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Synthesis of short and long chain cardiolipins

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Abstract—A phosphoramidite approach using 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite was utilized for the first time to synthesize short chain cardiolipins. The approach was extended to synthesize long chain and their ether analogue. Optically active 1,2-di-*O*-acyl-*sn*-glycerol or 1,2-di-*O*-myristyl-*sn*-glycerol was coupled with phosphoramidite reagent and 2-benzyloxy-1,3-propanediol in presence of 1*H*-tetrazole, followed by in situ oxidation, to give the corresponding protected cardiolipin analogues. The above intermediates were converted into cardiolipin analogues in two steps by deprotection of cyanoethyl and benzyl groups.

1. Introduction

A need for synthetic phospholipids has developed, in part, their use in liposomes, which have become useful carriers of active therapeutic agents, enzymes, antibiotics, antigens, hormones, and anticancer drugs.[1] Cardiolipin (also known as diphosphatidyl glycerol; Fig. 1) constitutes a class of complex anionic phospholipids, typically purified from cell membranes of tissues associated with high metabolic activity, including the mitochondria of heart and skeletal muscles.[2] However, known chromatographic purification techniques cannot resolve cardiolipin into discrete molecular species. As a result, the use of this component in drug formulations has limited potential because the resulting formulations are not homogeneous. In animal tissues cardiolipin contains up to 90% linoleic acid ($C_{18:2}$). Yeast cardiolipin differs in having more oleic ($C_{18:1}$) and palmitoleic ($C_{16:1}$) fatty acids, while the bacterial lipid contains saturated and monoenoic fatty acids with 14–18 carbons. Liposomes containing cardiolipin encapsulate a broad spectrum of therapeutic agents ranging from difficult-to-formulate, water-insoluble drugs to delivery of molecules to intracellular targets. The encapsulation of chemotherapeutic agents[3,4] in a macromolecular carrier, such as liposomes, significantly reduces the volume of distribution in normal tissues and thereby increases the concentration of drug in the tumor. This results in a decrease in toxicities and an increase in therapeutic efficacy. The potential effects of the length and nature of cardiolipin fatty acid chains (i.e., saturated or unsaturated) on liposome aggregation have not been elucidated. However, cardiolipin having short chain fatty acids are unknown till now. Recognizing the need for the development of short chain cardiolipins to improve drug delivery, we undertook a program to design and synthesize a new class of short chain cardiolipins ($C_{8:0}$–$C_{12:0}$), long chain cardiolipin ($C_{14:0}$), and their ether analogue ($C_{14:0}$).

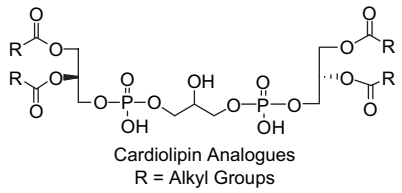

Figure 1. General structure of cardiolipin.

The known methodologies for synthesizing cardiolipin are mainly divided into two groups: (a) coupling the primary hydroxyl groups of a 2-protected glycerol with 1,2-*O*-diacyl-*sn*-glycerol using a phosphorylating agent and (b) condensation at both primary hydroxyl groups of a 2-protected glycerol with phosphatidic acid in the presence of 2,4,6-triisopropylbenzenesulfonylchloride.[5] Cardiolipin has also been generated via a reaction between the silver salt of diacylglycerophosphoric acid benzyl ester[6] with 1,3-diiodopropanol benzyl ether or 1,3-diiodopropanol *t*-butyl ether. Although the schemes were suitable for the preparation of small quantities of cardiolipin, these were unattractive for the routine preparation of larger quantities due to the many steps involved, the requirement for careful purification of intermediates, the use of highly photosensitive silver salt derivatives, and unstable iodo intermediates.

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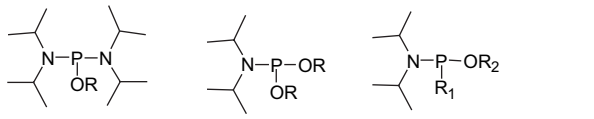


1. R = CH_3
2. R = CH_2Ph
3. R = C_2H_4CN
4. R = $C_4H_8NHCOCF_3$

5. R = CH_3
6. R = CH_2Ph
7. R = t-C_4H_9

8. R_1 = Cl, R_2 = CH_3
9. R_1 = Cl, R_2 = CH_2Ph
10. R_1 = Cl, R_2 = C_2H_4CN

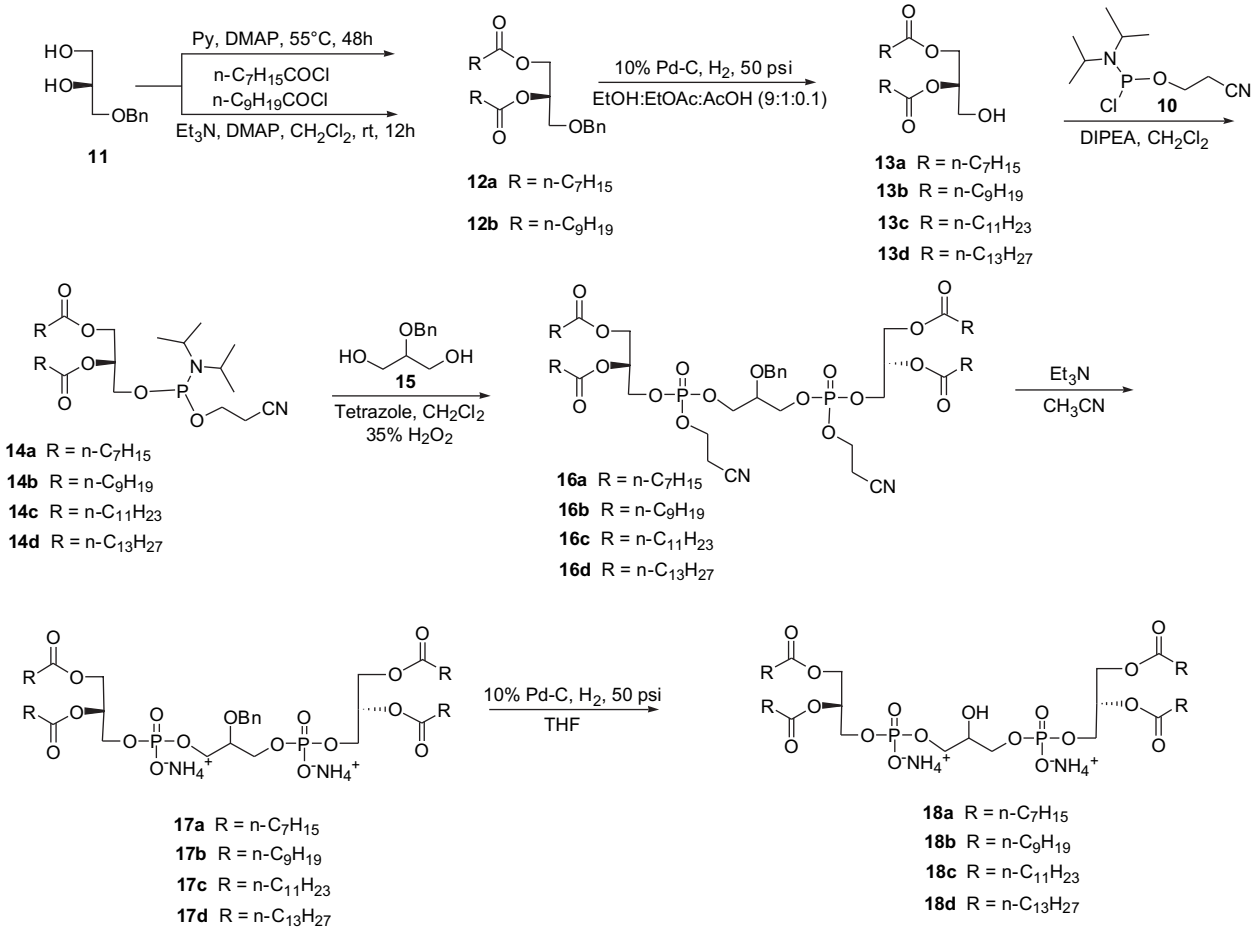
Figure 2. Phosphoramidite reagents.

Several phosphoramidite reagents (Fig. 2) such as methyl-*N*,*N*,*N*,*N*-tetraisopropyl phosphorodiamidite[7] **1**, benzyl-*N*,*N*,*N*,*N*-tetraisopropyl phosphorodiamidite[8] **2**, 2-cyanoethyl *N*,*N*,*N*,*N*-tetraisopropyl phosphorodiamidite[9] **3**, (*N*-trifluoroacetylamino)butyl-*N*,*N*,*N*,*N*-tetraisopropyl phosphoramidite[10] **4**, dimethyl-*N*,*N*-diisopropyl phosphoramidite[11] **5**, dibenzyl-*N*,*N*-diisopropyl phosphoramidite[12] **6**, di-*t*-butyl-*N*,*N*-diisopropyl phosphoramidite[13] **7**, methyl-*N*,*N*-diisopropylchlorophosphoramidite[14] **8**, benzyl-*N*,*N*-diisopropylchlorophosphoramidite[15] **9**, and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite[16] **10** have been used extensively in the synthesis of oligonucleotides,[16] phosphatidylinositols[15] [PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3], and to a lesser extent, in phospholipids' synthesis. Although the use of phosphate triesters and phosphoramidite esters in preparing phospholipids is known,[17] their usage in the synthesis of cardiolipin analogues having varying fatty acid chain lengths is not well established.

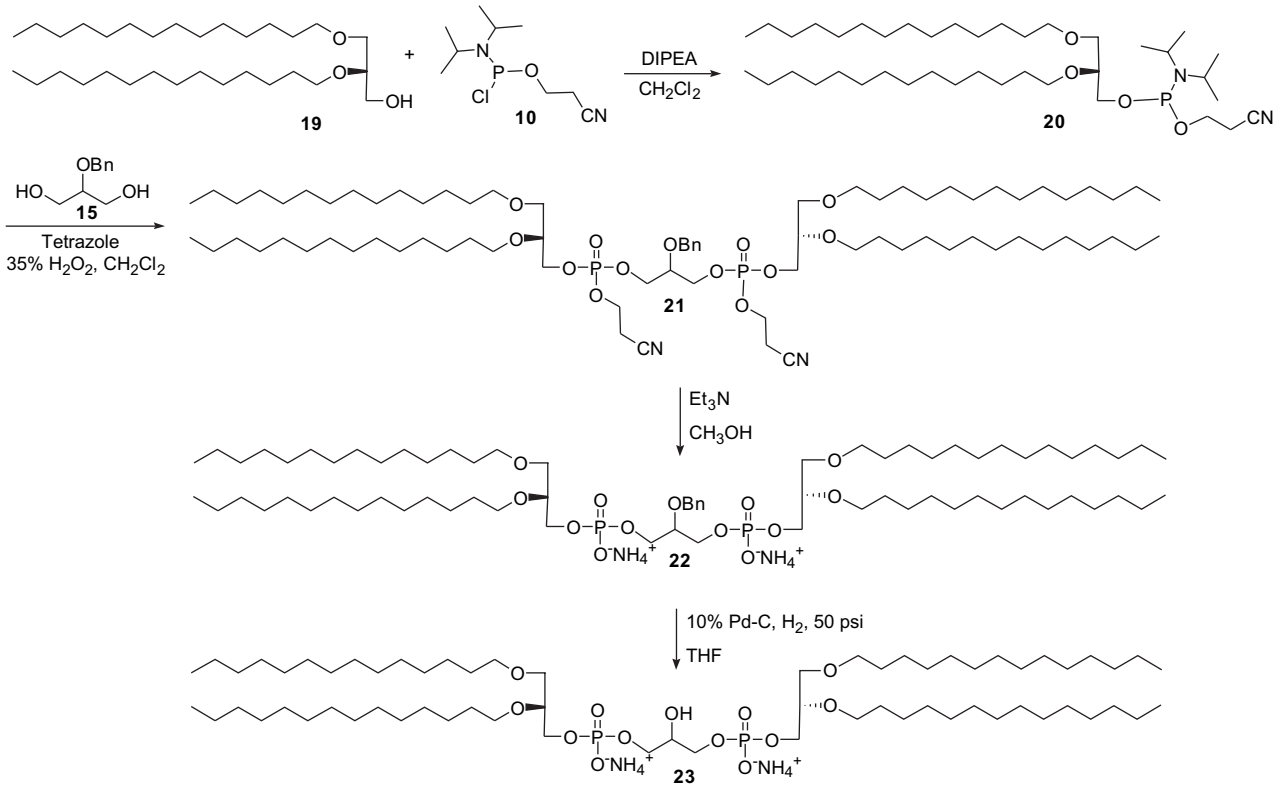
New synthetic methods are needed that can be used to prepare large quantities of cardiolipin analogue species having varying fatty acid chain length, particularly 'short chain cardiolipins'. Such methods would increase the availability of a wider variety of cardiolipin species and would diversify the lipids available for the development of new liposomal formulations containing active agents, which will have more defined compositions than those currently available. The short chain cardiolipin may also be useful for cosmetics and skin care products. As part of ongoing research, we developed new synthetic methods to synthesize cardiolipin and its analogues using the phosphoramidite approach[18,19a] as well as *O*-chlorophenyl dichlorophosphate.[19b] Herein, we report for the first time the use of novel phosphoramidite reagent, 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **10** to prepare short chain cardiolipins, long chain cardiolipin, and cardiolipin ether analogues.

2. Synthesis

The synthesis of cardiolipin analogues **18a**, **18b**, **18c**, and **18d** are outlined in Scheme 1. The synthetic methodology that we have developed involves the application of



Scheme 1. Synthesis of cardiolipin-ester analogues.



Scheme 2. Synthesis of cardiolipin-ether analogues.

chlorophosphoramidite **10** as the phosphitylating agent to build a cardiolipin core structure through a phosphotriester approach. The method is attractive because phosphorus(III) reagents are generally more reactive than phosphorus(V) reagents. Commercially available (*R*)-(+)-3-*O*-benzyl-1,2-propanediol **11** was treated with octanoyl chloride in pyridine at 55 °C to obtain 1,2-di-*O*-octanoyl-3-*O*-benzyl-*sn*-glycerol **12a**. The glycerol **11** on esterification with decanoyl chloride and triethylamine in dichloromethane afforded 1,2-di-*O*-decanoyl-3-*O*-benzyl-*sn*-glycerol **12b**. Debenzylation of **12a** and **12b** via hydrogenation over 10% Pd–C catalyst in ethanol/ethyl acetate/acetic acid (9:1:0.1) gave the corresponding glycerols **13a** and **13b**, respectively. 1,2-Di-*O*-octanoyl-*sn*-glycerol **13a** and 1,2-di-*O*-decanoyl-*sn*-glycerol **13b** were reacted with the bifunctional phosphitylating reagent 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **10** in dichloromethane in the presence of diisopropylethylamine for 1 h to afford 1,2-diacyl-*sn*-glycerol-*N*,*N*-diisopropylaminophosphoramidite intermediates **14a** and **14b**, respectively. Similarly commercially available 1,2-di-*O*-lauroyl-*sn*-glycerol **13c** and 1,2-di-*O*-myristoyl-*sn*-glycerol **13d** were reacted with the bifunctional phosphitylating reagent **10**, in dichloromethane in the presence of diisopropylamine, for 1 h to afford 1,2-di-*O*-acyl-*sn*-glycerol-*N*,*N*-diisopropylaminophosphoramidite intermediates **14c** and **14d**, respectively. The crude products were used as such for the next reaction without any purification. The phosphoramidite intermediates **14a–d** on coupling with 2-benzyloxy-1,3-propanediol **15** in the presence of 1*H*-tetrazole gave phosphite triester intermediates, which was oxidized in situ with 35% H_2O_2 to afford protected cardiolipin analogues **16a–d**, respectively. Deprotection of the cyanoethyl group in the presence of triethylamine by β-elimination yields benzyl protected cardiolipin analogues **17a–d**. Finally, **17a–d** subjected to hydrogenolysis with Pd–C, H_2, at 50 psi in tetrahydrofuran at room temperature for 6 h furnished short chain cardiolipins **18a**, **18b**, **18c**, and long chain cardiolipin **18d**, respectively.

The synthesis of cardiolipin ether analogue **23** is outlined in Scheme 2. Commercially available (*R*)-(+)-3-*O*-benzyl-1,2-propanediol was converted in two steps into 1,2-di-*O*-myristyl-*sn*-glycerol[20] **19**. The bifunctional phosphitylating reagent 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **10** mixed with **19** in dichloromethane in the presence of diisopropylethylamine for 2 h afforded 1,2-bis-tetradecyloxy-*sn*-glycerol-*N*,*N*-diisopropylaminophosphoramidite intermediate **20**, which was used as such for the next reaction without any purification. The phosphoramidite intermediate **20**, on coupling with 2-benzyloxy-1,3-propanediol **15** in the presence of 1*H*-tetrazole gave phosphite triester intermediate, which was oxidized in situ with hydrogen peroxide to produce the desired protected cardiolipin ether analogue **21**. Deprotection of the cyanoethyl group of 2-*O*-benzyl-1,3-bis[(1,2-di-*O*-myristyl-*sn-glycero*-3)-phosphoryl] glycerol dicyanoethyl ester **21** followed by hydrogenolysis gave cardiolipin ether analogue **23**.

3. Conclusions

We used commercially available, low cost, and efficient phosphoramidite reagent, such as 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, to produce cardiolipin analogues in higher yield. The 2-cyanoethyl group provided three advantages.[17c] First, the high reactivity of the

2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite ensured that a bulky primary alcohol could form efficient new O–P bonds. Second, it has been known that the 2-cyanoethyl phosphite derivatives were more stable to chromatography steps than the corresponding benzyl phosphites.[15c] Third, not only could the 2-cyanoethyl group serve as the source of the 3-amino-propyl linker, but also it could be removed by β-elimination using a base to provide high yield of the phosphodiesters. The deprotection can be accomplished by mild basic conditions and catalytic hydrogenolysis. The routes are short and proceed in good overall yield.

4. Experimental

4.1. General

^{1}H NMR spectra were recorded on a Varian Inova NMR spectrometer at 500 MHz. ^{1}H chemical shifts are reported in parts per million from internal tetramethylsilane. Mass spectral analyses [electron spray ionization (ESI)] were carried out on a Triple Quadruple LC/MS/MS mass spectrometer API 4000 (Applied Biosystems). Accurate mass measurements (HRMS–ESI) were done at University of Minnesota on a Bruker Daltonics BioTOF II spectrometer. Melting points were determined at atmospheric pressure and uncorrected. Infrared (IR) spectra were recorded on a Nicolet Nexus 470 FTIR. Samples were prepared by ATR method. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates (250 μm) and developed with the appropriate solvents. The TLC spots were visualized either by UV light or by heating the plates sprayed with a solution of phosphomolybdic acid (5% ethanolic solution). Flash column chromatography was carried out on silica gel (230–400 mesh). All chemicals and anhydrous solvents purchased from Aldrich Chemical Co. (Milwaukee, WI), except 1,2-di-*O*-lauroyl-*sn*-glycerol and 1,2-di-*O*-myristoyl-*sn*-glycerol (Genzyme Pharmaceutical, Cambridge, MA). All of the extracts were dried over anhydrous Na_2SO_4. Anhydrous dichloromethane, acetonitrile, ethanol, and tetrahydrofuran were used as such without further drying.

4.1.1. 1,2-Di-*O*-octanoyl-3-*O*-benzyl-*sn*-glycerol (12a). To an oven dried, 100 mL three-neck round bottom flask equipped with a stir bar, condenser, heating mantle, addition funnel, and a temperature probe under an argon atmosphere were added (*R*)-(+)-3-*O*-benzyl-1,2-propanediol **11** (4.5 g, 24.7 mmol) and anhydrous pyridine (45 mL). The solution was mixed moderately while octanoyl chloride (10.0 g, 61.5 mmol) was added dropwise via an addition funnel over 15 min maintaining the reaction temperature below 40 °C. To this reaction mixture was added 4-(dimethylamino) pyridine (0.320 g, 2.47 mmol) all at once. The reaction mixture was stirred vigorously for 48 h while refluxing at 55 °C. Then pyridine was removed under reduced pressure to afford a deep red oil. To this oil was added ethyl acetate (300 mL) and extracted with water (3×100 mL), 0.5 N HCl (3×100 mL), and brine solution (3×100 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford 13.5 g of oil. The oil was purified as such using flash chromatography (SiO_2) and eluted (3–10% ethyl acetate/hexane) to afford **12a** (5.4 g, 50%) as a colorless oil. TLC (SiO_2) R_f=0.34 (hexane/ethyl acetate, 9:1). ^{1}H NMR δ ($CDCl_3$, 500 MHz) 0.87 (t, *J*=7.0 Hz, 6H), 1.22–1.34 (m, 16H), 1.52–1.66 (m, 4H), 2.22–2.34 (m, 4H), 3.58 (d, *J*=4.2 Hz, 2H), 4.18 (dd, *J*=6.4 and 11.9 Hz, 1H), 4.34 (dd, *J*=6.4 and 11.9 Hz, 1H), 4.51 (d, *J*=12.2 Hz, 1H), 4.57 (d, *J*=12.2 Hz, 1H), 5.21–5.28 (m, 1H), 7.28–7.36 (m, 5H, Ar–*H*). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.2, 172.1, 137.2, 137.2, 128.5, 128.1, 128.1, 128.1, 128.0, 75.7, 75.8, 70.4, 67.4, 34.3, 34.1, 31.7, 31.6, 29.1, 29.0, 28.9, 28.9, 24.9, 22.6, 22.6, 14.0. HRMS (ESI) Calcd for $C_{26}H_{42}O_5$ ($M+Na^+$) 457.2924; found 457.2947.

4.1.2. 1,2-Di-*O*-decanoyl-3-*O*-benzyl-*sn*-glycerol (12b). To an oven dried, 250 mL three-neck round bottom flask equipped with rubber septum, argon inlet, and ice bath were added (*R*)-(+)-3-*O*-benzyl-1,2-propanediol **11** (7.38 g, 40.51 mmol), triethylamine (18.4 g, 182.39 mmol, 24.5 mL), and anhydrous CH_2Cl_2 (110 mL) with mixing. To the cooled solution was added dropwise decanoyl chloride (10.0 g, 91.15 mmol) over 15 min, followed by the addition of 4-(dimethylamino) pyridine (0.495 g, 4.05 mmol) with mixing. The reaction mixture was stirred at room temperature for 12 h, diluted with CH_2Cl_2 (200 mL), washed successively with water (3×100 mL), and (3×100 mL) brine, dried over anhydrous Na_2SO_4, and concentrated down to deep red oil. The oil was purified as such using flash chromatography (SiO_2) and eluted with step gradient from 3 to 10%, ethyl acetate/hexane to give **12b** (6.4 g, 32%) as an oil. TLC (SiO_2) R_f=0.34 (hexane/ethyl acetate, 9:1). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (t, *J*=6.8 Hz, 6H), 1.22–1.34 (m, 24H), 1.52–1.66 (m, 4H), 2.22–2.34 (m, 4H), 3.58 (d, *J*=4.2 Hz, 2H), 4.18 (dd, *J*=6.4 and 11.9 Hz, 1H), 4.34 (dd, *J*=6.4 and 11.9 Hz, 1H), 4.51 (d, *J*=12.2 Hz, 1H), 4.57 (d, *J*=12.2 Hz, 1H), 5.21–5.28 (m, 1H), 7.28–7.36 (m, 5H, Ar–*H*). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.9, 172.5, 137.2, 128.4, 128.3, 127.8, 127.7, 127.6, 75.9, 75.7, 71.1, 67.3, 33.1, 33.1, 35.5, 31.0, 30.0, 30.0, 29.7, 25.4, 23.1, 14.1. HRMS (ESI) Calcd for $C_{30}H_{50}O_5$ ($M+Na^+$) 513.3555; found 513.3571.

4.1.3. 1,2-Di-*O*-octanoyl-*sn*-glycerol (13a). To a solution of 1,2-dioctanoyl-3-*O*-benzyl-*sn*-glycerol **12a** (5.4 g, 12.42 mmol) in EtOH/EtOAc/AcOH (9:1:0.1, 24 mL) in a pressure vessel containing a stir bar was added palladium catalyst (0.7 g). The reaction mixture was stirred for 18 h at 40 psi of hydrogen before filtering the reaction over Celite to remove the catalyst. The solvent was removed under reduced pressure to afford an oil. The oil was purified as such using flash chromatography (SiO_2) and eluted with a step gradient of ethyl acetate/hexane (1:1 to 4:1). The solvents were removed and the resulting oil was kept under high vacuo for 4 h to afford **13a** (4.0 g, 94%) as pure oil. The compound was used as such for further reactions. TLC (SiO_2) R_f=0.51 (ethyl acetate/hexane, 1:1). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (*J*=7.0 Hz, 6H), 1.22–1.34 (m, 16H), 1.52–1.66 (m, 4H), 2.12 (t, *J*=6.2 Hz, 1H, –OH), 2.32 (t, *J*=7.6 Hz, 2H), 2.35 (t, *J*=7.6 Hz, 2H), 3.73 (t, *J*=6.0 Hz, 2H), 4.22 (dd, *J*=5.8 and 11.9 Hz, 1H), 4.33 (dd, *J*=5.8 and 11.9 Hz, 1H), 5.08 (quintet, *J*=5.1 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6, 173.3, 77.9, 67.2, 63.3, 34.2, 34.1, 31.7, 31.7, 29.1, 29.0, 28.9, 28.9, 24.9, 24.8, 22.6, 22.6, 14.0. HRMS (ESI) Calcd for $C_{19}H_{35}O_5$ ($M+Na^+$) 367.2455; found 367.2442.

4.1.4. 1,2-Di-*O*-decanoyl-*sn*-glycerol (13b). To a solution of 1,2-di-*O*-decanoyl-3-*O*-benzyl-*sn*-glycerol **12b** (6.4 g, 13.04 mmol) in EtOH/EtOAc/AcOH (9:1:0.1, 55 mL) was added palladium on carbon catalyst (1.92 g). The reaction mixture was stirred vigorously for 18 h under 40 psi of hydrogen. The catalyst was filtered over a Celite bed, and the solvent was removed to afford oil. The oil was purified as such using flash chromatography (SiO_2) with a step gradient of ethyl acetate/hexane (1:1 to 4:1), concentrated, and kept under high vacuo to yield **13b** (4.9 g, 94%) as a oil. TLC (SiO_2) R_f=0.39 (hexane/ethyl acetate, 3:2). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.87 (t, J=7.0 Hz, 6H), 1.22–1.34 (m, 24H), 1.52–1.66 (m, 4H), 2.03 (t, J=6.2 Hz, 1H, –OH), 2.32 (t, J=7.6 Hz, 2H), 2.35 (t, J=7.6 Hz, 2H), 3.73 (t, J=6.0 Hz, 2H), 4.22 (dd, J=5.8 and 11.9 Hz, 1H), 4.33 (dd, J=5.8 and 11.9 Hz, 1H), 5.08 (quintet, J=5.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.4, 172.3, 78.1, 67.0, 63.5, 33.9, 33.6, 32.5, 32.4, 30.3, 30.3, 30.1, 30.1, 30.0, 30.0, 29.8, 29.7, 25.4, 25.4, 23.4, 23.1, 14.0. HRMS (ESI) Calcd for $C_{23}H_{44}O_5$ (M+Na$^+$) 423.3081; found 423.3093.

4.1.5. 2-*O*-Benzyl-1,3-bis[(1,2-di-*O*-octanoyl-*sn*-glycero-3)-phosphoryl] glycerol dicyanoethyl ester (16a). To a solution of 1,2-di-*O*-octanoyl-*sn*-glycerol **13a** (4.0 g, 11.6 mmol) in anhydrous dichloromethane (30 mL) were added diisopropylethylamine (1.65 g, 12.76 mmol) and dropwise 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **10** (3.02 g, 12.76 mmol) under a steady stream of dry argon. The reaction mixture was stirred moderately at room temperature under an argon atmosphere for 1 h. To this stirring solution were added 1*H*-tetrazole (0.97 g, 13.91 mmol, 30.9 mL of 0.45 M solution in acetonitrile) and dropwise a solution of 2-benzyloxy-1,3-propanediol **15** (0.95 g, 5.23 mmol) in anhydrous dichloromethane (30 mL). The reaction mixture was stirred at room temperature for 3 h and cooled to −40 °C while hydrogen peroxide (0.51 g, 15.11 mmol, 1.34 mL of 35 wt % H_2O_2) was added dropwise. After stirring the reaction at −40 °C for 15 min, the reaction was allowed to come to room temperature over 2 h, diluted with CH_2Cl_2 (200 mL), and washed with 10% sodium thiosulfate solution (50 mL). The organic layer was extracted with water (2×50 mL), brine (2×50 mL), then dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum to yield 6.7 g as a colorless syrup. The residue was purified on a (SiO_2) column using a step gradient from 50% ethyl acetate/hexane to 100% ethyl acetate, which gave **16a** (5.6 g, 85%). TLC (SiO_2) R_f=0.35 (EtOAc/CH_2Cl_2, 1:3, v/v). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, J=7.0 Hz, 12H, –CH_3), 1.26–1.29 (m, 32H, –CH_2), 1.58–1.62 (m, 8H), 2.28–2.34 (m, 8H), 2.66–2.73 (m, 4H), 3.85–3.88 (m, 1H), 4.07–4.32 (m, 16H), 4.67 (s, 2H), 5.22–5.25 (m, 2H), 7.35–7.36 (m, 5H, Ar–H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 173.1, 172.8, 137.2, 137.1, 128.5, 128.1, 128.1, 128.1, 128.0, 127.9, 127.9, 127.9, 116.3, 77.2, 77.0, 76.7, 75.4, 75.4, 75.3, 72.3, 69.2, 69.2, 66.0, 65.9, 65.9, 62.2, 62.1, 61.5, 34.1, 33.9, 31.6, 29.0, 28.9, 28.8, 24.7, 22.5, 19.5, 19.4, 14.0. HRMS (ESI) Calcd for $C_{54}H_{90}N_2O_{17}P_2$ (M+Na$^+$) 1123.5607; found 1123.5610.

4.1.6. 2-*O*-Benzyl-1,3-bis[(1,2-di-*O*-decanoyl-*sn*-glycero-3)-phosphoryl] glycerol dicyanoethyl ester (16b). Compound **16b** was synthesized from compound **13b** in an 86% yield as a colorless syrup following the procedure used for the synthesis of **16a**. TLC (SiO_2) R_f=0.38 (EtOAc/CH_2Cl_2, 1:3). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.26–1.28 (m, J=10.5 Hz, 48H), 1.58–1.61 (m, J=6.0 Hz, 8H), 2.28–2.34 (m, J=2.0 Hz, 8H), 2.70–2.72 (m, J=6.0 Hz, 4H), 3.83–3.88 (m, 1H), 4.13–4.31 (m, 16H), 4.67–4.68 (s, 2H), 5.22–5.24 (m, 2H), 7.35–7.37 (m, 5H, Ar–*H*). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 173.1, 172.8, 172.7, 137.2, 137.2, 128.5, 128.1, 128.0, 127.9, 116.3, 77.2, 77.0, 76.7, 75.4, 75.3, 72.3, 69.2, 69.1, 66.0, 66.0, 65.9, 65.8, 62.2, 62.2, 62.1, 61.5, 34.1, 33.9, 31.8, 29.4, 29.2, 29.2, 29.1, 29.0, 24.8, 22.6, 19.6, 19.5, 19.4, 14.0. HRMS (ESI) Calcd for $C_{62}H_{106}N_2O_{17}P_2$ (M+Na$^+$) 1235.6879; found 1235.6893.

4.1.7. 2-*O*-Benzyl-1,3-bis[(1,2-di-*O*-lauroyl-*sn*-glycero-3)-phosphoryl] glycerol dicyanoethyl ester (16c). Compound **16c** was synthesized from **13c** in an 87% yield as a colorless syrup by following the procedure used for the synthesis of **16a**. TLC (SiO_2) R_f=0.48 (EtOAc/CH_2Cl_2, 1:3). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, J=7.0 Hz, 12H), 1.25–1.28 (m, 64H), 1.58–1.60 (m, 8H), 2.28–2.34 (m, 8H), 2.66–2.72 (m, 4H), 3.83–3.88 (m, 1H), 4.05–4.34 (m, 16H), 4.66–4.67 (s, 2H), 5.22–5.24 (m, 2H), 7.35–7.36 (m, 5H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 172.8, 137.2, 130.8, 128.8, 128.6, 128.5, 128.2, 128.1, 128.1, 128.0, 127.9, 127.7, 116.3, 77.2, 77.0, 76.7, 75.4, 75.3, 70.6, 70.5, 69.2, 68.1, 66.0, 65.9, 62.2, 62.2, 62.1, 61.6, 61.5, 38.7, 34.1, 33.9, 31.9, 30.3, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 29.1, 28.9, 24.8, 23.7, 22.9, 22.6, 19.6, 19.5, 14.1, 14.0. HRMS (ESI) Calcd for $C_{64}H_{122}N_2O_{17}P_2$ (M+Na$^+$) 1241.7585; found 1241.7566.

4.1.8. 2-*O*-Benzyl-1,3-bis[(1,2-di-*O*-myristoyl-*sn*-glycero-3)-phosphoryl] glycerol dicyanoethyl ester (16d). Compound **16d** was synthesized from compound **13d** in an 87% yield as a colorless oil following the procedure used for the synthesis of **16a**. TLC (SiO_2) R_f=0.26 (EtOAc/CH_2Cl_2, 1:3). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.25 (m, 80H), 1.58–1.59 (m, 8H), 2.27–2.33 (m, 8H), 2.64–2.75 (m, 4H), 3.90 (m, 1H), 4.13–4.17 (m, 16H), 4.67 (s, 2H), 5.19–5.28 (m, 2H), 7.28–7.38 (m, 5H, Ar–H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 173.2, 173.2, 138.0, 128.4, 127.7, 127.6, 77.2, 77.0, 76.7, 70.3, 70.3, 66.7, 63.6, 62.7, 34.3, 34.1, 31.9, 29.7, 29.7, 29.7, 29.7, 29.6, 29.6, 29.4, 29.3, 29.2, 29.1, 29.1, 24.9, 24.9, 22.7, 14.0. HRMS (ESI) Calcd for $C_{78}H_{138}N_2O_{17}P_2$ (M+Na$^+$) 1459.9363; found 1459.9394.

4.1.9. 1,3-Bis[(1,2-di-*O*-octanoyl-*sn*-glycero-3)-phosphoryl]-2-*O*-benzylglycerol diammonium salt (17a). A solution of the protected cardiolipin analogue **16a** (1.95 g, 1.77 mmol), Et_3N (1.36 g, 13.48 mmol), and water (0.1 mL) in acetonitrile (10 mL) was stirred overnight. The reaction mixture was evaporated to dryness. The residue was converted into ammonium salt by adding 2 mL NH_4OH and purified on SiO_2 column (15% MeOH in CH_2Cl_2 containing 1% NH_4OH) to give **17a** (606 mg, 65%) as a colorless syrup that slowly solidified. TLC (SiO_2) R_f~0.46 ($CHCl_3$/MeOH/NH_4OH, 6.5:2.5:0.5). ^{1}H NMR δ ($CDCl_3$, 500 MHz) 0.88 (t, J=7.0 Hz, 12H), 1.22–1.39 (m, 32H), 1.56–1.63 (m, 8H), 2.22–2.34 (m, 8H), 3.66–3.76 (m, 1H), 3.82–4.06 (m, 8H), 4.08–4.18 (m, 2H), 4.26–4.37 (m, 2H), 4.60 (s,

2H), 5.14–5.26 (m, 2H), 7.22–7.36 (m, 5H), 7.49 (br, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.2, 173.1, 172.8, 172.7, 128.4, 127.7, 127.6, 77.2, 77.0, 76.7, 71.4, 70.4, 63.7, 62.7, 45.8, 34.3, 34.1, 31.7, 29.1, 29.0, 28.9, 24.9, 24.8, 22.6, 14.0. HRMS (ESI) Calcd for $C_{48}H_{90}N_2O_{17}P_2$ $(M+2NH_4^+)^{2-}$ 496.2519; found 496.2519.

4.1.10. 1,3-Bis[(1,2-di-*O*-decanoyl-*sn*-glycero-3)-phosphoryl]-2-*O*-benzylglycerol diammonium salt (17b). Compound **17b** was synthesized from **16b** in an 88% yield as a colorless syrup following the procedure used for the synthesis of **17a**. TLC (SiO_2) R_f=0.47 ($CHCl_3$/MeOH/NH_4OH, 65:25:5, v/v). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.25–1.28 (m, J=16 Hz, 48H), 1.56 (m, 8H), 2.24–2.30 (m, J=3.0 Hz, 8H), 3.72 (m, 1H), 3.89–4.00 (m, J=6.5 Hz, 8H), 4.10–4.14 (m, J=4.0 Hz, 2H), 4.32–4.34 (m, 2H), 4.61 (s, 2H), 5.19 (m, 2H), 7.24–7.33 (m, 5H, Ar–H), 7.55 (br, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 173.2, 138.1, 128.3, 127.6, 77.2, 77.0, 76.7, 71.5, 70.4, 70.3, 63.5, 63.4, 62.7, 45.7, 34.2, 34.0, 31.8, 29.5, 29.4, 29.3, 29.1, 24.9, 24.8, 22.6, 14.0. HRMS (ESI) Calcd for $C_{56}H_{106}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 552.3145; found 552.3167.

4.1.11. 1,3-Bis[(1,2-di-*O*-lauroyl-*sn*-glycero-3)-phosphoryl]-2-*O*-benzylglycerol diammonium salt (17c). Compound **17c** was synthesized from **16c** in an 89% yield as a colorless syrup by following the procedure used for the synthesis of **17a**. TLC (SiO_2) R_f=0.34 ($CHCl_3$/MeOH/NH_4OH, 65:25:5). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.25–1.30 (m, J=7.5 Hz, 64H), 1.56 (m, 8H), 2.24–2.29 (m, J=4.0 Hz, 8H), 3.70–3.72 (m, 1H), 3.89–3.91 (m, 8H), 4.09–4.14 (m, 2H), 4.32–4.34 (m, 2H), 4.61 (s, 2H), 5.17–5.20 (m, 2H), 7.23–7.33 (m, 5H, Ar–H), 7.25 (br s, 8H, NH_4^+). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 173.2, 138.0, 128.3, 127.7, 127.6, 77.2, 77.0, 76.7, 76.2, 76.1, 71.5, 70.4, 70.3, 63.6, 63.5, 62.7, 34.3, 35.0, 31.9, 29.7, 29.6, 29.5, 29.3, 29.3, 29.2, 29.1, 24.9, 24.8, 22.6, 14.0. HRMS (ESI) Calcd for $C_{64}H_{122}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 609.3821; found 609.3831.

4.1.12. 1,3-Bis[(1,2-di-*O*-myristoyl-*sn*-glycero-3)-phosphoryl]-2-*O*-benzylglycerol diammonium salt (17d). Compound **17d** was synthesized from compound **16d** in an 85% yield as a colorless oil following the procedure used for the synthesis of **17a**. TLC (SiO_2) R_f=0.54 ($CHCl_3$/MeOH/NH_4OH, 65:25:5). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.25–1.31 (m, 80H), 1.56 (m, 8H), 2.23–2.30 (m, 8H), 3.71 (m, 1H), 3.90–3.98 (m, 8H), 4.10–4.14 (m, 2H), 4.31–4.34 (m, 2H), 4.60 (s, 2H), 5.18–5.20 (m, 2H), 7.24–7.32 (m, 5H, Ar–H), 7.51 (br, 8H, NH_4^+). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 173.2, 138.0, 128.4, 127.7, 127.6, 77.2, 77.0, 76.7, 71.5, 70.4, 70.3, 63.5, 62.7, 34.3, 34.0, 31.9, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 29.3, 29.3, 29.2, 29.1, 24.0, 24.8, 22.7, 22.6, 14.0. HRMS (ESI) Calcd for $C_{72}H_{138}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 665.4447; found 665.4443.

4.1.13. 1,3-Bis[(1,2-di-*O*-octanoyl-*sn*-glycero-3)-phosphoryl] glycerol diammonium salt (18a, tetraoctanoyl cardiolipin diammonium salt). A solution of benzyl protected cardiolipin analogue **17a** (1.33 g, 1.29 mmol) in tetrahydrofuran (15 mL) was hydrogenated at 50 psi over 10% Pd–C (665 mg) for 10 h. The catalyst was filtered off over Celite bed and concentrated. The residue was dissolved in $CHCl_3$, filtered through a 0.25 μ filter, and precipitated with acetone to give tetraoctanoyl ($C_{8:0}$) cardiolipin **18a** (0.99 g, 85%) as a white semi solid. TLC (SiO_2) R_f~0.43 ($CHCl_3$/MeOH/NH_4OH, 6.5:2.5:0.5). ^{1}H NMR δ ($CDCl_3$, 500 MHz) 0.88 (t, J=7.0 Hz, 12H), 1.22–1.34 (br s, 32H), 1.52–1.64 (m, 8H), 2.26–2.34 (m, 8H), 3.82–3.98 (m, 9H), 4.12–4.18 (m, 2H), 4.35–4.42 (m, 2H), 5.14–5.24 (m, 2H), 7.41 (br, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 173.3, 77.2, 77.0, 76.7, 62.6, 34.2, 34.1, 31.7, 31.6, 29.1, 29.0, 28.9, 24.8, 22.6, 22.5, 14.0. FTIR (ATR) 3250, 3015, 2945, 2922, 2840, 1746, 1473, 1371, 1198, 1082, 1069 cm^{-1}. HRMS (ESI) Calcd for $C_{41}H_{84}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 451.2324; found 451.2266.

4.1.14. 1,3-Bis[(1,2-di-*O*-decanoyl-*sn*-glycero-3)-phosphoryl] glycerol diammonium salt (18b, tetradecanoyl cardiolipin diammonium salt). Compound **18b** was synthesized from **17b** in a 97% yield as a white solid by following the procedure used for the synthesis of **18a**. Mp 177–179 °C. TLC (SiO_2) R_f=0.38 ($CHCl_3$/MeOH/NH_4OH, 65:25:5, v/v). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.88 (t, J=7.0 Hz, 12H), 1.26 (br s, 48H), 1.58–1.59 (m, J=6.0 Hz, 8H), 2.27–2.33 (m, J=7.0 Hz, 8H), 3.62 (br, 1H), 3.90–3.91 (m, J=4.5 Hz, 9H), 4.13–4.17 (m, J=7.0 Hz, 2H), 4.35–4.37 (m, J=10 Hz, 2H), 5.19–5.20 (m, J=2.0 Hz, 2H), 7.44 (br, 8H, NH_4^+). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 173.3, 77.2, 77.0, 76.7, 62.6, 34.3, 34.1, 31.9, 31.8, 29.5, 29.3, 29.2, 29.1, 24.2, 24.8, 22.6, 14.0. FTIR (ATR) 3228, 3022, 2946, 2914, 2850, 1744, 1466, 1387, 1212, 1095, 1070 cm^{-1}. HRMS (ESI) Calcd for $C_{49}H_{100}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 507.2962; found 507.2953.

4.1.15. Synthesis of 1,3-bis[(1,2-di-*O*-lauroyl-*sn*-glycero-3)-phosphoryl] glycerol diammonium salt (18c, tetralauroyl cardiolipin diammonium salt). Compound **18c** was synthesized from **17c** in a 98% yield as a white solid by following the procedure used for the synthesis of **18a**. Mp 156–158 °C. TLC (SiO_2) R_f=0.31 ($CHCl_3$/MeOH/NH_4OH, 65:25:5). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H), 1.26 (m, 64H), 1.58–1.59 (m, 8H), 2.27–2.33 (m, 8H), 3.90–3.92 (m, 9H), 4.13–4.17 (m, 2H), 4.36–4.38 (m, 2H), 5.20–5.21 (m, 2H), 7.41 (br, 8H, NH_4^+). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.6, 173.3, 167.7, 132.4, 130.8, 128.8, 70.3, 70.3, 69.5, 68.1, 66.6, 63.6, 63.5, 62.7, 38.7, 34.3, 34.1, 31.9, 30.3, 29.7, 29.6, 29.4, 29.3, 29.2, 28.9, 24.9, 24.8, 23.7, 22.9, 22.6, 14.0. FTIR (ATR) 3207, 3035, 2956, 2918, 2850, 1737, 1467, 1378, 1206, 1092, 1067 cm^{-1}. HRMS (ESI) Calcd for $C_{57}H_{116}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 563.3553; found 563.3540.

4.1.16. Synthesis of 1,3-bis[(1,2-di-*O*-myristoyl-*sn*-glycero-3)-phosphoryl] glycerol diammonium salt (18d, tetramyristoyl cardiolipin diammonium salt). Compound **18d** was synthesized from compound **17d** in a 98% yield as a white solid following the procedure used for the synthesis of **18a**. Mp 181–182 °C. TLC (SiO_2) R_f=0.29 ($CHCl_3$/MeOH/NH_4OH, 65:25:5). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, J=7.0 Hz, 12H, –CH_3), 1.25 (br, 80H, –CH_2–), 1.58–1.59 (m, 8H), 2.27–2.33 (m, 8H), 3.06 (br, 1H), 3.90 (m, 9H), 4.13–4.17 (m, 2H), 4.36–4.38 (m, 2H), 5.20–5.21 (m, 2H), 7.41 (br, 8H, NH_4^+). ^{13}C NMR ($CDCl_3$,

125 MHz) δ 173.6, 173.3, 77.2, 77.0, 76.7, 70.34, 70.3, 66.7, 63.6, 62.7, 34.3, 34.1, 31.9, 29.7, 29.6, 29.4, 29.2, 29.1, 24.9, 24.8, 22.7, 14.0. FTIR (ATR) 3207, 3035, 2956, 2918, 1737, 1461, 1378, 1206, 1092, 1067 cm^{-1}. HRMS (ESI) Calcd for $C_{65}H_{132}N_2O_{17}P_2$ $(M-2NH_4^+)^{2-}$ 619.4199; found 619.4173.

4.1.17. Synthesis of 2-*O*-benzyl-1,3-bis[(1,2-di-*O*-myristyl-*sn*-*glycero*-3)-phosphoryl] glycerol dicyanoethyl ester (21). To a 250-mL three-neck round bottom flask equipped with stir bar, rubber septum, and argon inlet was added 1,2-di-*O*-myristyl-*sn*-glycerol[19] **19** (9.2 g, 19.01 mmol) in anhydrous dichloromethane (150 mL). To the mixture was added diisopropylethylamine (5.46 g, 42.26 mmol) and stirred for 10 min. To this mixture was added dropwise 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite **10** (5.0 g, 21.13 mmol), and the reaction mixture was stirred moderately at room temperature. The reaction mixture was stirred for 2 h before adding 1*H*-tetrazole (1.59 g, 22.77 mmol of 0.45 M solution in acetonitrile) and dropwise a solution of 2-benzyloxy-1,3-propanediol **15** (1.55 g, 8.54 mmol) in anhydrous dichloromethane (20 mL). The reaction mixture was stirred for 3 h, and then cooled to −20 °C while 35 wt % H_2O_2 (0.42 g, 12.38 mmol, 1.09 mL) was added dropwise. After stirring the reaction mixture at −20 °C for 15 min, the reaction was allowed to come to room temperature over 2 h, diluted with CH_2Cl_2 (200 mL), and washed with 10% sodium thiosulfate solution (50 mL). The organic layer was extracted with water (2×50 mL), brine (2× 50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum to afford a colorless syrup. The residue was purified as such by flash chromatography on a (SiO_2) column using a step gradient from 50 to 70% ethyl acetate/petroleum ether to yield **21** (7.1 g, 61%). TLC (SiO_2) R_f=0.27 (ethyl acetate/hexane, 3:1). ^1H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, *J*=7.0 Hz, 12H), 1.25–1.31 (br, *J*=7.5, 4.5, and 9.5 Hz, 80H), 1.53–1.54 (m, 8H), 2.23–2.27 (m, 8H), 2.70–2.71 (m, 4H), 3.01 (m, 1H), 4.05–4.11 (m, 4H), 4.16–4.23 (m, 4H), 4.24–4.30 (m, 4H), 4.61–4.63 (d, 2H), 4.67–4.69 (m, 2H), 7.34–7.36 (m, 5H, Ar–H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 128.5, 128.4, 128.0, 127.9, 127.8, 77.5, 77.2, 77.0, 76.7, 72.1, 71.8, 70.7, 70.6, 69.3, 69.3, 67.7, 61.9, 61.8, 60.8, 31.9, 30.0, 29.9, 29.6, 29.5, 29.4, 29.3, 26.0, 22.6, 19.5, 19.45, 14.0. HRMS (ESI) Calcd for $C_{78}H_{146}N_2O_{13}P_2$ $(M+Na^+)$ 1404.0197; found 1404.0234.

4.1.18. 2-*O*-Benzyl-1,3-bis[(1,2-di-*O*-myristyl-*sn*-glycerol-3)-phosphoryl] glycerol diammonium salt (22). To a (250 mL) round bottom flask were added 2-*O*-benzyl-1,3-bis[(1,2-di-*O*-myristyl-*sn*-*glycero*-3)-phosphoryl] glycerol dicyanoethyl ester **21** (3.48 g, 2.51 mmol) and anhydrous acetonitrile (50 mL). The solution was mixed vigorously while triethylamine (2.18 g, 21.52 mmol, 3.0 mL) was added. The reaction mixture was stirred vigorously for 24 h at room temperature. The solvents were removed under reduced pressure, and the crude oil was kept under high vacuo for 4 h. The residue was purified by flash chromatography on a (SiO_2) column using a step gradient of ($CHCl_3$/MeOH/NH_4OH, 100:15:1, v/v) and ($CHCl_3$/MeOH/NH_4OH, 65:15:1, v/v) to yield **22** (2.8 g, 88%). TLC (SiO_2) R_f=0.43 ($CHCl_3$/MeOH/NH_4OH, 65:25:5, v/v). ^1H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, *J*=7.0 Hz, 12H), 1.25 (br, 80H), 1.52 (m, 8H), 3.00–3.02 (d, 1H), 3.39–3.44 (m, 4H), 3.49–3.51 (d, 4H), 3.56 (m, 4H), 3.83 (m, 6H), 3.98 (br, 4H), 4.60 (s, 2H), 7.28–7.31 (m, 5H, Ar–H), 7.56 (br s, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 128.3, 127.6, 77.2, 77.0, 76.7, 71.8, 71.4, 70.6, 31.9, 30.10, 29.8, 29.7, 29.7, 29.6, 29.4, 26.1, 22.7, 14.1. HRMS (ESI) Calcd for $C_{72}H_{146}N_2O_{13}P_2$ $(M-2NH4+)^{2-}$ 637.4901; found 637.4901.

4.1.19. 1,3-Bis[(1,2-di-*O*-myristyl-*sn*-glycerol-3)-phosphoryl] glycerol diammonium salt (23, tetradecyloxy cardiolipin diammonium salt). To a solution of 2-*O*-benzyl-1,3-bis-[(1,2-di-*O*-myristyl-*sn*-*glycero*-3)-phosphoryl]-2-*O*-benzylglycerol diammonium salt **22** (1.2 g, 0.91 mmol) in tetrahydrofuran (30 mL) in a pressure vessel, was added palladium on carbon catalyst (600 mg). The reaction mixture was stirred under 50 psi of hydrogen at room temperature for 6 h. The palladium catalyst was filtered over Celite, washed with chloroform/methanol (1:1, 100 mL), and concentrated under reduced pressure to afford white solids. The product was purified on a flash chromatography (SiO_2) column using a step gradient of ($CHCl_3$/MeOH/NH_4OH, 100:15:1, v/v) and ($CHCl_3$/MeOH/NH_4OH, 65:15:1, v/v) to yield **23** (1.1 g, 98%). Mp 174–176 °C. TLC (SiO_2) R_f=0.34 ($CHCl_3$/MeOH/NH_4OH, 65:25:5, v/v). ^1H NMR ($CDCl_3$, 500 MHz) δ 0.86–0.89 (t, *J*=7.0 Hz, 12H), 1.25–1.27 (br, 80H), 1.31 (br, 8H), 1.54–1.55 (m, 8H), 3.42–3.43 (m, 4H), 3.53–3.54 (m, 4H), 3.57–3.58 (m, 4H), 3.85 (br, 4H), 7.43 (br s, 8H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 77.2, 77.0, 76.7, 71.8, 31.9, 30.0, 29.8, 29.8, 29.7, 29.6, 29.4, 26.2, 26.1, 22.7, 14.1. HRMS (ESI) Calcd for $C_{65}H_{140}N_2O_{13}P_2$ $(M-2NH_4^+)^{2-}$ 591.4613; found 591.4597.

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