Nucleoside-Based Phospholipids and Their Liposomes Formed in Water

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Phospholipids and liposomes have been the subjects of considerable attention because of their importance in biological systems. We have efficiently synthesized novel nucleoside-based phospholipids in six-step sequences starting from their corresponding nucleosides. These nucleoside-based phospholipids self-assemble into liposome-like structures in aqueous solutions. We have analyzed the structures of these liposomes by dynamic light scattering, transmission electron microscopy, and confocal microscopy.

Introduction

Phospholipids are major constituents of cell membranes.[1] When certain phospholipids are dispersed in water, spherical bilayer vesicles, known as liposomes, form spontaneously. Modification of different parts of a phospholipid's structure can affect its activity in biological processes.[2] Such modifications are generally limited to its hydrophobic tail and hydrophilic head groups, or to structural variants of its glycerol unit.[3] Recently, the synthesis and physical characterization of novel carbohydrate-based phospholipids have been reported; these phospholipids self-assemble into liposome-like structures in aqueous solution.[4] An example is the synthesis and physicochemical study of one such uridine-based phospholipid.[5] The authors demonstrated that this modified phospholipid exhibits physical properties that differ from those of its glycerol-based analogues; this indicates the importance that the constitution of the backbone has for the nature of the bilayer structure. The morphologies of some phosphatidyl nucleosides have already been reported by Yanagawa[6] and Luisi,[7] who were the first to investigate the self-association of this family of lipids. Intrigued by these interesting results, we have designed and synthesized novel nucleoside-based phospholipids. Nucleoside phospholipids have one more structural element for molecular recognition—namely, the nucleobase—than carbohydrate phospholipids.[4,8]

Results and Discussion

Here we present an efficient synthesis of novel phospholipids based on the nucleosides uridine, adenosine, and inosine (Scheme 1), their molecular recognition, and the morphologies of their self-assembled liposome structures in aqueous solution. Modifications of phospholipid structure are currently limited to the hydrophobic tails, hydrophilic headgroups, and structural variations of the glycerol backbone.[3j,9] Consequently, alteration of the conventional glycerol backbone by complete substitution provides new opportunities for: 1) assessing supramolecular structure formation, and 2) attaching macromolecules or ligands for biological targeting.

The first step in the synthesis of the nucleoside-based phospholipids involved protecting the primary hydroxy groups of uridine, adenosine, and inosine with 4-methoxytrityl chloride (MMTrCl) to yield **1** (97%), **2** (62%), and **3** (74%), respectively, after chromatography on silica gel. Next, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl (EDC)/*N*,*N*-dimethylaminopyridine (DMAP) coupling individually with decanoic, lauric, myristic, palmitic, and stearic acids in CH_2Cl_2 afforded a series of diesters. Without purification, each of these intermediates was dissolved in 80% aqueous AcOH at 60°C to remove its protecting groups. Compounds **4** (88–98% from **1**), **5** (85–98% from **2**), and **6** (77–88% from **3**) were purified by chromatography on silica gel, and compounds **7**–**9** were then prepared by treating **4**–**6** with 2-chloro-1,3,2-dioxaphospholane, oxidizing the intermediate phosphorus(III) compounds with Br_2 to give the phosphorus(V) products, and subsequently introducing the phosphocholine group by treatment with Me_3N. Compounds **7**–**9** were isolated after chromatography on silica gel. The overall yields for the last three steps (d–f) were 37–58% (**7**), 18–33% (**8**), and 19–27% (**9**).[10]

The first feature of nucleoside-based phospholipids that we studied was the hydrodynamic radii of their vesicles. We heated 0.3 mM aqueous solutions of each nucleoside-based phospholipid to 60°C and then sonicated them for 30 min.[11–13] The liposomes could then be prepared by using a high-pressure extrusion technique, which allows liposomes of small and uniform size to be obtained. Extrusion of the nucleoside-based phospholipids five times through a 200 nm poly-

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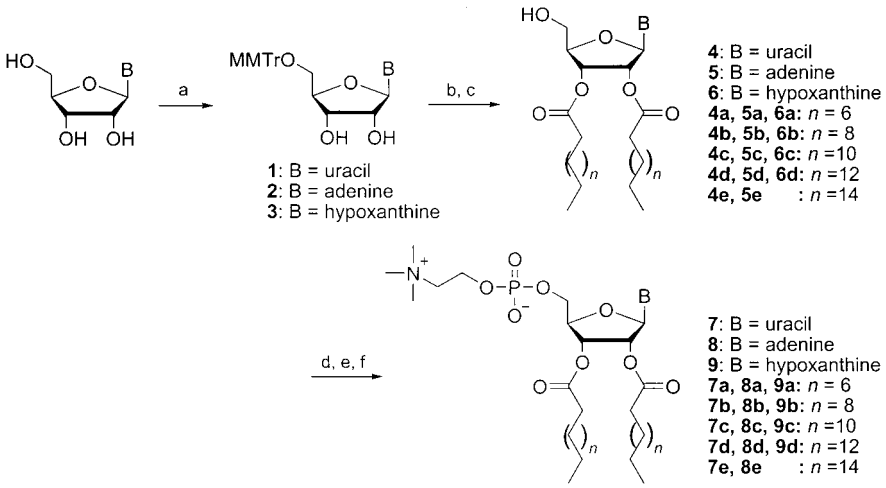

Scheme 1. *Reagents and conditions: a) MMTr-Cl, pyridine, RT, 9 h (uridine), 20 h (adenosine), 18 h (inosine). b) EDC, DMAP, decanoic/lauric/myristic/palmitic/stearic acid, CH_2Cl_2, RT, 4 h. c) 80% acetic acid, 60°C, 3 h. d) DIPEA, 2-chloro-1,3,2-dioxaphospholane, THF, RT, 15 min. e) Br_2, 0°C, 1 h. f) 40% Me_3N, $CHCl_3$/iPrOH/CH_3CN (3:5:5), RT, 3 days.*

carbonate filter at 25 °C provided vesicles with average particle hydrodynamic radii of 39–70 nm when analyzed with a dynamic light scattering (DLS) apparatus. Generally, the hydrodynamic radii of the vesicles increase with increasing length of their alkyl chains.

We next used transmission electron microscopy (TEM) to observe the morphologies of the vesicles. We prepared liposomes using a buffer solution comprising 30 mM Tris/HCl, 20 mM KCl, and 0.1 mM EDTA that was adjusted to pH 8 at room temperature. Again, the lipid dispersion (0.3 mM) was passed repeatedly through a 200 nm polycarbonate filter before being analyzed by TEM, by the negative-staining technique;[14,15] Figure 1 displays the resulting micrographs. From these images, it is clear that the liposomes aggregate into spherical closed liposomes. In the previous studies performed by Yanagawa[6] and Luisi,[7] phosphatidyl nucleosides were demonstrated to aggregate and to have morphologies such as super-helical strands, rings, and flat disks. We observed the same spherical morphologies for all the liposomes of nucleoside-based phospholipids, even though they feature different nucleobases. From these results, we conclude that the morphologies of the nucleoside-based phospholipids are the same (i.e., independent of the nucleobase), but the most significant effect on the liposomes' structures arises from changes in the nature of the nonpolar alkyl chain. The longer the alkyl chains they possess, the greater bending rigidity. Again, this finding stresses that the molecular structure of the lipid has an important effect on the morphology and physical properties of the aggregates.

Next, to confirm the location of nucleobases within the liposomes and to study their molecular recognition properties, we prepared liposomes through the self-assembly of **7**, **8**, and **9**. To study these effects, we chose to treat the dispersions of liposomes with functionalized dyes that should be specific for certain base sequences, and to view their association by confocal microscopy. Because of the resolution of confocal microscopy (> ca. 1 μm), it was necessary for us to prepare relatively large phospholipid vesicles.[16,17] To obtain these samples, we heated 0.3 mM aqueous solutions of nucleoside-based phospholipids to 60 °C and then sonicated them for 30 min. The complementary base pairing of nucleic acids is a biological recognition process based on hydrogen bonding.[18] We wondered whether the bases of the nucleosides were presented at the surfaces of the spherical vesicles and, if so, whether they were capable of hydrogen bonding with short oligonucleotide sequences. To answer these questions, we synthesized a fluorescein isothiocyanate-2′-deoxynucleoside trimer (FITC-trimer, **10**) and a hexachlorofluorescein-2′-deoxynucleoside trimer (Hex-trimer, **11**), from commercially available phosphoamidite monomers, using a DNA synthesizer (Scheme 2).

Figure 2 indicates that such hydrogen bonding indeed occurs and, additionally, that it occurs in a selective manner. Figures 2a and b indicate that the liposome formed from **7c** binds both to the FITC-linked and to the Hex-linked adenosine trimers (i.e., both of these dyes can be accommodated by the liposome). To rule out the possibility that this binding might be indiscriminate (e.g., that it is the dye that binds and not the adenosine trimer unit), we irradiated a solution containing the liposome of **7c** and a mixture of the dyed oligonucleotide trimers FTIC-AAA, Hex-CCC, and Hex-GGG. Figure 2c indicates that this liposome displays only green fluorescence, while Figure 2d indicates that the liposome does not display red fluorescence from this mixture, which suggests that the liposome selectively recognizes the AAA unit, rather than the CCC unit or the GGG unit (i.e., that there is selective hydrogen bonding between the uracil and adenine bases). From these initial finding, we believe that liposomes of uridine-based phospholipids should recognize the poly(A) tail of mRNA.[19]

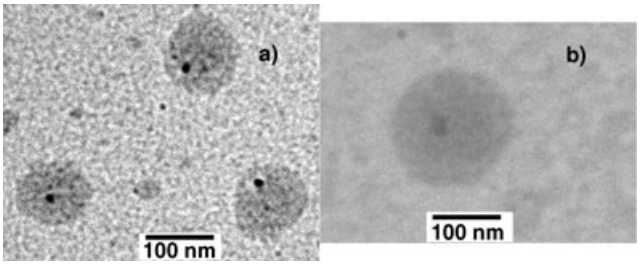

Figure 1. *TEM images of negatively stained liposomes formed from dispersions of nucleoside-based phospholipids (0.3 mM) prepared in Tris-HCl (30 mM)/KCl (20 mM)/EDTA (0.1 mM) at pH 8. Micrographs of: a) adenosine-based liposome **8b**, and b) uridine-based liposome **7b**.*

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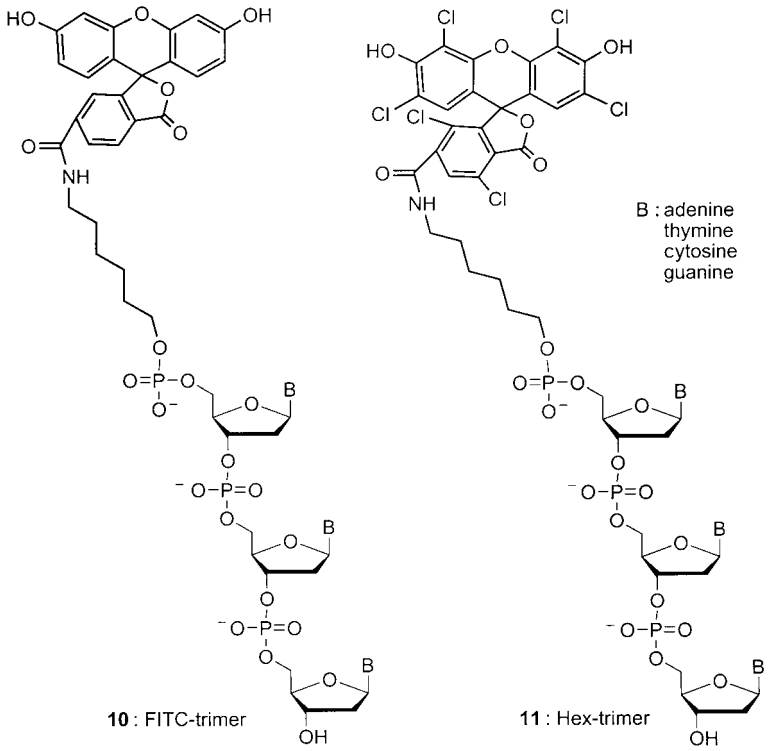

Scheme 2. *Fluorescein-tagged 2′-deoxynucleoside trimers.*

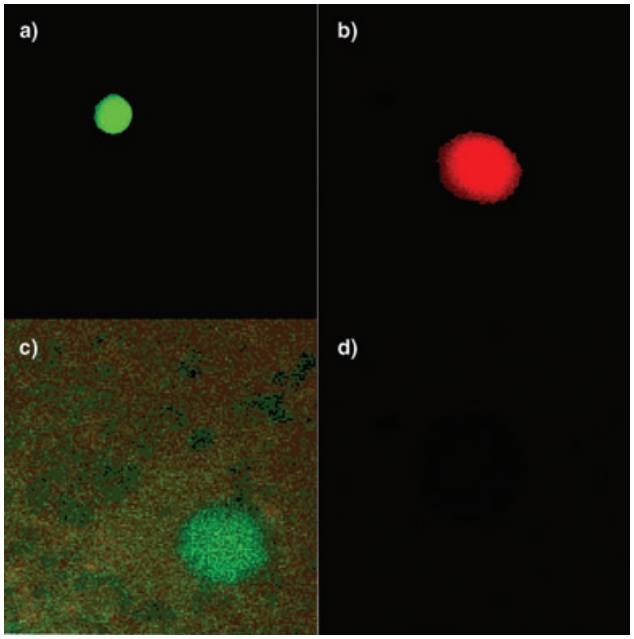

Figure 2. *Confocal microscopy images of uridine-based liposomes formed from* ***7c*** *associating on a glass surface with labeled oligonucleotide trimers. a) Mixture of the liposome of* ***7c*** *and FITC-AAA, irradiated with laser light (λ_{ex} = 488 nm) that causes green fluorescence of the FITC moieties. b) Mixture of the liposome of* ***7c*** *and Hex-AAA irradiated with laser light (λ_{ex} = 543 nm) that causes red fluorescence of the Hex moieties. c) Mixture of the liposome of* ***7c*** *and FITC-AAA, Hex-CCC, and Hex-GGG, irradiated simultaneously both at 488 and at 543 nm. d) Mixture of the liposome of* ***7c*** *and Hex-CCC and Hex-GGG.*

Conclusion

These nucleoside-based phospholipids have large backbones that increase the spacing between the head and tail units, and they have increased hydrodynamic radii relative to other phospholipids with different backbones. Like the phosphatidyl nucleosides,[6,7] the monomers of these nucleoside-based phospholipids can aggregate together, but the morphologies of these aggregates differ substantially. The liposomes of these nucleoside-based phospholipids have spherical morphologies, which we believe are affected primarily by the lengths of the alkyl chains of nucleoside-based phospholipid monomers. The ability of the liposomes to recognize oligonucleotides through hydrogen bonding makes them attractive alternatives to glycerol- and carbohydrate-based phospholipid liposomes. These results may provide new insight into the tailoring of vesicle properties for specific pharmaceutical and industrial applications.

Experimental Section

Materials and instruments: All starting materials were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran (THF) was distilled under nitrogen from sodium/benzophenone, and CH_2Cl_2 and pyridine were distilled under nitrogen from calcium hydride, immediately prior to use. Reactions were executed under an inert atmosphere of dry argon, and the glassware was flame-dried under vacuum. Flash chromatography was performed on Merck silica gel 60 (230–400 mesh; ASTM). Melting points are uncorrected and were obtained with an Electrothermal IA 9000 series apparatus. Infrared (IR) spectra were recorded on a Bruker model FT-IR PS55+ spectrometer. Low- and high-resolution FAB mass spectra were obtained on a Jeol JMS-AX505WA (FAB) spectrometer. An LSM510 (Zeiss) apparatus was used for confocal microscopy. A Malvern 4700 series photon correlation spectrometer was used for dynamic light scattering (DLS) to measure particle size. Transmission electron microscopy (TEM) measurements were obtained on a Hitachi-7600 instrument. ^{1}H, ^{13}C, and ^{31}P NMR spectra were obtained on a Bruker Aspect 3000 spectrometer. Chemical shifts in ^{1}H and ^{13}C NMR spectra are reported in parts per million downfield of tetramethylsilane (TMS) as the internal standard. ^{31}P NMR chemical shifts are reported in ppm downfield relative to phosphoric acid as the external standard. Coupling constants are reported in hertz.

Uridine-based phospholipids

*5′-O-(4-Methoxytrityl)uridine (**1**)*: MMTr-Cl (1.55 g, 5.03 mmol) was added to a solution of uridine (1.02 g, 4.19 mmol) in dry pyridine (50 mL) and the mixture was stirred at room temperature for 9 h. The solvent was evaporated under high vacuum, and the resultant oil was dissolved in CH_2Cl_2 and washed with water. The organic layer was separated and then dried (Na_2SO_4). Chromatographic purification (SiO_2; CH_2Cl_2/MeOH 50:1) yielded **1** (2.11 g, 97%). m.p. 117.2–119.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.01 (d, J = 9.0 Hz, 1H), 7.40–6.82 (m, 15H), 5.89 (d, J = 3.0 Hz, 1H), 5.32 (t, J = 7.5 Hz, 1H), 4.44 (t, J = 6.0 Hz, 1H), 4.35 (t, J = 3.0 Hz, 1H), 4.17–4.15 (m, 1H), 3.76 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.2, 158.9, 151.4, 144.1, 143.9, 140.6, 134.9, 130.7, 128.6, 128.5, 128.2, 127.5,

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113.5, 102.5, 90.5, 87.5, 83.8, 75.6, 69.8, 62.1, 55.4 ppm; IR (NaCl): $\tilde{\nu}$ = 3392, 3059, 2930, 1697, 1509, 1463, 1393, 1252 cm^{-1}; MS-FAB (*m/z*): found 517.14 $[M+H]^+$; $C_{29}H_{28}N_2O_7$ calcd 517.19.

*2′,3′-O-Didecanoyluridine (**4a**)—General Procedure*: Decanoic acid (1.78 g, 10.4 mmol) was added to a solution of **1** (2.15 g, 4.17 mmol), EDC (2.00 g, 10.4 mmol), and DMAP (615 mg, 5.00 mmol) in dry CH_2Cl_2 (40 mL). The mixture was stirred at room temperature for 4 h, extracted with water, and then concentrated to dryness. The residue was dissolved in acetic acid (80%, 50 mL) and was then heated at 60 °C for 3 h. The reaction mixture was washed with water and CH_2Cl_2, the organic layer was separated and dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. Chromatographic purification (SiO_2; CH_2Cl_2/MeOH 60:1) afforded **4a** (2.23 g, 96%). m.p. 98.5–106.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45 (s, 1H), 7.71 (d, *J* = 9.0 Hz, 1H), 6.04 (t, *J* = 3.0 Hz, 1H), 5.79 (dd, J_1 = 6.0 Hz, J_2 = 3.0 Hz, 1H), 5.49 (t, *J* = 4.5 Hz, 2H), 4.20 (d, *J* = 6.0 Hz, 1H), 3.99–3.85 (m, 2H), 2.40–2.31 (m, 4H), 1.67–1.57 (m, 5H), 1.27 (br, 24H), 0.89 ppm (t, *J* = 7.5 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.8, 172.4, 162.5, 150.2, 140.5, 109.5, 103.2, 88.0, 83.5, 72.7, 70.9, 61.9, 34.0, 33.8, 31.8, 29.4, 29.2, 29.1, 29.0, 24.7, 22.0, 14.0 ppm; IR (NaCl): $\tilde{\nu}$ = 3452, 2925, 2854, 1748, 1652, 1384, 1269 cm^{-1}; MS-FAB (*m/z*): found 553.30 $[M+H]^+$; $C_{29}H_{48}N_2O_8$ calcd 552.70.

*2′,3′-O-Dilauroyluridine (**4b**)*: Yield: 89%. m.p. 105.8–106.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.73 (d, *J* = 9.0 Hz, 1H), 6.03 (t, *J* = 3.0 Hz, 1H), 5.78 (d, *J* = 9.0 Hz, 1H), 5.47 (d, *J* = 3.0 Hz, 2H), 4.21 (s, 1H), 3.97–3.89 (m, 2H), 2.39–2.30 (m, 4H), 1.27 (br, 32H), 0.88 ppm (t, *J* = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.8, 172.4, 162.5, 150.2, 140.5, 109.4, 103.2, 88.0, 83.5, 72.8, 70.8, 61.9, 34.0, 33.8, 31.9, 29.6, 29.4, 29.3, 29.2, 29.1, 29.0, 24.8, 24.7, 22.6, 14.0 ppm; IR (NaCl): $\tilde{\nu}$ = 3479, 2955, 2850, 1739, 1653, 1386, 1255 cm^{-1}; MS-FAB (*m/z*): found 609.40 $[M+H]^+$; $C_{33}H_{56}N_2O_8$ calcd 609.40.

*2′,3′-O-Dimyristoyluridine (**4c**)*: Yield: 98%. m.p. 104.0–105.2 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.71 (d, *J* = 9.0 Hz, 1H), 6.03 (d, *J* = 6.0 Hz, 1H), 5.78 (d, *J* = 9.0 Hz, 1H), 5.47 (d, *J* = 6.0 Hz, 2H), 4.21 (s, 1H), 3.96–3.81 (m, 2H), 2.39–2.30 (m, 4H), 1.66–1.59 (m, 4H), 1.26 (br, 40H), 0.88 ppm (t, *J* = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.5, 173.1, 163.1, 150.8, 141.2, 103.9, 88.7, 84.2, 73.5, 71.5, 62.6, 34.7, 34.5, 32.6, 30.3, 30.2, 30.0, 29.9, 29.8, 29.7, 25.5, 25.4, 23.4, 14.7 ppm; IR (NaCl): $\tilde{\nu}$ = 3454, 2954, 2850, 1738, 1652, 1386, 1255 cm^{-1}; MS-FAB (*m/z*): found 665.40 $[M+H]^+$; $C_{37}H_{64}N_2O_8$ calcd for 665.47.

*2′,3′-O-Dipalmitoyluridine (**4d**)*: Yield: 96%. m.p. 111.6–112.7 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.74 (d, *J* = 9.0 Hz, 1H), 6.05 (t, *J* = 4.5 Hz, 1H), 5.79 (d, *J* = 9.0 Hz, 1H), 5.47 (d, *J* = 3.0 Hz, 2H), 4.20 (s, 1H), 3.90 (dd, J_1 = 7.5 Hz, J_2 = 3.0 Hz, 2H), 2.39–2.29 (m, 4H), 1.63–1.58 (m, 4H), 1.26 (br, 48H), 0.88 ppm (t, *J* = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.5, 173.1, 163.3, 150.9, 141.3, 110.1, 103.8, 88.6, 84.2, 73.6, 71.6, 62.6, 34.7, 34.5, 32.6, 30.4, 30.3, 30.2, 30.0, 29.9, 29.8, 29.7, 25.5, 25.4, 23.4, 14.7 ppm; IR (NaCl): $\tilde{\nu}$ = 3421, 2917, 2849, 1652 cm^{-1}; MS-FAB (*m/z*): found 721.50 $[M+H]^+$; $C_{41}H_{72}N_2O_8$ calcd 721.51.

*2′,3′-O-Distearoyluridine (**4e**)*: Yield: 96%. m.p. 111.7–112.7 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.71 (d, *J* = 6.0 Hz, 1H), 6.85 (d, *J* = 9.0 Hz, 1H), 6.03 (t, *J* = 3.0 Hz, 1H), 5.78 (d, *J* = 6.0 Hz, 1H), 5.48 (t, *J* = 3.0 Hz, 1H), 4.21 (s, 1H), 4.00–3.86 (m, 2H), 2.40–2.31 (m, 5H), 1.26 (br, 56H), 0.89 ppm (t, *J* = 6.0 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): spectrum not obtained because of poor solubility; IR (NaCl): $\tilde{\nu}$ = 3421, 2917, 2849, 1645 cm^{-1}; MS-FAB (*m/z*): found 799.60 $[M+Na]^+$; $C_{45}H_{80}N_2O_8$ calcd 799.59.

*5′-O-Phosphatidylcholine-2′,3′-O-didecanoyluridine (**7a**)—General Procedure*: 2-Chloro-1,3,2-dioxaphospholane (245 μL, 2.75 mmol) was added to a solution of **4a** (1.01 g, 1.83 mmol) and diisopropylethylamine (384 μL, 2.20 mmol) in dry THF (20 mL), and the mixture was then stirred for 15 min at room temperature. Br_2 (282 μL, 5.50 mmol) in dry THF (20 mL) was slowly added while stirring at 0 °C for 1 h. The solvent was evaporated, and the residue was dissolved in $CHCl_3$/*i*PrOH/CH_3CN (3:5:5). Aqueous trimethylamine (40%, 10 mL) was added. The mixture was stirred for 3 days at room temperature, and the solvents were then evaporated. Purification was performed by flash chromatography (SiO_2; CH_2Cl_2/MeOH/H_2O 14:5:0.1 to 140:55:8). Silica gel was removed by filtration through celite, and **7a** was obtained upon evaporation of the solvent (668 mg, 51%). m.p. > 152.3 °C (dec.); ^{1}H NMR (300 MHz; $CDCl_3$/CD_3OD 1:1): δ = 7.86 (d, *J* = 9.0 Hz, 1H), 6.22 (d, *J* = 6.0 Hz, 1H), 5.92 (d, *J* = 9.0 Hz, 1H), 5.48 (d, *J* = 6.0 Hz, 1H), 5.31 (t, *J* = 6.0 Hz, 1H), 4.47 (s, 2H), 4.28 (s, 1H), 4.20 (s, 2H), 3.71 (s, 2H), 3.23 (s, 9H), 2.38 (t, *J* = 6.0 Hz, 2H), 2.28 (t, *J* = 6.0 Hz, 2H), 1.62–1.54(m, 5H), 1.26 (br, 24H), 0.86 ppm (t, *J* = 6.0 Hz, 6H); ^{13}C NMR (75 MHz; $CDCl_3$/CD_3OD 1:1): δ = 173.2, 172.9, 165.2, 151.1, 141.2, 103.2, 85.9, 82.5, 82.4, 73.2, 71.3, 66.7, 65.2, 59.7, 54.5, 34.1, 33.8, 31.9, 29.5, 29.3, 29.2, 29.1, 24.9, 24.7, 22.7, 13.9 ppm; ^{31}P NMR (121 MHz; $CDCl_3$/CD_3OD 1:1): δ = −0.5845 ppm; IR (NaCl): $\tilde{\nu}$ = 3446, 2955, 2853, 1739, 1652, 1617, 1457, 1378 cm^{-1}; MS-FAB (*m/z*): found 718.40 $[M+H]^+$; $C_{34}H_{60}N_3O_{11}P$ calcd 718.40; HRMS-FAB (*m/z*): found 718.4047 $[M+H]^+$; $C_{34}H_{60}N_3O_{11}P$ calcd 718.4044.

*5′-O-Phosphatidylcholine-2′,3′-O-dilauroyluridine (**7b**)*: Yield: 42%. m.p. > 145.3 °C (dec.); ^{1}H NMR (300 MHz; $CDCl_3$/CD_3OD 1:1): δ = 7.91 (d, *J* = 9.0 Hz, 1H), 6.24 (d, *J* = 6.0 Hz, 1H), 5.92 (d, *J* = 9.0 Hz, 1H), 5.50 (dd, J_1 = 6.0 Hz, J_2 = 3.0 Hz, 1H), 5.32 (t, *J* = 7.5 Hz, 1H), 4.54 (s, 2H), 4.31 (s, 1H), 4.25 (s, 2H), 3.73 (s, 2H), 3.24 (s, 9H), 2.39 (t, *J* = 7.5 Hz, 2H), 2.29 (t, *J* = 7.5 Hz, 2H), 1.63–1.55 (m, 5H), 1.27 (br, 32H), 0.87 ppm (t, *J* = 6.0 Hz, 6H); ^{13}C NMR (75 MHz; $CDCl_3$/CD_3OD 1:1): δ = 172.9, 172.6, 165.2, 150.9, 141.1, 102.8, 85.8, 82.4, 73.1, 71.3, 66.6, 65.1, 59.6, 54.3, 31.8, 29.5, 29.4, 29.3, 29.2, 29.0, 28.9, 24.7, 24.5, 22.5, 13.9 ppm; ^{31}P NMR (121 MHz; $CDCl_3$/CD_3OD 1:1): δ = −0.4853 ppm; IR (NaCl): $\tilde{\nu}$ = 3448, 2955, 2853, 1739, 1652, 1457, 1378, 1244 cm^{-1}; MS-FAB (*m/z*): found 774.46 $[M+H]^+$; $C_{38}H_{68}N_3O_{11}P$ calcd 774.46; HRMS-FAB (*m/z*): found 774.4670 $[M+H]^+$; $C_{38}H_{68}N_3O_{11}P$ calcd 774.4670.

*5′-O-Phosphatidylcholine-2′,3′-O-dimyristoyluridine (**7c**)*: Yield: 37%. m.p. > 147.4 °C (dec.); ^{1}H NMR (300 MHz; $CDCl_3$/CD_3OD 1:1): δ = 7.75 (d, *J* = 9.0 Hz, 1H), 6.08 (d, *J* = 6.0 Hz, 1H), 5.78 (d, *J* = 9.0 Hz, 1H), 5.35 (dd, J_1 = 6.0 Hz, J_2 = 3.0 Hz, 1H), 5.18 (t, *J* = 6.0 Hz, 1H), 4.38 (s, 2H), 4.16 (s, 1H), 4.12 (s, 2H), 3.58 (s, 2H), 3.08 (s, 9H), 2.25 (t, *J* = 7.5 Hz, 2H), 2.14 (t, *J* = 7.5 Hz, 2H), 1.48–1.40 (m, 5H), 1.12 (br, 40H), 0.72 ppm (t, *J* = 6.0 Hz, 6H); ^{13}C NMR (75 MHz; $CDCl_3$/CD_3OD 1:1): δ = 172.9, 172.7, 165.3, 151.0, 141.2, 102.9, 85.7, 82.3, 73.1, 71.3, 66.5, 65.2, 59.6, 54.3, 31.8, 29.6, 29.4, 29.3, 29.2, 29.1, 29.0, 24.8, 24.5, 22.5, 13.8 ppm; ^{31}P NMR (121 MHz; $CDCl_3$/CD_3OD 1:1): δ = −0.5202 ppm; IR (NaCl): $\tilde{\nu}$ = 3452, 1749, 1652, 1456 cm^{-1}; MS-FAB (*m/z*): found 830.40 $[M+H]^+$; $C_{42}H_{76}N_3O_{11}P$ calcd 830.52; HRMS-FAB (*m/z*): found 830.5293 $[M+H]^+$; $C_{42}H_{76}N_3O_{11}P$ calcd 830.5296.

*5′-O-Phosphatidylcholine-2′,3′-O-dipalmitoyluridine (**7d**)*: Yield: 53%. m.p. > 146.5 °C (dec.); ^{1}H NMR (300 MHz; $CDCl_3$/CD_3OD 1:1): δ = 7.91 (d, *J* = 9.0 Hz, 1H), 6.24 (d, *J* = 6.0 Hz, 1H), 5.93 (d, *J* = 9.0 Hz, 1H), 5.50 (d, *J* = 6.0 Hz, 1H), 5.33 (t, *J* = 7.5 Hz, 1H), 4.53 (s, 2H), 4.31 (s, 1H), 4.25 (s, 2H), 3.73 (s, 2H), 3.24 (s, 9H), 2.39 (t, *J* = 7.5 Hz, 2H), 2.29 (t, *J* = 7.5 Hz, 2H), 1.63–1.56 (m, 5H), 1.26 (br, 48H), 0.86 ppm (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (75 MHz; $CDCl_3$/CD_3OD 1:1): δ = 172.9, 172.6, 165.3, 151.0, 141.1, 109.4, 102.9, 85.9, 82.4, 73.2,

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71.3, 66.5, 65.1, 59.6, 54.3, 33.6, 31.8, 29.6, 29.5, 29.4, 29.2, 29.0, 24.5, 22.5, 13.7 ppm; ^{31}P NMR (121 MHz; $CDCl_3/CD_3OD$ 1:1): $\delta = -0.9707$ ppm; IR (NaCl): $\tilde{\nu} = 3452, 1733, 1652, 1456, 772\ cm^{-1}$; MS-FAB (*m/z*): found 886.59 $[M+H]^+$; $C_{46}H_{84}N_3O_{11}P$ calcd 886.58; HRMS-FAB (*m/z*): found 886.5922 $[M+H]^+$; $C_{46}H_{83}N_3O_{11}P$ calcd 886.5922.

*5′-O-Phosphatidylcholine-2′,3′-O-distearoyluridine (**7e**)*: Yield: 58%. m.p. >103.2 °C (dec.); ^{1}H NMR (300 MHz; $CDCl_3/CD_3OD$ 1:1): $\delta =$ 7.87 (d, $J = 9.0$ Hz, 1H), 6.19 (d, $J = 6.0$ Hz, 1H), 5.99 (d, $J = 9.0$ Hz, 1H), 5.45 (t, $J = 3.0$ Hz, 1H), 5.28 (t, $J = 6.0$ Hz, 1H), 4.99 (s, 2H), 4.27 (s, 1H), 4.21 (s, 2H), 3.69 (s, 2H), 3.19 (s, 9H), 2.35 (t, $J = 7.5$ Hz, 2H), 2.25 (t, $J = 7.5$ Hz, 2H), 1.59–1.51 (m, 5H), 1.22 (br, 56H), 0.83 ppm (d, $J = 6.0$ Hz, 6H); ^{13}C NMR (75 MHz; $CDCl_3/CD_3OD$ 1:1): $\delta = 173.6$, 173.3, 165.9, 151.7, 141.8, 103.5, 86.5, 83.0, 73.8, 72.0, 67.3, 65.8, 60.4, 54.9, 34.3, 32.5, 30.3, 30.2, 30.1, 29.9, 29.8, 29.7, 25.4, 25.2, 23.2, 14.3 ppm; ^{31}P NMR (121 MHz; $CDCl_3/CD_3OD$ 1:1): $\delta =$ 1.7925 ppm; IR (NaCl): $\tilde{\nu} = 3409, 2916, 2849, 1692, 1467, 1252\ cm^{-1}$; MS-FAB (*m/z*): found 942.66 $[M+H]^+$; $C_{50}H_{92}N_3O_{11}P$ calcd 942.65; HRMS-FAB (*m/z*): found 942.6550 $[M+H]^+$; $C_{50}H_{92}N_3O_{11}P$ calcd 942.6548.

Adenosine-based Phospholipids

*5′-O-(4-Methoxytrityl)adenosine (**2**)*: MMTr-Cl (2.14 g, 6.31 mmol) was added to a solution of adenosine (1.30 g, 4.86 mmol) in dry pyridine (60 mL) and the mixture was stirred at room temperature for 20 h. The solvent was evaporated under high vacuum, and the resultant oil was dissolved in CH_2Cl_2 and then washed with water. The organic layer was dried over anhydrous Na_2SO_4. Chromatographic purification (SiO_2; CH_2Cl_2/MeOH 50:1) yielded **2** (1.62 g, 62%). m.p. 159–160 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.09$ (s, 1H), 8.02 (s, 1H), 7.26–7.08 (m, 14H), 6.67 (d, $J = 8.3$ Hz, 2H), 5.89 (d, $J =$ 3.9 Hz, 1H), 5.19 (m, 1H), 4.46 (m, 1H), 4.29 (m, 1H), 3.65 (s, 3H), 3.25 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 159.0$, 156.1, 152.9, 149.3, 144.4, 144.3, 139.3, 135.5, 130.8, 128.8, 128.2, 127.4, 119.8, 113.5, 89.7, 87.3, 84.6, 75.45, 71.0, 63.5, 55.5 ppm; IR (NaCl): $\tilde{\nu} =$ 3353, 2926, 2855, 1665, 1605, 1443 cm^{-1}; MS-FAB (*m/z*): found 540.00 $[M+H]^+$; $C_{30}H_{29}N_5O_5$ calcd 539.22.

*2′,3′-O-Didecanoyladenosine (**5a**)—General Procedure*: Decanoic acid (1.13 g, 7.87 mmol) was added to a solution of **2** (1.70 g, 3.15 mmol), EDC (1.51 g, 7.87 mmol), and DMAP (461 mg, 3.78 mmol) in dry CH_2Cl_2 (40 mL). The mixture was stirred at room temperature for 4 h and was then extracted with water and concentrated to dryness. The residue was dissolved in acetic acid (80%, 50 mL) and was then heated at 60 °C for 3 h. The reaction mixture was washed with water and CH_2Cl_2, and the organic layer was separated, dried (Na_2SO_4), and then evaporated under reduced pressure. Column chromatographic purification (SiO_2; CH_2Cl_2/MeOH 60:1) afforded **5a** (1.60 g, 95%). m.p. 150–151 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.34$ (s, 1H), 7.82 (s, 1H), 6.75 (d, $J = 9.2$ Hz, 1H), 6.00 (m, 2H), 5.71 (m, 1H), 4.34 (m, 1H), 3.96 (m, 2H), 2.40 (t, $J = 7.4$ Hz, 2H), 2.24 (t, $J = 7.4$ Hz, 2H), 1.66 (br, 2H), 1.52 (br, 2H), 1.32–1.23 (br, 24H), 0.90–0.84 ppm (br, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 173.1$, 172.4, 156.8, 153.5, 149.4, 140.7, 121.8, 89.2, 87.2, 73.4, 73.2, 63.2, 34.8, 34.3, 32.5, 32.5, 30.1, 30.0, 29.9, 29.9, 29.9, 29.8, 29.8, 29.7, 25.6, 25.3, 23.3, 23.3, 14.7 ppm; IR (NaCl): $\tilde{\nu} = 3442$, 2923, 2851, 1742, 1641 cm^{-1}; MS-FAB (*m/z*): found 576.20 $[M+H]^+$; $C_{30}H_{49}N_5O_6$ calcd 575.37.

*2′,3′-O-Dilauroyladenosine (**5b**)*: Yield: 88%. m.p. 148–149 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.36$ (s, 1H), 7.81 (s, 1H), 6.70 (d, $J =$ 10.3 Hz, 1H), 6.06–5.98 (m, 2H), 5.89 (s, 2H), 5.71 (m, 1H), 4.35 (s, 1H), 4.01–3.82 (m, 2H), 2.40 (t, $J = 7.4$ Hz, 2H), 2.24 (t, $J = 7.3$ Hz, 2H), 1.89 (s, 1H), 1.66 (m, 2H), 1.52 (m, 2H), 1.27–1.22 (br, 32H), 0.89–0.85 ppm (br, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 173.2$, 172.5, 156.7, 153.4, 149.3, 140.7, 121.7, 89.2, 87.2, 73.3, 73.2, 63.2, 34.8, 34.3, 32.5, 30.2, 30.1, 30.0, 29.9, 29.8, 29.8, 29.6, 25.5, 25.2, 23.3, 14.7 ppm; IR (NaCl): $\tilde{\nu} = 3423, 2924, 2852, 1733, 1653\ cm^{-1}$; MS-FAB (*m/z*): found 632.20 $[M+H]^+$; $C_{34}H_{57}N_5O_6$ calcd 631.43.

*2′,3′-O-Dimyristoyladenosine (**5c**)*: Yield: 98%. m.p. 147–148 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.31$ (s, 1H), 7.79 (s, 1H), 6.70 (d, $J =$ 10.2 Hz, 1H), 6.06 (s, 2H), 6.02–5.96 (m, 2H), 5.71 (m, 1H), 4.37 (m, 1H), 3.98–3.85 (m, 2H), 2.37 (t, $J = 7.5$ Hz, 2H), 2.21 (t, $J = 7.3$ Hz, 2H), 1.64 (m, 2H), 1.49 (m, 2H), 1.29–1.20 (br, 40H), 0.87–0.82 ppm (br, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 172.8$, 172.1, 156.4, 153.1, 140.3, 121.4, 88.8, 86.9, 73.0, 72.9, 62.9, 34., 34.02, 32.2, 30.0, 29.9, 29.9, 29.8, 29.7, 29.6, 29.5, 29.5, 29.3, 24.9, 22.9, 14.4 ppm; IR (NaCl): $\tilde{\nu} = 3439, 2920, 2858, 1745, 1652\ cm^{-1}$; MS-FAB (*m/z*): found 688.30 $[M+H]^+$; $C_{38}H_{65}N_5O_6$ calcd 687.49.

*2′,3′-O-Dipalmitoyladenosine (**5d**)*: Yield: 85%. m.p. 140–141 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.80$ (s, 1H), 6.72 (s, 1H), 6.16 (d, $J =$ 10.2 Hz, 1H), 5.47 (m, 2H), 5.25 (s, 2H), 5.16 (m, 1H), 3.80 (m, 1H), 3.42–3.32 (m, 2H), 1.85 (t, $J = 7.4$ Hz, 2H), 1.67 (t, $J = 7.6$ Hz, 2H), 1.26 (s, 1H), 1.17 (m, 2H), 0.98 (m, 2H), 0.77–0.68 (br, 48H), 0.35–0.31 ppm (br, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 173.1$, 172.4, 156.6, 153.5, 140.3, 121.4, 89.3, 87.3, 78.1, 73.2, 63.3, 34.8, 34.3, 32.6, 30.3, 30.3, 30.2, 30.1, 30.0, 29.9, 29.8, 29.7, 25.6, 25.3, 23.3, 14.7 ppm; IR (NaCl): $\tilde{\nu} = 3438, 2925, 2855, 1747, 1640\ cm^{-1}$; MS-FAB (*m/z*): found 744.40 $[M+H]^+$; $C_{42}H_{73}N_5O_6$ calcd 743.56.

*2′,3′-O-Distearoyladenosine (**5e**)*: Yield: 81.5%. m.p. 141–142 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.35$ (s, 1H), 7.98 (s, 1H), 6.70 (d, $J =$ 10.7 Hz, 1H), 6.00 (s, 2H), 5.73 (m, 2H), 4.35 (m, 1H), 4.01–3.86 (m, 2H), 2.40 (t, $J = 7.4$ Hz, 2H), 2.24 (t, $J = 7.4$ Hz, 2H), 1.69 (m, 2H), 1.52 (m, 2H), 1.25 (br, 56H), 0.86 ppm (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 173.6$, 172.8, 157.06, 153.9, 149.7, 141.2, 122.3, 89.8, 87.8, 78.5, 73.7, 63.7, 35.2, 34.7, 33.0, 30.8, 30.7, 30.6, 30.5, 30.4, 30.3, 30.3, 30.1, 26.0, 25.7, 23.8, 15.2 ppm; IR (NaCl): $\tilde{\nu} = 3430, 2916,$ 2849, 1740, 1646 cm^{-1}; MS-FAB (*m/z*): found 800.30 $[M+H]^+$; $C_{46}H_{81}N_5O_6$ calcd 799.62.

*5′-O-Phosphatidylcholine-2′,3′-O-didecanoyladenosine (**8a**)—General Procedure*: 2-Chloro-1,3,2-dioxaphospholane (158 μL, 1.78 mmol) was added to a solution of **5a** (620 mg, 1.19 mmol) and diisopropylethylamine (248 μL, 1.42 mmol) in dry THF (20 mL), and the mixture was then stirred for 15 min at room temperature. Br_2 (183 μL, 3.57 mmol) in dry THF (20 mL) was then slowly added with stirring at 0 °C for 1 h. The solvents were evaporated, and the residue was dissolved in $CHCl_3$/*i*PrOH/CH_3CN (3:5:5). Aqueous trimethylamine (40%, 10 mL) was added, the mixture was stirred for 3 days at room temperature, and the solvents were then evaporated. Purification was accomplished by flash column chromatography (CH_2Cl_2/MeOH/H_2O 14:5:0.1 to 140:55:8). Removal of silica gel was accomplished by filtration through celite, and the solvent was then evaporated to afford **8a** (260 mg, 29.4%). m.p. 240–241 °C; ^{1}H NMR (300 MHz, CD_3COOD): $\delta = 11.56$ (s, 2H), 8.66 (s, 1H), 8.39 (s, 1H), 6.44 (d, $J = 6.5$ Hz, 1H), 5.73 (m, 1H), 5.71 (m, 1H), 4.56–4.51 (m, 3H), 4.35 (s, 2H), 3.77 (s, 2H), 2.28 (s, 9H), 2.49 (t, $J = 7.0$ Hz, 2H), 2.31 (t, $J = 7.0$ Hz, 2H), 1.72 (m, 2H), 1.55 (m, 2H), 1.34–1.27 (br, 24H), 0.93 ppm (m, 6H); ^{13}C NMR (75 MHz, CD_3COOD): $\delta = 173.2$, 172.7, 153.9, 149.7, 148.9, 140.9, 117.9, 85.86, 83.1, 83.0, 74.6, 71.7, 66.5, 65.3, 59.9, 54.2, 33.9, 33.6, 31.9, 30.0, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 24.9, 24.6, 22.69, 20.0, 13.6 ppm; ^{31}P NMR (121 MHz; $CDCl_3/CD_3OD$ 1:1): $\delta = 1.8332$ ppm; IR (NaCl): $\tilde{\nu} = 3428, 2923, 2853,$ 1747, 1643 cm^{-1}; HRMS-FAB (*m/z*): found 741.4316 $[M+H]^+$; $C_{35}H_{62}N_6O_9P$ calcd 741.4316.

*5′-O-Phosphatidylcholine-2′,3′-O-dilauroyladenosine (**8b**)*: Yield: 33%. m.p. 239–240 °C; ^{1}H NMR (300 MHz, CD_3COOD): $\delta = 11.57$ (s, 2H),

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8.68 (s, 1H), 8.41 (s, 1H), 6.45 (d, J=6.7 Hz, 1H), 5.79 (m, 2H), 5.72 (m, 1H), 4.57–4.52 (m, 3H), 4.36 (s, 2H), 3.78 (s, 2H), 3.29 (s, 9H), 2.50 (t, J=7.3 Hz, 2H), 2.32 (t, J=7.2 Hz, 2H), 1.73 (m, 2H), 1.56 (m, 2H), 1.34–1.29 (br, 32H), 0.94 ppm (m, 6H); ^{13}C NMR (75 MHz, CD_3COOD): δ=173.1, 172.6, 153.6, 149.4, 141.0, 140.9, 117.9, 85.8, 83.0, 74.5, 71.6, 66.4, 65.2, 59.8, 54.1, 33.5, 33.5, 31.9, 29.9, 29.7, 29.6, 29.5, 29.4, 29.3, 29.2, 29.0, 24.8, 24.6, 22.6, 13.5 ppm; ^{31}P NMR (121 MHz; $CDCl_3/CD_3OD$ 1:1): δ=1.8615; IR (NaCl): $\tilde{\nu}$=3426, 2922, 2852, 1741, 1643 cm^{-1}; HRMS-FAB (*m/z*): found 797.4940 $[M+H]^+$; $C_{39}H_{70}N_6O_9P$ calcd 797.4942.

5′-O-Phosphatidylcholine-2′,3′-O-dimyristoyladenosine (**8c**): Yield: 28%. m.p. 246–247°C; ^{1}H NMR (300 MHz, CD_3COOD): δ=11.47 (s, 2H), 8.66 (s, 1H), 6.44 (d, J=6.6 Hz, 1H), 5.77 (m, 1H), 5.71 (m, 1H), 4.55 (m, 3H), 4.35 (s, 2H), 3.76 (s, 2H), 3.28 (s, 9H), 2.49 (t, J=7.4 Hz, 2H), 2.31 (t, J=7.4 Hz, 2H), 1.72 (m, 2H), 1.55 (m, 2H), 1.31–1.28 (m, 40H), 0.95–0.93 ppm (m, 6H); ^{13}C NMR (75 MHz, CD_3COOD): δ=173.1, 172.7, 153.6, 149.6, 141.0, 140.7, 117.5, 85.8, 83.0, 74.5, 71.6, 66.4, 65.2, 59.8, 54.1, 33.8, 33.5, 31.9, 29.7, 29.4, 29.0, 24.9, 24.6, 22.6, 13.5 ppm; ^{31}P NMR (121 MHz; $CDCl_3/CD_3OD$ 1:1): δ=1.8497 ppm; IR (NaCl): $\tilde{\nu}$=3443, 2923, 2854, 1738, 1644 cm^{-1}; HRMS-FAB (*m/z*): found 853.5570 $[M+H]^+$; $C_{43}H_{78}N_6O_9P$ calcd 853.5568.

5′-O-Phosphatidylcholine-2′,3′-O-dipalmitoyladenosine (**8d**): Yield: 22%. m.p. 235–236°C; ^{1}H NMR (300 MHz, CD_3COOD): δ=8.61 (s, 1H), 8.35 (s, 1H), 6.38 (d, J=6.2 Hz, 1H), 5.72 (m, 1H), 4.49 (m, 3H), 4.29 (s, 2H), 3.70 (s, 2H), 3.22 (s, 9H), 2.43 (t, J=6.9 Hz, 2H), 2.26 (t, J=6.9 Hz, 2H), 1.66 (m, 2H), 1.50 (m, 2H), 1.26 (br, 48H), 0.87 ppm (m, 6H); ^{13}C NMR (75 MHz, CD_3COOD): δ=173.1, 172.6, 153.5, 149.2, 148.9, 141.1, 117.8, 85.9, 83.1, 82.9, 74.5, 71.6, 66.4, 65.2, 59.9, 54.1, 33.8, 33.5, 31.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.3, 29.2, 29.0, 24.8, 24.6, 22.6, 13.5 ppm; ^{31}P NMR (121 MHz; $CDCl_3/CD_3OD$ 1:1): δ=1.8413 ppm; IR (NaCl): $\tilde{\nu}$=3433, 2917, 2849, 1747, 1644 cm^{-1}; HRMS-FAB (*m/z*): found 909.6198 $[M+H]^+$; $C_{47}H_{86}N_6O_9P$ calcd 909.6194.

5′-O-Phosphatidylcholine-2′,3′-O-distearoyladenosine (**8e**): Yield: 18.6%. m.p. 238–239°C; ^{1}H NMR (300 MHz, CD_3COOD): δ=11.74 (s, 2H), 8.61 (s, 1H), 8.34 (s, 1H), 6.38 (d, J=6.6 Hz, 1H), 5.71 (m, 1H), 5.65 (m, 1H), 4.51 (m, 3H), 4.30 (s, 2H), 3.71 (s, 2H), 3.22 (s, 9H), 2.43 (t, J=7.5 Hz, 2H), 2.25 (t, J=7.4 Hz, 2H), 1.66 (m, 2H), 1.49 (m, 2H), 1.26–1.22 (br, 56H), 0.87 ppm (m, 6H); ^{13}C NMR (75 MHz, CD_3COOD): δ=173.8, 172.8, 153.8, 149.7, 148.5, 141.2, 117.7, 85.3, 83.2, 82.7, 76.8, 74.3, 71.5, 66.5, 65.3, 59.8, 54.2, 33.8, 33.5, 31.8, 29.6, 29.5, 29.5, 29.4, 29.2, 29.2, 29.1, 28.9, 24.7, 24.5, 22.5, 20.3, 20.0, 13.7 ppm; ^{31}P NMR (121 MHz; $CDCl_3/CD_3OD$ 1:1): δ=1.8856 ppm; IR (NaCl): $\tilde{\nu}$=3430, 2916, 2849, 1747, 1646 cm^{-1}; HRMS-FAB (*m/z*): found 965.6820 $[M+H]^+$; $C_{51}H_{93}N_6O_9P$ calcd 965.6820.

Inosine-based Phospholipids

5′-O-(4-Methoxytrityl)inosine (**3**): MMTr-Cl (2.96 g, 9.60 mmol) was added to a solution of inosine (2.15 g, 8.00 mmol) in dry pyridine/DMSO (1:1, 50 mL), and the mixture was stirred at room temperature for 18 h. The solvent was evaporated under high vacuum, and residual DMSO was removed by extraction with water and CH_2Cl_2. The organic layer was dried (Na_2SO_4) and concentrated to dryness. Column chromatographic purification (SiO_2; CH_2Cl_2/MeOH 50:1) yielded **3** (2.00 g, 74%). m.p. 208.8–210.1°C; ^{1}H NMR (300 MHz; $CDCl_3/CD_3OD$ 5:1): δ=7.84 (s, 1H), 7.67 (s, 1H), 7.18 (d, J=7.02 Hz, 1H), 7.08–6.94 (m, 8H), 6.58 (d, J=9.1 Hz, 2H), 5.80 (d, J=5.2 Hz, 1H), 4.48 (t, J=5.1 Hz, 1H), 4.18 (t, J=4.6 Hz, 1H), 4.04 (q, J=3.9 Hz, 1H), 3.54 (s, 3H), 3.24–3.12 ppm (m, 2H); ^{13}C NMR (75 MHz; $CDCl_3/CD_3OD$ 5:1): δ=159.3, 158.2, 149.1, 145.7, 144.7, 144.7, 139.4, 135.9, 130.9, 129.0, 128.3, 127.5, 125.6, 113.7, 89.5, 87.4, 84.9, 75.2, 71.5, 64.2, 55.6 ppm; MS-FAB (*m/z*): 541.1 $[M+H]^+$.

2′,3′-O-Didecanoylinosine (**6a**)*—General Procedure*: Decanoic acid (798 mg, 4.63 mmol) was added to a solution of **3** (1.00 g, 1.85 mmol), EDC (888 mg, 24.6 mmol), and DMAP (271 mg, 2.20 mmol) in dry CH_2Cl_2 (40 mL). The mixture was stirred at room temperature for 4 h and was then extracted with water and concentrated to dryness. The residue was dissolved in acetic acid (80%, 50 mL) and was then heated at 60°C for 3 h. The reaction mixture was washed with water and CH_2Cl_2, the organic layer was separated and dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. Column chromatographic purification (SiO_2; CH_2Cl_2/MeOH 60:1) afforded **6a** (897 mg, 84%). m.p. 169.7–171.9°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=13.21 (s, 1H), 8.52 (s, 1H), 8.05 (s, 1H), 6.06 (d, J=7.5 Hz, 1H), 5.94 (t, J=5.4 Hz, 1H), 5.69 (d, J=5.3 Hz, 1H), 4.35 (s, 1H), 3.98 (t, J=15.3 Hz, 2H), 2.40 (t, J=7.5 Hz, 2H), 2.26 (t, J=7.4 Hz, 2H), 1.64 (br, 2H), 1.53 (br, 2H), 1.32–1.22 (m, 24H), 0.90–0.82 ppm (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=172.7, 172.0, 158.4, 148.0, 146.5, 140.0, 126.2, 88.1, 86.2, 73.3, 72.4, 62.5, 34.2, 33.7, 32.0, 31.9, 29.5, 29.5, 29.4, 29.4, 29.3, 29.3, 29.1, 25.0, 24.7, 22.8, 22.7 14.2 ppm; MS-FAB (*m/z*): 599.3 $[M+H]^+$; elemental analysis calcd (%) for $C_{30}H_{48}N_4O_7$: C 62.48, H 8.39, N 9.71; found C 62.54, H 8.21, N 9.80.

2′,3′-O-Dilauroylinosine (**6b**): Yield: 87%. m.p. 164.0–165.9°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.38 (s, 1H), 7.97 (s, 1H), 6.03 (d, J=7.5 Hz, 1H), 5.94 (t, J=5.4 Hz, 1H), 5.68 (d, J=5.4 Hz, 1H), 4.36 (s, 1H), 3.95 (dd, J_1=19.5 Hz, J_2=12.0 Hz, 2H), 2.41 (t, J=7.4 Hz, 2H), 2.26 (t, J=7.4 Hz, 2H), 1.65 (br, 2H), 1.54 (br, 2H), 1.33–1.24 (m, 32H), 0.91–0.85 (m, 6H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ=172.7, 172.0, 158.7, 148.0, 145.9, 140.1, 126.8, 88.5, 86.3, 73.2, 72.4, 62.7, 34.3, 33.8, 32.1, 29.8, 29.7, 29.6, 29.5, 29.4, 29.4, 29.2, 25.1, 24.8, 22.8, 14.2 ppm; IR (NaCl): $\tilde{\nu}$=3468, 2923, 2853, 1743, 1719, 1595, 1555, 1467, 1422, 1162 cm^{-1}; MS-FAB (*m/z*): 655.4 $[M+Na]^+$; elemental analysis calcd (%) for $C_{34}H_{56}N_4O_7$: C 64.53, H 8.92, N 8.85; found C 64.74, H 8.72, N 8.76.

2′,3′-O-Dimyristoylinosine (**6c**): Yield: 77%. m.p.: 162.2–163.6°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ=12.94 (s, 1H), 8.36 (s, 1H), 7.98 (s, 1H), 6.01 (d, J=7.6 Hz, 1H), 5.91 (t, J=5.4 Hz, 1H), 5.66 (d, J=5.2 Hz, 1H), 4.33 (s, 1H), 3.92 (dd, J_1=19.7 Hz, J_2=12.5 Hz, 2H), 2.38 (t, J=7.4 Hz, 2H), 2.23 (t, J=7.4 Hz, 2H), 1.64 (br, 2H), 1.51 (br, 2H), 1.24 (br, 40H), 0.86–0.82 ppm (m, 6H); ^{13}C NMR (75 MHz; $CDCl_3/CD_3OD$ 5:1): δ=172.8, 172.2, 157.5, 147.8, 145.6, 139.6, 126.0, 88.6, 85.7, 73.4, 72.1, 62.1, 34.1, 33.7, 31.9, 29.6, 29.5, 29.4, 29.3, 29.2, 29.2, 29.0, 24.9, 24.6, 22.6, 14.0 ppm; MS-FAB (*m/z*): 689.2 $[M+H]^+$; elemental analysis calcd (%) for $C_{38}H_{64}N_4O_7$: C 66.25, H 9.36, N 8.13; found C 66.44, H 9.38, N 8.09.

2′,3′-O-Dipalmitoylinosine (**6d**): Yield: 85%. m.p. 160.6–163.9°C; ^{1}H NMR (300 MHz; $CDCl_3/CD_3OD$ 5:1): δ=13.15 (s, 1H), 8.47 (s, 1H), 8.01 (s, 1H), 6.05 (d, J=7.5 Hz, 1H), 5.94 (t, J=5.4 Hz, 1H), 5.70 (d, J=5.1 Hz, 1H), 4.36 (s, 1H), 3.96 (dd, J_1=18.9 Hz, J_2=12.3 Hz, 2H), 2.41 (t, J=7.5 Hz, 2H), 2.26 (t, J=7.5 Hz, 2H), 1.66 (br, 2H), 1.53 (br, 2H), 1.33–1.23 (m, 48H), 0.89–0.85 ppm (m, 6H); ^{13}C NMR (75 MHz; $CDCl_3/CD_3OD$ 5:1): δ=172.8, 172.2, 157.4, 147.8, 145.5, 139.6, 126.2, 87.7, 85.7, 73.4, 72.1, 62.2, 34.1, 33.7, 31.9, 29.7, 29.7, 29.5, 29.5, 29.4, 29.3, 29.2, 29.1, 25.0, 24.7, 22.7, 14.0 ppm; MS-FAB (*m/z*): 745.4 $[M+H]^+$; elemental analysis calcd (%) for $C_{42}H_{72}N_4O_7$: C 67.71, H 9.74, N 7.52; found: C 67.88, H 9.79, N 7.32.

5′-O-Phosphatidylcholine-2′,3′-O-didecanoylinosine (**9a**)*—General Procedure*: 2-Chloro-1,3,2-dioxaphospholane (117 μL, 1.31 mmol) was added to a solution of **6a** (500 mg, 0.87 mmol) and diisopropylethylamine (303 μL, 1.74 mmol) in dry THF (20 mL), and the mix-

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ture was then stirred for 15 min at room temperature. Br_2 (130 μL, 2.61 mmol) in dry THF (20 mL) was then slowly added with stirring at 0 °C for 1 h. The solvent was evaporated, and the residue was dissolved in $CHCl_3$/*i*PrOH/CH_3CN (3:5:5). Aqueous trimethylamine (40%, 10 mL) was added, the mixture was stirred for 3 days at room temperature, and the solvents were evaporated. Purification was accomplished by flash chromatography (SiO_2; CH_2Cl_2/MeOH/H_2O 14:5:0.1 to 140:55:8). Removal of silica gel was accomplished by filtration through celite, and the solvent was then evaporated to afford **9a** (173 mg, 27%). m.p. >197.0 °C (dec.); ^{1}H NMR (300 MHz; $CDCl_3$/CD_3COOD 5:1): δ = 8.54 (s, 1H), 8.21 (s, 1H), 6.28 (d, J = 6.1 Hz, 1H), 5.83 (t, J = 5.7 Hz, 1H), 5.67 (m, 1H), 4.46 (br, 3H), 4.30 (br, 2H), 3.70 (br, 2H), 3.23 (s, 9H), 2.43 (t, J = 7.2 Hz, 2H), 2.30 (t, J = 7.4 Hz, 2H), 1.67 (br, 2H), 1.54 (br, 2H), 1.26 (m, 24H), 0.88 ppm (m, 6H); ^{13}C NMR (75 MHz; $CDCl_3$/CD_3COOD 5:1): δ = 173.1, 172.8, 158.9, 149.4, 146.0, 140.2, 123.9, 86.6, 83.3, 74.6, 71.8, 68.0, 66.9, 65.1, 60.0, 54.8, 34.3, 34.1, 32.2, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 29.4, 25.2, 25.0, 22.3, 14.2 ppm; ^{31}P NMR (121 MHz; $CDCl_3$/CD_3OD 5:1): δ = −0.1918 ppm; MS-FAB (*m/z*): 742.2 $[M+H]^+$; HRMS-FAB (*m/z*): found 742.4156 $[M+H]^+$; $C_{35}H_{60}N_5O_{10}P$ calcd 742.4156;.

5′-O-Phosphatidylcholine-2′,3′-O-dilauroylinosine (**9b**): Yield: 15%. m.p. >179.1 °C (dec.); ^{1}H NMR (300 MHz; $CDCl_3$/CD_3COOD 5:1): δ = 8.41 (s, 1H), 8.08 (s, 1H), 6.17 (d, J = 6.0 Hz, 1H), 5.72 (t, J = 5.6 Hz, 1H), 5.56 (br, 1H), 4.34 (br, 3H), 4.17 (br, 2H), 3.56 (br, 2H), 3.13 (s, 9H), 2.32 (t, J = 7.2 Hz, 2H), 2.19 (t, J = 7.3 Hz, 2H), 1.54 (br, 2H), 1.44 (br, 2H), 1.17 (m, 32H), 0.79 ppm (t, J = 5.4 Hz, 6H); ^{13}C NMR (75 MHz; $CDCl_3$/CD_3OD 5:1): δ = 173.0, 172.6, 158.7, 149.3, 145.9, 139.9, 123.9, 86.3, 83.1, 74.3, 71.6, 66.7, 65.0, 59.9, 54.6, 34.2, 33.9, 32.1, 29.8, 29.7, 29.7, 29.5, 29.5, 29.4, 29.3, 25.1, 24.8, 22.8, 14.1 ppm; ^{31}P NMR (121 MHz; $CDCl_3$/CD_3OD 5:1): δ = 4.9022 ppm; MS-FAB (*m/z*): 798.3 $[M+H]^+$; HRMS-FAB (*m/z*): found 798.4779 $[M+H]^+$; $C_{39}H_{68}N_5O_{10}P$ calcd 798.4782.

5′-O-Phosphatidylcholine-2′,3′-O-dimyristoylinosine (**9c**): Yield: 20%. m.p. >184.8 °C (dec.); ^{1}H NMR (300 MHz; $CDCl_3$/CD_3COOD 5:1): δ = 8.42 (s, 1H), 8.11 (s, 1H), 6.17 (d, J = 6.1 Hz, 1H), 5.72 (t, J = 5.6 Hz, 1H), 5.57 (m, 1H), 4.35 (br, 3H), 4.19 (br, 2H), 3.60 (br, 2H), 3.12 (s, 9H), 2.33 (t, J = 7.3 Hz, 2H), 2.19 (t, J = 7.3 Hz, 2H), 1.54 (br, 2H), 1.44 (br, 2H), 1.17 (m, 40H), 0.78 ppm (t, J = 6.2 Hz, 6H); ^{13}C NMR (75 MHz; $CDCl_3$/CD_3OD 5:1): δ = 173.2, 172.8, 158.9, 149.5, 146.3, 140.3, 124.0, 86.7, 83.3, 74.7, 71.9, 67.0, 65.3, 60.2, 54.8, 34.4, 34.1, 32.4, 30.1, 30.1, 30.0, 29.9, 29.8, 29.7, 29.7, 29.5, 29.3, 25.1, 23.1, 14.2 ppm; ^{31}P NMR (121.50 Hz; $CDCl_3$/CD_3OD 5:1): δ = −0.2444 ppm; MS-FAB (*m/z*): 854.4 $[M+H]^+$; HRMS-FAB (*m/z*): found 854.5412 $[M+H]^+$; $C_{43}H_{76}N_5O_{10}P$ calcd 854.5408.

5′-O-Phosphatidylcholine-2′,3′-O-dipalmitoylinosine (**9d**): Yield: 20%. m.p. >195.0 °C (dec.); ^{1}H NMR (300 MHz; $CDCl_3$/CD_3COOD 5:1): δ = 8.53 (s, 1H), 8.20 (s, 1H), 6.27 (d, J = 6.2 Hz, 1H), 5.83 (t, J = 5.6 Hz, 1H), 5.67 (m, 1H), 4.46 (br, 3H), 4.30 (br, 2H), 3.71 (br, 2H), 3.23 (s, 9H), 2.43 (t, J = 7.3 Hz, 2H), 2.29 (t, J = 7.4 Hz, 2H), 1.67 (br, 2H), 1.54 (br, 2H), 1.28 (m, 48H), 0.88 ppm (t, J = 6.3 Hz, 6H); ^{13}C NMR (75 MHz; $CDCl_3$/CD_3COOD 5:1): δ = 173.1, 172.7, 158.9, 149.4, 146.0, 140.2, 123.9, 86.6, 83.2, 74.6, 71.8, 66.9, 65.2, 60.0, 54.7, 34.3, 34.1, 32.2, 30.0, 29.9, 29.8, 29.7, 29.6, 29.6, 29.4, 25.2, 25.0, 23.0, 14.1 ppm; ^{31}P NMR (121 MHz; $CDCl_3$/CD_3OD 5:1): δ = −0.2332 ppm; MS-FAB (*m/z*): 910.5 $[M+H]^+$; HRMS (*m/z*): found 910.6033 $[M+H]^+$; $C_{49}H_{84}N_5O_{10}P$ calcd 910.6034; elemental analysis calcd (%) for $C_{49}H_{84}N_5O_{10}P$: C 62.02, H 9.30, N 7.69; found C 62.16, H 9.02, N 7.38.

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