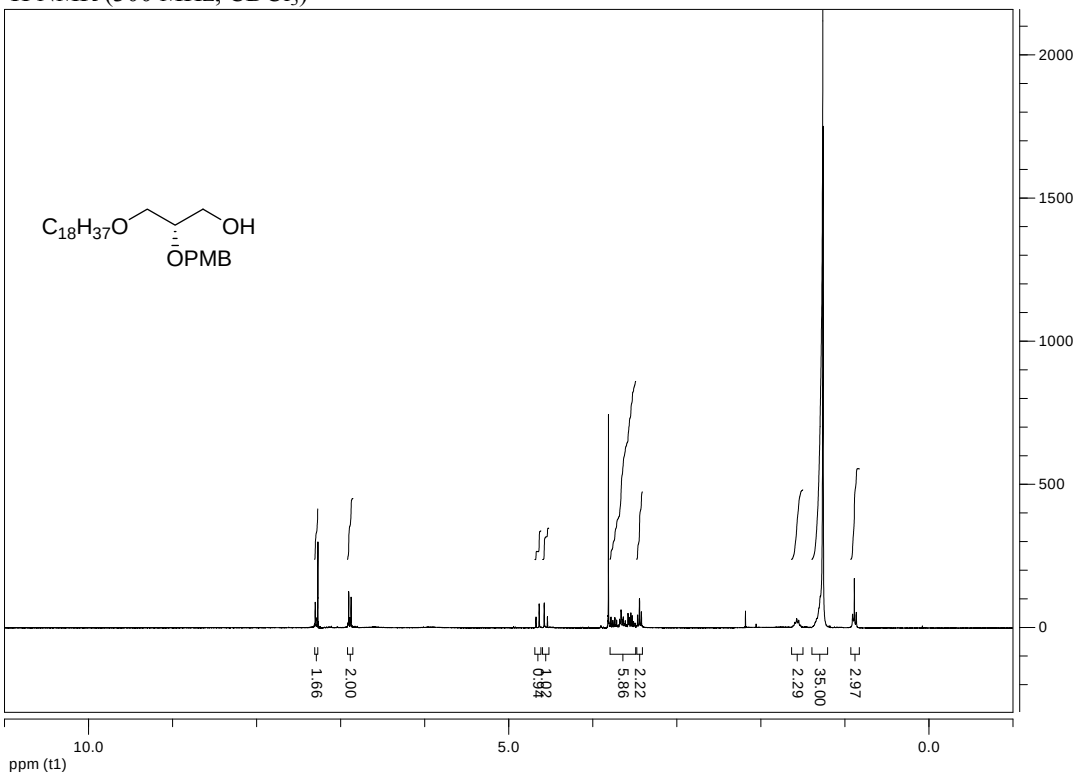


^{13}C NMR (75 MHz, $CDCl_3$)

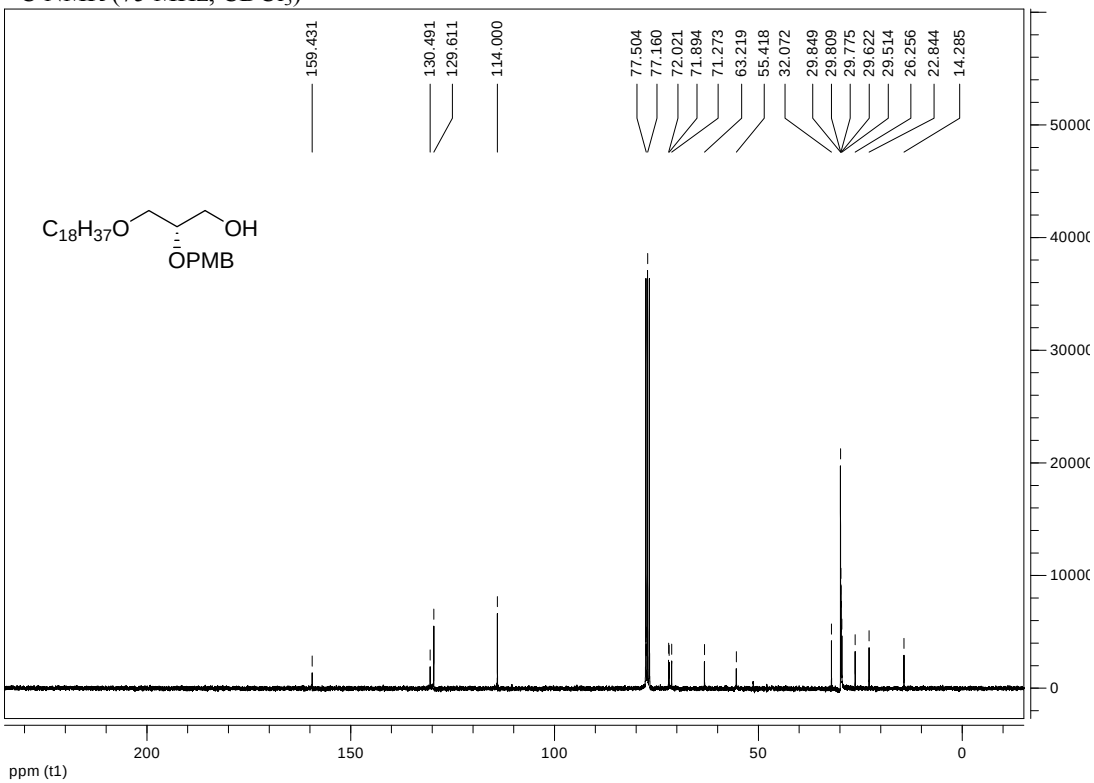


(*S*)-1-*O*-Octadecyl-2-*O*-(4-methoxybenzyl)-glycerol (6b)

^{1}H NMR (300 MHz, $CDCl_3$)

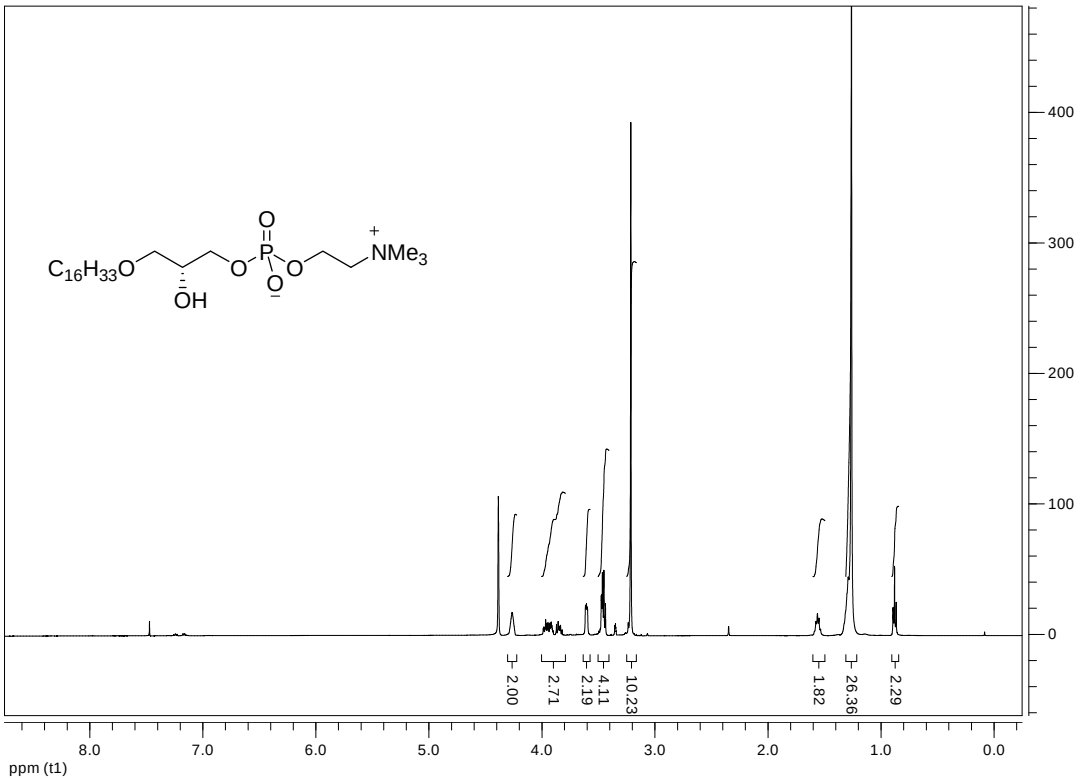


^{13}C NMR (75 MHz, $CDCl_3$)



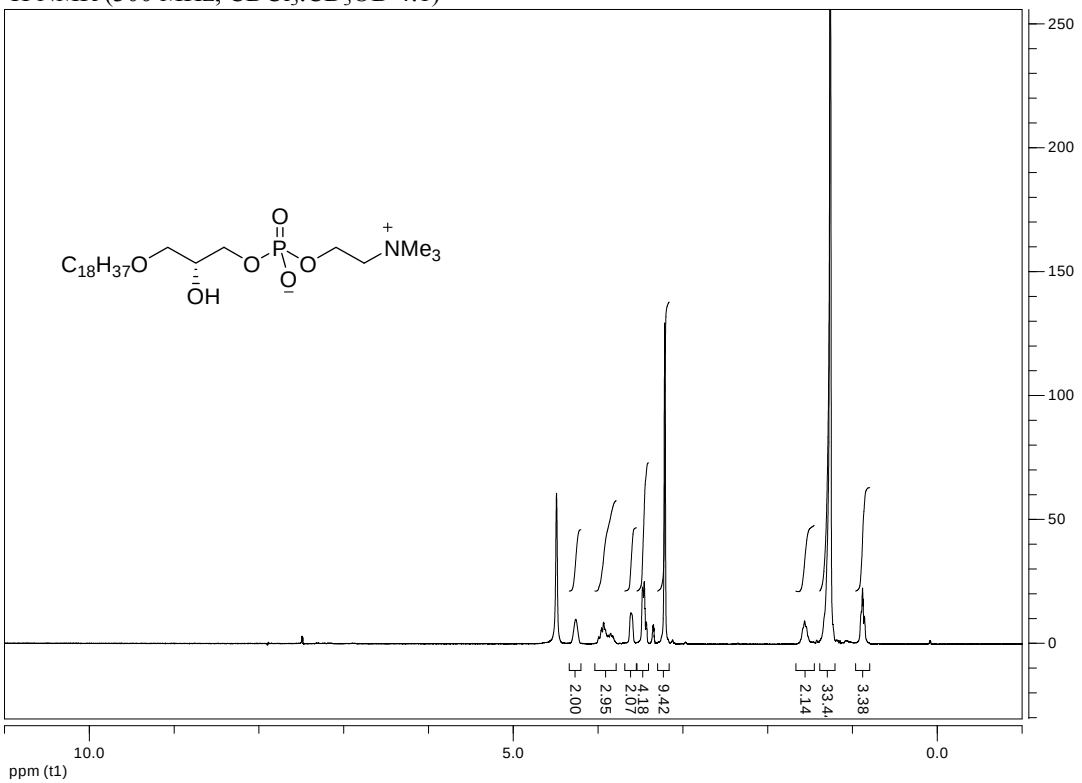
1-*O*-Hexadecyl-2-lyso-*sn*-glycero-3-phosphocholine (7a)

^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD 4:1)



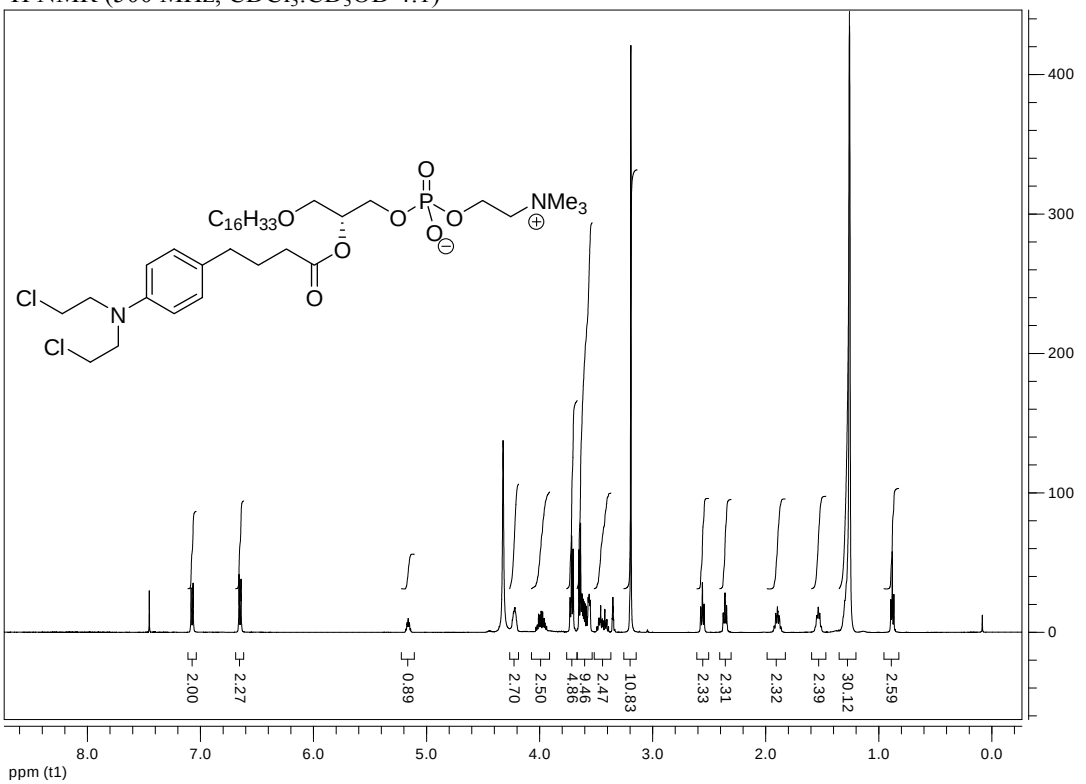
1-*O*-Octadecyl-2-lyso-*sn*-glycero-3-phosphocholine (7b)

^{1}H NMR (300 MHz, $CDCl_3$:CD_3OD 4:1)

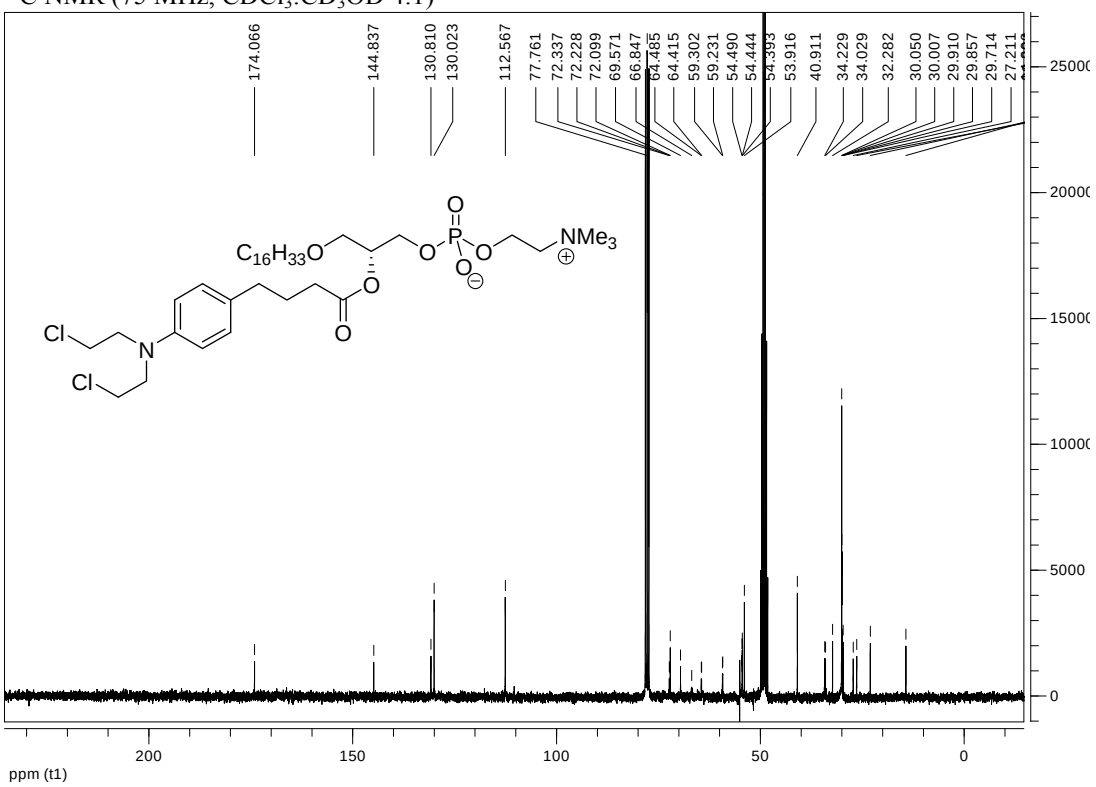


1-*O*-Hexadecyl-2-(4-(4-(*bis*-(2-chloroethyl)-amino)-phenyl)-butanoyl)-*sn*-glycero-3-phosphocholine (1a)

^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD 4:1)

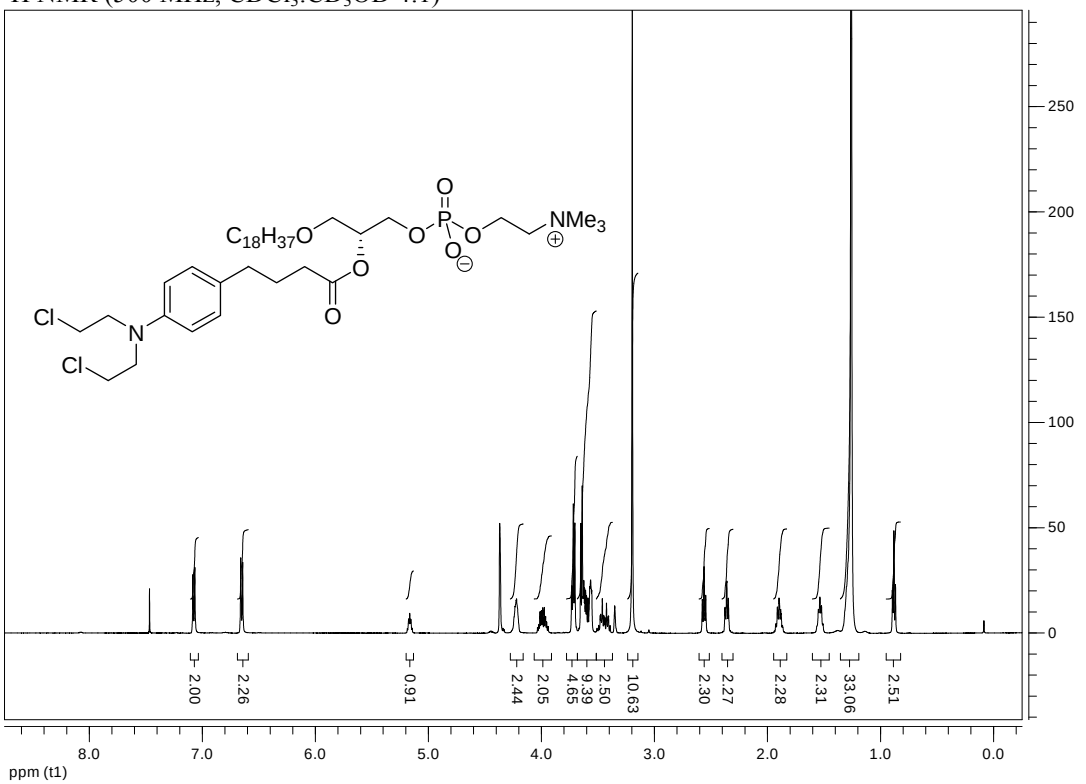


^{13}C NMR (75 MHz, $CDCl_3$:CD_3OD 4:1)

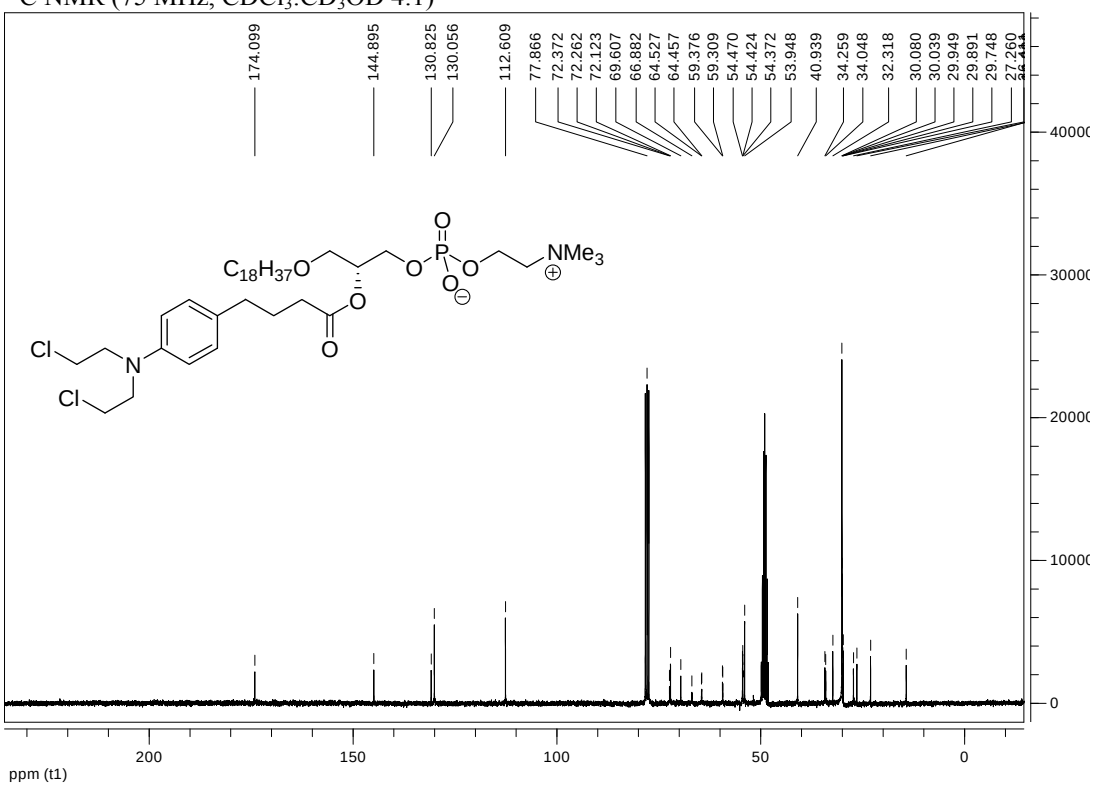


1-*O*-Octadecyl-2-(4-(4-(*bis*-(2-chloroethyl)-amino)-phenyl)-butanoyl)-*sn*-glycero-3-phosphocholine (1b)

^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD 4:1)

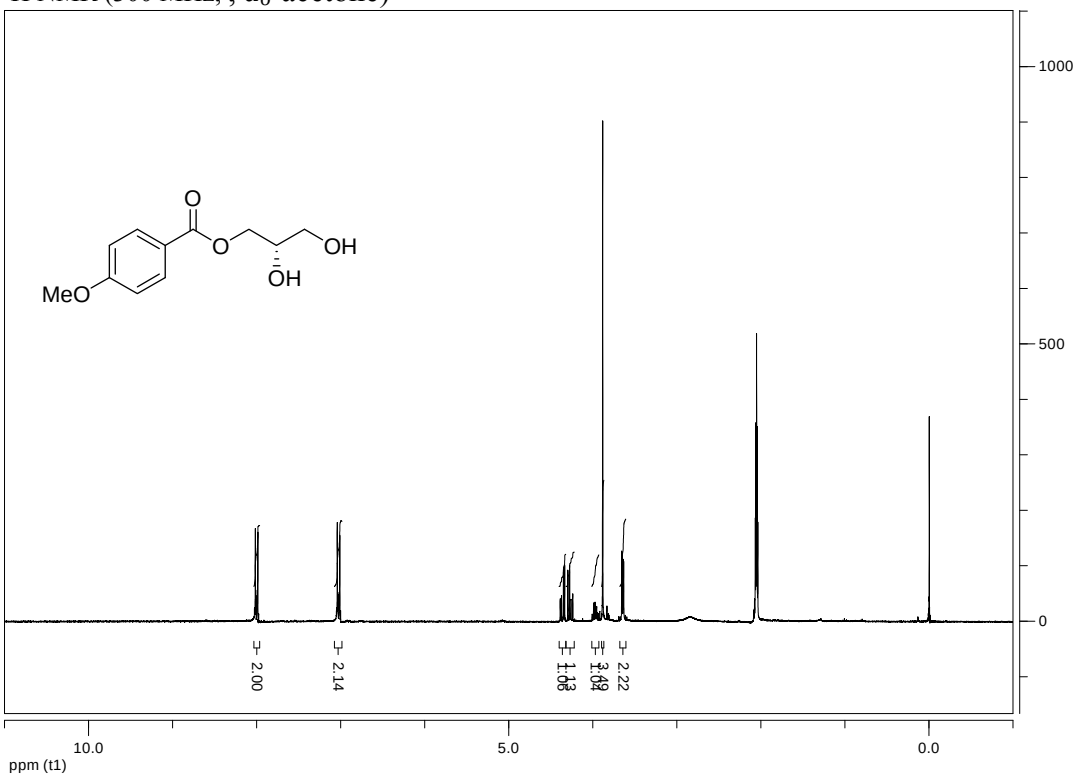


^{13}C NMR (75 MHz, $CDCl_3$:CD_3OD 4:1)



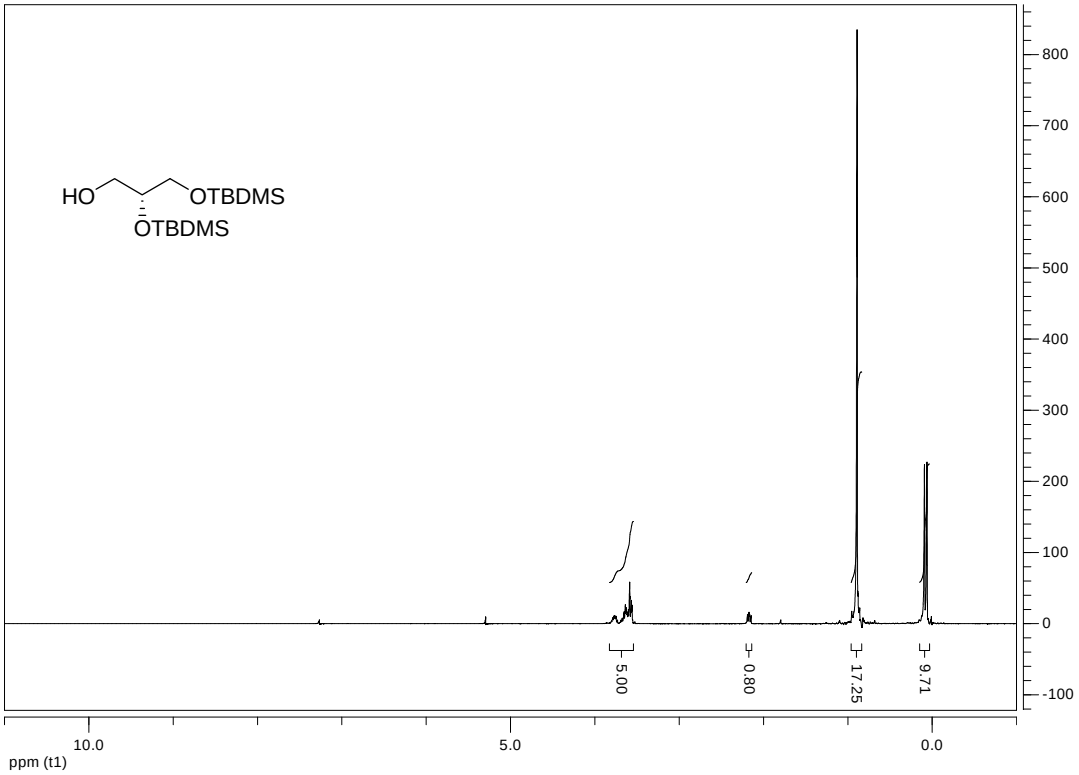
(*S*)-1-*O*-(4-Methoxybenzoyl)-glycerol (9)

^{1}H NMR (300 MHz, , d_6-acetone)

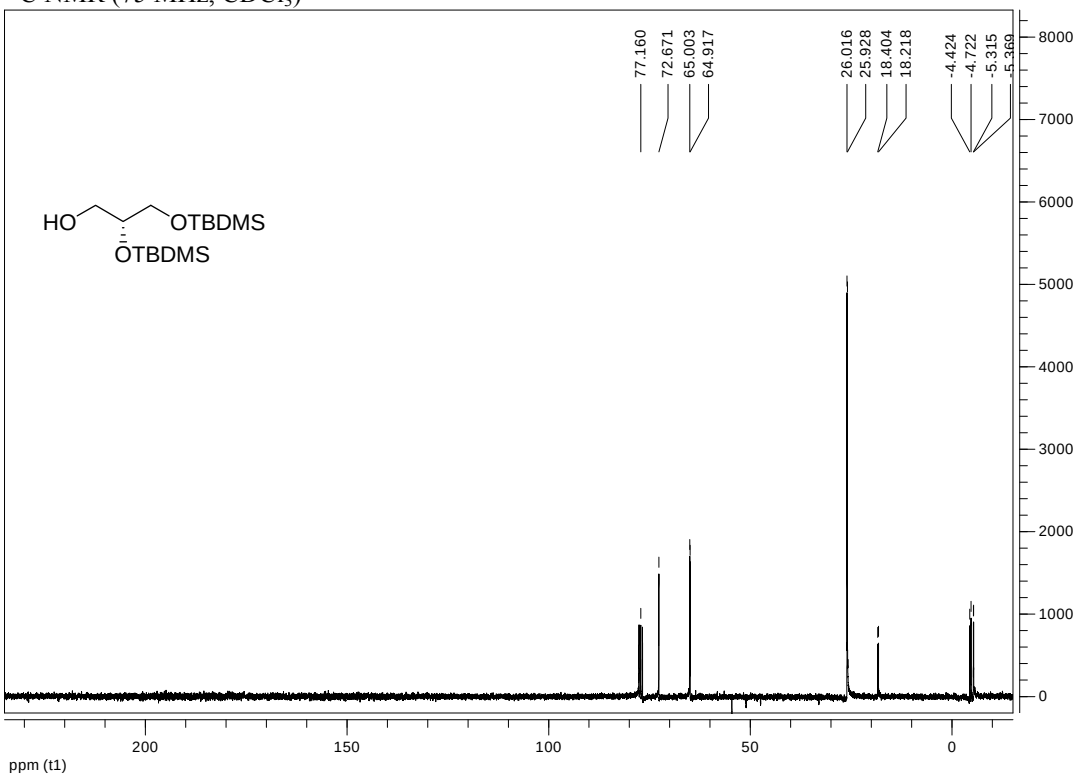


(*R*)-1,2-di-*O*-*tert*-butyldimethylsilyl-glycerol (10)

^{1}H NMR (300 MHz, $CDCl_3$)

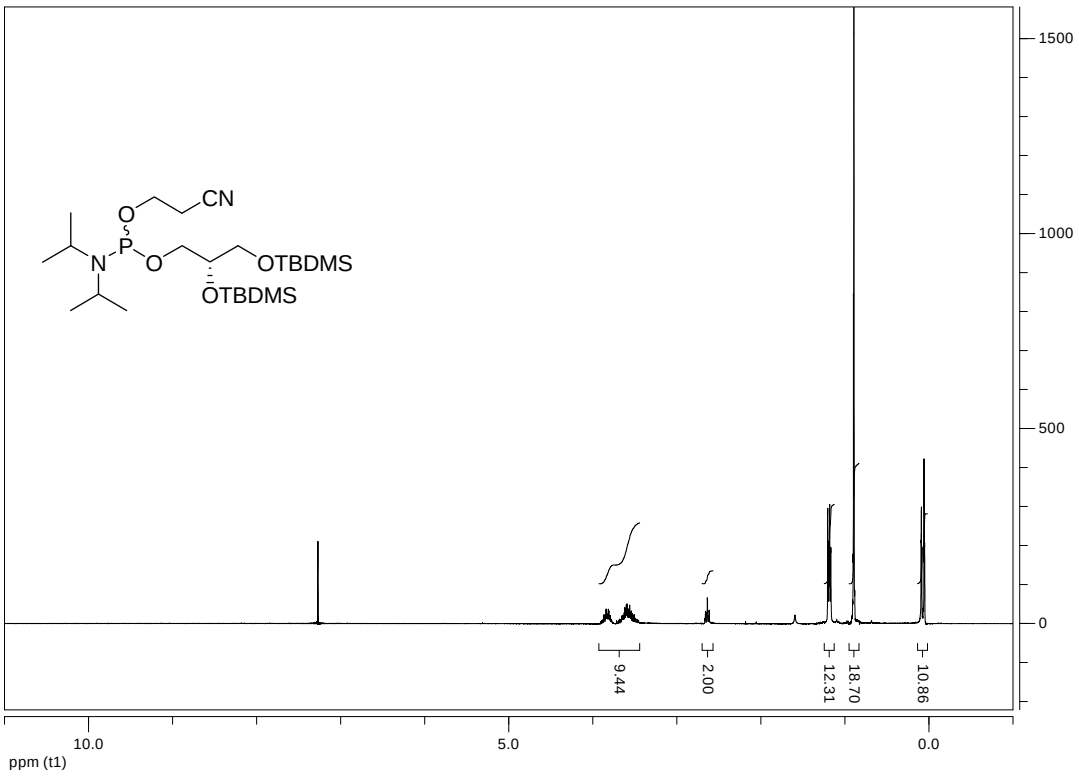


^{13}C NMR (75 MHz, $CDCl_3$)

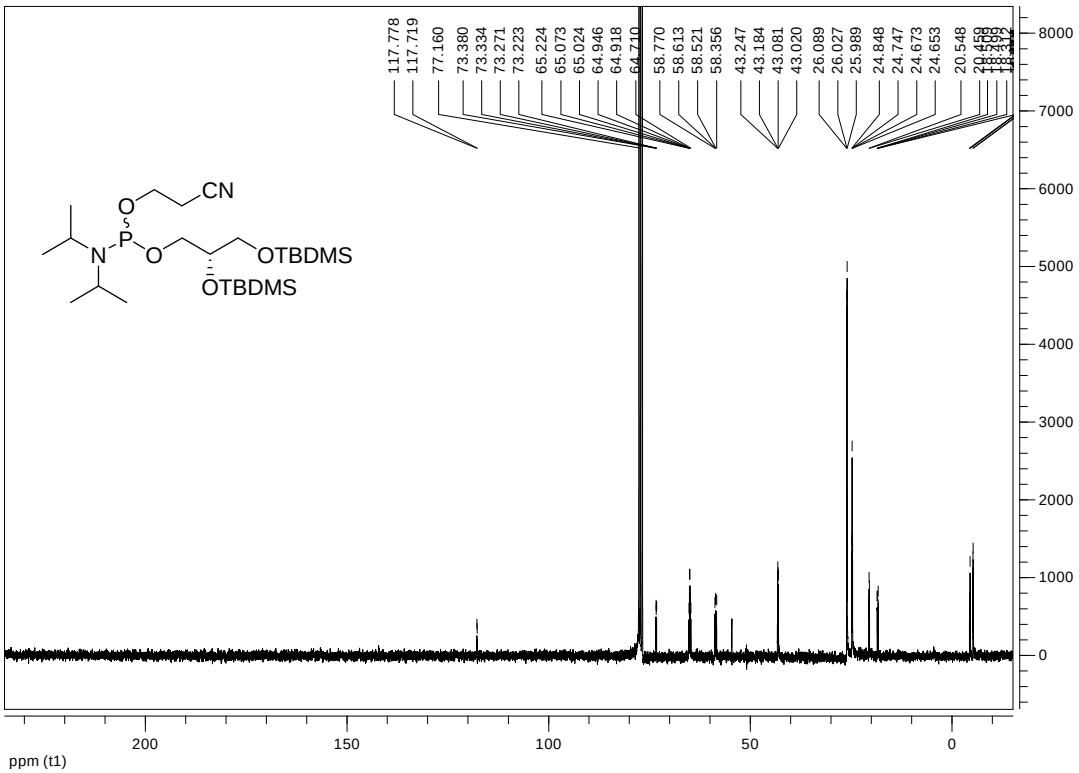


(*S*)-(2,3-di-*O*-*tert*-butyldimethylsilyl)-glyceryl 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidite (11)

^{1}H NMR (300 MHz, $CDCl_3$)

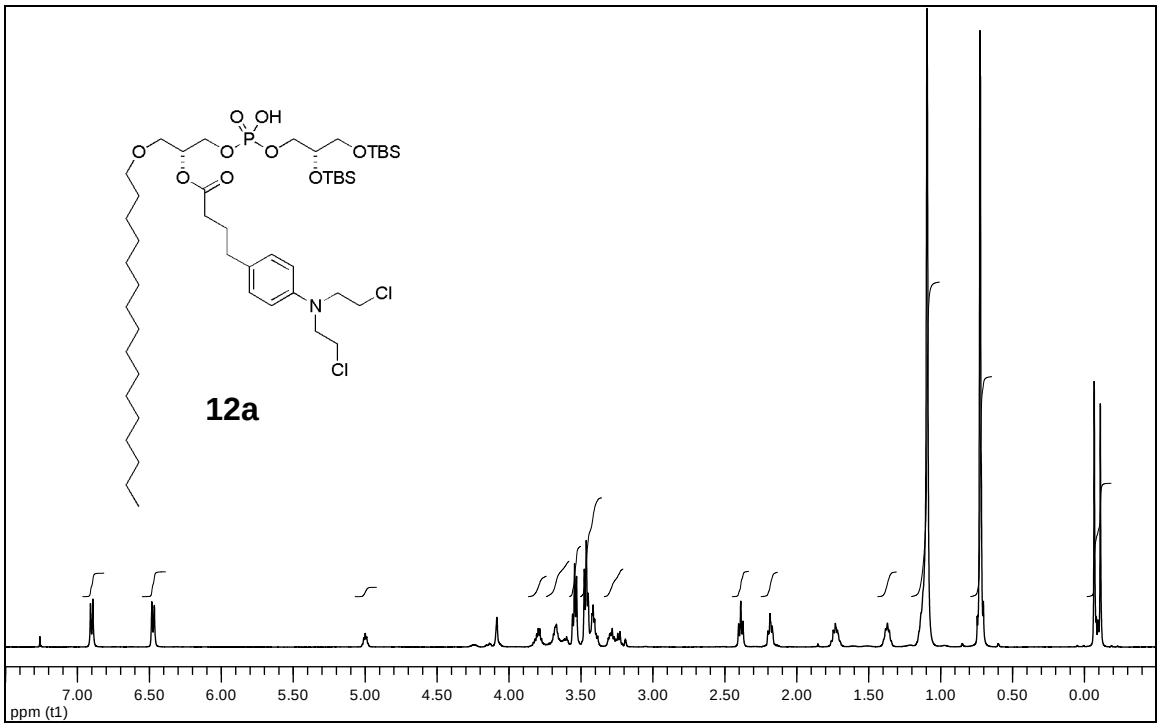


^{13}C NMR (75 MHz, $CDCl_3$)

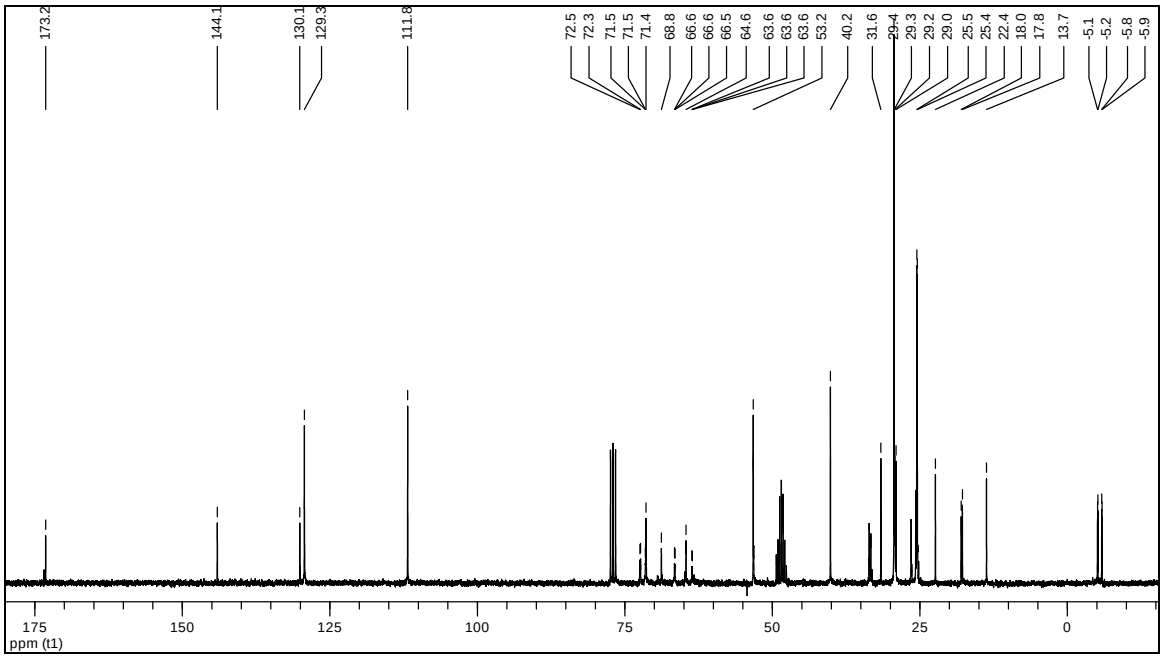


1-*O*-Hexadecyl-2-(4-(4-(*bis*-(2-chloroethyl)-amino)-phenyl)-butanoyl)-*sn*-glycero-3-(2-cyanoethyl-phospho)-(*S*)-2,3-di-*O*-*tert*-butyldimethylsilyl-glycerol (12a)

^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD 3:1)

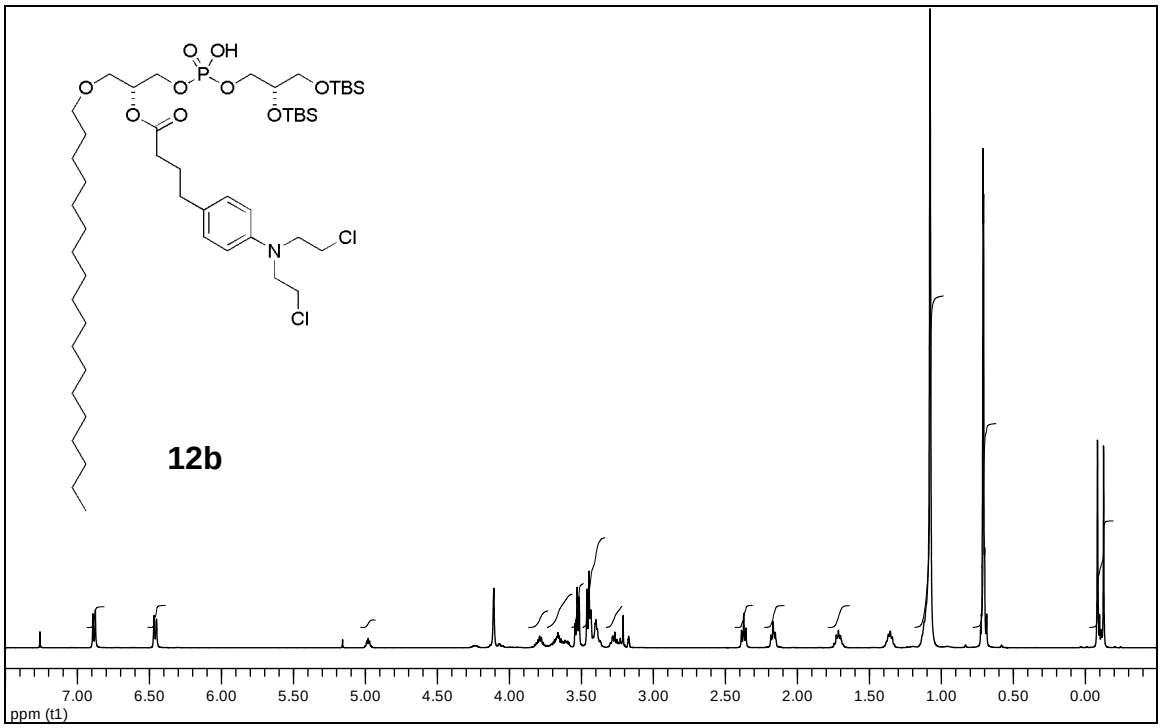


^{13}C NMR (75 MHz, $CDCl_3$:CD_3OD 3:1)

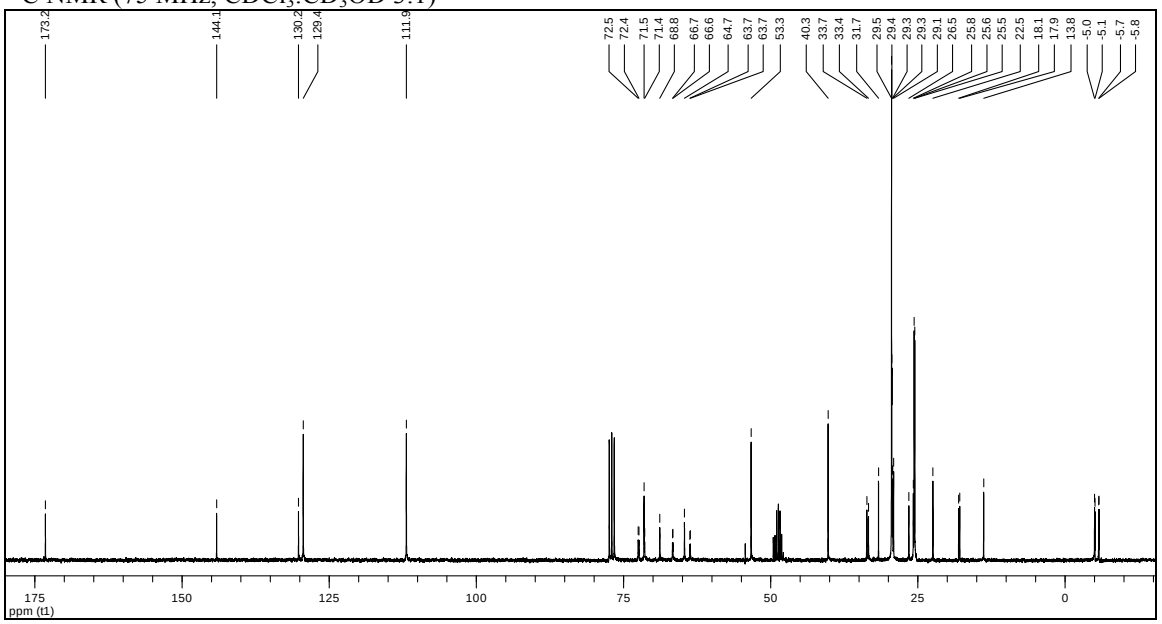


1-*O*-Octadecyl-2-(4-(4-(*bis*-(2-chloroethyl)-amino)-phenyl)-butanoyl)-*sn*-glycero-3-(2-cyanoethyl-phospho)-(*S*)-2,3-di-*O*-*tert*-butyldimethylsilyl-glycerol (12b)

^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD 3:1)

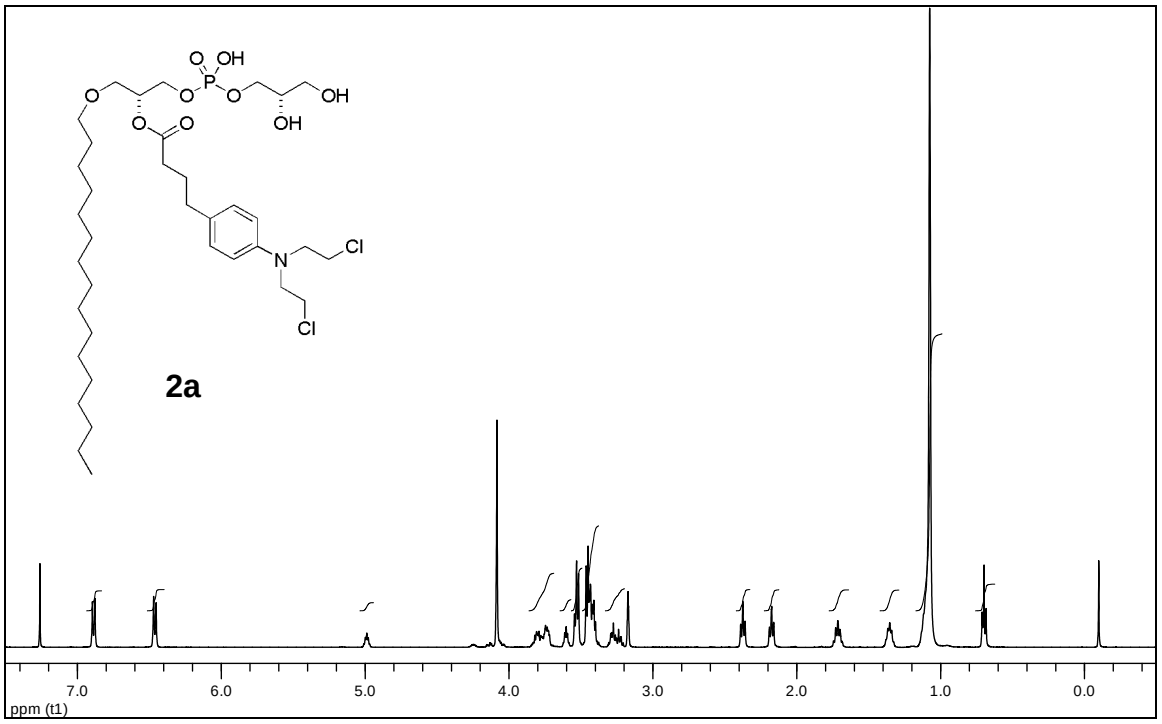


^{13}C NMR (75 MHz, $CDCl_3$:CD_3OD 3:1)

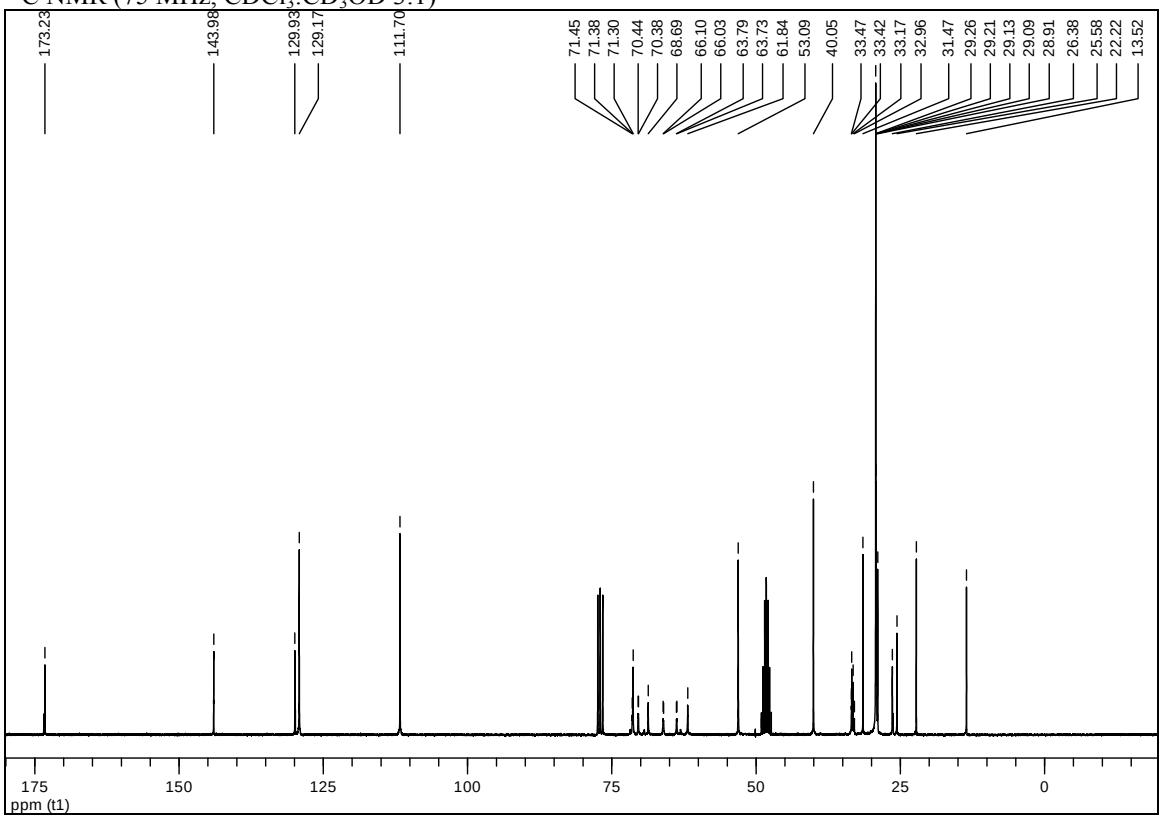


1-*O*-Hexadecyl-2-(4-(4-(*bis*-(2-chloroethyl)-amino)-phenyl)-butanoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol (2a)

^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD 3:1)

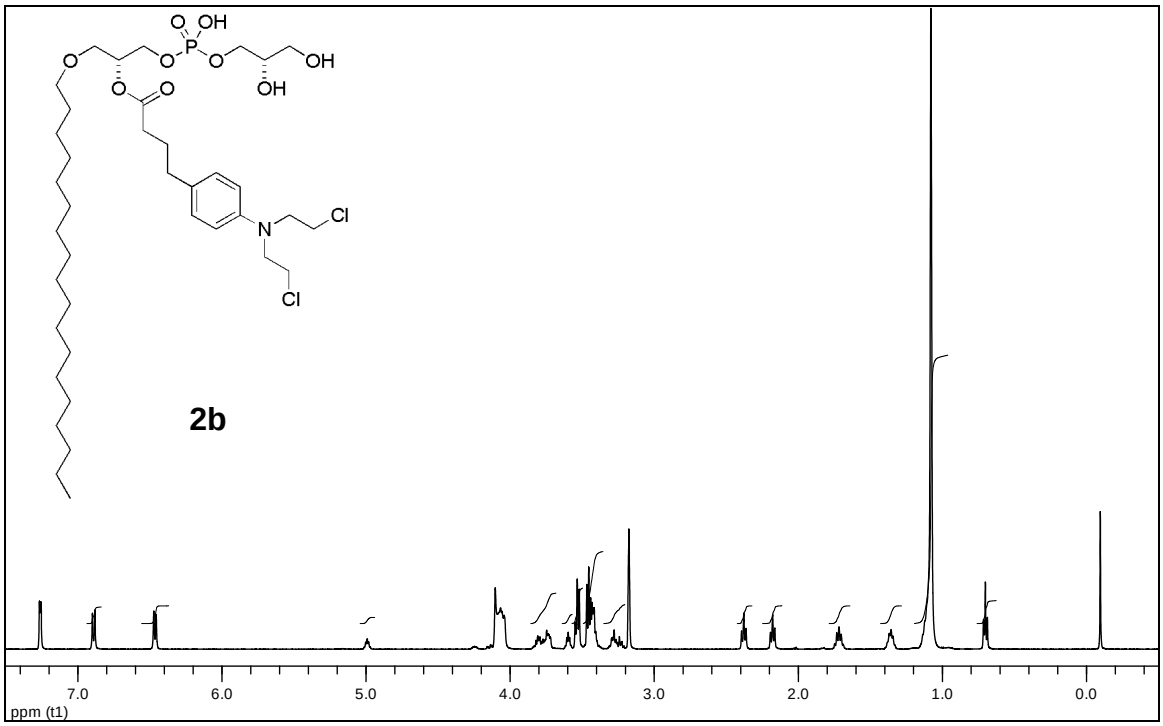


^{13}C NMR (75 MHz, $CDCl_3$:CD_3OD 3:1)



1-*O*-Octadecyl-2-(4-(4-(*bis*-(2-chloroethyl)-amino)-phenyl)-butanoyl)-*sn*-glycero-3-phospho-(*S*)-glycerol (2b)

^{1}H NMR (500 MHz, $CDCl_3$:CD_3OD 3:1)



^{13}C NMR (75 MHz, $CDCl_3$:CD_3OD 3:1)