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ARTICLE *in* BIOCONJUGATE CHEMISTRY · JULY 2006

Impact Factor: 4.51 · DOI: 10.1021/bc050310c · Source: PubMed

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Dendritic Cationic Lipids with Highly Charged Headgroups for Efficient Gene Delivery

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Gene therapy is expected to lead to powerful new approaches for curing many diseases, a potential that is currently explored in worldwide clinical trials. Nonviral DNA delivery systems are desirable to overcome the inherent problems of viral vectors, but their current efficiency requires improvement and the understanding of their mechanism of action is incomplete. We have synthesized new multivalent cationic lipids with highly charged dendritic headgroups to probe the structure−transfection efficiency relationships of cationic liposome (CL)−DNA complexes, a prevalent nonviral vector. The lipid headgroups are constructed from ornithine cores and ornithine or carboxyspermine endgroups. The dendritic lipids were prepared on a gram scale, using a synthetic scheme that permits facile variation of the lipid building blocks headgroup, spacer, and hydrophobic moiety. They carry four to sixteen positive charges in their headgroups. Complexes of DNA with mixtures of the dendritic lipids and neutral 1,2-dioleoyl-*sn*-glycero phosphatidylcholine (DOPC) exhibit novel structures at high contents of the highly charged lipids, while the well-known lamellar phase is formed at high contents of DOPC. DNA complexes of the new dendritic lipids efficiently transfect mammalian cells in culture without cytotoxicity and, in contrast to lamellar complexes, maintain high transfection efficiency over a broad range of composition.

INTRODUCTION

Gene therapy is based on the use of DNA as a therapeutic agent. This novel medical approach is being investigated in numerous current clinical trials (*1*, *2*). Inherited diseases and cancers feature most prominently as targets, but cardiovascular and a large range of other diseases are amenable to gene therapeutic treatment as well (*3*). In addition, new therapeutic applications for the delivery of nucleic acids have been opening up (*4*) due to discoveries of novel pathways of gene expression and regulation, such as that of RNA interference (RNAi) (*5*−*8*).

Efficient and safe DNA carriers (vectors) are essential for the success of gene therapy. While engineered viruses are very efficient (*9*, *10*) both in vitro and in vivo, their carrying capacity is limited, their preparation is comparatively complex and concerns about their safety have recently been intensified by a few severe setbacks (*11*). Thus, there has been increasing interest in nonviral or synthetic vectors (*12*−*14*). Typically, these are complexes of DNA with cationic macroions, such as cationic liposomes (CLs) (*15*−*17*) or cationic polyelectrolytes (*18*, *19*). Synthetic vectors are facile to prepare and often allow a variation of the vector properties simply by modifying their composition; they lack immunogenic protein components and can transport DNA of unlimited length. However, their efficiency needs to be improved to render them viable for widespread therapeutic use (*20*).

Dendrimers are highly branched synthetic molecules typically based on AB_2 monomers. In contrast to branched polymers, however, dendrimers are monodisperse and typically assembled by adding generations of monomer to a core molecule (*21*−*23*). Some commercially available dendrimers with protonatable amino groups, such as poly(amidoamine) (PAMAM) and poly(propylenimine) (PPI) dendrimers, have been used as gene delivery vectors (*19*, *24*−*28*), and the structure of their DNA complexes has been investigated (*29*). Conjugates of dendritic molecules to PEG have also been used for gene delivery (*30*).

A large number of cationic and other lipids have been synthesized in efforts to improve the efficiency of CL−DNA complexes. Earlier work has been reviewed in detail (*31*−*33*), and several more recent reviews with a smaller scope have also appeared (*34*−*36*). Some lipid vectors have been commercialized, and many are able to efficiently transfect cultured mammalian cells. Nevertheless, establishing structure−efficiency relationships of CL−DNA complexes has turned out to be challenging. This is mainly due to the large number of relevant parameters, such as lipid structure, lipid composition, lipid/DNA charge ratio, and complex structure. To date, only a few unifying concepts regarding structure−efficiency relationships have emerged. For example, it has recently been shown that the transfection behavior of cationic lipids exhibits universal trends when properties of the self-assembled system as a whole, such as the membrane charge density σ_M, are used to plot the data from various lipids (*37*, *38*). The lipids described in this paper were designed to further explore this finding: their highly charged, dendritic headgroups allow for very high membrane charge densities.

The nanostructure of CL−DNA complexes has a significant impact on their mechanism and efficiency of transfection (*39*). Optimized complexes of either of the two well-known phases of CL−DNA complexes, the inverted hexagonal (H_{II}^C) (*40*), and the lamellar (L_α^C) (*41*) liquid crystal phase, show equally

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10.1021/bc050310c CCC: $33.50
Published on Web 07/01/2006

high transfection efficiency. However, the transfection mechanisms of the two phases vary greatly as a consequence of their structures (*42*−*44*). A third, hexagonal phase of CL−DNA complexes (H_I^C), which also transfects cells efficiently, has recently been described (*45*). Which CL−DNA complex phase a particular mixture of lipids forms with DNA is governed by the composition and the rigidity of the membrane (*40*). Molecularly, the shape and corresponding preferred curvature of a lipid, encoded in the lipid structure, is a crucial parameter. DOPE with its cone-shaped structure, for example, favors formation of the H_{II}^C phase. Similarly, a complex structure with positive curvature should result from using lipids with large headgroups (corresponding to an inverted cone molecular shape). Multivalent lipids with 3 to 5 cationic charges in the headgroup, however, still form L_α^C complexes with DNA (*38*, *46*). Thus, we further increased the headgroup charge and size by synthesizing the lipids with dendritic headgroups described in this paper. Their headgroups have an ornithine-based core that is terminated by ornithine or carboxyspermine moieties, resulting in a maximum headgroup charge of +16 e at complete protonation. These lipids naturally extend the level of headgroup charge and, therefore, the accessible range of membrane charge density for multivalent lipids (*16*, *47*, *48*). They also bridge the gap to previously reported lipid−polymer and lipid−dendrimer hybrid materials (*49*−*53*).

The lipids have been prepared by an efficient synthetic scheme that permits their gram-scale synthesis. Modular assembly of the headgroup, spacer, and hydrophobic moieties allows facile variation of these components in the final lipid. Measurements of the headgroup charge of the lipids in complexes with DNA via an ethidium bromide displacement assay and their zeta potential show complete protonation of the headgroups. Mixtures of the dendritic lipids with DOPC at various ratios were used to prepare their DNA complexes. The structure of the complexes was investigated with X-ray diffraction, and their transfection efficiency was assessed using a luciferase reporter gene assay. X-ray diffraction shows formation of the L_α^C phase (*41*) for lipids with lower headgroup charge (4+) or lower contents of dendritic lipid in the membrane. For high contents of highly charged (8+, 16+) dendritic lipids, the observed scattering patterns are not consistent with any of the previously reported phases of CL−DNA complexes, except for a relatively narrow composition window that corresponds to the H_I^C phase (*45*). DNA complexes of the dendritic lipids efficiently transfect mammalian cells, at mol fractions of cationic lipid as low as 10%. For optimal transfection, a slightly higher lipid to DNA charge ratio than for the standard cationic lipid 2,3-dioleyloxy-propyl-trimethylammonium chloride (DOTAP) (*54*) is required. DNA complexes of the dendritic lipids are less cytotoxic than those of DOTAP, and no cytotoxicity is observed at the amounts required for transfection. In contrast to the lamellar complexes of less highly charged multivalent lipids, complexes of the dendritic lipids remain efficiently transfecting over a broad range of lipid composition, including high contents of cationic lipid (i.e. high membrane charge densities), pointing to differences in the transfection mechanism.

EXPERIMENTAL PROCEDURES

General Methods. NMR spectroscopy was carried out on a Bruker Avance 200 MHz spectrometer. MALDI-TOF mass spectrometry was performed on a Dynamo spectrometer from Thermal BioAnalysis Ltd. using 2,5-dihydroxybenzoic acid as the matrix and 30 vol.-% water in acetonitrile as the solvent. For thin-layer chromatography, silica covered plastic sheets with fluorescence indicator (Macherey-Nagel) were used. Detection of spots was achieved with UV light and ninhydrin reagent (300 mg in 95 mL of 2-propanol and 5 mL of acetic acid). By addition of methanol to a 4:2:1 (v/v/v) mixture of chloroform, methanol, and 25% ammonia until the mixture became homogeneous, solvent mixture A was obtained. Similarly, solvent mixture B was prepared starting with a 1:1:1 (v/v/v) mixture of the same solvents. Silica gel from Fisher Scientific with a mesh size of 200−425 was used for flash chromatography (*55*). Zeta potential measurements were performed as described (*56*).

Lipid Solutions. Stock solutions of the dendritic lipids were prepared in chloroform/methanol (9:1, v/v). DOPC and DOTAP were purchased as a solution in chloroform from Avanti Lipids. These lipid solutions were combined at the desired ratio of lipids and dried, first by a stream of nitrogen and subsequently in a vacuum for 8 to 12 h. To the residue, high conductivity (18.2 MΩ) water was added and the mixture was incubated at 37 °C for at least 12 h to give solutions of a final concentration of 30 mM for X-ray samples (15 mM for MVLBisG2). For transfection, aqueous lipid solutions were prepared at 0.6 mM. Lipid mixtures containing higher mol fractions of DOPC formed opaque suspensions, which were sonicated to clarity and filtered through 0.2 μm syringe filters. The lipid solutions were stored at 4 °C until use.

Ethidium Bromide (EtBr) Displacement Assay. Lipid−DNA complexes were prepared in duplicate in a 96 well plate. Each well contained 2.4 μg HPCT DNA, 0.28 μg EtBr, and the desired amount of lipid in a final volume of 250 μL. The emission at 605 nm was measured on a Cary Eclipse fluorescence spectrophotometer, following excitation at 519 nm.

Transfection. Mouse fibroblast L-cells were cultured at 37 °C in supplemented cell medium (Dulbecco's Modified Eagle's Medium (DMEM) with 1% penicillin−streptomycin and 5% fetal bovine serum, v/v; from Gibco BRL) in an atmosphere containing 5% CO_2 and were reseeded approximately every 72 h to maintain subconfluency. Measurements were performed in duplicate and are displayed as mean plus/minus standard deviation. The day before the transfection experiment, cells were seeded in 24-well plates. At the time of transfection, the cells were approximately 70% confluent. Luciferase plasmid DNA (pGL3 Control Vector, Promega) was prepared using a Qiagen Giga Kit. For each well, 0.4 μg of Luciferase plasmid DNA was used. Liposomes (0.6 mM) were added to the DNA, and the mixture was diluted to a final volume of 0.1 mL with nonsupplemented DMEM. The cells were incubated with this solution for 6 h, rinsed three times with phosphate-buffered saline (PBS, Invitrogen), and incubated with supplemented cell media for an additional 24 h. Luciferase gene expression was measured with the Luciferase Assay System (Promega), and light output readings were taken on a Berthold AutoLumat luminometer. Transfection efficiency, measured as relative light units (RLU), was normalized to the weight of total cellular protein determined using Bio-Rad Protein Assay Dye Reagent (Bio-Rad).

Cytotoxicity. Cytotoxicity was assayed by measuring extracellular levels of lactate dehydrogenase. At 18 h prior to treatment, cells were seeded in 96-well plates at 15 000 cells per well. Lipid−DNA complexes were prepared as above and added to the wells, adding a total of 0.08 μg DNA per well. After incubation for 6 h, 75 μL of CytoTox-ONE Homogeneous Membrane Integrity Assay (Promega) and 35 μL of culture medium were added to each well. Fluorescence was quantified on a Bio-Rad PharosFX Molecular Imager system and converted to percent cytotoxicity as described in the manufacturer's instructions.

Synthesis. Chemicals were from Fisher Scientific and at least of analytical grade unless otherwise indicated. They were used as received. Our syntheses of 3,4-dioleylbenzoic acid (DOB) (*59*) and of **MVLBG2** and the intermediates leading to it

(**Boc_2Orn**, **CbxSpr(Boc)$_4$**, **BocG2OMe**, **G2OMe**, **MVLBG2**, **BocBG2OMe**, **BocBG2En**) have been published previously (*45*).

General Procedure for TBTU-Mediated Coupling (dendron synthesis; amine compound with *n* amino groups). To a mixture of 1.1*n* mmol of acid component, 1.1*n* mmol of *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU; Bachem), and 1.1*n* mmol of 1-hydroxybenzotriazole (HOBt) hydrate (Peptides International) in dimethylformamide (DMF) (peptide synthesis grade, Applied Biosystems) was added 2*n* mmol of *N*,*N*-diisopropylethylamine (DIEA; Aldrich). After 10 min, a mixture of 1 mmol of amine compound (hydrochloride or hydrotrifluoroacetate form) and *n* mmol of DIEA in DMF was added to the resulting solution with stirring. Stirring was continued at room temperature for 2 h, and chloroform and water were added with vigorous stirring after evaporation of most of the DMF (*57*). The phases were separated, and after extracting two more times with chloroform, the organic phases were combined and washed twice with 2 M HCl, twice with 2 M NaOH, and once with water. After drying (Na_2SO_4), the solvents were evaporated.

General Procedure for the Aminolysis of Methyl Esters with Diamines. The methyl ester was dissolved in methanol, if necessary, by addition of some chloroform. Twenty equivalents of diamine were added in one portion, and the mixture was stirred at room temperature until the reaction appeared complete by TLC. The solvent was evaporated, and water and chloroform were added. After agitation, the phases were separated, and the organic layer was washed two more times with water, dried (Na_2SO_4), and evaporated. The residue was then purified as required.

***N*1-(2-Aminoethyl)-(2*S*)-2,5-di[(1*S*)-1,4-di(*tert*-butyl-carbamoyl-amino)butyl]carboxamidopentanamide (BocG2En).** To a solution of 1.90 g (2.45 mmol) of **BocG2OMe** in 5 mL of methanol, 3.3 mL (2.96 g, 49.3 mmol) of ethylenediamine (Fluka) was added, and the mixture was stirred for 16 h at room temperature. After the addition of 100 mL water, the mixture was stirred vigorously at room temperature until the formed precipitate crystallized. Filtration and drying of the residue yielded 1.7 g (2.1 mmol, 86%) of **BocG2En** as colorless crystals: $R_f = 0.33$ ($CHCl_3$/MeOH 6:1, 1% concentrated NH_4OH); ^{1}H NMR ($CDCl_3$): δ = 0.8−2.1 (m, C−CH_2−C), 1.35 (s, C(CH_3)$_3$), 2.34 (b, 2 H, NH_2) 2.76 (b, 2 H, CH_2−N), 2.9−3.5 (bm, 10 H, CH_2−N), 3.95−4.55 (2 bm, 3 H, C*H*), 4.9−5.7 (3 m, 2 H, C(O)N*H*), 5.7−6.3 (2 b, 2 H, C(O)N*H*), 7.2−7.7 (bm, 3 H, C(O)N*H*); ^{13}C NMR ($CDCl_3$): δ = 25.6, 25.9 (CH$_2$−CH_2−CH$_2$), 28.3, 28.4 (C(**C**H$_3$)$_3$), 29.4, 29.9, 30.3 (CH$_2$−CH_2−CH), 38.4, 39.6, 39.8, 41.0, 41.8 (CH_2−N), 53.0, 53.6, 54.1 (**C**H), 78.9, 79.8, 79.9 (**C**(CH$_3$)$_3$), 156.1, 156.2 (O−*C*(O)−N), 171.9, 173.1, 173.2 (C−*C*(O)).

***N*1-2-[2-(2-Aminoethoxy)ethoxy]ethyl-(2*S*)-2,5-di[(1*S*)-1,4-di(*tert*-butyl-carbamoyl-amino)butyl]carboxamidopentanamide (BocG2NTEG).** As described in the general procedure for aminolysis, 2.00 g (2.58 mmol) of **BocG2OMe** was reacted with 2,2′-(Ethylenedioxy)diethylamine (NTEG, Aldrich) for 60 h. Extraction and evaporation yielded 1.89 g (2.13 mmol, 83%) of **BocG2NTEG** as a colorless solid: $R_f = 0.33$ ($CHCl_3$/MeOH 6:1, 1% concentrated NH_4OH); ^{1}H NMR ($CDCl_3$): δ = 0.8−2.0 (m), 1.35 (s) (48 H, C−CH_2–C, C(CH_3)$_3$), 2.80 ("t", J = 5.0 Hz, 2 H, CH_2−NH$_2$), 2.9−3.2 (m, 4 H, CH_2−NBoc), 3.2−3.7 (m, 12 H, CH_2−N, CH_2−O), 4.0−4.5 (2 bm, 3 H, C*H*), 4.9−5.4 (2 m, 2 H, C(O)N*H*), 5.5−6.0 (2 b, 2 H, C(O)N*H*), 7.1−7.7 (m, 3 H, C(O)N*H*); ^{13}C NMR ($CDCl_3$): δ = 25.5, 25.9, 26.0 (CH$_2$−CH_2−CH$_2$), 28.3, 28.4 (C(*C*H$_3$)$_3$), 29.3, 30.0, 30.2 (CH$_2$−CH_2−CH), 38.6, 39.1, 39.7, 39.8, 41.5 (*C*H$_2$−N), 52.6, 53.7, 53.9 (*C*H), 69.5, 70.0, 70.1, 73.0 (*C*H$_2$O), 78.9, 79.8 (*C*(CH$_3$)$_3$), 155.9, 156.1, 156.2 (O−*C*(O)−N), 171.5, 172.7, 173.1 (C−*C*(O)).

Methyl (2*S*)-2,5-Di[((1*S*)-1,4-di[(1*S*)-1,4-di(*tert*-butyl-carbamoyl-amino)butyl]carboxamidobutyl)carboxamido]-pentanoate (BocG3OMe). As described in the general procedure for dendron synthesis, 6.34 g (19.1 mmol) of **Boc_2Orn** was reacted for 3 h with 3.60 g (4.33 mmol) of **G2OMe**. For workup, the reaction mixture was evaporated and 600 mL of diethyl ether was added. The precipitate was isolated by filtration, and dichloromethane and 5% $NaHCO_3$ were added. The phases were separated, and the organic phase was washed once more with 5% $NaHCO_3$, twice with 5% citric acid, and once with water. It was then dried (Na_2SO_4) and evaporated to yield 5.51 g (3.38 mmol, 78%) of **BocG3OMe** as a colorless solid: $R_f = 0.72$ ($CHCl_3$/MeOH 6:1, 1% concentrated NH_4OH); ^{1}H NMR ($CDCl_3$): δ = 0.7−2.2 (m), 1.37 (s) (100 H, C−CH_2−C, C(CH_3)$_3$), 2.9−3.4, 3.4−3.7 (2 m, 18 H, CH_2−N, OCH_3), 3.8−4.7 (bm, 7 H, C*H*), 4.7−6.2 (several bm, 6 H, N*H*), 7.0−8.0 (4 m, 8 H, N*H*); ^{13}C NMR ($CDCl_3$): δ = 25.2, 25.8, 29.3, 30.0 (all b, C−CH_2−C), 28.1, 28.2 (C(*C*H$_3$)$_3$), 38.4, 39.6 (b, CH_2−N), 51.7, 52.2, 52.6, 53.7, 54.1 (O*C*H$_3$, *C*H), 78.8 (b, 4), 79.6 (b,4) (*C*(CH$_3$)$_3$),155.8, 156.2 (2 b, overlap, 6) (O−*C*-(O)−N), 171.9 (b, 2), 172.4, 172.9 (b,4) (C−*C*(O)).

***N*1-(2-Aminoethyl)−(2*S*)-2,5-di[((1*S*)-1,4-di[(1*S*)-1,4-di-(*tert*-butyl-carbamoyl-amino)butyl]carboxamidobutyl)carboxamido]pentanamide (BocG3En).** As described in the general procedure for aminolysis, 2.50 g (1.53 mmol) of **BocG3OMe** was reacted with ethylenediamine for 7 days. Extraction and evaporation yielded 1.58 g (0.95 mmol, 62%) of **BocG3En** as a colorless solid: $R_f = 0.40$ ($CHCl_3$/MeOH 6:1, 1% concentrated NH_4OH); ^{1}H NMR ($CDCl_3$/MeOH-d_4): δ = 0.7−1.9 (m), 1.10 (s) (100 H, C−CH_2−C, C(CH_3)$_3$), 2.43 ("t", J = 5.7 Hz, 2 H), 2.55−3.1 (m, 16 H) (CH_2−N), 3.69, 3.99 (2 b, 7 H, C*H*); ^{13}C NMR ($CDCl_3$/MeOH-d_4,): δ = 25.0, 25.5 (CH$_2$−CH_2−CH$_2$), 27.5, 27.6 (C(*C*H$_3$)$_3$), 28.4, 28.8, 29.3 (CH$_2$−CH_2−CH), 38.0, 39.2, 40.2, 41.5 (CH_2−N), 52.6, 53.0, 53.8, 54.4 (*C*H), 78.5, 79.3, 79.5 (*C*(CH$_3$)$_3$), 155.8, 156.2, 156.5 (O−*C*(O)−N), 171.8, 171.9, 172.2, 173.1 (C−*C*(O)).

***N*1-2-[2-(2-Aminoethoxy)ethoxy]ethyl-(2*S*)-2,5-di[((1*S*)-1,4-di[(1*S*)-1,4-di(*tert*-butyl-carbamoyl-amino)butyl]carboxamidobutyl)carboxamido]pentanamide (BocG3NTEG).** As described in the general procedure for aminolysis, 2.43 g (1.49 mmol) of **BocG3OMe** was reacted with NTEG for 11 days. Extraction and evaporation yielded 1.67 g (0.96 mmol, 64%) of **BocG3NTEG** as a colorless solid: $R_f = 0.40$ ($CHCl_3$/MeOH 6:1, 1% concentrated NH_4OH); ^{1}H NMR (MeOH-d_4): δ = 0.8−2.1 (m), 1.44 (s) (100 H, C−CH_2−C, C(CH_3)$_3$), 2.7−2.9 (m, 2 H, CH_2−NH$_2$), 2.95−3.75 (several m, 24 H, CH_2−N, CH_2−O), 3.85−4.15 (b, 4 H), 4.2−4.5 (bm, 3 H) (C*H*); ^{13}C NMR (MeOH-d_4): δ = 26.8, 27.1, 27.5, 27.6 (CH$_2$−CH_2−CH$_2$), 28.9 (C(*C*H$_3$)$_3$), 30.3, 30.7, 31.0 (CH$_2$−CH_2−CH), 39.8, 40.5, 41.0, 42.2 (*C*H$_2$−N), 54.3, 54.4, 54.5, 56.0 (*C*H), 70.6, 71.4 (2), 73.6 (*C*H$_2$O), 80.0, 80.7, 80.9 (*C*(CH$_3$)$_3$), 157.9, 158.0, 158.2, 158.6 (O−*C*(O)−N), 173.9, 174.0 (dbl peak), 175.2 (C−*C*(O)).

Methyl (2*S*)-2,5-Di[((1*S*)-1,4-di[(*tert*-butyl-carbamoyl)(3-[*tert*-butyl-carbamoyl-amino]propyl)] aminobutyl)carboxamido]pentanoate (BocBG1OMe). As described in the general procedure for dendron synthesis (but using diethyl ether in place of chloroform), 5.25 g (8.12 mmol) of **Bis(Boc)$_4$** was reacted with 870 mg (3.85 mmol) of ornithine methyl ester dihydrochloride to yield 4.97 g (3.54 mmol, 92%) of **BocBG1OMe** as a colorless solid: $R_f = 0.58$ ($CHCl_3$/MeOH 9:1, 1% concentrated NH_4OH); ^{1}H NMR ($CDCl_3$): δ = 0.8−2.2 (m, 92 H, C−CH_2−C, C(CH_3)$_3$), 2.5−3.5 (m, 22 H, CH_2−N), 3.59 (s, 3 H, OCH_3), 3.9−4.6 (bm, 3 H, C*H*), 4.6−5.6 (bm, 3 H), 6.4−7.2 (2 b, 2 H), (N*H*); ^{13}C NMR ($CDCl_3$): δ = 24.8, 25.2, 26.0, 27.9, 28.9, 29.8 (C−CH_2−C), 28.2 (C(*C*H$_3$)$_3$), 37.2 (2), 37.9 (2), 38.5, 42.8,

43.4, 44.2, 46.1, 46.3 (b, 2) (CH_2−N), 51.7, 52.2, 58.4 (b) (OCH_3, *C*H), 78.7, 79.4, 80.6, 80.7 (*C*(CH_3)$_3$), 155.4 (b), 155.9 (O−*C*(O) −N), 171.4 (b), 172.0 (C−*C*(O)); MALDI-MS: *m*/*z* = 1425.9 (M + Na^+).

***N*1-(2-Aminoethyl)-(2*S*)-2,5-di[((1*S*)-1,4-di[(*tert*-butyl-carbamoyl)(3-[*tert*-butyl-carbamoyl-amino]propyl)]aminobutyl-)carboxamido]pentanamide (BocBG1En).** As described in the general procedure for aminolysis, 1.51 g (1.07 mmol) of **BocBG1OMe** was reacted with ethylenediamine for 16 h. The residue was purified by flash chromatography on 120 g of silica gel using chloroform/methanol (11:1, changed to 6:1 once the product started to elute) as the eluent to yield 1.30 g (0.90 mmol, 84%) of **BocBG1En** as a colorless solid: R_f = 0.51 ($CHCl_3$/MeOH 6:1, 1% concentrated NH_4OH); ^{1}H NMR ($CDCl_3$): δ = 0.9−2.0 (m), 1.32, 1.34, 1.36 (3 s), (92 H, C−CH_2−C, C(CH_3)$_3$), 2.70 (("t", *J* = 5.3 Hz, 2 H, CH_2−NH_2), 2.8−3.45 (m, 26 H, CH_2−N), 3.95−4.45 (m, 3 H, C*H*), 5.42 (b, NH); ^{13}C NMR ($CDCl_3$): δ = 25.2, 25.9, 27.8, 29.6 (C−CH_2−C), 28.08, 28.16 (C(*C*H_3)$_3$), 37.3, 37.9, 40.6, 41.3, 43.2, 43.7, 44.3, 46.4 (CH_2−N), 52.3, 58.3, 59.2 (*C*H), 78.8, 79.6, 80.7, 80.8 (*C*(CH_3)$_3$), 155.8, 156.1 (O−*C*(O)−N), 171.4, 171.9 (C−*C*(O)); MALDI-MS: *m*/*z* = 1433.5 (M + H^+), 1455.3 (M + Na^+).

***N*1-2-[2-(2-Aminoethoxy)ethoxy]ethyl-(2*S*)-2,5-di[((1*S*)-1,4-di[(*tert*-butyl-carbamoyl)(3-[*tert*-butyl-carbamoyl-amino]propyl)]aminobutyl)carboxamido]pentanamide (BocBG1NTEG).** As described in the general procedure for aminolysis, 1.51 g (1.07 mmol) of **BocBG1OMe** was reacted with NTEG for 40 h. The residue was purified by flash chromatography on 120 g of silica gel using chloroform/methanol (11:1, changed to 6:1 once the product started to elute) as the eluent to yield 1.25 g (0.82 mmol, 77%) of **BocBG1NTEG** as a colorless solid: R_f = 0.51 ($CHCl_3$/MeOH 6:1, 1% concentrated NH_4OH); ^{1}H NMR (MeOH-d_4): δ = 0.9−2.1 (m), 1.44, 1.47, 1.49 (3 s) (92 H, C−CH_2−C, C(CH_3)$_3$), 2.84 ("t", *J* = 5.3 Hz, 2 H, CH_2−NH_2), 3.04 ("t", *J* = 6.5 Hz), 2.9−3.45 (m), 24 H, CH_2−N), 3.55 ("t", *J* = 5.3 Hz), 3.63 (s) (8 H, CH_2−O), 4.2−4.6 (bm, 3 H, C*H*); ^{13}C NMR (MeOH-d_4): δ = 26.3, 26.9, 27.8, 30.3, 30.9 (C−CH_2−C), 28.97, 29.02 (C(CH_3)$_3$), 39.0, 39.3, 40.0, 42.1, 44.8, 45.7, 46.2, 48.0 (CH_2−N), 54.3, 60.2 (b) (*C*H), 70.6, 71.4, 73.0 (CH_2O), 79.9, 81.1, 81.8, 82.0 (*C*(CH_3)$_3$), 157.4, 158.4 (O−*C*(O)−N), 173.4, 174.0 (C−*C*(O)); MALDI-MS: *m*/*z* = 1519.0 (M + H^+), 1541.4 (M + Na^+).

***N*1-2-[2-(2-Aminoethoxy)ethoxy]ethyl-(2*S*)-2,5-di((1*S*)-1,4-di[((1*S*)-1,4-di(*tert*-butyl-carbamoyl)(3-[*tert*-butyl-carbamoyl-amino]propyl)aminobutyl)carboxamido]butylcarboxamido)-pentanamide (BocBG2NTEG).** As described in the general procedure for aminolysis, 2.00 g (0.69 mmol) of **BocBG2OMe** was reacted with NTEG for 10 days. The residue was purified by flash chromatography on 120 g of silica gel using chloroform/methanol (15:1, changed to 6:1 once the product started to elute) as the eluent to yield 1.23 g (0.41 mmol, 59%) of **BocBG2NTEG** as a colorless solid: R_f = 0.57 ($CHCl_3$/MeOH 6:1, 1% concentrated NH_4OH);

General Procedure for the Synthesis of the Boc-Protected Lipids. A solution of 1.1 mmol of DOB (46,59) in dichloromethane was added to a mixture of 1.1 mmol of TBTU and 1.1 mmol of HOBt hydrate in DMF. Following the addition of 3 mmol of DIEA, the mixture was left to stand for 10 min and a solution of 1 mmol of amine compound in DMF was added. After standing for 4 h, the solvents were evaporated. Following the addition of chloroform and 5% citric acid and agitation, the phases were separated and the organic phase was washed one more time with 5% citric acid, twice with 10% $NaHCO_3$, and once with water. After drying (Na_2SO_4), the solvent was evaporated and the residue was purified as required.

***N*1-2-[((1*S*)-1,4-Di[(1*S*)-1,4-di(*tert*-butyl-carbamoyl-amino)-butyl]carboxamidobutyl)carboxamido]ethyl-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (BocMVLG2).** As described in the general procedure, 1.01 g (1.25 mmol) of **BocG2En** was coupled with DOB. The product was purified by flash chromatography on 150 g of silica gel using chloroform/methanol (19:1) as the eluent (*58*) to yield 1.55 g (1.08 mmol, 86%) of **BocMVLG2** as a colorless solid: R_f = 0.28 ($CHCl_3$/MeOH 19:1); ^{1}H NMR ($CDCl_3$): δ = 0.79 ("t", 6 H, CH_2−CH_3), 0.9−2.2 (several m, lg. peaks @ 1.32, 1.23, 1.18, 104 H, C−CH_2−CH_2, C(CH_3)$_3$), 2.7−3.7 (m, 10 H, N−CH_2), 3.9−4.5 (m, 7 H, O−CH_2, C*H*), 5.0−5.75 (m, 8 H, =C*H*−, N*H*), 6.76 (d, ^3J = 8.3 Hz, 1 H, H_{ar} *m*-C(O)), 7.1−7.7 (m, 6 H, H_{ar}, N*H*); ^{13}C NMR ($CDCl_3$): δ = 14.0 (*C*H_3), 22.5 (*C*H_2−CH_3), 28.2, 28.2, 28.3 (C(*C*H_3)$_3$), 25.9, 25.9, 26.0, 27.1, 29.2, 29.3, 29.4, 29.6, 30.0, 31.8 (CH_2−*C*H_2−C), 38.3, 39.3, 39.8, 40.2, 40.9 (*C*H_2−N), 53.0, 53.8, 54.5 (*C*H), 68.9, 69.2 (*C*H_2−O), 78.9, 79.7, 79.9 (*C*(CH_3)$_3$), 112.1, 112.9, 120.1 (C_{ar}H), 126.6 (C_{ar}−C(O)N), 129.6, 129.8 (=*C*H−CH_2), 148.6, 151.7 (C_{ar}−O), 156.0, 156.2, 156.3 (O−*C*(O) −N), 167.6, 172.4, 172.9, 173.0 (C−*C*(O)).

***N*1-[2-((1*S*)-1,4-Di[((1*S*)-1,4-di[(1*S*)-1,4-di(*tert*-butyl-carbamoyl-amino)butyl]carboxamidobutyl)carboxamido]-butylcarboxamido)ethyl]-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (BocMVLG3).** As described in the general procedure, 1.475 g (889 μmol) of **BocG3En** was coupled with DOB. The product was purified by flash chromatography on 150 g of silica gel using chloroform/methanol (15:1) as the eluent (58) to yield 1.85 g (806 μmol, 91%) of **BocMVLG3** as a colorless solid: R_f = 0.14 ($CHCl_3$/MeOH 19:1); ^{1}H NMR ($CDCl_3$/MeOH-d_4 = 9:1): δ = 0.70 ("t", 6 H, CH_2−CH_3), 0.7−2.1 (several m, lg. peaks @ 1.10, 1.15, 1.25, 1.35, 156 H, C−CH_2−C, C(CH_3)$_3$), 2.7−3.45 (m, 18 H, CH_2−N), 3.6−4.3 (m, 11 H, O−CH_2, C*H*), 5.05−5.35 (m, 4 H, =C*H*−), 6.70 (d, ^3J = 8.4 Hz, 1 H, H_{ar} *m*-C(O)), 7.1−7.3 (m, 2 H, H_{ar}); ^{13}C NMR ($CDCl_3$/MeOH-d_4 = 9:1): δ = 13.7 (*C*H_3), 22.3 (*C*H_2−CH_3), 27.9, 28.0 (C(*C*H_3)$_3$), 25.0, 25.2, 25.7, 26.9, 29.0, 29.2, 29.4, 29.5, 31.6 (CH_2−*C*H_2−C), 38.1, 38.3, 39.2, 39.5 (all b, CH_2−N), 52.8 (3), 53.9 (2), 54.4 (2) (*C*H), 68.9, 69.2 (*C*H_2−O), 78.9, 79.6, 79.8 (*C*(CH_3)$_3$), 112.1, 112.9, 120.2 (C_{ar}H), 126.3 (C_{ar}−C(O)N), 129.5, 129.6 (=*C*H−CH_2), 148.4, 151.7 (C_{ar}−O), 155.9, 156.1, 156.5 (O−*C*(O)−N), 168.1 (1), 171.9 (2), 172.5 (1), 173.2 (4, b) (C−*C*(O)).

***N*1-[2-((1*S*)-1,4-Di[((1*S*)-1,4-di[(*tert*-butyl-carbamoyl)(3-[*tert*-butyl-carbamoyl-amino]propyl)amino]butyl)carboxamido]butylcarboxamido)ethyl]-3,4-di[(*Z*)-9-octadecenyloxy]-benzamide (BocMVLBG1).** As described in the general procedure, 1.02 g (712 μmol) of **BocBG1En** was coupled with DOB. The product was purified by flash chromatography on 100 g of silica gel using chloroform/methanol (25:1) as the eluent (58) to yield 1.3 g (0.63 mmol, 88%) of **BocTMVLBG1** as a colorless solid: R_f = 0.38 ($CHCl_3$/MeOH 19:1); ^{1}H NMR ($CDCl_3$): δ = 0.76 ("t", 6 H, CH_2−CH_3), 0.9−2.3 (several m, lg. peaks @ 1.16, 1.21, 1.32, 1.33, 148 H, C−CH_2−C, C(CH_3)$_3$), 2.7−3.7 (m, 26 H, N−CH_2), 3.8−4.55 (m, 7 H, O−CH_2, C*H*), 4.75−5.7 (m, 8 H, =C*H*−, N*H*), 6.5−7.15 (m, 3 H), 7.15−7.7 (m, 4 H) (H_{ar}, N*H*); ^{13}C NMR ($CDCl_3$): δ = 13.9 (*C*H_3), 22.5 (*C*H_2−CH_3), 25.4, 25.8, 27.0, 29.1, 29.2, 29.3, 29.6, 31.7 (C−CH_2−C), 28.2, 28.3 (C(*C*H_3)$_3$), 37.4 (b), 37.9 (b), 39.5, 40.4, 43.2 (b), 43.5 (b), 44.2 (b), 46.4 (b) (*C*H_2−N), 52.1, 59.1 (very b, *C*H), 68.9, 69.1, (*C*H_2−O), 78.8, 79.5, 80.7, 80.8 (*C*(CH_3)$_3$), 112.1, 112.8, 119.8 (C_{ar}H), 126.5 (C_{ar}−C(O)N), 129.6, 129.7 (=*C*H−CH_2), 148.6, 151.7 (C_{ar}−O), 155.9, 156.0 (b, O−*C*(O)−N), 167.5, 171.3, 172.4 (C−*C*(O)).

***N*1-[2-(2−2-[((1*S*)-1,4-Di[(1*S*)-1,4-di(tert-butyl-carbamoyl-amino)butyl]carboxamidobutyl)carboxamido]ethoxyethoxy)-ethyl]-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (BocTMV-LG2).** As described in the general procedure, 1.10 g (1.23 mmol) of **BocG2NTEG** was coupled with DOB. The product was purified by flash chromatography on 150 g of silica gel

using chloroform/methanol (19:1) as the eluent (58) to yield 1.63 g (1.07 mmol, 87%) of **BocTMVLG2** as a colorless solid: R_f = 0.28 ($CHCl_3$/MeOH 19:1); ^{1}H NMR ($CDCl_3$): δ = 0.77 ("t", 6 H, CH_2–CH_3), 0.9−2.4 (104 H, C−CH_2−C, C(CH_3)$_3$), 2.8−3.7 (m, 18 H, N−CH_2, O−CH_2), 3.8−4.5 (m, 7 H, O−CH_2, C*H*), 4.9−6.1 (m, 8 H, =C*H*−, N*H*), 6.74 (d, ^3J = 8.3 Hz, 1 H, H_{ar} *m*-C(O)), 7.1−7.8 (m, 6 H, H_{ar}, N*H*); ^{13}C NMR ($CDCl_3$): δ = 13.9 (*C*H_3), 22.5 (*C*H_2−CH_3), 28.2, 28.3 (C(*C*H_3)$_3$), 25.9, 27.0, 29.1, 29.2, 29.3, 29.6, 29.9, 30.1, 31.7 (CH_2−*C*H_2−C), 38.6, 39.0, 39.7 (C*H*$_2$−N), 52.7, 53.7, 53.8 (*C*H), 68.9, 69.1, 69.4, 69.8, 69.9, 70.0 (*C*H_2−O), 78.8, 79.7, 79.8 (*C*(CH$_3$)$_3$), 112.1, 113.0, 119.6 (C_{ar}H), 126.8 (C_{ar}−C(O)N), 129.6, 129.7 (=*C*H−CH_2), 148.7, 151.7 (C_{ar}−O), 155.9, 156.1 (O−*C*(O)−N), 167.2, 171.4, 172.8, 173.2 (C−*C*(O)).

***N*1-(2−2-[2-((1*S*)-1,4-Di[((1*S*)-1,4-di[(1*S*)-1,4-di(*tert*-butyl-carbamoyl-amino)butyl]carboxamidobutyl)carboxamido]-butylcarboxamido)ethoxy]ethoxyethyl)-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (BocTMVLG3).** As described in the general procedure, 1.565 g (895 μmol) of **BocG3NTEG** was coupled with DOB. The product was purified by flash chromatography on 150 g of silica gel using chloroform/methanol (15:1) as the eluent (58) to yield 1.73 g (716 μmol, 81%) of **BocTMVLG3** as a colorless solid: R_f = 0.18 ($CHCl_3$/MeOH 19:1); ^{1}H NMR ($CDCl_3$): δ = 0.78 ("t", 6 H, CH_2–CH_3), 0.9−2.4 (several m, lg. peaks @ 1.17, 1.22, 1.32, 156 H, C−CH_2−C, C(CH_3)$_3$), 2.75−3.7 (m, 26 H, O−CH_2, CH_2−N), 3.8−4.5 (m, 11 H, O−CH_2, C*H*), 4.9−6.5 (m, 12 H, =C*H*−, N*H*), 6.76 (d, ^3J = 8.4 Hz, 1 H, H_{ar} *m*-C(O)), 6.9−8.0 (m, 10 H, H_{ar}, N*H*); ^{13}C NMR ($CDCl_3$): δ = 13.9 (*C*H_3), 22.5 (*C*H_2−CH_3), 28.3 (C(*C*H_3)$_3$), 25.3, 25.9, 27.0, 29.1, 29.2, 29.3, 29.6, 31.7 (CH_2−*C*H_2−C), 38.6, 39.1, 39.6 (all b, C*H*$_2$−N), 52.5, 53.8, 54.2 (*C*H), 68.9, 69.2, 69.4, 69.7, 70.0 (*C*H_2−O), 78.8, 79.7 (*C*(CH$_3$)$_3$), 112.1, 113.0, 119.6 (C_{ar}H), 126.7 (C_{ar}−C(O)N), 129.6, 129.7 (=*C*H−CH_2), 148.7, 151.7 (C_{ar}−O), 156.1 (O−*C*(O)−N), 167.2, 171.5, 171.7, 172.9, 173.1 (C−*C*(O)).

***N*1-(2−2-[2-((1*S*)-1,4-Di[((1*S*)-1,4-di[(*tert*-butyl-carbamoyl)-(3-[*tert*-butyl-carbamoyl-amino]propyl)amino]butyl)carboxamido]butylcarboxamido)ethoxy]ethoxyethyl)-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (BocTMVLBG1).** As described in the general procedure, 1.10 g (723 μmol) of **BocBG1NTEG** was coupled with DOB. The product was purified by flash chromatography on 100 g of silica gel using chloroform/methanol (19:1) as the eluent (58) to yield 1.3 g (0.60 mmol, 83%) of **BocTMVLBG1** as a colorless solid: R_f = 0.28 ($CHCl_3$/MeOH 19:1); ^{1}H NMR ($CDCl_3$): δ = 0.75 ("t", 6 H, CH_2−CH_3), 0.9−2.3 (several m, lg. peaks @ 1.14, 1.19, 1.31, 1.32, 148 H, C−CH_2−C, C(CH_3)$_3$), 2.7−3.7 (m, 34 H, O−CH_2, N−CH_2), 3.8−4.5 (m, 7 H, O−CH_2, C*H*), 4.75−5.7 (m, 8 H, =C*H*−, N*H*), 6.5−7.5 (several m, 7 H), H_{ar}, N*H*); ^{13}C NMR ($CDCl_3$): δ = 13.9 (*C*H_3), 22.5 (*C*H_2−CH_3), 25.3, 25.8, 27.0, 29.1, 29.3, 29.6, 31.7 (C−*C*H_2−C), 28.1, 28.2 (C(*C*H_3)$_3$), 38.0 (b), 38.4 (b), 39.1, 39.6, 43.1 (b), 43.6 (b), 44.2 (b), 46.4 (b) (*C*H_2−N), 52.1, 58.6, 59.2 (all b, *C*H), 68.9, 69.1, 69.4, 69.8, 70.0 (*C*H_2−O), 78.7, 79.5, 80.6, 80.8 (*C*(CH$_3$)$_3$), 112.1, 112.9, 119.6 (C_{ar}H), 126.7 (C_{ar}−C(O)N), 129.6, 129.7 (=*C*H−CH_2), 148.7, 151.7 (C_{ar}−O), 155.9 (b) (O−*C*(O)−N), 167.1, 171.3, 171.7 (C−*C*(O)).

***N*1−2-[2-(2-[(1*S*)-1,4-Di((1*S*)-1,4-di[(*tert*-butyl-carbamoyl)-(3-[*tert*-butyl-carbamoyl-amino]propyl)amino]butyl)carboxamido]butylcarboxamido)butylcarboxamido]ethoxy)ethoxy]-ethyl-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (BocTMVLBG2).** As described in the general procedure, 1.05 g (349 μmol) of **BocBG2NTEG** was coupled with DOB. The product was purified by flash chromatography on 100 g of silica gel using chloroform/methanol (19:1) as the eluent (58) to yield 1.06 g (0.29 mmol, 84%) of **BocTMVLBG2** as a colorless solid: R_f = 0.24 ($CHCl_3$/MeOH 19:1); ^{1}H NMR ($CDCl_3$): δ = 0.75 CH_2−CH_3), 0.85−2.3 (several m, lg. peaks @ 1.14, 1.19, 1.30, 1.32, 244 H, C−CH_2−C, C(CH_3)$_3$), 2.7−3.7 (m, 58 H, N−CH_2, O−CH_2), 3.89 (("t", 4 H, O−CH_2), 4.0−4.5 (bm, 7 H, C*H*), 4.7−5.7 (m, 12 H, =C*H*−, N*H*), 6.5−7.7 (several m and b, 11 H), H_{ar}, N*H*); ^{13}C NMR ($CDCl_3$): δ = 13.9 (*C*H_3), 22.4 (*C*H_2−CH_3), 25.3 (very b), 25.8, 27.0, 29.1, 29.2, 29.3, 29.5, 31.7 (C−CH_2−C), 28.2 (C(*C*H_3)$_3$), 37.2, 38.0, 39.1, 39.6, 43.5, 44.1, 46.3 (*C*H_2−N), 52.4, 54.7, 58.6, 59.3 (very b, *C*H), 68.9, 69.1, 69.3, 69.7, 69.9, 70.0 (*C*H_2−O), 78.7, 79.4, 80.5, 80.7 (*C*(CH$_3$)$_3$), 112.1, 112.9, 119.6 (C_{ar}H), 126.7 (C_{ar}−C(O)N), 129.6, 129.7 (=*C*H−CH_2), 148.6, 151.7 (C_{ar}−O), 155.3, 155.4, 155.9 (O−*C*(O)−N), 167.1, 171.4, 171.7 (C−*C*(O)).

General Procedure for the Deprotection of Boc-Protected Lipids. The protected lipid (0.9−1.6 g) was dissolved in 10 mL of 95% TFA (5% water), which had been saturated with nitrogen and cooled on ice. The mixture was left to stand at room temperature for 25 min with occasional swirling, and most of the TFA was then evaporated in vacuo. A mixture of diethyl ether/pentane (1:1, 30 mL/g protected lipid) was added with stirring, and the precipitate isolated by decanting. This was repeated twice, and the precipitate was dried in vacuo.

***N*1−2-[((1*S*)-1,4-Di[(1*S*)-1,4-diaminobutyl]carboxamidobutyl)carboxamido]ethyl-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (MVLG2).** Deprotecting 1.25 g (868 μmol) of **BocMVLG2** as described in the general procedure yielded 1.06 g (712 μmol, 82%) of **MVLG2** as a colorless solid: R_f = 0.31 (solvent mixture A); ^{1}H NMR (MeOH-d_4): δ = 0.88 ("t", 6 H, CH_3), 1.0−2.2 (m, lg. peaks at 1.28, 1.35, 68 H, C−CH_2−C), 2.8−3.7 (m, 10 H, N−CH_2), 3.8−4.1 (m, 6 H, O−CH_2, C*H*), 4.25−4.5 (m, 1 H, C*H*), 5.2−5.45 (m, 4 H, =C*H*−), 6.96 (d, ^3J = 9.1 Hz, 1 H, H_{ar} *m*-C(O)), 7.3−7.5 (m, 2 H, H_{ar}), 7.8−8.8 (m, N*H*); ^{13}C NMR (MeOH-d_4): δ = 14.7 (*C*H_3), 23.9, 24.2, 26.7, 28.3, 29.5, 29.7, 30.5, 30.6, 30.8, 30.9, 31.0, 33.2 (C−*C*H_2−C), 39.7, 40.1, 40.5 (C*H*$_2$−N), 53.7, 54.1, 54.5 (*C*H), 70.2, 70.7 (*C*H_2–O), 113.8, 114.3, 122.3 (C_{ar}H), 118.2 (q, J = 294 Hz, *C*F), 127.7 (C_{ar}−C(O)N), 130.9, 131.0 (=*C*H−CH_2), 150.2, 153.9 (C_{ar}−O), 163.2 (q, J = 35 Hz, TFA *C*(O)), 170.0, 170.16, 170.24, 174.3 (*C*(O)); MALDI-MS: m/z = 1039.6 (M + H$^+$).

***N*1-[2-((1*S*)-1,4-Di[((1*S*)-1,4-di[(1*S*)-1,4-diaminobutyl]carboxamidobutyl)carboxamido]butylcarboxamido)ethyl]-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (MVLG3).** Deprotecting 1.55 g (675 μmol) of **BocMVLG3** as described in the general procedure yielded 1.61 g (668 μmol, 99%) of **MVLG3** as a colorless solid: R_f = 0.27 (2:2:1 $CHCl_3$/ MeOH/ 25% NH_4-OH); ^{1}H NMR (MeOH-d_4): δ = 0.88 ("t", CH_3), 0.75−2.3 (m, lg. peaks @ 1.28, 1.35, C−CH_2−C) (90 H), 2.8−3.7 (m, 18 H, N−CH_2), 3.85−4.25 (m, 8 H, O−CH_2, C*H*), 4.25−4.6 (2 m, 3 H, C*H*), 5.2−5.45 (m, 4 H, =C*H*−), 6.96 (d, ^3J = 8.6 Hz, 1 H, H_{ar} *m*-C(O)), 7.4−7.55 (m, 2 H, H_{ar}), 7.9−8.9 (m, N*H*); ^{13}C NMR (MeOH-d_4): δ = 14.7 (*C*H_3), 23.9, 24.2, 26.7, 27.5, 28.3, 29.6, 30.5, 30.6, 30.8, 30.9, 30.99, 31.04, 33.2 (C−*C*H_2−C), 39.8, 40.2 (C*H*$_2$−N), 53.7, 54.1, 54.6, 55.1 (*C*H), 70.2, 70.7 (*C*H_2−O), 113.8, 114.3, 122.3 (C_{ar}H), 118.1 (q, J = 293 Hz, *C*F), 127.6 (C_{ar}−C(O)N), 130.9, 131.0 (=*C*H−CH_2), 150.2, 153.9 (C_{ar}−O), 163.0 (q, J = 35 Hz, TFA *C*(O)), 170.08, 170.16, 170.25, 170.32 (*C*(O)); MALDI-MS: m/z = 1494.8 (M + H$^+$).

***N*1-(2-[(1*S*)-1,4-Di((1*S*)-1,4-di[(3-aminopropyl)amino]butylcarboxamido)butyl]carboxamidoethyl)-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (MVLBG1).** Deprotecting 1.15 g (556 μmol) of **BocMVLBG1** as described in the general procedure yielded 1.02 g (468 μmol, 84%) of **MVLBG1** as a colorless solid: R_f = 0.51 (solvent mixture B); ^{1}H NMR (MeOH-d_4): δ = 0.88 ("t", CH_3), 0.8−2.3 (several m, lg. peaks at 1.29, 1.35, C−CH_2−C) (82 H), 2.9−3.7 (m, 26 H, N−CH_2), 3.8−4.2 (m, 6 H, O−CH_2, C*H*), 4.3−4.55 (m, 1 H, C*H*), 5.2−5.45 (m, 4 H, =C*H*−), 6.96 (d, ^3J = 8.6 Hz, 1 H, H_{ar} *m*-C(O)), 7.35−7.55

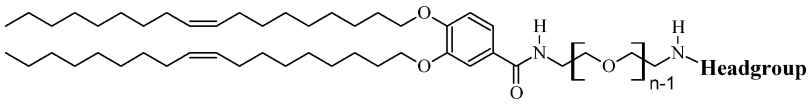


Figure 1. General structure of the new cationic lipids with dendritic headgroups; spacer lengths are $n = 1$ and $n = 3$. See Table 1 for headgroup structures and lipid names.

(m, 2 H, H_{ar}), 7.9−9.1 (m, N*H*); ^{13}C NMR (MeOH-d_4): δ = 14.7 (*C*H$_3$), 22.4, 22.9, 23.9, 25.4, 26.6, 27.5, 28.3, 30.5, 30.6, 30.8, 31.0, 33.2 (C−C*H*$_2$−C), 38.0, 38.5, 40.1, 40.5, 44.9, 45.8, 46.0, 48.0 (*C*H$_2$−N), 54.8, 61.0, 61.4 (*C*H), 70.2, 70.8, (*C*H$_2$–O), 113.9, 114.4, 122.4 (C_{ar}H), 118.2 (q, J = 294 Hz, *C*F), 127.7 (C_{ar}−C(O)N), 130.9, 131.0 (=*C*H−CH$_2$), 150.2, 154.0 (C_{ar}−O), 163.2 (q, J = 34 Hz, TFA *C*(O)), 168.66, 168.70, 170.2, 174.1 (*C*(O)); MALDI-MS: m/z = 1268.3 (M + H$^+$).

***N*1-[2-(2−2-[((1*S*)-1,4-Di[(1*S*)-1,4-diaminobutyl]carboxamidobutyl)carboxamido]ethoxyethoxy)ethyl]-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (TMVLG2).** Deprotecting 1.48 g (967 μmol) of **BocTMVLG2** as described in the general procedure yielded 1.44 g (909 μmol, 94%) of **TMVLG2** as a colorless solid: R_f = 0.31 (solvent mixture A); ^{1}H NMR (MeOH-d_4): δ = 0.88 ("t", 6 H, C*H*$_3$), 1.0−2.2 (m, lg. peaks at 1.28, 1.34, 68 H, C−C*H*$_2$−C), 2.8−3.8 (3 m, 18 H, N−C*H*$_2$, O−C*H*$_2$), 3.8−4.1 (m, 6 H, C_{ar}-O−C*H*$_2$, C*H*), 4.25−4.5 (m, 1 H, C*H*), 5.2−5.45 (m, 4 H, =C*H*−), 6.95 (d, ^3J = 9.1 Hz, 1 H, H_{ar} *m*-C(O)), 7.3−7.5 (m, 2 H, H_{ar}), 7.8−8.8 (m, N*H*); ^{13}C NMR (MeOH-d_4): δ = 14.7 (*C*H$_3$), 23.9, 24.2, 26.7, 27.5, 28.4, 29.5, 29.6, 30.5, 30.6, 30.8, 30.9, 31.0, 31.1, 33.2 (C−*C*H$_2$−C), 40.1, 40.3, 40.9 (C*H*$_2$−N), 53.7, 54.0, 54.6 (*C*H), 70.2, 70.4, 70.6, 70.8, 71.2, 71.4 (*C*H$_2$−O), 113.8, 114.3, 122.2 (C_{ar}H), 118.2 (q, J = 293 Hz, *C*F), 127.8 (C_{ar}−C(O)N), 130.9, 131.0 (=*C*H−CH$_2$), 150.2, 153.8 (C_{ar}−O), 163.0 (q, J = 35 Hz, TFA *C*(O)), 169.9, 170.0, 170.1, 174.0 (*C*(O)); MALDI-MS: m/z = 1127.5 (M + H$^+$).

***N*1-(2−2-[2-((1*S*)-1,4-Di[((1*S*)-1,4-di[(1*S*)-1,4-diaminobutyl]carboxamidobutyl)carboxamido]butylcarboxamido)ethoxy]-ethoxyethyl)-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (TMVLG3).** Deprotecting 1.55 g (650 μmol) of **BocTMVLG3** as described in the general procedure yielded 1.57 g (631 μmol, 97%) of **TMVLG3** as a colorless solid: R_f = 0.26 (2:2:1 CHCl$_3$/ MeOH/ 25% NH$_4$OH); ^{1}H NMR (MeOH-d_4): δ = 0.88 ("t", C*H*$_3$), 0.8−2.3 (m, lg. peaks @ 1.28, 1.35, C−C*H*$_2$−C) (90 H), 2.85−3.85 (m, 26 H, N−C*H*$_2$, O−C*H*$_2$), 3.85−4.25 (m, 8 H, C_{ar}-O−C*H*$_2$, C*H*), 4.25−4.6 (m, 3 H, C*H*), 5.2−5.45 (m, 4 H, =C*H*−), 6.96 (d, ^3J = 9.1 Hz, 1 H, H_{ar} *m*-C(O)), 7.4−7.55 (m, 2 H, H_{ar}), 7.9−8.9 (m, N*H*); ^{13}C NMR (MeOH-d_4): δ = 14.7 (*C*H$_3$), 23.9, 24.2, 26.7, 27.5, 28.3, 29.6, 30.5, 30.6, 30.8, 31.0, 33.2 (C−*C*H$_2$−C), 0.39.7, 40.1, 40.9 (C*H*$_2$−N), 53.7, 54.1, 54.5, 55.1 (*C*H), 70.2, 70.7, 70.8, 71.3 (*C*H$_2$−O), 113.8, 114.3, 122.2 (C_{ar}H), 118.1 (q, J = 292 Hz, *C*F), 127.7 (C_{ar}−C(O)N), 130.9, 131.0 (=*C*H−CH$_2$), 150.2, 153.9 (C_{ar}−O), 163.0 (q, J = 35 Hz, TFA *C*(O)), 170.06, 170.17, 170.21, 170.3, 173.9, 174.2 (*C*(O)); MALDI-MS: m/z = 1583.2 (M + H$^+$).

***N*1-2-[2-(2-[(1*S*)-1,4-Di((1*S*)-1,4-di[(3-aminopropyl)amino]-butylcarboxamido)butyl]carboxamidoethoxy)ethoxy]ethyl-3,4-di[(*Z*)-9-octadecenyloxy]benzamide (TMVLBG1).** Deprotecting 1.15 g (533 μmol) of **BocTMVLBG1** as described in the general procedure yielded 0.96 g (421 μmol, 79%) of **TMVLBG1** as a colorless solid: R_f = 0.49 (solvent mixture B); ^{1}H NMR (MeOH-d_4): δ = 0.88 ("t", C*H*$_3$), 0.7−2.5 (several m, lg. peaks at 1.28, 1.35, C−C*H*$_2$−C) (82 H), 2.85−3.8 (m, 34 H, N−C*H*$_2$, O−C*H*$_2$), 3.8−4.25 (m, 6 H, C_{ar}-O−C*H*$_2$, C*H*), 4.3−4.55 (m, 1 H, C*H*), 5.2−5.45 (m, 4 H, =C*H*−), 6.96 (d, ^3J = 8.6 Hz, 1 H, H_{ar} *m*-C(O)), 7.35−7.55 (m, 2 H, H_{ar}), 7.9−9.1 (m, N*H*); ^{13}C NMR (MeOH-d_4): δ = 14.7 (*C*H$_3$), 22.4, 22.9, 23.9, 25.5, 26.6, 27.5, 28.3, 30.5, 30.6, 30.76, 30.82, 30.97, 31.03, 33.2 (C−C*H*$_2$−C), 38.0, 38.5, 40.5, 40.9, 45.0, 45.8, 46.0, 48.1 (*C*H$_2$−N), 54.9, 61.0, 61.4 (*C*H), 70.2, 70.4, 70.7, 71.3, 71.4 (*C*H$_2$−O), 113.8, 114.3, 122.2 (C_{ar}H), 118.3 (q, J = 293 Hz, *C*F), 127.8 (C_{ar}−C(O)N), 130.9, 131.0 (=*C*H−CH$_2$), 150.2, 153.8 (C_{ar}−O), 163.2 (q, J = 35 Hz, TFA *C*(O)), 168.56, 168.64, 170.0, 173.9 (*C*(O)); MALDI-MS: m/z = 1355.7 (M + H$^+$).

***N*1-[2-(2−2-[((1*S*)-1,4-Di[(1*S*)-1,4-di((1*S*)-1,4-di[(3-aminopropyl)amino]butylcarboxamido)butyl]carboxamidobutyl)-carboxamido]ethoxyethoxy)ethyl]-3,4-di[(*Z*)-9-octadecenyloxy]-benzamide (TMVLBG2).** Deprotecting 910 mg (250 μmol) of **TMVLBG2** as described in the general procedure yielded 910 mg (235 μmol, 94%) of **TMVLBG2** as a colorless solid: R_f = 0.06 (solvent mixture B); ^{1}H NMR (MeOH-d_4): δ = 0.88 ("t", C*H*$_3$), 0.75−2.4 (several m, lg. peaks at 1.28, 1.35, C−C*H*$_2$−C) (106 H), 2.85−3.8 (m, 58 H, N−C*H*$_2$, O−C*H*$_2$), 3.8−4.2 (m, 8 H, C_{ar}-O−C*H*$_2$, C*H*), 4.25−4.6 (m, 3 H, C*H*), 5.2−5.45 (m, 4 H, =C*H*−), 6.97 (d, ^3J = 9.1 Hz, 1 H, H_{ar} *m*-C(O)), 7.35−7.55 (m, 2 H, H_{ar}), 8.0−9.2 (m, N*H*); ^{13}C NMR (MeOH-d_4): δ = 14.6 (*C*H$_3$), 22.5, 22.9, 23.8, 25.5, 26.7, 27.4, 28.3, 28.5, 30.5, 30.6, 30.7, 30.8, 30.95, 31.00, 33.2 (C−C*H*$_2$−C), 38.0, 38.5, 40.1, 40.4, 40.9, 45.0, 45.9, 46.0, 48.2 (*C*H$_2$−N), 54.7, 55.1, 61.0, 61.4 (*C*H), 70.2, 70.5, 70.7, 71.4 (*C*H$_2$−O), 113.9, 114.3, 122.3 (C_{ar}H), 118.2 (q, J = 293 Hz, *C*F), 127.8 (C_{ar}−C(O)N), 130.9, 131.0 (=*C*H−CH$_2$), 150.2, 153.9 (C_{ar}−O), 163.3 (q, J = 34 Hz, TFA *C*(O)), 168.6, 168.7, 168.8, 168.9, 170.1, 173.7, 173.8, 174.3 (*C*(O)); MALDI-MS: m/z = 2040.2 (M + H$^+$).

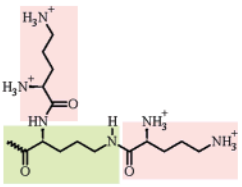
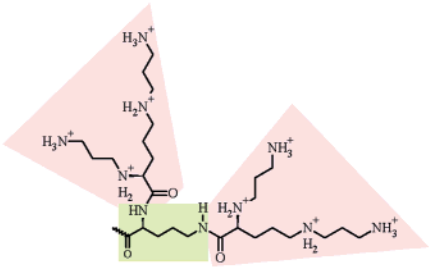
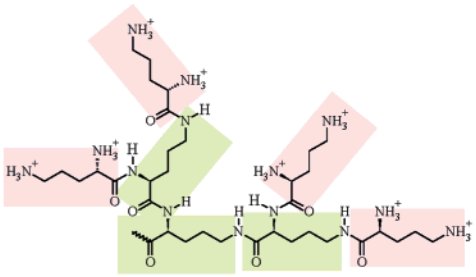
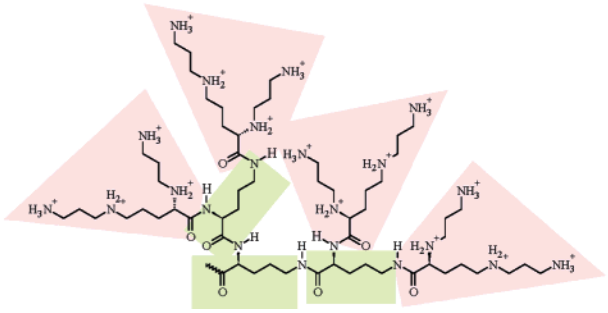
RESULTS AND DISCUSSION

Lipid Design and Synthesis. Figure 1 shows the general structure of the new dendritic lipids. Their names, headgroup structures, spacer lengths, and charges are compiled in Table 1. All lipids share a hydrophobic moiety based on 3,4-dioleyloxybenzoic acid (DOB) (*46*, *59*). The oleyl chains prevent chain crystallization in the bilayer and confer stable membrane anchoring and miscibility with DOPC and DOPE. The amino acid ornithine is used as an AB$_2$ branching building block in the core of the headgroups. Using a core made up of one or three (1+2) ornithine molecules, and ornithine or carboxyspermine (*45*, *60*, *61*) as end groups, lipids bearing four to 16 amino groups in the headgroup have been prepared. An ethylendiamine- or triethyleneglycol-based linker connects headgroup and DOB. Thus, the lipids enable investigations of the effect of spacer length, high charge density, and charge arrangement (two headgroups bear eight charges, but in different geometry) on complex structure and transfection efficiency.

The synthesis of the dendritic headgroup building blocks is shown in Figure 2. It proceeds similarly to that of multiple antigenic peptides (MAPs) (*62*, *63*) and poly(ethylene glycol)-dendritic oligo-lysine block copolymers (*64*), doubling the number of available amino groups for each generation of ornithine that is coupled onto itself. Ornithine methyl ester is first coupled with 2.2 equiv of Boc-protected ornithine. After deprotection of the amino groups, this process is repeated. Alternatively, the initial or second coupling was performed using Boc-protected carboxyspermine (*45*, *60*, *61*) to yield headgroups of higher charge.

The synthesis of the lipids is illustrated in Figure 3, using MVLG2 and TMVLG2 as examples. The methyl ester of the headgroup building block is subjected to aminolysis with an

Table 1. Abbreviated Names and Spacer Lengths of the Synthesized Lipids, Their Charge, and the Chemical Structures of Their Headgroups

Lipid name	Charge / e		Headgroup structure
	Z_{max} [a]	Z_{exp} [b]	
MVLG2 (n=1) TMVLG2 (n=3)	+ 4	3.98 ± 0.24	
MVLBG1 (n=1) TMVLBG1 (n=3)	+ 8	8.00 ± 0.10	
MVLG3 (n=1) TMVLG3 (n=3)	+ 8	7.93 ± 0.26	
MVLBG2 (n=1) TMVLBG2 (n=3)	+ 16	15.9 ± 1.0	

The lipids have the general structure shown in Figure 1. [a] Charge at full protonation. [b] Charge in complex determined by an ethidium bromide displacement assay: see text.

excess of ethylenediamine or 2-[2-(2-aminoethoxy)ethoxy]-ethanamine (α,ω-diaminotriethyleneglycol, NTEG) to yield an amine-functionalized spacer-headgroup building block. This was coupled with DOB (*46*, *59*), and the resulting Boc-protected lipids were purified by flash chromatography and deprotected with TFA. This synthetic route has been carried out on a gram scale and allows facile variation of the spacer length and the headgroup charge by employing other diamines and multivalent headgroup building blocks (*38*, *43*), respectively.

Determination of the Headgroup Charge. We determined the headgroup charge of the lipids effective in DNA complexation with an ethidium bromide (EtBr) displacement assay (*38*, *65*−*67*) and zeta potential measurements. Adding lipid to a mixture of DNA and EtBr reduces the measured fluorescence as EtBr is expelled from the DNA during complex formation and self-quenches in solution. Thus, increasing amounts of lipid were added to a constant amount of DNA and EtBr, recording the resulting fluorescence and normalizing it to the fluorescence without added lipid. The data from this assay is shown in Figure 4: initially, a steep drop in fluorescence with increasing amount of added lipid is observed. The fluorescence then reaches a baseline value. The isoelectric point is obtained from analysis of these data (*68*−*70*), yielding the lipid headgroup charges Z_{exp} documented in Table 1. These data show that the lipids essentially bear their maximal charge when incorporated in the complex. While the lipid charge is calculated from the onset of the baseline value, it is also evident from the data that this final value of the fluorescence varies with the headgroup charge: higher charge corresponds to a lower value for the final fluorescence intensity. Zeta potential measurements confirm the headgroup charges determined by the EtBr displacement assay. This is illustrated in Figure 5, which shows the zeta potential of MVLBG1−DNA complexes as a function of the lipid/DNA charge ratio ρ_{chg}. The zeta potential of the complexes changes sign when ρ_{chg}, calculated using Z_{exp} for the lipid, is unity. Similar results were obtained for lipids of different headgroup charge (MVLG2 and MVLBG2) and for mixtures of the cationic lipids with DOPC (data not shown).

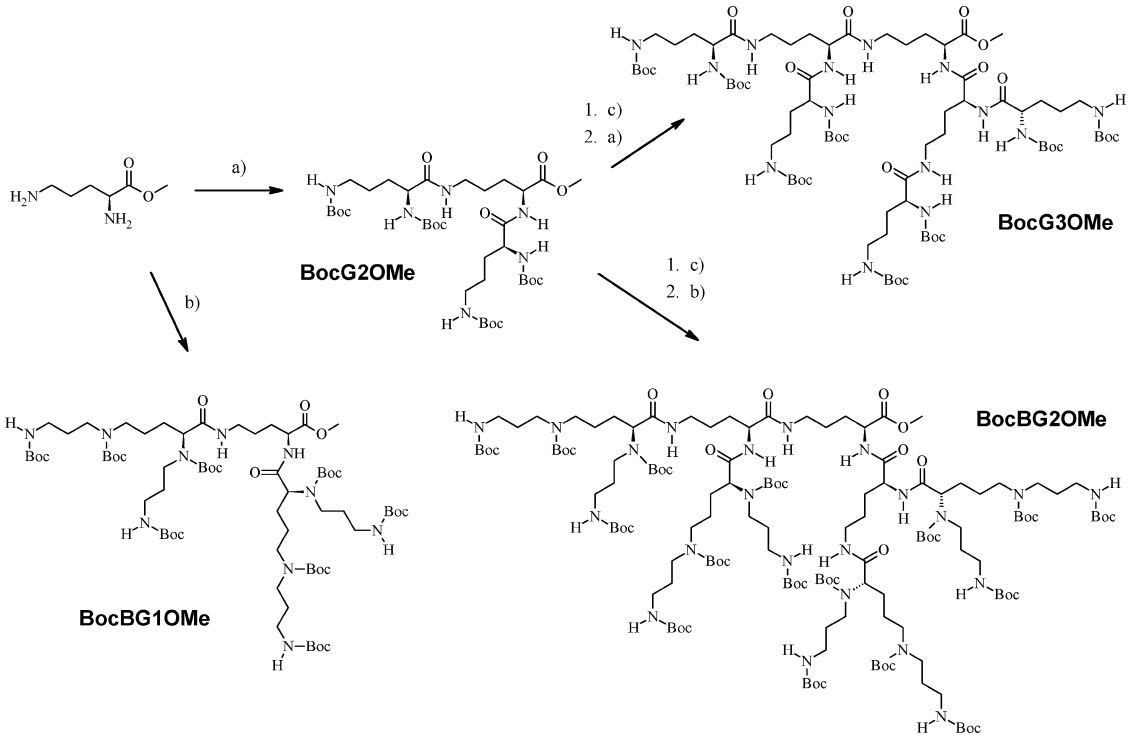


Figure 2. Synthesis of the dendritic headgroup building blocks, starting from ornithine methyl ester. Reaction conditions: (a) Boc-ornithine, TBTU, HOBt, DIEA; (b) Boc-carboxyspermine, TBTU, HOBt, DIEA; (c) TFA. HOBt: 1-hydroxybenzotriazole hydrate; DIEA: *N*,*N*-diisopropylethylamine; TBTU: *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; TFA: trifluoroacetic acid.

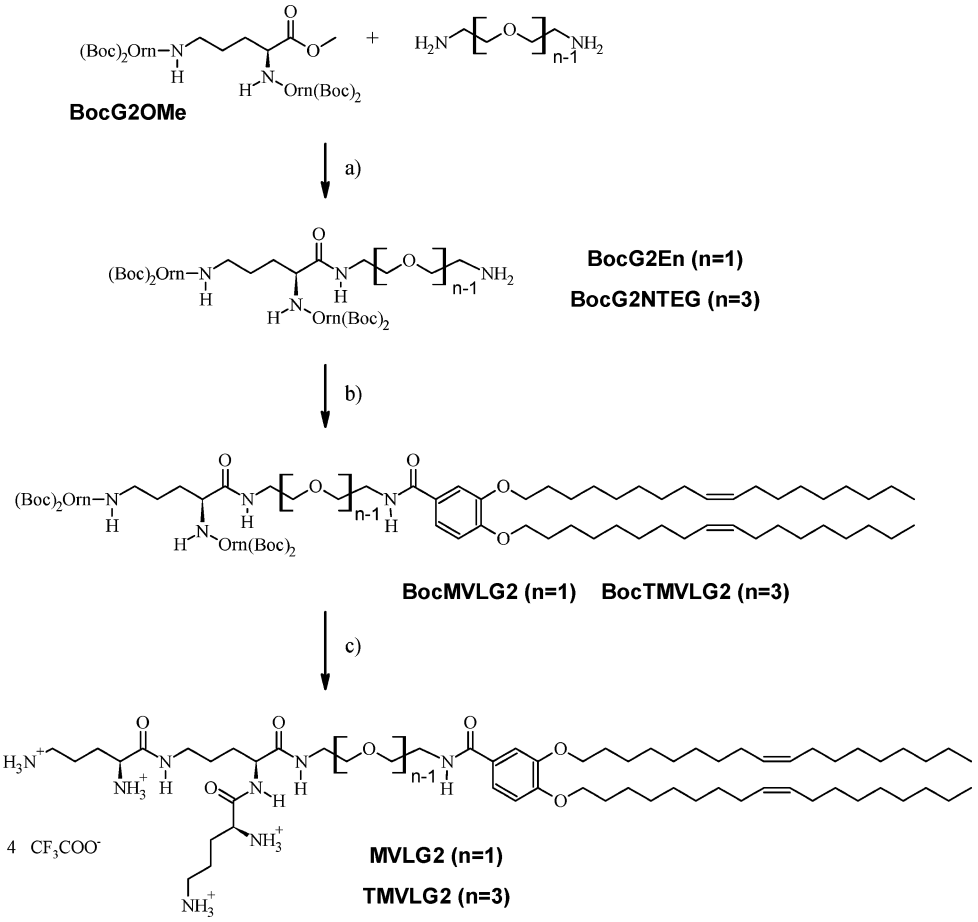


Figure 3. Synthesis of the dendritic cationic lipids **MVLG2** ($n = 1$) and **TMVLG2** ($n = 3$); $Orn(Boc)_2 = C(O)CH(NHBoc)(CH_2)_3NHBoc$. Reaction conditions: (a) excess diamine (20×) in MeOH; b) DOB, TBTU, HOBt, DIEA; (c) TFA. DOB: 3,4-dioleyloxybenzoic acid; HOBt: 1-hydroxybenzotriazole hydrate; DIEA: *N*,*N*-diisopropylethylamine; TBTU: *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; TFA: trifluoroacetic acid.

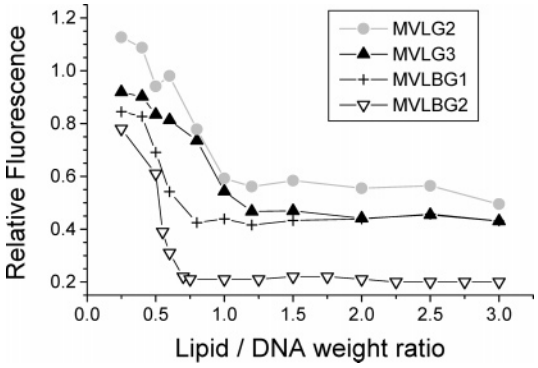


Figure 4. Data from an ethidium bromide (EtBr) displacement assay, used to measure the charge of the dendritic lipids in complexes with DNA. EtBr fluoresces when intercalated into DNA but is expelled by lipid−DNA complex formation, which results in reduced fluorescence intensity due to self-quenching of EtBr in solution. The fluorescence intensity is normalized to that of DNA with EtBr and no lipid.

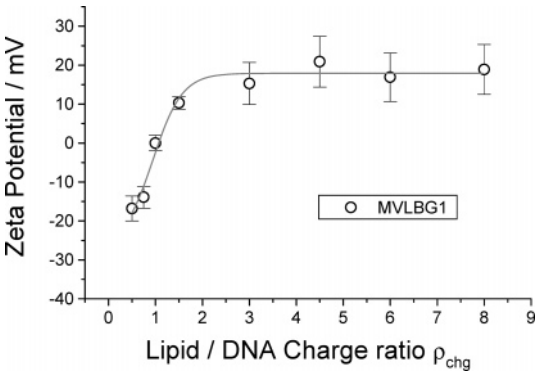


Figure 5. Zeta potential of MVLBG1-DNA complexes as a function of ρ_{chg}. The lipid/DNA charge ratio was calculated using Z_{exp} from the EtBr displacement assay. The line (sigmoidal fit) is a guide to the eye. Note that the zeta potential changes sign at approximately $\rho_{chg} = 1$.

Characterization of CL−DNA complexes. CL−DNA complexes were formed by combining DNA and aqueous lipid preparations consisting of mixtures of DOPC and a dendritic lipid or DOTAP. In addition to performing transfection experiments, we employed small-angle X-ray scattering (SAXS) to characterize the nanoscale internal structure of DNA complexes of the dendritic lipids. All SAXS experiments were performed in the presence of DMEM, the medium used in the transfection experiments. While their structure changes significantly with varying ratio of neutral to cationic lipid (see below), it is not affected by the lipid to DNA charge ratio (ρ_{chg}) above the isoelectric point (*71*). Lipids with identical headgroups but different spacer (MVLs vs TMVLs) and lipids of identical charge but different headgroup structure (G3 vs BG2) yield DNA complexes of similar structure (*71*). The characteristic scattering pattern of the lamellar phase is observed for all complexes of the least highly charged dendritic lipid MVLG2 (charge: 4+) as well as for complexes containing mol fractions below and including 50% and 15% of the lipids with G3/BisG1 (8+) and BisG2 (16+) headgroups, respectively (data not shown). Complexes at certain intermediate contents of highly charged dendritic lipids in the membrane exhibit SAXS patterns indicative of the H_I^C phase (*45*). However, the scattering patterns at the remaining compositions do not correspond to any previously described structure. A detailed investigation of the structures of these complexes is in progress and will be described elsewhere.

Under low ionic strength conditions, all lipid DNA complexes (at $\rho_{chg} \neq 1$) form particles of about 200 nm size. These aggregate in the higher salt conditions of, e.g., cell medium, resulting in μm-sized particles that vary in size over time (*56*).

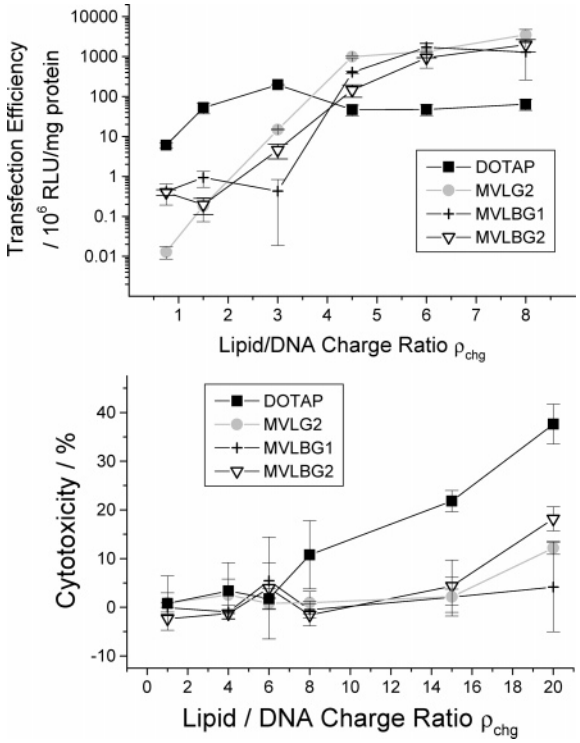


Figure 6. Transfection efficiency (top) and cytotoxicity (bottom) of DNA complexes containing 60 mol % cationic lipid at various lipid/DNA charge ratios. Note the difference in scale of the charge ratio axes. The amount of DNA is constant for all data points.

Consistent with this, fluorescence microscopy of dendriticlipid−DNA complexes shows colocalization of lipid and DNA and complex particles with dimensions on the μm scale, consisting of aggregates of globular submicron particles (*45*).

Cell Transfection and Cytotoxicity. We have measured the transfection efficiencies of DNA complexes of the dendritic lipids in mouse L cells using a luciferase reporter gene assay and compare them to the standard monovalent lipid DOTAP. The headgroup charge dominates the transfection behavior of the lipids, while neither the spacer length nor the headgroup architecture have a significant effect (*71*). Thus, in the following we show data for three lipids representing the three different headgroup charges, MVLG2 (4+), MVLBG1 (8+), and MVLBG2 (16+). In these experiments, both the lipid to DNA charge ratio ρ_{chg} and the ratio of cationic lipid and neutral DOPC in the membrane were varied.

For DOTAP, TE increases with ρ_{chg} up to a saturation value; this behavior is independent from the composition of the membrane (*72*). A similar trend is observed for the dendritic lipids, but with saturation occurring at a higher charge ratio: $\rho_{chg} = 3$ lies in the saturated regime for DOTAP, while all dendritic lipids require at least $\rho_{chg} = 4.5$ (*71*). This is illustrated in Figure 6 (top), which shows transfection efficiencies of complexes with 60 mol % cationic lipid for DOTAP, MVLG2, MVLBG1, and MVLBG2 at various values of ρ_{chg}. Cell toxicities of the complexes were measured using a commercially available assay that probes cell membrane integrity. Figure 6 (bottom) shows the cytotoxicity of CL−DNA complexes as a function of ρ_{chg}, at 60 mol % cationic lipid. Note the different scale of the ρ_{chg}-axis when comparing with the transfection efficiency plot. For all lipids, only charge ratios exceeding those required for efficient transfection produce any notable toxicity. Furthermore, the onset of the curve is much delayed for the dendritic lipids in comparison with DOTAP, demonstrating their reduced cytotoxicity.

In Figure 7 (top), TE of complexes at $\rho_{chg} = 6$ is plotted against the composition of the membrane. The plots for all lipids

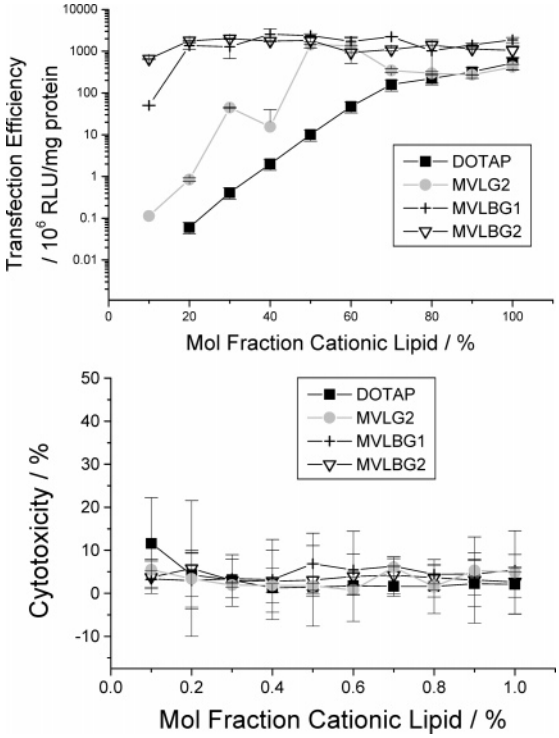


Figure 7. Transfection efficiency (top) and cytotoxicity (bottom) of DNA complexes of DOTAP, MVLG2, MVLBG1, and MVLBG2 plotted against the mol fraction of cationic lipid in mixtures with DOPC. All data points were taken at a lipid/DNA charge ratio of 6, using a constant amount of DNA.

show an initial, exponential rise with increasing mol fraction of cationic lipid. The higher the headgroup charge of the lipid, the lower the mol fraction of cationic lipid where this rise occurs, consistent with our previous findings on the relevance of membrane charge density (*37*, *38*). Thus, optimal transfection can be achieved with mol fractions as small as 10% (MVLBG2) or 20% (MVLBG1). Since the cationic lipid is the more expensive lipid component in CL−DNA complexes, this is advantageous for potential applications. Interestingly, the highly charged lipids MVLBG1 and MVLBG2 do not show the drop in TE at higher mol fractions (very high charge densities) that has been observed for multivalent lipids of charge 2+ to 5+ (*38*). This deviation coincides with a structural transition, away from the lamellar complex structure (*71*). As expected from the data shown in Figure 6, no significant cytotoxicity is observed for any of the complexes (Figure 7 (bottom)). This further confirms that the dendritic lipids are a safe and efficient DNA delivery vector. Of note, DNA complexes of MVLBG2 were shown to be much superior over those of DOTAP in a "hard to transfect" mouse embryonic fibroblast cell line (*45*).

Currently, we are conducting further experiments with the dendritic lipids. The high headgroup charge of multivalent lipids appears to be particularly advantageous for transfections with siRNA, which require very high lipid to DNA charge ratios.

ACKNOWLEDGMENT

This work was supported by NIH GM59288. Our NMR characterization work made use of MRL Central Facilities supported by the MRSEC Program of the National Science Foundation under award No. DMR00-80034.

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BC050310C