Synthesis and Transfection Efficiencies of New Lipophilic Polyamines

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A homologous series of lipophilic polyamines was synthesized and evaluated for DNA delivery and transfection efficiency. The series contained 1,4-butanediamine, 1,8-octanediamine, 2-[2-(2-amino-ethoxy)-ethoxy]-ethylamine, homospermidine, and homospermine covalently attached via their N^1 terminus to a 3,4-bis(oleyloxy)-benzyl motif. In addition, homospermidine and homospermine were also attached via amide linkers. The homospermidine derivatives (i.e., benzyl tether **25** and benzamide tether **27**) showed a 3-fold and 4-fold respective enhancement in delivery of AlexaFluor-488-labeled DNA over the butanediamine analogue **22**. Homospermine derivative **26** was shown to inhibit ^{14}C-spermine uptake (IC_{50} ∼10 μM), which implied that **26** is able to compete effectively for polyamine recognition sites on the cell surface. This study demonstrated that the number *and* position of the positive charges along the polyamine scaffold plays a key role in DNA delivery and in determining the transfection efficiency.

Introduction

With the information gained by the sequencing of the human genome,[1,2] gene therapy now holds promise for the treatment of hereditary diseases and cancers.[3,4] It is now possible to silence a bad gene,[5] turn on a needed gene,[6] or install a new gene to address particular cellular defects.[7] However, a key requirement for successful gene therapy is the efficient transfer of DNA to specific cell types *in vivo*. Viral vectors have proven to be very efficient transfection agents and allow for the insertion of foreign DNA into many cell types.[8,9] However, the inherent problems with viral vectors such as immunogenicity and the limited size of the DNA plasmid that can be transferred has led to interest in developing efficient nonviral vectors.[10−14]

Nonviral vectors are ideal because of their expected low toxicity and immunogenicity, ability to transfer large strands of DNA, and simpler synthetic preparation. Typically, these vectors consist of a lipophilic component attached to a positively charged, polar headgroup, through the use of a spacer or linking motif, and are a mixture of neutral and positively charged lipids. A wide range of cationic liposomes have been synthesized and recently reviewed.[15−17] The cationic headgroups typically found in these liposome systems include quaternary ammonium salts,[10] polylysine,[18] polyguanadinium salts,[19] polyarginine,[20] and polyamines.[21]

Polyamines were introduced into liposomes by Behr,[22] when it was realized that the naturally occurring polyamines such as spermidine and spermine (Figure 1) could efficiently compact DNA. Behr went on to synthesize one of the first transfection vectors, **1** (DOGS[11]) and incorporated a branched polyamine as the polar headgroup (Figure 2).[11]

Following this work, a number of research groups have looked at the effect of changing the positively charged, polyamine headgroup on cationic liposomes with respect to their efficiency at transfecting DNA.[15,16,21,23,24] Byk et al. showed that when assayed in HeLa cells, compound **2**,[21] whose configuration of the polar head is linear, was 5−10 times more effective at transfection than those with branched-, globular- or T-shaped polar domains.[21] Safinya et al.[24] demonstrated with **3**[24] that lipids containing a higher number of positive charges had better transfection efficiency within a series of liposomes containing branched-polyamine headgroups. Other popular vectors **4**−**6**[18b] are shown in Figure 2. Commercially available Lipofectamine (LFA[a]) is a 3:1 mixture of **4** and **6**.[18c]

Although a large number of cationic lipids have been surveyed, a systematic study of how linear polyamine architectures effect transfection efficiency is still needed, especially in light of the molecular recognition elements required to use the polyamine transporter (PAT) for cellular entry.[25−32] Our goal was to perform the key crossover experiments needed to tie these two fields together. Indeed, understanding how cationic motifs are transported across the cell membrane is critical to both enterprises.

Targeting a specific cellular transporter could provide cell-selective transfection. For example, rapidly proliferating cancer cells could be targeted via their PAT, which is often up-regulated.[28] Indeed, the ability to transfect a specific cell type would have profound impact on a multitude of gene therapy strategies.

Since many cancer cell lines have active polyamine transporters, it is possible to target these cells using the molecular recognition events involved in polyamine import.[28] For example, an anthracene−homospermidine conjugate was shown to be 10−30 fold more toxic to B16 melanoma cells than to 'normal' melanocytes (Mel-A cells).[28] A multitude of polyamine structures were previously screened for their high PAT selectivity in Chinese hamster ovary cells (i.e., CHO and CHO-MG cells).[28] Several linear polyamine architectures were identified, which selectively targeted the PAT-active CHO cell line over its PAT-inactive CHO-MG mutant.[25−32] The discovery of homospermidine, a 4,4-triamine, as a cell-selective 'vector' motif provided the means to test the PAT-delivery system as a conduit for gene delivery.[26,28]

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[a] Abbreviations: PAT, polyamine transporter; BOC_2O, di-*tert*-butyl dicarbonate; LFA, Lipofectamine; DOSPA, 2,3-bis(oleyloxy)-*N*-2-(sperminecarboxamido)ethyl-*N*,*N*-diethyl-1-propanaminium trifluoroacetate; DOTMA, 1,2−bis(oleyloxy)propyl-3-trimethylammonium bromide; DOPE, dioleoylphosphatidylethanolamine.

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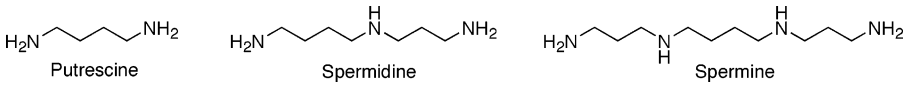


Figure 1. Native polyamines.

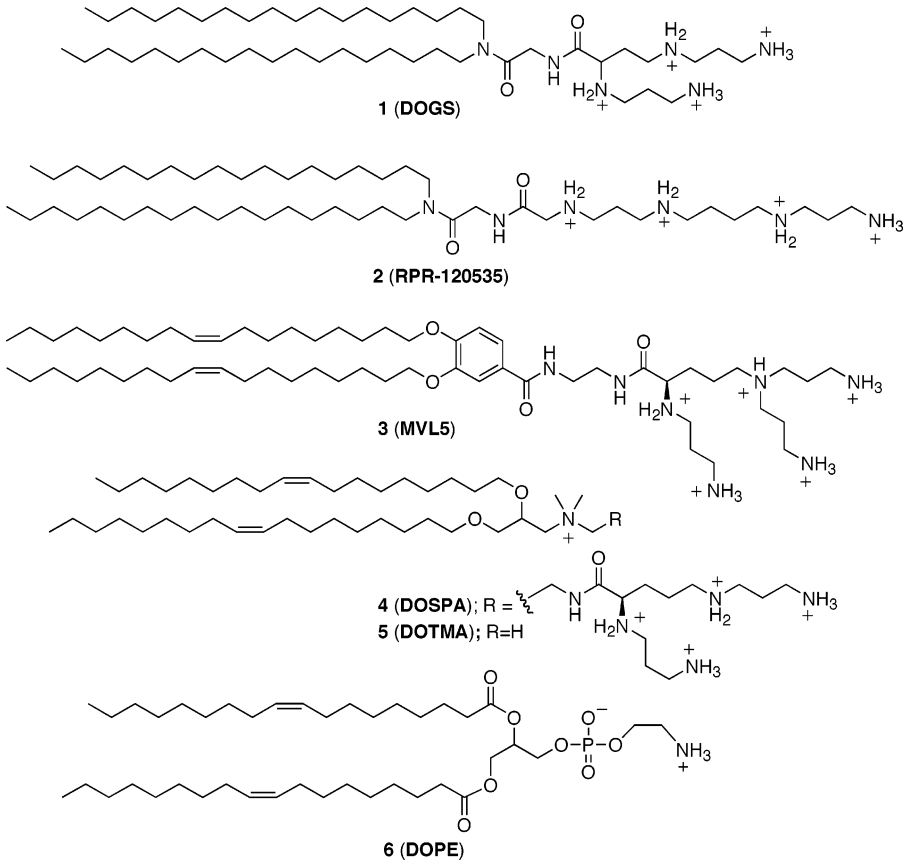
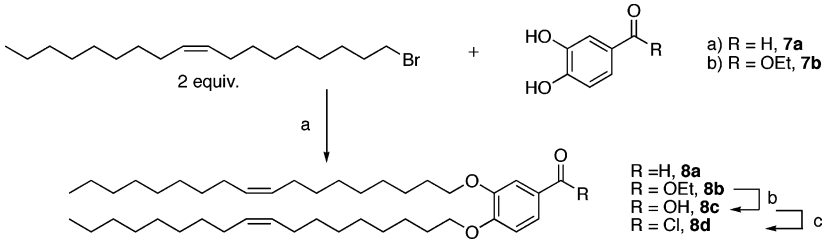


Figure 2. Structures of transfection lipids.

Scheme 1[a]



[a] Reagents: (a) K_2CO_3, KI, cyclohexanone; (b) KOH, EtOH; (c) oxalyl chloride.

In short, our aim was to combine these two areas of research (PAT targeting and gene delivery) by attaching PAT-targeting polyamine sequences[28] to the aryl lipid motif (e.g., **3**[24]) in order to facilitate DNA plasmid uptake. These materials were then evaluated for their DNA-transporting ability as well as transfection efficacy and compared to the commercially available transfection reagent, Lipofectamine 2000.[33]

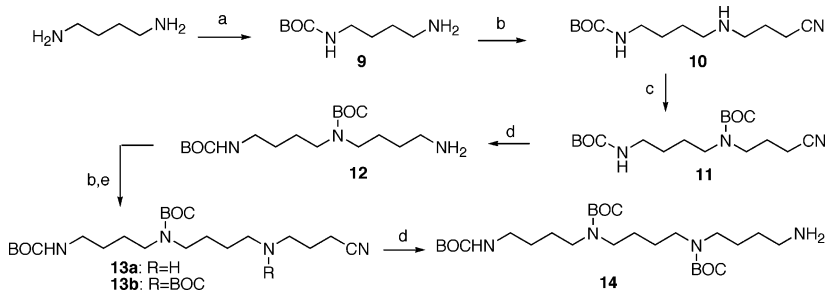
Results and Discussion

Synthesis. A series of derivatives were synthesized with a variety of linear polyamine headgroups. These cationic headgroups contained from two to four positive charges. The convergent synthetic route involved (a) the synthesis of the hydrophobic lipid moiety, (b) the synthesis of the BOC-protected amine head groups, and (c) coupling of the two separate components followed by (d) the deprotection of the polyamine moiety to provide the final compounds as HCl salts.

The 3,4-disubstituted benzene-containing lipids were chosen due to the significant success of these lipids in earlier transfection studies by Safinya and others.[10,23,24] Indeed, this structural element is present in the published transfection agent **3** (Figure 2).[10,23,24] The unsaturated unit within the C18 chain was shown to prevent side-chain recrystallization and conferred a high degree of flexibility upon the cationic liposomes. These side chains were attached to the central benzene core via ether linkages. An aldehyde or acid chloride in position 1 allowed for attachment of the polyamine component. The polyamine scaffolds and controls were identified in earlier investigations of PAT-mediated drug delivery.[25−29]

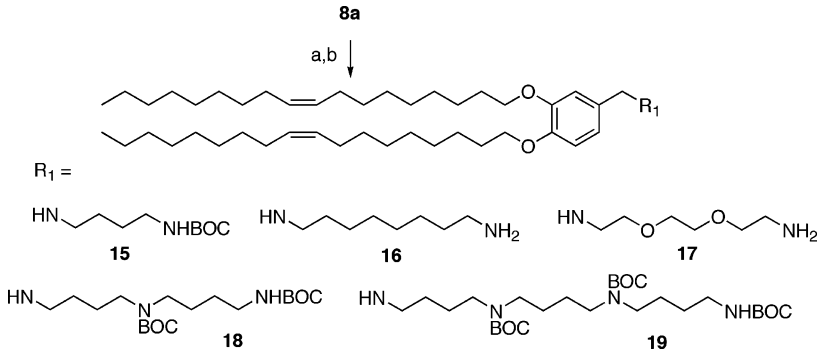
The first step was the synthesis of 3,4-bis(oleyloxy)benzaldehyde **8a** by the *O*-alkylation of 3,4-dihydroxy benzaldehyde **7a** with oleyl bromide (Scheme 1). Similarly, the bis-*O*-

Scheme 2[a]



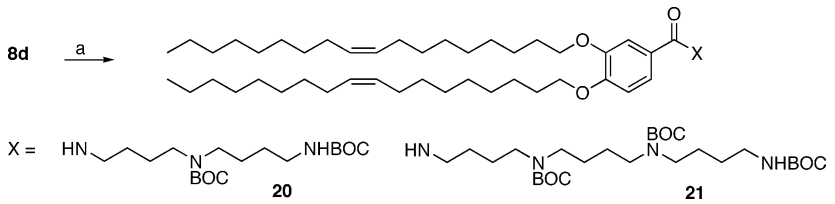
[a] Reagents: (a) BOC_2O (0.33 equiv), MeOH, NEt_3; (b) $Br(CH_2)_3CN$, K_2CO_3, CH_3CN; (c) BOC_2O (1.5 equiv), MeOH, NEt_3; (d) H_2, Raney Ni, EtOH, NH_4OH; (e) BOC_2O, THF.

Scheme 3. Synthesis of the Lipid−Polyamine Conjugates **15**−**19**[a]



[a] Reagents: (a) respective amine, CH_2Cl_2, MeOH; (b) $NaBH_4$.

Scheme 4[a]



[a] Reagents: (a) **12** or **14**, CH_2Cl_2, 1 M NaOH.

alkylation of ethyl 3,4-dihydroxybenzoate **7b** with oleyl bromide was used to form ethyl 3,4-bis(oleyloxy)benzoate **8b** using the method of Safinya et al.[23,24] Subsequent cleavage of **8b** with KOH was carried out to give the acid **8c** (Scheme 1) followed by treatment with oxalyl chloride to give the desired acid chloride **8d**.

As shown in Scheme 2, the next step in the synthesis involved the generation of the BOC-protected polyamines: **9**, **12**,[34] and **14**. The mono BOC protection of diaminobutane gave the amine **9** in a good yield. Sequential addition of bromobutyronitrile and BOC protection of the newly formed secondary amine, followed by reduction of the nitrile with Raney Ni, gave masked triamine **12**.[34] Repetition of these three steps on **12** gave tri-BOC-protected tetraamine **14** (via nitriles **13a** and **13b**).

The coupling of the aldehyde **8a** to a range of polyamines was based on previous procedures for coupling of amines to benzaldehyde derivatives.[25−28] As shown in Scheme 3, the reductive amination of **8a** was achieved in two steps via *in situ* generation of the imine (with a series of amines) followed by reduction using $NaBH_4$ to give the respective secondary amines (**15**−**19**).

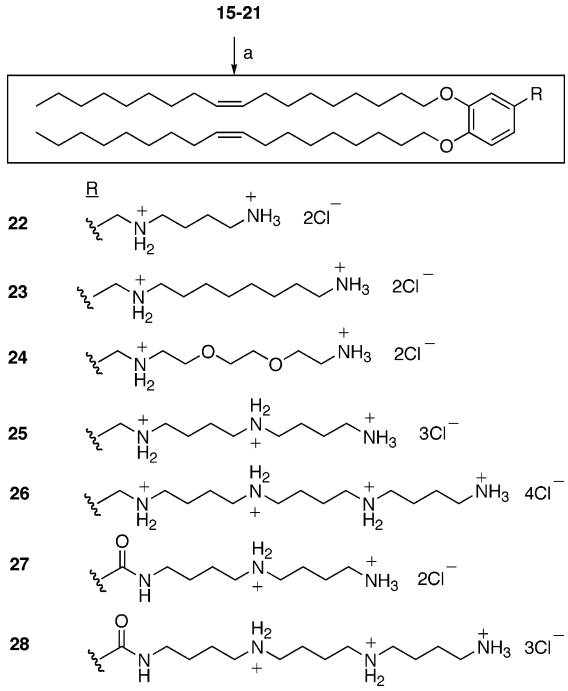
As shown in Scheme 4, the respective polyamines **12**[34] and **14** were coupled to acid chloride **8d** to provide the BOC-protected benzamide systems, **20** and **21**.

The final step to produce the desired lipid−polyamine conjugates involved acidification of the amines with a solution of anhydrous HCl in ethyl acetate.[23,24] As shown in Scheme 5, treatment of the penultimate compounds **15**−**21** with anhydrous HCl/EtOAc provided the target HCl salts **22**−**28**. By design, the lipid portion of the conjugate (boxed structure in Scheme 5) was held constant throughout the series. This feature allowed for later comparisons and an understanding of how the polyamine component influenced DNA delivery.

Biological Evaluation. Before conducting the transfection studies, the series of conjugates (**22**−**28**) were first evaluated for cytotoxicity in Chinese hamster ovary (CHO) cells. This was an important step in determining what dose of lipid−polyamine conjugate could be tolerated by the cell line. Ideally, one would use a dose of the conjugate that is not cytotoxic to the cell line to be transfected. This is an important caveat in evaluating DNA delivery systems.

As expected, there were significant differences in the aqueous solubility of these new conjugates. DMSO was added in portions to provide aqueous solutions of **22**−**28**. Since DMSO itself is toxic to CHO cells above 40 μM, stock solutions of each conjugate were made in such a manner so that the total DMSO concentration remained below 40 μM. This constraint limited the amount of conjugate that could be dosed. Poorly soluble

Scheme 5[a]



[a] Reagents: (a) anhydrous HCl in ethyl acetate.

materials required higher DMSO levels, which in turn limited the amount of material that could be dosed to cells in our toxicity screen.

Taking these factors into account, cytotoxicity screens were performed to investigate the relative toxicity of each system. Most materials were relatively nontoxic with 48 h IC_{50} values ≥ 20 μM. Armed with this insight, cells were treated with ≤ 6.4 μM of the polyamine conjugate so as to avoid significant toxic effects from the delivery agent itself. Indeed, at this dose, $\geq 90\%$ of cells survived transfection for all compounds except **28**. Indeed, compound **28** was very toxic (after 24 h incubation), and even at the lowest dose used for transfection (0.5 μg/mL; 0.5 μM **28**) killed 95% of the cells. As such, **28** was too toxic for efficient transfection at the doses surveyed, and its data is strongly biased by the few remaining cells, which survived.

It should be noted that the cytotoxicity screen was performed with free lipopolyamine. The above data ($\geq 90\%$ cell survival) suggests that complex formation with DNA significantly attenuates the cationic lipid cytotoxicity. This observation is particularly relevant in the context of their use as transfection agents.

Gene Transfer Studies. Armed with knowledge of the cytotoxicity range of the series **22**−**28**, CHO-K1 cells were evaluated for DNA uptake using a fluorescently labeled DNA (Alexa Fluor-488-DNA). As shown in Figure 3, cells were dosed with the Alexa Fluor-488-DNA in the presence of increasing concentrations (i.e., 0.5, 2.5, 5 μg/mL) of the respective conjugate, **22**−**28**. Each conjugate was as good (**24**, **25**) or better (**26, 27**) than the lipofectamine control (LFA, except **22** and **23**) and facilitated uptake of the fluorescent DNA probe in a concentration-dependent manner (μg/mL).

While the molecular weights in this series do range from 784 (**22**), 840 (**23**), 844 (**24**), 891 (**25**), 999 (**26**), 869 (**27**), and 976 g/mol (**28**), they are relatively close (within 10−22%) and allow for general comparisons, especially in light of the large differences observed in activity. For example, tetraamine **26**, which has the highest molecular weight of the series (999 g/mol), was at a slightly lower concentration (5 μM) than **22** (6.4 μM) at

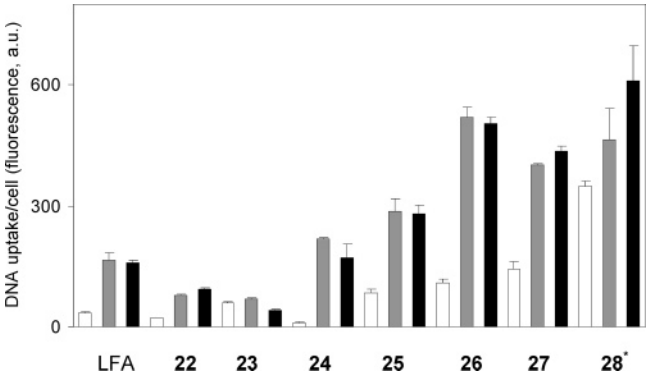


Figure 3. DNA uptake activity by novel cationic lipids **22**−**28** (compared with Lipofectamine, LFA). CHO K1 cells were grown to confluence in 24-well plates, rinsed with medium, and incubated for 4 h at 37 °C with 1 μg/mL Alexa Fluor-488-DNA complexed with 0.5 μg /mL (white bars), 2.5 μg /mL (gray bars), or 5 μg /mL (black bars) of the respective cationic lipid. Cells were then extensively washed with PBS and treated with trypsin, and cell pellets were again extensively washed with PBS 1% BSA to completely remove nonspecific fluorescence from the cell exterior. Finally, cells were analyzed for Alexa Fluor-488-DNA uptake by flow cytometry on a FACSCalibur (BD Biosciences) operated by Cell-Quest software. (*) During this relatively short incubation (4 h), cells remained viable with **28**. Note: in converting from μg/mL to μM, the concentrations used were typically approximately 0.6, 3.0, and 6 μM, respectively, and are tabulated specifically in the Supporting Information. The negative control with DNA only gave 3.8 $\pm$ 0.6 a.u. (data not shown).

the 5 μg/mL dose. Nevertheless, DNA uptake experiments with tetraamine **26** had over a 5-fold increase in DNA uptake (as measured by fluorescence of the imported DNA probe) than those conducted with diamine **22**. Clearly, compound **26** was more efficient in facilitating DNA delivery to cells.

Interestingly, conjugate **27**, which represents a butanediamine motif similar to **22** except with the diamine placed further away from the lipid tail, was a more efficient DNA delivery agent than **22**. In contrast, compound **23**, which separated the ammonium centers via an octanediamine had similar or lower activity as **22** (depending on the dose). Insertion of the polyether motif present in **24** maintained this eight-atom spacer, yet nearly doubled the DNA delivery (DNA probe fluorescence) observed.

The PAT-selective homospermidine motif present in **25** resulted in a 3-fold increase in DNA delivery. However, this outcome may simply be due to the presence of the additional charge provided by the triamine motif present in **25**. Indeed, significant increases in DNA delivery were observed across the homologous series **22**, **25**, and **26** which at 5 μg/mL gave 94, 281, and 504 fluorescence absorbance units (a.u.), respectively.

While the average amount of DNA taken up/cell shows wide variations between the different compounds (Figure 3), all compounds were able to deliver significant amounts of DNA to virtually the entire cell population (Figure 4). However, in terms of gene therapy, simple DNA delivery to the cell is insufficient. There are other cellular barriers, which must be traversed.

GFP Expression Studies. In order for the 'therapy' to be effective, the DNA must escape from the endosome and enter the cell's nucleus, be transcribed to a regulatory, noncoding RNA (RNAi) or to mRNA that is translated into its coded protein. Therefore, we investigated the conjugate-assisted expression of an eGFP DNA plasmid encoding for the green fluorescent protein (GFP). CHO cells, which were successfully transfected, were easily identified by their green fluorescence. Control experiments conducted with only the eGFP DNA plasmid (and no lipid carrier) gave virtually no fluorescence.

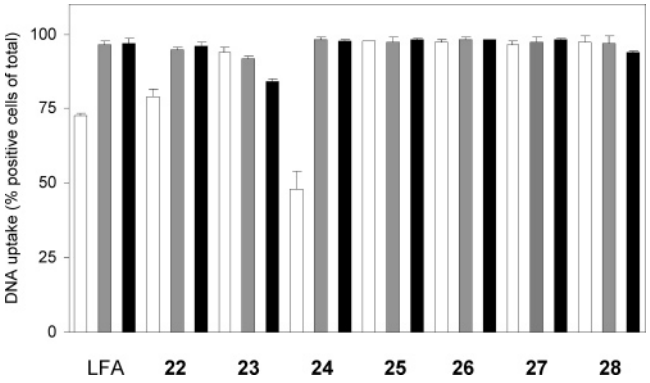


Figure 4. The results obtained from the experiment described by Figure 3 were analyzed using a gated channel to determine the percentage of cells that had internalized significant amounts of DNA as compared with cells incubated with DNA only. A gate was set just above the threshold signal for control cells (DNA only with no lipid conjugate, gate value = 10.5). Note: Alexa Fluor-488-DNA complexed with 0.5 μg /mL (white bars), 2.5 μg /mL (gray bars), or 5 μg /mL (black bars) of the respective cationic lipid was used, as indicated in Figure 3. Data are presented as the mean ± SD. Lipofectamine is denoted as LFA.

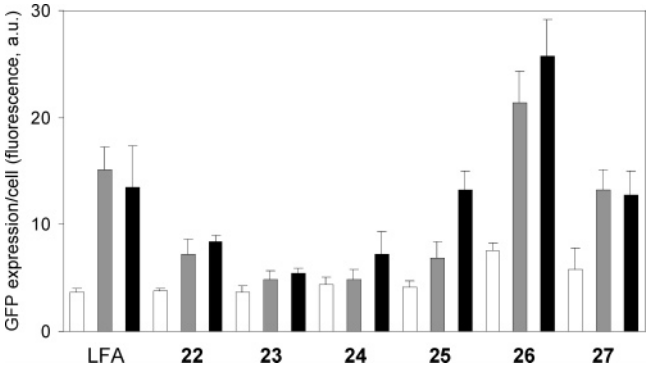


Figure 5. GFP expression results from transfection experiments. CHO K1 cells were grown to approximately 50% subconfluence in 24-well plates, rinsed with medium, and incubated for 4 h at 37 °C with 1 μg/mL eGFP DNA plasmid complexed with 0.5 μg /mL (white bars), 2.5 μg /mL (gray bars), or 5 μg /mL (black bars) of the respective cationic lipid, as indicated above. Medium was then changed, and cells were incubated for another 24 h to allow for GFP plasmid expression. Cells were detached by trypsin treatment and analyzed for GFP expression by FACS. Lipofectamine is denoted as LFA. The value obtained with DNA plasmid only was subtracted from each entry above (i.e., 1.2 ± 0.7 GFP expression with DNA only).

A gate or instrumental threshold was set based upon this low background fluorescence. Fluorescence detected above this background was considered a positive response.

The interpretation of the data in Figures 5 and 6 can be quite subtle. For example, Figure 5 relates the amount of GFP expression per cell and is a summary measurement of how well the DNA 'message' was delivered and read and protein (GFP) produced. While the magnitudes are different, the relative trends are consistent with those observed in Figure 3. In this regard, DNA delivery correlated with GFP expression.

Figure 6 revealed the % GFP positive cells observed in the total cell population after the transfection experiment. This information is a direct measure of transfection efficiency. Using the 5 μg/mL dose for comparisons, **22** (38%), **25** (49%), and **27** (40%) were all comparable to the LFA control (41%) in terms of transfection efficiency. Both **23** (23%) and **24** (25%) gave lower values. The lone standout was tetraamine **26** (67%), which had over 50% higher transfection efficiency than the LFA control (41%).

Cells treated with conjugate **23** at the high dose (5 μg/mL,

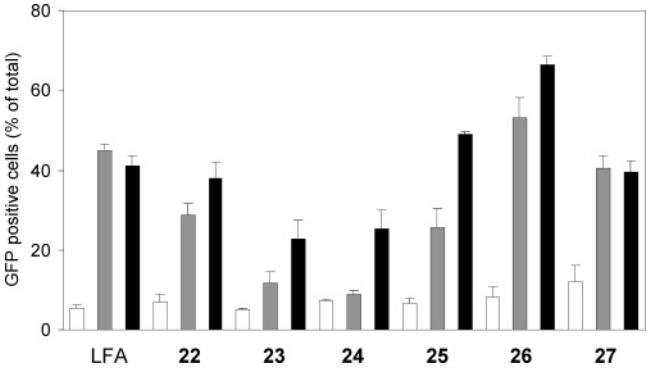


Figure 6. The results obtained from the experiment described in Figure 5 were analyzed using a gated channel to determine the percentage of cells that expressed significant levels of GFP as compared with cells incubated with DNA plasmid only. A gate was set just above the threshold measured with no DNA plasmid and no conjugate added (gate value = 5.5). All cells, which exhibited a fluorescence signal above that level, were considered positive. Data are presented as the mean ± SD. Lipofectamine is denoted as LFA.

black bars in Figure 4) gave 84% positive cells of the total cell population remaining after the DNA uptake experiment; whereas all the others (LFA, **22**, and **24**−**28**) gave typically >94% positive cells. Using these data as benchmarks of DNA import via each conjugate, the significantly lower % GFP positive cells (23−67%) observed in Figure 6 suggests that intracellular processing and nuclear delivery of DNA also depend on the structure of the cationic lipid.

A closer analysis of the data revealed just how important these latter two parameters (i.e., intracellular processing and nuclear delivery) are for successful gene delivery. For example, although **26** was capable of delivering 3-fold more DNA to the cell than the control LFA (black bars in Figure 3: **26**: 504; LFA: 158 a.u.), the relative GFP expression/cell was only 2-fold higher in Figure 5 (25 vs 13 a.u.) and the % of GFP positive cells was only 1.5 fold higher in Figure 6 (67% vs 41%).

It cannot be excluded that maximum GFP expression occurs at varying time points post-transfection for the different compounds. Nevertheless, the data reflect an interesting correlation between intracellular processing of internalized DNA and the cationic lipid structure.

In summary, while a significant number of cells imported the fluorescent DNA probe in the presence of the synthetic conjugates (**22**−**28**), the amount of the DNA probe entering each cell varied depending upon the conjugate used. The % GFP positive cells of the total cell population roughly correlated with the GFP expression/cell. Cells with low %GFP positive cells (Figure 6: **23**, 23%; **24**, 25%) had low GFP expression/cell (Figure 5: **23**, 5; **24**, 7). Cells with high %GFP positive cells (Figure 6: **26**, 67%) had high GFP expression/cell (Figure 5: **26**, 25%). Indeed, the additional 'intracellular barriers' associated with successful GFP expression (as shown in Figure 6 in terms of % GFP positive cells) seemed to moderate the large differences seen in the earlier DNA delivery study (Figure 3).

We speculated that **26** may be using the polyamine transporter, PAT, for cellular entry. Indeed, as shown in Figure 7, tetraamine **26** was shown to be a potent inhibitor of spermine uptake ($IC_{50} \sim 10$ μM) using a ^{14}C-radiolabeled spermine competition assay. This observation implied that **26** was able to compete for the polyamine recognition sites on the cell surface (e.g., PAT). Therefore, it is possible that certain lipophilic polyamines, which present the correct polyamine 'message', may be able to recognize and target cells expressing high levels

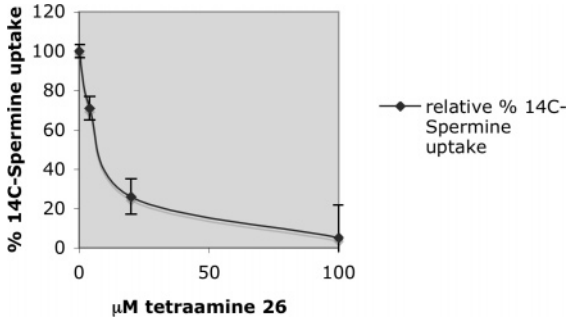


Figure 7. [^{14}C]Spermine uptake inhibition by **26**. Tetraamine **26** is an efficient inhibitor of polyamine uptake. CHO-K1 cells were incubated with 1 μM [^{14}C]spermine (31 Ci/mol) for 20 min in serum free medium supplemented with varying concentrations of tetraamine **26** as indicated in the figure. After extensive rinsing, imported [^{14}C]spermine was determined by scintillation counting on cell lysates.

of polyamine transporters on their cell surface (e.g., cancer cells, rapidly dividing tumors, etc.). However, as shown in this report, delivery into the cell is just one step in successful gene transfection. Nevertheless, our findings are an important first step in developing 'smart' transfection agents.

Conclusions

This study demonstrated that the number *and* position of the positive charges along the polyamine scaffold plays a key role in DNA delivery and in determining the intracellular outcome of the DNA import event, i.e., the transfection efficiency. Although the Lipofectamine (LFA: 75% **4** and 25% **6** mixture) has five positive charges (presumably a DNA delivery enhancing feature) in one of its components (e.g., **4**), it also has a neutral component **6** present, which makes structural comparisons to this control difficult beyond the dose-to-dose comparisons made above. Future studies will look at the effect of neutral lipids (like **6**) in terms of transfection efficiency of polyamine−lipid conjugates. Indeed, **6** has been shown to play a role in endosome disruption and to facilitate transfection.[35]

In addition, this paper presented the first study that probed the transfection phenomena with a PAT-selective homospermidine motif[28] attached to a lipid cargo, e.g., **25**. As expected, compound **25** showed a 3-fold enhancement in DNA delivery over its butanediamine analogue **22**. Unfortunately, the DNA delivery enhancement observed with **25** seemed to be muted by the latter steps of the transfection process (e.g., intracellular trafficking, nuclear delivery, etc.). As a result, **25** had only slightly higher transfection efficiency than **22** (e.g., **25** and **22** gave 49% and 38% GFP positive cells in Figure 6, respectively).

This phenomenon was also observed with the two diamines, **27** and **22**. Compound **27** showed greater than 4-fold enhancement in DNA delivery over its butanediamine analogue **22**. Evidently, moving the diamine 'message' further away from the lipid component enhanced the delivery characteristics of the conjugate (Figure 3). Again, this potential delivery enhancement by **27** was tempered by the latter steps of the transfection process. Alas, nearly identical transfection efficiency was observed for **27** and **22** (40% and 38% GFP positive cells in Figure 6, respectively).

The ability of these polyamine transfection agents to both target the polyamine transporter and deliver DNA was demonstrated for tetraamine **26**. However, cellular DNA delivery alone was insufficient to predict successful transfection. Understanding how different polyamine structures[21,36] and neutral lipids like **6**[35] enhance the intracellular trafficking and nuclear delivery of plasmid DNA is critical for the future design of efficient polyamine transfection agents.

Experimental Section

Materials and Methods. Silica gel (32−63 μm) and chemical reagents were purchased from commercial sources and used without further purification. All solvents were distilled prior to use. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, unless otherwise noted. TLC solvent systems are based on volume %, and NH_4OH refers to concentrated aqueous NH_4OH. High-resolution mass spectrometry was performed at the University of Florida Mass Spectrometry facility.

ULYSIS AlexaFluor-488 (Molecular Probes) was used for labeling of DNA as recommended by the manufacturer. All fine chemicals were from Sigma. Lipofectamine 2000 was purchased from Invitrogen. [^{14}C]Spermine was purchased from Amersham International, UK. Wild-type Chinese hamster ovary cells (CHO-K1) were obtained from the ATCC (Manassas, VA). CHO cells were routinely cultured in F12K nutrient mixture supplemented with 10% (v/v) fetal calf serum, 2 mM l-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin (growth medium) in a humidified 5% CO_2, 37 °C incubator.

DNA Uptake Studies. AlexaFluor-488-labeled DNA with or without cationic lipid was mixed in serum-free F12K and added to extensively rinsed cells grown in 24-well plates. Cells were then incubated for 4 h at 37 °C. After removal of the incubation medium and being rinsed with PBS, cells were detached with trypsin followed by extensive washing with ice-cold PBS, 1% (w/v) bovine serum albumin (BSA), to remove nonspecific fluorescence. Finally, cells were suspended in PBS, 1% BSA, and analyzed for DNA uptake by flow cytometry on a FACSCalibur (BD Biosciences) operated by Cell-Quest software. Cells remained viable with all compounds tested, including **28**.

GFP Transfection Experiments. EGFP-encoding DNA plasmid with or without cationic lipid was mixed in F12K media and incubated with prerinsed, subconfluent cells in 24-well plates for 4 h, followed by another incubation period of 24 h in fresh growth medium. Cells were then washed with PBS, detached by trypsin treatment, dissolved in PBS, 1% BSA, and analyzed by FACS for GFP expression. Note: severe toxicity was noted for compound **28** after the 24 h incubation period.

Spermine Uptake Study in the Presence of 26. Subconfluent CHO K1 cells (growing in 24-well plates) were rinsed three times with F12K medium. The respective wells were dosed with the corresponding dose of the spermine conjugate **26** and then incubated for 20 min with [^{14}C]spermine (31 Ci/mol). At this time point, the cells were rinsed twice with F12K medium, three times with 1 mM spermine (in F12K medium), and rinsed once with F12K medium. The cells were lysed in 0.5 M NaOH for 1 h at 37 °C, and an aliquot was removed and neutralized with 0.5 M HCl for scintillation counting.[37]

Cell Cytotoxicity Studies. CHO cells were grown in RPMI medium supplemented with 10% fetal calf serum, glutamine (2 mM), penicillin (100U/mL), and streptomycin (50 μg/mL). Cells were grown at 37 °C under a humidified 5% CO_2 atmosphere. Aminoguanidine (2 mM) was added to the culture medium to prevent oxidation of the drugs by the enzyme (bovine serum amine oxidase) present in calf serum. Trypan blue staining was used to determine cell viability before the initiation of a cytotoxicity experiment. L1210 cells in early to mid log-phase were used. Cell growth was assayed in sterile 96-well microtiter plates (Becton-Dickinson, Oxnard, CA). CHO cells were plated at 2e^3 cells/mL. Drug solutions (10 μL per well) of appropriate concentration were added after an overnight incubation for the CHO cells. After exposure to the drug for 48 h, cell growth was determined by measuring formazan formation from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium using a Titertek Multiskan MCC/340 microplate reader for absorbance (540 nm) measurements.[38] These experiments allowed for a quick assessment of conjugate cytotoxicity and facilitated determination of the proper dosing for the latter

transfection and delivery experiments. Note: the cytotoxicity data presented were performed with free lipopolyamine. Complex formation with DNA significantly attenuated the cationic lipid toxicity, which is relevant to their use as transfection agents.

3,4-Bis-octadec-9-enyloxy-benzaldehyde (8a). To a solution of oleyl bromide (1.8 g, 5.4 mmol) in cyclohexanone (20 mL) was added protocatachualdehyde (0.341 g, 2.5 mmol), potassium carbonate (1.02 g, 7.4 mmol), and potassium iodide (0.05 g, 0.3 mmol). The suspension was stirred at 100 °C for 18 h under nitrogen. Due to the light sensitivity of the oleyl bromide, the flask was covered in aluminum foil. TLC (10% MeOH/$CHCl_3$) showed the reaction was complete. The hot reaction mixture was filtered to remove some of the particulates, and the solvent was removed *in vacuo*. The residue was dissolved in $CHCl_3$ (100 mL) and washed with water (2 × 75 mL). The $CHCl_3$ layer was dried over anhydrous sodium sulfate and filtered and the solvent removed *in vacuo* to give a dark yellow oil. The oily residue was purified by flash column chromatography (5% EtAc/hexane) to yield the product **8a** as a white solid (1.08 g, 69%), $R_f = 0.3$ (5% EtAc/hexane). ^{1}H NMR ($CDCl_3$) δ 9.80 (s, 1H, CHO), 7.37 (m, 2H, phenyl), 6.93 (d, 1H, phenyl), 5.33 (t, 4H, olefinic), 4.06 (t, 2H, OCH_2), 4.04 (t, 2H, OCH_2), 2.01 (m, 8H, $CH_2C{=}C$), 1.85 (m, 4H, CH_2CH_2O), 1.50−1.18 (m, 44H, 22 × CH_2), 0.88 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 191.00, 155.34, 149.95, 130.14, 130.12, 129.97, 126.79, 111.89, 111.04, 69.36, 32.29, 30.16, 29.92, 29.90, 29.88, 29.72, 29.64, 29.62, 29.44, 29.36, 27.60, 26.37, 26.34, 23.09, 14.53; HRMS (FAB): theory for $C_{43}H_{75}O_3$ (M + 1), 639.5716; found (M + 1), 639.5755. Anal. ($C_{43}H_{74}O_3$): C, H.

3,4-Bis-octadec-9-enyloxy-benzoic Acid (8c). To a solution of oleyl bromide (1.8 g, 5.4 mmol) in cyclohexanone (20 mL) was added ethyl 3,4-dihydroxybenzoate **7b** (0.45 g, 2.5 mmol), potassium carbonate (1.02 g, 7.4 mmol), and potassium iodide (0.05 g, 0.3 mmol). The suspension was stirred at 100 °C for 18 h under nitrogen. Due to the light sensitivity of the oleyl bromide, the flask was covered in aluminum foil. The hot reaction mixture was filtered to remove some of the particulates, and the solvent was removed *in vacuo*. The residue containing ester **8b** was dissolved in a solution of ethanol (20 mL) containing potassium hydroxide (0.8 g, 20 mmol) and refluxed for 4 h. The hot reaction mixture was added to water (30 mL), and acidifying with 1 M HCl to pH 1 resulted in the precipitation of a white solid. The solid **8c** was filtered off and washed several times with water. The crude acid was recrystallized from ethanol (10 mL) to give the product **8c** as a white solid (1.14 g, 70%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (dd, 1H, phenyl), 7.58 (d, 1H, phenyl), 6.89 (d, 1H, phenyl), 5.35 (t, 4H, olefinic), 4.05 (2 × t, 4H, 2 × OCH_2), 2.01 (m, 8H, $CH_2C{=}C$), 1.85 (m, 4H, CH_2CH_2O), 1.48 (quin, 4H, 2 × CH_2), 1.41−1.19 (m, 40H, 20 × CH_2), 0.88 (t, 6H, CH_3).

Acid Chloride 8d. See compound **20** for experimental details.

(4-Amino-butyl)-carbamic Acid *tert*-Butyl Ester (9).[34] 1,4-Diaminobutane (4.4 g, 0.05 mol) was dissolved in a solution of triethylamine and methanol (10% TEA in MeOH, 110 mL). A solution of di-*tert*-butyl dicarbonate (3.63 g, 0.017 mol) in methanol (10 mL) was added dropwise to this mixture with vigorous stirring. The mixture was stirred at RT overnight. The *tert*-butoxycarbonylation was complete as shown by TLC (4% NH_4OH/MeOH). The excess 1,4-diaminobutane, methanol, and TEA were removed *in vacuo* to yield an oily residue that was dissolved in dichloromethane (100 mL) and washed with a solution of sodium carbonate (10% aq, 2 × 100 mL). The organic layer was dried over anhydrous sodium sulfate and filtered, the solvent removed *in vacuo*, and the oily residue purified by flash column chromatography (1:10:89 NH_4OH:MeOH:$CHCl_3$) to give the product **9** as a clear oil (2.23 g, 71%), $R_f = 0.38$ (1:10:89 NH_4OH:MeOH:$CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 4.72 (br s, 1H, NHCO), 3.12 (q, 2H, CH_2), 2.70 (t, 2H, CH_2), 1.57−1.30 (m, 13H, 2 × CH_2, 3 × CH_3).

[4-(3-Cyano-propylamino)-butyl]-carbamic Acid *tert*-Butyl Ester (10).[34] To a solution of the BOC protected diamine **9** (2.10 g, 0.01 mol) in anhydrous acetonitrile (50 mL) was added potassium carbonate (5.14 g), and the suspension was stirred at RT for 10 min. A solution of 4-bromobutyronitrile (1.65 g, 0.01 mol) in acetonitrile (25 mL) was added, and the resulting mixture stirred at 50 °C for 24 h. TLC (1:10:89 NH_4OH:MeOH:$CHCl_3$) showed that the reaction was 95% complete. The mixture was filtered to remove most of the inorganic salts, and the acetonitrile was removed *in vacuo* to give a solid/oily residue that was purified by flash column chromatography (1:5:94 NH_4OH:MeOH:$CHCl_3$) to yield the product **10** as a clear oil (1.74 g, 61%), $R_f = 0.5$ (1:10:89 NH_4OH:MeOH:$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.79 (br s, 1H, NHCO), 3.12 (q, 2H, CH_2), 2.74 (t, 2H, CH_2), 2.60 (t, 2H, CH_2), 2.46 (t, 2H, CH_2), 1.81 (quin, 2H, CH_2), 1.57−1.37 (m, 13H, 2 × CH_2, 3 × CH_3).

(4-*tert*-Butoxycarbonylamino-butyl)-(3-cyano-propyl)-carbamic Acid *tert*-Butyl Ester (11).[34] The amino-nitrile **10** (1.74 g, 6.8 mmol) was dissolved in a solution of triethylamine and methanol (10% TEA in MeOH, 40 mL). A solution of di-*tert*-butyl dicarbonate (3.63 g, 0.017 mol) in methanol (20 mL) was added dropwise to this mixture with vigorous stirring. The mixture was stirred at rt overnight. TLC (1:10:89 NH_4OH:MeOH:$CHCl_3$) showed the *tert*-butoxycarbonylation was complete. The methanol and TEA were removed *in vacuo* to yield an oily residue that was dissolved in dichloromethane (100 mL) and washed with a solution of sodium hydroxide (2.5 M, 3 × 30 mL) and water (2 × 30 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, the solvent removed *in vacuo* and the oily residue purified by flash column chromatography (40% EtAc/hexane) to give the product **11** as a clear oil (2.10 g, 87%), $R_f = 0.45$ (40% EtAc/hexane); ^{1}H NMR ($CDCl_3$) δ 4.60 (br s, 1H, NHCO), 3.28 (t, 2H, CH_2), 3.14 (m, 4H, 2 × CH_2), 2.35 (t, 2H, CH_2), 1.88 (quin, 2H, CH_2), 1.58−1.28 (m, 22H, 2 × CH_2, 6 × CH_3).

(4-Amino-butyl)-(4-*tert*-butoxycarbonylamino-butyl)-carbamic Acid *tert*-Butyl Ester (12).[34] The nitrile **11** (2.00 g, 5.6 mmol) was dissolved in ethanol (100 mL). NH_4OH (10 mL) and Raney nickel (8 g) were added, and ammonia gas was bubbled through the solution for 20 min at 0 °C. The suspension was hydrogenated at 50 psi for 24 h. Air was bubbled through the solution, and the Raney nickel was removed by filtering through a sintered glass funnel, keeping the Raney nickel residue moist with solvent (EtOH) at all times. The ethanol and NH_4OH were removed *in vacuo*, and the oily residue was dissolved in CH_2Cl_2 and washed with 10% aq, Na_2CO_3 (3 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and filtered and the solvent removed *in vacuo* to give the product **12** as a clear oil without further purification (1.92 g, 95%), $R_f = 0.4$ (1:10:89 NH_4OH:MeOH:$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.65 (br s, 1H, NHCO), 3.14 (m, 6H, 3 × CH_2), 2.69 (t, 2H, CH_2), 2.35 (t, 2H, CH_2), 1.58−1.26 (m, 26H, 4 × CH_2, 6 × CH_3). The ^{1}H NMR matched the literature spectrum of **12**.[34]

(4-{*tert*-Butoxycarbonyl-[4-(3-cyano-propylamino)-butyl]-amino}-butyl)-carbamic Acid *tert*-Butyl Ester (13a). To a solution the amine **12**[34] (1.32 g, 3.68 mmol) in anhydrous acetonitrile (20 mL) was added potassium carbonate (1.7 g), and the suspension was stirred at rt for 10 min. A solution of 4-bromobutyronitrile (0.54 g, 3.68 mmol) in acetonitrile (10 mL) was added and the resulting mixture stirred at 50 °C under nitrogen for 24 h. TLC (1:10:89 NH_4OH:MeOH:$CHCl_3$) showed the reaction was 95% complete. The mixture was filtered to remove most of the inorganic salts, and the acetonitrile was removed *in vacuo* to give a solid/oily residue. The oil was redissolved in CH_2Cl_2 (70 mL) and washed with saturated Na_2CO_3 (50 mL). The organic layer was separated out, dried over Na_2SO_4, and filtered, the solvent was removed *in vacuo*, and the oily residue purified by flash column chromatography using 7% MeOH/1% NH_4OH/CH_2Cl_2 to give the product **13a** as a clear oil in 55% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.60 (br s, 1H, NHCO), 3.15 (m, 6H, 3 × CH_2), 2.74 (t, 2H, CH_2), 2.62 (t, 2H, CH_2), 2.46 (t, 2H, CH_2), 1.83 (quin, 2H, CH_2), 1.55−1.39 (m, 26H, 4 × CH_2, 6 × CH_3); ^{13}C NMR ($CDCl_3$) δ 156.0, 155.6, 119.8, 79.3, 79.2, 53.6, 49.5, 48.1, 46.9, 40.4, 28.7, 28.6, 27.6, 27.3, 26.1, 25.9, 15.2.

(4-*tert*-Butoxycarbonylamino-butyl)-{4-[*tert*-butoxycarbonyl-(3-cyano-propyl)-amino]-butyl}-carbamic Acid *tert*-Butyl Ester (13b). A solution of di-*tert*-butyl dicarbonate (0.92 g, 0.42 mmol) in methanol (10 mL) was added dropwise to the stirring solution

of **13a** (0.12 g, 0.28 mmol) in 10% TEA/MeOH (10/80) at 0 °C with vigorous stirring. The mixture was stirred at rt overnight. TLC (1:10:89 NH_4OH:MeOH:$CHCl_3$) showed the *tert*-butoxycarbonylation was complete. The solvent was removed *in vacuo* to yield an oily residue that was dissolved in dichloromethane (100 mL) and washed with a solution of saturated sodium carbonate (3 × 30 mL). The organic layer was dried over anhydrous sodium sulfate and filtered, the solvent removed *in vacuo*, and the oily residue purified by flash column chromatography ($CHCl_3$/MeOH 99:1) to give the product **13b** as a clear oil (95% yield). R_f = 0.5 ($CHCl_3$/MeOH 99:2); ^{1}H NMR ($CDCl_3$) δ 4.63 (br s, 1H, NHCO), 3.28 (t, 2H, CH_2), 3.14 (m, 8H, 4 × CH_2), 2.64 (t, 2H, CH_2), 1.88 (quin, 2H, CH_2), 1.58−1.35 (m, 35H, 4 × CH_2, 9 × CH_3); ^{13}C NMR: δ 155.9, 155.5, 79.9, 79.3, 79.0, 46.7, 45.8, 40.3, 28.6, 28.5, 27.5, 26.0, 25.7, 24.6, 14.9.

{4-[(4-Amino-butyl)-*tert*-butoxycarbonyl-amino]-butyl}-(4-*tert*-butoxycarbonyl-amino-butyl)-carbamic Acid *tert*-Butyl Ester (14). The nitrile **13b** (1.33 g, 2.5 mmol) was dissolved in ethanol (100 mL). Concentrated aq NH_4OH (10 mL) and Raney nickel (6 g) were added, and ammonia gas was bubbled through the solution for 20 min at 0 °C. The suspension was then hydrogenated at 50 psi for 24 h. Air was bubbled through the solution, and the Raney nickel was removed by filtering through a sintered glass funnel (keeping the Raney nickel residue moist at all times). The ethanol and NH_4OH were removed *in vacuo*, and the oily residue dissolved in CH_2Cl_2 and washed with 10 wt % aq sodium carbonate (3 × 50 mL). The organic layer was dried over anhydrous sodium sulfate and filtered and the solvent removed *in vacuo*. The oil was purified by flash column chromatography (1:10:89 NH_4OH:MeOH:$CHCl_3$) to give the product **14** as a clear oil (0.85 g, 64%), R_f = 0.5 (1:10:89 NH_4OH:MeOH:$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.65 (s, 1H, NHCO), 3.15 (m, 10H, 5 × CH_2), 2.70 (t, 2H, CH_2NH$_2$), 1.58−1.39 (m, 39H, 6 × CH_2, 9 × CH_3); ^{13}C NMR ($CDCl_3$): δ 155.9, 155.4, 79.2, 79.1, 47.0, 46.7, 40.3, 28.6, 28.5, 27.5, 26.1, 25.7; HRMS (FAB): theory for $C_{27}H_{55}N_4O_6$ (M + 1), 531.4122; found (M + 1), 531.4111; Anal. Calcd ($C_{27}H_{54}N_4O_6$·0.1H_2O): C, H, N.

[4-(3,4-Bis-octadec-9-enyloxy-benzylamino)-butyl]-carbamic Acid *tert*-Butyl Ester (15). To a vigorously stirred solution of the amine **9** (0.071 g, 0.38 mmol, 1.2 equiv) in CH_2Cl_2/MeOH (3:1, 5 mL) was added a solution of the aldehyde **8a** (0.20 g, 0.33 mmol) in CH_2Cl_2/MeOH (3:1, 5 mL), dropwise over 20 min. The resulting mixture was stirred at rt under an atmosphere of nitrogen overnight. ^{1}H NMR showed the reaction to be complete when there was no aldehyde peak present. The solvent was removed *in vacuo* and the crude imine dissolved in CH_2Cl_2/MeOH (1:1, 10 mL). The solution was cooled to 0 °C, and $NaBH_4$ (60 mg, 1.58 mmol) was added in 15 mg portions over 30 min. TLC (10% EtAc/hexane) showed the reaction to be complete after stirring overnight. The solvent was removed *in vacuo* and the crude oil dissolved in CH_2Cl_2 (50 mL) and washed with sodium carbonate (10% aq, 3 × 40 mL). The CH_2Cl_2 layer was dried over Na_2CO_3 and filtered, the solvent removed *in vacuo*, and the oily residue purified by flash column chromatography (4% MeOH/$CHCl_3$) to give the product **15** as a clear oil (0.18 g, 78%), R_f = 0.2 (3% MeOH/$CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.85 (s, 1H, aryl), 6.80 (m, 2H, aryl), 5.34 (t, 4H, olefinic), 4.78 (br s, 1H, NHCO), 3.97 (q, 4H, OCH_2), 3.69 (s, 2H, benzylic), 3.13 (m, 2H, CH_2) 2.63 (t, 2H, CH_2NH), 2.01 (m, 8H, CH_2C═C), 1.80 (m, 4H, CH_2CH$_2$O), 1.55 (m, 4H), 1.49−1.12 (m, 57H, 24 × CH_2, 3 × CH_3), 0.89 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 156.17, 149.34, 148.29, 133.15, 130.11, 130.00, 120.67, 114.18, 114.08, 79.23, 69.69, 69.52, 54.12, 49.22, 40.80, 32.28, 30.16, 29.92, 29.82, 29.71, 29.67, 28.81, 28.27, 27.68, 27.60, 26.44, 23.09, 14.54; Anal. ($C_{52}H_{94}N_2O_4$): C, H, N.

***N*^{1}-(3,4-Bis-octadec-9-enyloxy-benzyl)-octane-1,8-diamine (16).** To a vigorously stirred solution of diaminooctane (0.23 g, 1.56 mmol, 5 equiv) in CH_2Cl_2/MeOH (3:1, 5 mL) was added a solution of the aldehyde **8a** (0.20 g, 0.33 mmol) in CH_2Cl_2/MeOH (3:1, 5 mL), dropwise over 1 h. The resulting mixture was stirred at RT under an atmosphere of nitrogen overnight. NMR showed the reaction to be complete when there was no aldehyde peak present in the NMR spectrum. The solvent was removed *in vacuo* and the crude imine dissolved in CH_2Cl_2/MeOH (1:1, 10 mL). The solution was cooled to 0 °C, and sodium borohydride (60 mg, 1.58 mmol) was added in 15 mg portions over 30 min. TLC (10% EtAc/hexane) showed the reaction to be complete after stirring overnight. The solvent was removed *in vacuo* and the crude solid dissolved in CH_2Cl_2 (50 mL) and washed with sodium carbonate (10% aq, 3 × 40 mL). The CH_2Cl_2 layer was dried over sodium sulfate and filtered, the solvent was removed *in vacuo*, and the solid residue purified by flash column chromatography (1:10:89 NH_4OH:MeOH:$CHCl_3$) to give the product **16** as a white solid (0.15 g, 63%), R_f = 0.5 (1:10:89 NH_4OH:MeOH:$CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.85 (s, 1H, aryl), 6.80 (m, 2H, aryl), 5.34 (t, 4H, olefinic), 3.97 (q, 4H, OCH_2), 3.69 (s, 2H, benzylic), 2.67 (t, 2H, CH_2NH$_2$), 2.60 (t, 2H, CH_2NH$_2$), 2.01 (m, 8H, CH_2C═C), 1.80 (m, 4H, CH_2CH$_2$O), 1.54−1.18 (m, 56H, 28 × CH_2), 0.88 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 149.31, 148.20, 133.55, 130.11, 130.00, 120.59, 114.14, 114.07, 69.70, 69.50, 54.24, 49.83, 42.60, 34.20, 32.28, 30.46, 30.16, 29.92, 29.81, 29.71, 29.67, 27.73, 27.60, 27.22, 26.44, 23.08, 14.53; HRMS (FAB): theory for $C_{51}H_{95}N_2O_2$ (M + 1), 767.7394; found (M + 1), 767.7377. Anal. ($C_{51}H_{94}N_2O_2$·0.3H_2O): C, H, N.

2-{2-[2-(3,4-Bis-octadec-9-enyloxy-benzylamino)-ethoxy]-ethoxy}-ethylamine (17). To a vigorously stirred solution of diaminodioxaoctane (0.23 g, 1.56 mmol, 5 equiv) in CH_2Cl_2/MeOH (3:1, 5 mL) was added a solution of the aldehyde **8a** (0.20 g, 0.33 mmol) in CH_2Cl_2/MeOH (3:1, 5 mL), dropwise over 1 h. The resulting mixture was stirred at rt under an atmosphere of nitrogen overnight. NMR showed the reaction to be complete when there was no aldehyde peak present in the NMR spectrum. The solvent was removed *in vacuo* and the crude imine dissolved in CH_2Cl_2/MeOH (1:1, 10 mL). The solution was cooled to 0 °C, and $NaBH_4$ (60 mg, 1.58 mmol) was added in 15 mg portions over 30 min. TLC (10% EtAc/hexane) showed the reaction to be complete after stirring overnight. The solvent was removed *in vacuo* and the crude oil dissolved in CH_2Cl_2 (50 mL) and washed with sodium carbonate (10% aq, 3 × 40 mL). The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and filtered, the solvent was removed *in vacuo*, and the oily residue purified by flash column chromatography (1:10:89 NH_4OH:MeOH:$CHCl_3$) to give the product **17** as a clear oil (0.168 g, 70%), R_f = 0.45 (1:10:89 NH_4OH:MeOH:$CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.87 (s, 1H, aryl), 6.81 (m, 2H, aryl), 5.34 (t, 4H, olefinic), 3.97 (q, 4H, OCH_2), 3.72 (s, 2H, benzylic), 3.61 (m, 6H, OCH_2), 3.61 (t, 2H, OCH_2), 2.86 (t, 2H, CH_2NH$_2$), 2.81 (t, 2H, CH_2NH$_2$), 2.01 (m, 8H, CH_2C═C), 1.80 (m, 4H, CH_2CH$_2$O), 1.54−1.17 (m, 44H, 22 × CH_2), 0.89 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 149.31, 148.25, 133.11, 130.09, 129.99, 120.73, 114.26, 114.07, 73.57, 70.85, 70.57, 70.52, 69.69, 69.51, 54.02, 48.93, 42.01, 32.28, 30.15, 29.91, 29.80, 29.70, 29.67, 27.59, 26.45, 26.42, 23.07, 14.53; HRMS (FAB): theory for $C_{49}H_{91}N_2O_4$ (M + 1), 771.6979; found (M + 1), 771.6995; Anal. ($C_{49}H_{90}N_2O_4$): C, H, N.

(4-{[4-(3,4-Bis-octadec-9-enyloxy-benzylamino)-butyl]-*tert*-butoxycarbonyl-amino}-butyl)-carbamic Acid *tert*-Butyl Ester (18). To a vigorously stirred solution of the amine **12** (0.135 g, 0.38 mmol, 1.2 equiv) in CH_2Cl_2/MeOH (3:1, 5 mL) was added a solution of the aldehyde **8a** (0.20 g, 0.33 mmol) in CH_2Cl_2/MeOH (3:1, 5 mL), dropwise over 20 min. The resulting mixture was stirred at RT under an atmosphere of nitrogen overnight. NMR showed the reaction to be complete when there was no aldehyde peak present in the NMR spectrum. The solvent was removed *in vacuo* and the crude imine dissolved in CH_2Cl_2/MeOH (1:1, 10 mL). The solution was cooled to 0 °C, and sodium borohydride (60 mg, 1.58 mmol) was added in 15 mg portions over 30 min. TLC (10% EtAc/hexane) showed the reaction to be complete after stirring overnight. The solvent was removed *in vacuo* and the crude oil dissolved in CH_2Cl_2 (50 mL) and washed with sodium carbonate (10% aq, 3 × 40 mL). The CH_2Cl_2 layer was dried over sodium sulfate and filtered, the solvent removed *in vacuo*, and the oily residue purified by flash column chromatography (3% MeOH/$CHCl_3$) to give the product **18** as a clear oil (0.24 g, 78%), R_f = 0.3 (3% MeOH/$CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 6.85 (s, 1H, aryl), 6.80 (m, 2H, aryl), 5.34 (t, 4H, olefinic), 4.60 (br s, 1H, NHCO), 3.97 (q, 4H, OCH_2), 3.69 (s, 2H, benzylic), 3.13 (m, 6H, CH_2) 2.63 (t, 2H, CH_2NH),

2.01 (m, 8H, $CH_2C{=}C$), 1.80 (m, 4H, CH_2CH_2O), 1.67−1.15 (m, 70H, 26 × CH_2, 6 × CH_3), 0.89 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 156.17, 155.71, 149.33, 148.25, 133.31, 130.11, 130.00, 120.62, 114.15, 114.08, 79.45, 69.70, 69.51, 54.13, 49.42, 47.34, 46.89, 40.58, 32.28, 30.14, 29.91, 29.80, 29.70, 29.67, 28.85, 28.79, 27.79, 27.70, 27.59, 26.42, 23.07, 14.53; HRMS (FAB): theory for $C_{61}H_{112}N_3O_6$ (M + 1), 982.8551; found (M + 1), 982.8510; Anal. ($C_{61}H_{111}N_3O_6$): C, H, N.

(4-{[4-(3,4-Bis-octadec-9-enyloxy-benzylamino)-butyl]-*tert*-butoxycarbonyl-amino}-butyl)-(4-*tert*-butoxycarbonylamino-butyl)-carbamic Acid *tert*-Butyl Ester (19). To a vigorously stirred solution of the amine **14** (0.20 g, 0.38 mmol, 1.2 equiv) in CH_2Cl_2/MeOH (3:1, 5 mL) was added a solution of the aldehyde **8a** (0.20 g, 0.33 mmol) in CH_2Cl_2/MeOH (3:1, 5 mL), dropwise over 20 min. The resulting mixture was stirred at RT under an atmosphere of nitrogen overnight. NMR showed the reaction to be complete when there was no aldehyde peak present in the spectrum. The solvent was removed *in vacuo* and the crude imine dissolved in CH_2Cl_2/MeOH (1:1, 10 mL). The solution was cooled to 0 °C, and sodium borohydride (60 mg, 1.58 mmol) was added in 15 mg portions over 30 min. TLC (10% EtAc/hexane) showed the reaction to be complete after stirring overnight. The solvent was removed *in vacuo* and the crude oil dissolved in CH_2Cl_2 (50 mL) and washed with sodium carbonate (10% aq, 3 × 40 mL). The CH_2Cl_2 layer was dried over sodium sulfate and filtered, the solvent removed *in vacuo*, and the oily residue purified by flash column chromatography (3% MeOH/$CHCl_3$) to give the product **19** as a clear oil (0.319 g, 88%), R_f = 0.25 (3% MeOH/$CHCl_3$). ^1H NMR ($CDCl_3$) δ 6.85 (s, 1H, aryl), 6.80 (m, 2H, aryl), 5.34 (t, 4H, olefinic), 4.60 (br s, 1H, NHCO), 3.97 (q, 4H, OCH_2), 3.70 (s, 2H, benzylic), 3.14 (m, 10H, 5 × CH_2) 2.64 (t, 2H, CH_2NH), 2.01 (m, 8H, $CH_2C{=}C$), 1.80 (m, 4H, CH_2CH_2O), 1.59−1.14 (m, 83H, 28 × CH_2, 9 × CH_3), 0.89 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 156.12, 155.69, 149.33, 148.25, 133.28, 130.10, 129.99, 120.61, 114.14, 114.07, 79.40, 69.69, 69.50, 54.15, 49.47, 47.24, 46.98, 40.57, 32.27, 30.14, 29.90, 29.80, 29.69, 29.66, 28.85, 28.79, 27.77, 27.58, 26.44, 23.07, 14.53; HRMS (FAB): theory for $C_{70}H_{129}N_4O_8$ (M + 1), 1153.9810; found (M + 1), 1153.9840; Anal. Calcd ($C_{70}H_{128}N_4O_8$): C, H, N.

(4-{[4-(3,4-Bis-octadec-9-enyloxy-benzoylamino)-butyl]-*tert*-butoxycarbonyl-amino}-butyl)-carbamic Acid *tert*-Butyl Ester (20). A solution of 3,4-bis-octadec-9-enyloxy-benzoic acid **8c** (0.2 g, 0.31 mmol) in 2:1 dichloromethane /benzene (15 mL) was stirred at 0 °C for 10 min. Anhydrous DMF (2 drops) and oxalyl chloride (0.3 mL) were added in sequence, and the mixture was stirred for 1 h at 0 °C. The solution was concentrated *in vacuo* to give the crude acid chloride, **8d**. Crude **8d** was dissolved in CH_2Cl_2 (10 mL) and added dropwise to a solution of the amine **12** (0.132 g, 0.37 mmol, 1.2 equiv) dissolved in CH_2Cl_2 (10 mL) and 1 M NaOH (10 mL) that had been cooled to 0 °C for 15 min. The reaction was stirred overnight under N_2 at room temperature. The water layer was separated off. The CH_2Cl_2 layer was washed with Na_2CO_3 (10% aq, 3 × 20 mL), dried over Na_2SO_4, filtered, and removed *in vacuo* and the oily residue purified by flash column chromatography (30% EtAc/hexane) to give the product **20** as a clear oil (0.261 g, 86%), R_f = 0.25 (30% EtAc/hexane); ^1H NMR (500 MHz, $CDCl_3$) δ 7.50−7.20 (m, 2H, phenyl), 6.90 (br s, 0.5H, NHCO), 6.81 (d, 1H, phenyl), 6.41 (br s, 0.5H, NHCO), 5.35 (t, 4H, olefinic), 4.68 (m, 1H, NHCO), 3.99 (2t, 4H, 2 × OCH_2), 3.44 (m, 2H, CH_2), 3.11 (m, 6H, 3 × CH_2), 2.00 (m, 8H, $CH_2C{=}C$), 1.79 (m, 4H, CH_2CH_2O), 1.53−1.17 (m, 70H, 26 × CH_2, 6 × CH_3), 0.88 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 167.32, 156.18, 151.84, 148.98, 130.11, 130.09, 127.32, 113.02, 112.39, 79.62, 79.32, 69.52, 69.36, 47.09, 40.49, 39.92, 32.26, 30.13, 29.89, 29.76, 29.68, 29.65, 29.62, 29.59, 29.50, 28.84, 28.77, 27.79, 27.58, 26.38, 26.36, 23.06, 14.51; HRMS (FAB): theory for $C_{61}H_{109}N_3O_7$ (M + Na), 1018.8158; found (M +Na), 1018.8123; Anal. ($C_{61}H_{109}N_3O_7$): C, H, N.

(4-{[4-(3,4-Bis-octadec-9-enyloxy-benzoylamino)-butyl]-*tert*-butoxycarbonyl-amino}-butyl)-(4-*tert*-butoxycarbonylamino-butyl)-carbamic Acid *tert*-Butyl Ester (21). A solution of 3,4-Bis-octadec-9-enyloxy-benzoic acid **8c** (0.2 g, 0.31 mmol) in 2:1 dichloromethane/benzene (15 mL) was stirred at 0 °C for 10 min. Anhydrous DMF (2 drops) and oxalyl chloride (0.3 mL) were added in sequence, and the mixture was stirred for 1 h at 0 °C. The solution was concentrated *in vacuo* to give the crude acid chloride **8d**. The acid chloride **8d** was dissolved in CH_2Cl_2 (10 mL) and added dropwise to a solution of the amine **14** (0.195 g, 0.37 mmol, 1.2 equiv) dissolved in CH_2Cl_2 (10 mL) and 1 M NaOH (10 mL) that had been cooled to 0 °C for 15 min. The reaction was stirred overnight under N_2 at room temperature. The water layer was separated off. The CH_2Cl_2 layer was washed with Na_2CO_3 (10% aq. 3 × 20 mL), dried over Na_2SO_4, filtered, and removed *in vacuo* and the oily residue purified by flash column chromatography (30% EtAc/hexane) to give the product **21** as a clear oil (0.295 g, 83%), R_f = 0.20 (30% EtAc/hexane); ^1H NMR (500 MHz, $CDCl_3$) δ 7.42−7.20 (m, 2H, phenyl), 6.82 (br s, 0.5 H, NHCO), 6.80 (d, 1H, phenyl), 6.39 (br s, 0.5 H, NHCO), 5.35 (t, 4H, olefinic), 4.65 (m, 1H, NHCO), 3.99 (2 × t, 4H, 2 × OCH_2), 3.42 (m, 2H, CH_2), 3.13 (m, 10H, 5 × CH_2), 2.00 (m, 8H, $CH_2C{=}C$), 1.79 (m, 4H, CH_2CH_2O), 1.53−1.17 (m, 83H, 28 × CH_2, 9 × CH_3), 0.88 (t, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 167.35, 156.16, 155.73, 151.88, 148.99, 130.13, 130.11, 127.37, 113.05, 112.41, 79.54, 69.54, 69.38, 47.03, 40.58, 39.97, 32.27, 30.14, 29.90, 29.77, 29.69, 29.66, 29.63, 29.60, 29.51, 28.85, 28.79, 27.78, 27.59, 26.40, 26.37, 23.07, 14.53; HRMS (FAB): theory for $C_{70}H_{126}N_4O_9$ (M + Na), 1189.9407; found (M + Na), 1189.9365; Anal. ($C_{70}H_{126}N_4O_8$): C, H, N.

***N*^{1}-(3,4-Bis-octadec-9-enyloxy-benzyl)-butane-1,4-diamine, Dihydrochloride Salt (22)**. A concentrated solution of the amine **15** (0.158 g, 0.19 mmol) in ethyl acetate was added cooled to 0 °C. A total of 4 mL of a freshly prepared saturated solution of HCl in ethyl acetate was added dropwise and the solution stirred for 1 h at room temperature during which time a white precipitate formed. The ethyl acetate was removed *in vacuo*, and the residue coevaporated with ethyl acetate and chloroform to give the product **22** as an off white powder (0.15 g, 98%); ^1H NMR (500 MHz, $CDCl_3$) δ 9.50 (br s, 2H, $R_2N^+H_2$), 8.22 (br s, 3H, RN^+H_3), 7.24 (s, 1H, aryl), 7.05 (d, 1H, aryl), 6.82 (d, 1H, aryl), 5.34 (m, 4H, olefinic), 4.02 (q, 4H, OCH_2), 3.93 (t, 2H, benzylic), 3.07 (m, 2H, CH_2) 2.81 (m, 2H, CH_2), 2.01 (m, 8H, $CH_2C{=}C$), 1.95 (m, 2H, CH_2), 1.85 (m, 2H, CH_2), 1.78 (m, 4H, CH_2CH_2O), 1.45 (m, 4H, 2 × CH_2), 1.38−1.20 (m, 40H, 20 × CH_2), 0.89 (t, 6H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 150.00, 149.37, 130.12, 130.09, 129.95, 122.58, 115.81, 113.49, 69.63, 69.38, 51.44, 51.06, 45.80, 39.63, 32.29, 30.24, 30.21, 30.16, 30.00, 29.93, 29.71, 27.61, 26.63, 26.49, 24.83, 23.75, 23.08, 14.54; HRMS (FAB): theory for $C_{47}H_{87}N_2O_2$ (M + 1), 711.6768; found (M + 1), 711.6827; Anal. ($C_{47}H_{88}Cl_2N_2O_4 \cdot 1.2H_2O$): C, H, N.

***N*^{1}-(3,4-Bis-octadec-9-enyloxy-benzyl)-octane-1,8-diamine, Dihydrochloride Salt (23).** A concentrated solution of the amine **16** (0.097 g, 0.13 mmol) in ethyl acetate was added cooled to 0 °C. A total of 4 mL of a freshly prepared saturated solution of HCl in ethyl acetate was added dropwise and the solution stirred for 1 h at room temperature during which time a white precipitate formed. The ethyl acetate was removed *in vacuo* and the residue coevaporated with ethyl acetate and chloroform to give the product **23** as an off white powder (0.104 g, 98%); ^1H NMR ($CDCl_3$) δ 7.24 (s, 1H, aryl), 7.05 (d, 1H, aryl), 6.85 (d, 1H, aryl), 5.35 (m, 4H, olefinic), 4.07 (q, 4H, OCH_2), 3.95 (t, 2H, benzylic), 3.07 (m, 2H, CH_2) 2.79 (m, 2H, CH_2), 2.05 (m, 8H, $CH_2C{=}C$), 1.82 (m, 8H, CH_2CH_2O, 2 × CH_2), 1.58−1.21 (m, 52H, 26 × CH_2), 0.89 (t, 6H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 149.36, 148.78, 130.85, 130.33, 129.74, 122.23, 115.56, 113.45, 69.56, 69.32, 51.56, 51.15, 45.96, 39.86, 32.53, 30.44, 30.44, 30.23, 30.12, 30.00, 29.71, 27.61, 27.51, 27.10, 26.83, 26.69, 24.82, 23.77, 23.08, 14.54; HRMS (FAB): theory for $C_{51}H_{95}N_2O_2$ (M + 1), 767.7394; found (M + 1), 767.7419; Anal. ($C_{51}H_{96}Cl_2N_2O_2 \cdot 0.3H_2O$): C, H, N.

2-{2-[2-(3,4-Bis-octadec-9-enyloxy-benzylamino)-ethoxy]-ethoxy}-ethylamine, Dihydrochloride Salt (24). A concentrated solution of the amine **17** (0.14 g, 0.18 mmol) in ethyl acetate was added cooled to 0 °C. A total of 4 mL of a freshly prepared saturated solution of HCl in ethyl acetate was added dropwise and the solution stirred for 1 h at room temperature. The ethyl acetate was removed *in vacuo* and the residue coevaporated with ethyl acetate and

chloroform to give the product **24** as an off white powder (0.15 g, 98%); ^{1}H NMR ($CDCl_3$) δ 7.27 (s, 1H, aryl), 7.00 (d, 1H, aryl), 6.82 (d, 1H, aryl), 5.33 (m, 4H, olefinic), 4.14 (m, 2H, benzylic), 4.01 (t, 2H, OCH_2), 3.95 (t, 2H, OCH_2), 3.87 (t, 2H, CH_2), 3.78 (t, 2H, CH_2), 3.70 (t, 4H, CH_2), 3.25 (t, 2H, CH_2) 2.93 (m, 2H, CH_2), 2.02 (m, 8H, CH_2C═C), 1.89 (m, 4H, CH_2CH_2O), 1.53−1.18 (m, 44H, 22 × CH_2), 0.89 (t, 6H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 149.91, 149.62, 130.12, 130.00, 123.28, 122.61, 115.46, 113.63, 70.22, 70.09, 69.64, 69.40, 66.50, 65.98, 51.06, 45.08, 40.11, 32.28, 30.15, 29.92, 29.70, 27.60, 26.54, 26.43, 23.07, 14.54; HRMS (FAB): theory for $C_{49}H_{91}N_2O_4$ (M + 1), 771.6973; found (M + 1), 771.6967; Anal. ($C_{49}H_{92}Cl_2N_2O_4$·0.4H_2O): C, H, N.

***N*-(4-Amino-butyl)-*N*′-(3,4-bis-octadec-9-enyloxy-benzyl)-butane-1,4-diamine, Trihydrochloride Salt (25).** A concentrated solution of the amine **18** (0.17 g, 0.17 mmol) in ethyl acetate was added cooled to 0 °C. A total of 4 mL of a freshly prepared saturated solution of HCl in ethyl acetate was added dropwise and the solution stirred for 1 h at room temperature during which time a white precipitate formed. The ethyl acetate was removed *in vacuo* and the residue coevaporated with ethyl acetate and chloroform to give the product **25** as a white powder (0.151 g, 98%); ^{1}H NMR ($CDCl_3$/CH_3OD 9:1) δ 7.13 (s, 1H, aryl), 7.02 (d, 1H, aryl), 6.85 (d, 1H, aryl), 5.33 (m, 4H, olefinic), 4.05 (m, 2H, benzylic), 3.97 (t, 2H, OCH_2), 3.04 (m, 6H, 3 × CH_2) 2.93 (m, 2H, CH_2), 2.02 (m, 8H, CH_2C═C), 1.90 (m, 8H, 4 × CH_2), 1.81 (m, 4H, CH_2CH_2O), 1.47 (m, 4H, 2 × CH_2), 1.40−1.17 (m, 40H, 20 × CH_2), 0.89 (t, 6H, CH_3); ^{13}C NMR (300 MHz, D_2O) δ 148.94, 148.33, 129.26, 129.19, 123.64, 122.52, 113.10, 68.50, 68.39, 49.96, 45.98, 45.43, 39.86, 31.28, 29.18, 29.11, 28.93, 28.86, 28.70, 28.62, 26.64, 26.59, 25.68, 25.62, 24.00, 22.54, 22.46, 22.11, 13.89; HRMS (FAB): theory for $C_{51}H_{96}N_3O_2$ (M + 1), 782.7497; found (M + 1), 782.7495; Anal. ($C_{51}H_{98}Cl_3N_3O_2$·0.6H_2O): C, H, N.

***N*-[4-(4-Amino-butylamino)-butyl]-*N*′-(3,4-bis-octadec-9-enyloxy-benzyl)-butane-1,4-diamine, Tetrahydrochloride Salt (26).** A concentrated solution of the amine **19** (0.253 g, 0.22 mmol) in ethyl acetate was added cooled to 0 °C. A total of 5 mL of a freshly prepared saturated solution of HCl in ethyl acetate was added dropwise and the solution stirred for 1 h at room temperature during which time a white precipitate formed. The ethyl acetate was removed *in vacuo* and the residue coevaporated with ethyl acetate and chloroform to give the product **26** as a white powder (0.215 g, 98%); ^{1}H NMR (CD_3OD) δ 7.13 (s, 1H, aryl), 7.04 (d, 1H, aryl), 6.98 (d, 1H, aryl), 5.33 (t, 4H, olefinic), 4.12 (m, 2H, benzylic), 4.03 (t, 2H, OCH_2), 3.99 (t, 2H, OCH_2), 3.08 (m, 10H, 5 × CH_2), 2.98 (t, 2H, CH_2), 2.02 (m, 8H, CH_2C═C), 1.89 (m, 16H, 8 × CH_2), 1.47 (m, 4H, 2 × CH_2), 1.42−1.19 (m, 40H, 20 × CH_2), 0.89 (t, 6H, CH_3); ^{13}C NMR (300 MHz, D_2O) δ 149.74, 148.90, 129.62, 129.46, 123.92, 123.71, 69.36, 68.88, 51.12, 46.68, 38.99, 32.14, 29.97, 29.81, 29.60, 27.36, 26.53, 24.69, 24.22, 23.14, 23.06, 22.84, 14.05; HRMS (FAB): theory for $C_{55}H_{105}N_4O_2$ (M + 1), 853.8238; found (M + 1), 853.8264; Anal. ($C_{55}H_{109}Cl_4N_4O_2$·0.6H_2O): C, H, N.

***N*-[4-(4-Amino-butylamino)-butyl]-3,4-bis-octadec-9-enyloxy-benzamide, Dihydrochloride Salt (27).** A concentrated solution the amide **20** (0.22 g, 0.22 mmol) in ethyl acetate was added cooled to 0 °C. A total of 5 mL of a freshly prepared saturated solution of HCl in ethyl acetate was added dropwise and the solution stirred for 1 h at room temperature during which time a white precipitate formed. The ethyl acetate was removed *in vacuo* and the residue coevaporated with ethyl acetate and chloroform to give the product **27** as a white powder (0.19 g, 98%); ^{1}H NMR ($CDCl_3$) δ 8.95 (br s, 2H, $R_2N^+H_2$), 8.20 (br s, 3H, RN^+H_3), 7.45 (m, 2H, aryl), 6.78 (m, 1H, aryl), 5.33 (m, 4H, olefinic), 3.95 (m, 4H, OCH_2), 3.38 (m, 2H, CH_2), 3.11 (m, 2H, CH_2), 3.01 (m, 4H, 2 × CH_2), 2.08−1.89 (m, 14H, 7 × CH_2), 1.71 (m, 6H, 3 × CH_2), 1.47−1.18 (m, 44H, 20 × CH_2), 0.89 (t, 6H, CH_3); HRMS (FAB): theory for $C_{51}H_{94}N_3O_3$ (M + 1), 796.7290; found (M + 1), 796.7249; Anal. ($C_{51}H_{95}Cl_2N_3O_3$·1.6H_2O): C, H, N.

***N*-{4-[4-(4-Amino-butylamino)-butylamino]-butyl}-3,4-bis-octadec-9-enyloxy-benzamide, Trihydrochloride Salt (28).** A concentrated solution of the amide **21** (0.22 g, 0.19 mmol) in ethyl acetate was added cooled to 0 °C. A total of 5 mL of a freshly prepared saturated solution of HCl in ethyl acetate was added dropwise and the solution stirred for 1 h at room temperature during which time a white precipitate formed. The ethyl acetate was removed *in vacuo* and the residue coevaporated with ethyl acetate and chloroform to give the product **28** as a white powder (0.18 g, 98%); ^{1}H NMR ($CDCl_3$/CH_3OD 9:1) δ 7.42 (m, 2H, aryl), 6.78 (m, 1H, aryl), 5.33 (m, 4H, olefinic), 4.03 (m, 4H, OCH_2), 3.41 (m, 2H, CH_2), 3.01 (m, 10H, 5 × CH_2), 2.08−1.66 (m, 24H, 12 × CH_2), 1.71 (m, 6H, 3 × CH_2), 1.56−1.13 (m, 44H, 20 × CH_2), 0.89 (t, 6H, CH_3); HRMS (FAB): theory for $C_{55}H_{103}N_4O_3$ (M + 1), 867.8025; found (M + 1), 867.8022; Anal. Calcd for ($C_{55}H_{105}Cl_3N_4O_3$·1.5H_2O): C, H, N.

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Supporting Information Available: Elemental analysis data for compounds **8a** and **14**−**28**, conversions of doses into μM concentrations for transfection experiments, sample fluorescent images from transfection studies with eGFP plasmid, and ^{1}H NMR spectra for **8a**, **8c**, and **15**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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