Synthesis of Novel Cationic Lipids: Effect of Structural Modification on the Efficiency of Gene Transfer

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A series of novel cationic lipids was designed and synthesized in an effort to understand the importance of the various structural features with respect to transfection efficiency. Particular attention has been paid to the hydrophobic domain and the cationic headgroup. An efficient method of synthesizing asymmetric diether lipids is described, using alkyl chains ranging from C_{12} to C_{18} and the unsaturated oleyl group. The ternary formulations including the diether lipid 3β-[*N*-(*N*′,*N*′-dimethylaminoethyl)carbamoyl]cholesterol (DC-Chol) and dioleoyl phosphatidylethanolamine (DOPE) were up to 10-fold more efficacious in in vitro assays than the DC-Chol/DOPE control. The shorter and most asymmetric diether lipids performed the best. The chemical nature and basicity of the headgroups have been varied by the coupling of the four naturally occurring amino acids with cationic side chains—arginine, histidine, lysine, and tryptophan. Transfection efficiency was highest for arginine/lysine derivatives, with binary formulations containing the amino acid derivative alone and DOPE proving superior.

Introduction

Gene therapy is an umbrella term covering the methods of introducing foreign genes into recipient cells for therapeutic gain.[1] A transfer vehicle or "vector" is usually required to increase the uptake of the DNA into the target cells. Most of the current vectors can be divided into two groups, viral and nonviral. Both have their intrinsic advantages and disadvantages. Viral vectors are particularly efficient since they have evolved with the express purpose of infecting mammalian cells with foreign genetic material. However, immunogenicity, recombination to form replication-competent virions, the potential for insertional mutagenesis, vector inactivation, and constraints on the size of genetic material that may be carried in the viral capsid have obstructed the development of these vectors.[2]

The main drawback to the development of nonviral vectors has been that they are not as efficient as viruses and so need to be presented to the target cells in high concentration.[2] There is also a problem with their rapid removal from circulation by leucocytes and the reticuloendothelial system, while a significant proportion of the nonviral vector dose is deposited in the large capillary bed of the lungs.[3] However, there are less of the safety and immunogenicity concerns inherent in viral systems. Also, nonviral vectors, particularly cationic lipids and polymers, are easier and cheaper to produce and can be obtained on a much larger scale. A greater degree of control can be exercised over the structure of nonviral vectors on a molecular level, and the products can be highly purified[3] without the risk of toxic contaminants, which are a cause for concern with viral vectors.

Cationic liposomes are the largest and most extensively studied group of nonviral vectors. They are large, spherical structures that form spontaneously when their constituent lipids are exposed to an aqueous environment. The structure of cationic lipids generally comprises a cationic headgroup attached via a linker to a large hydrophobic domain. The headgroup often consists of primary, secondary, or tertiary amines, but quaternary ammonium salts, guanidino, and imidazole groups have also been investigated.[4−11] The most common linkers used are ethers and esters, although amides and carbamates are also employed.[11−14] The hydrophobic domain is generally either a double chain hydrocarbon or a cholesterol derivative. The double chain hydrocarbons are normally 12−18 carbon units in length and either are completely saturated or contain one double bond (oleyl group). Cationic lipids are sometimes formulated into liposomes alone, but more often, they are mixed with a neutral colipid such as dioleoyl phosphatidylethanolamine (DOPE).[6,7,11−13,15−19] Even relatively small structural changes to these domains are known to affect transfection efficiency, sometimes drastically.[6,7,10−12,15,17−23]

In the work reported here, we have examined these domains and their influence on transfection efficiency in detail. The hydrophobic domain was considered first. There are few extensive reports in the literature focusing on the variation of alkyl chain length and still fewer with asymmetric lipids. Felgner et al. reported the synthesis and assay results for several series of homologous lipids.[18] These possess symmetric saturated hydrophobic moieties that ranged from 14 to 18 carbon atoms in length as well as the unsaturated oleyl chain. The order of efficacy was found to be $C_{14} > C_{oleyl} > C_{16} > C_{18}$. Balasubramaniam et al. synthesized a series that included some asymmetric lipids.[21] The in vitro activities of asymmetric lipids were usually superior to the best symmetric analogues, with the degree of improvement depending on the cell line. However, the degree of asymmetry was usually small (e.g., a C_{12} and a C_{14}).

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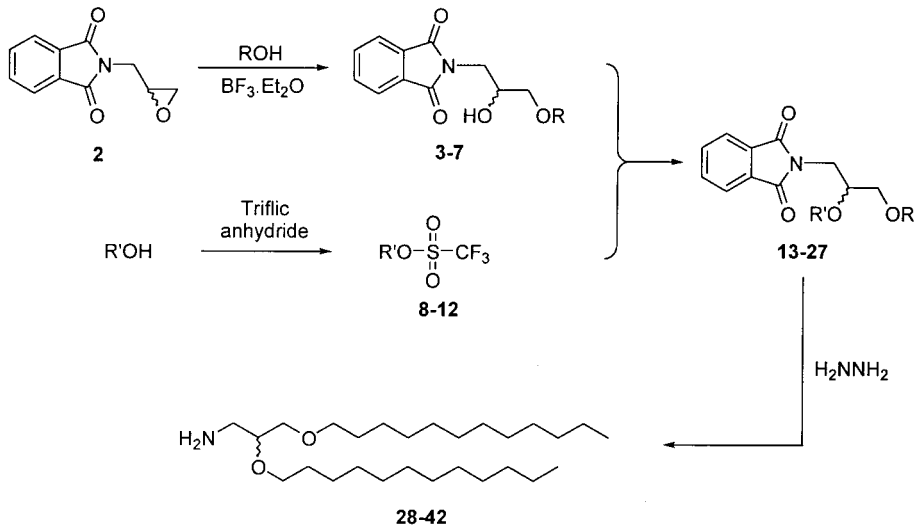

Figure 1. Scheme showing the general synthesis of the diether lipids. In all cases, R/R′ = either C_{12}, C_{14}, C_{16}, C_{18}, or C_{oleyl}. Example product shown is **28** (R = R′ = C_{12}).

The two compounds that were particularly asymmetric (C_8:C_{18} and C_{18}:C_8) performed poorly. The authors hypothesized that the C_8 chain was too short, and the compounds could not form bilayer structures. Byk et al. synthesized a series of four lipids with saturated double alkyl chain lengths of 12, 13, 14, or 18 carbon atoms.[23] When assayed in vitro, they reported that a structure containing two C_{18} chains produced the most efficient lipid and that transfection efficiency was reduced with decreasing chain length. In another in vitro study, the transfection efficiency of a series of phosphonolipids with alkyl chains of different length reported by Floch et al. was found to be $C_{14} > C_{oleyl} > C_{18} > C_{16} > C_{12}$.[24]

As much of this evidence is conflicting, we resolved to investigate further the effect of lipid chain length on transfection by synthesizing a series of dialkyl lipids possessing either C_{12}, C_{14}, C_{16}, C_{18}, or C_{oleyl} chains. In particular, it was considered desirable to develop a synthetic route that would allow the synthesis of asymmetric lipids. All possible combinations of chains were considered, and 15 target compounds of the possible 25 were synthesized. A primary amine was used as the cationic headgroup, to allow subsequent derivatization of the lipids. An ether linkage was utilized between the two domains since it is known to be particularly effective and stable in physiological conditions.[6,7,18,20]

The headgroups of the lipids were also studied, with respect to variations to the chemical nature of this group and the effect on transfection. A straightforward and interesting methodology was developed by coupling the four cationic amino acids (Arg, His, Lys, and Trp) to the two diether lipids that had performed best in the first series of lipids. This produced a further 8 compounds with guanidine, imidazole, primary amine, and indole headgroups. The lipids were assayed in vitro for their ability to transfect cells with the *lacZ* gene.

Chemical Syntheses. Diether Lipids. The diether lipids were synthesized via the synthetic route displayed in Figure 1. The chemistry of Carruthers et al. was slightly modified, such that allyl bromide was reacted with potassium phthalimide to give *N*-(2,3-propenyl)-phthalimide (**1**), which was then epoxidized to yield *N*-(2,3-epoxypropyl)phthalimide (**2**).[25] The first alkyl chains were introduced following the method reported by Guivisdalsky and Bittman.[26] The phthalimide **2** was refluxed with the corresponding alcohol of the alkyl chain in the presence of a catalytic amount of $BF_3 \cdot Et_2O$, yielding the protected monolipids **3**−**7**. The second alkyl chains were added by first preparing the relevant triflates (**8**−**12**), according to the protocol of Aoki and Poulter.[27] The triflates were then refluxed with protected monolipids in the presence of the hindered base proton sponge, as reported by Thomson et al.,[28] to afford the protected dilipids **13**−**27**. The final step of the synthesis was the removal of the phthalimide protecting group. This was achieved using the Ing−Manske procedure,[29] whereby the phthalimide is refluxed in the presence of a large excess of hydrazine, to afford the free amine lipids **28**−**42**. The final products synthesized are tabulated in Table 1.

Table 1. Diether Lipids Synthesized[a]

	R				
R′	C_{12}	C_{14}	C_{16}	C_{18}	*cis*-oleyl
C_{12}	**28**			**37**	**40**
C_{14}	**29**	**31**			
C_{16}	**30**	**32**	**35**		**41**
C_{18}		**33**	**36**	**38**	
cis-oleyl		**34**		**39**	**42**

[a] R/R′ are shown in Figure 1.

Alternative Head Group Lipids. The lipids that performed best in transfection assays, lipids **28** (C_{12}/C_{12}) and **37** (C_{12}/C_{18}), were then selected for coupling to the cationic amino acids. The amino acids, with an activated C-terminus and a protected side chain and N-terminus, were purchased if available or synthesized and coupled to the amino groups of **28** and **37**. The protecting groups were then removed to yield the amino acid derivatives. Figure 2 shows this general synthetic route, with the compounds synthesized tabulated in Table 2.

An *N*-hydroxysuccinimide Lys ester with both amines protected by the *tert*-butyloxycarbonyl (Boc) group was commercially available, and this was used to couple directly to **28** and **37**. This afforded the protected Lys lipids, **43** and **44**, and the Boc groups were then removed with trifluoroacetic acid (TFA). The products are initially in the form of TFA salts, but the ammonia content

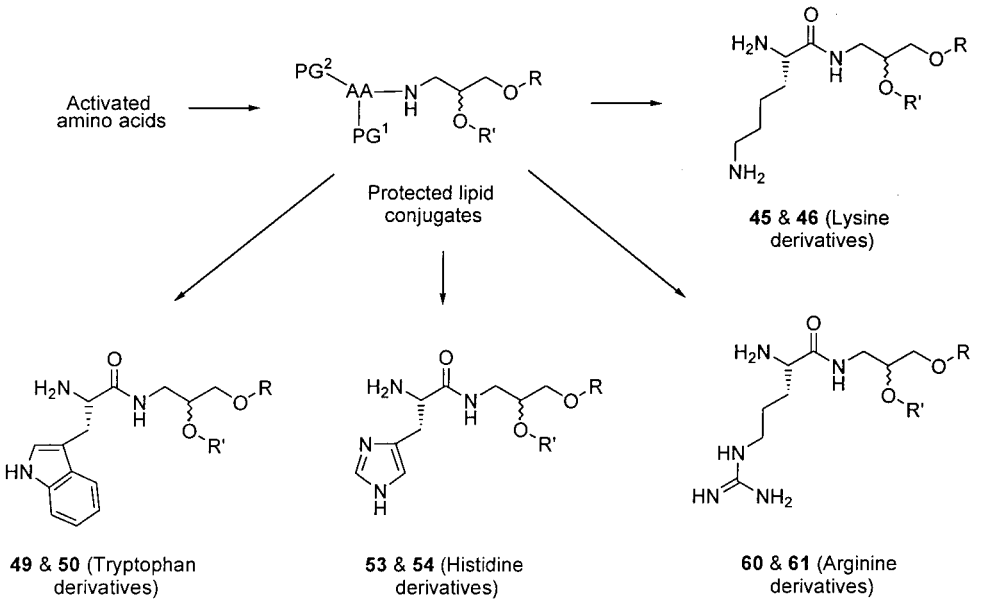

Figure 2. Scheme showing the general synthesis of the amino acid derivatives. AA = amino acid. R = C_{12} or C_{18}. R′ = C_{12}. PG = protecting group. Note: PG^1 is on the amino acid side chain and PG^2 on the N-terminus. Further information is displayed in Table 2.

Table 2. Cationic Amino Acid Derivatives of **28** and **37**

		substituents				
			protecting group		alkyl chain	
compound type	compound no.	amino acid (AA)	PG^1	PG^2	R	R′
protected lipid conjugates	**43**	Lys	Boc	Boc	C_{12}	C_{12}
	44	Lys	Boc	Boc	C_{18}	C_{12}
	47	Trp		Fmoc	C_{12}	C_{12}
	48	Trp		Fmoc	C_{18}	C_{12}
	52	His	Trt	Boc	C_{12}	C_{12}
	53	His	Trt	Boc	C_{18}	C_{12}
	56	Arg	Mtr	Fmoc	C_{12}	C_{12}
	57	Arg	Mtr	Fmoc	C_{18}	C_{12}
	58	Arg	Mtr		C_{12}	C_{12}
	59	Arg	Mtr		C_{18}	C_{12}
Lysine derivatives	**45**	Lys			C_{12}	C_{12}
	46	Lys			C_{18}	C_{12}
Tryptophan derivatives	**49**	Trp			C_{12}	C_{12}
	50	Trp			C_{18}	C_{12}
Histidine derivatives	**54**	His			C_{12}	C_{12}
	55	His			C_{18}	C_{12}
Arginine derivatives	**60**	Arg			C_{12}	C_{12}
	61	Arg			C_{18}	C_{12}

of column chromatography conditions drives the equilibrium to yield the free bases. This yielded the Lys lipids **45** and **46**.

The pentafluorophenyl (PFP) ester of Trp was commercially available, with the amine protected by the fluorenylmethoxycarbonyl (Fmoc) group. Protection of the indole side chain is unnecessary since the nucleophilicity of the NH group is significantly reduced by delocalization around the ring system. The activated ester was coupled to **28** and **37** by refluxing overnight in THF, to yield **47** and **48**. Fmoc removal was then carried out in a 20% solution of piperidine in dimethylformamide (DMF) to afford **49** and **50**.

A suitable activated histidine ester was not available, so the PFP ester **51** was prepared from the trityl/Boc-protected free acid.[30] 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimde hydrochloride (EDC) was used as the coupling agent since the urea byproduct is soluble in aqueous media and easily removed in the workup.[31] Two-dimensional thin-layer chromatography (TLC) showed **51** to be unstable, and it was used immediately to couple to **28** and **37** to yield **52** and **53**. Deprotection of the trityl and Boc groups with TFA afforded **54** and **55**. A percentage of triisopropylsilane (TIS) was included in the cleavage mixture. Highly stabilized cations such as those generated by the trityl group are not irreversibly removed from the solution by water or scavengers such as thiols, whereas trialkylsilanes are more effective.[32] Again, chromatography conditions give the products as the free bases.

A PFP ester of Arg was commercially available, although the NH_2 and side chain were orthogonally protected by Fmoc and 4-methoxy-2,3,6-trimethylbenzene-sulfonyl (Mtr) groups, respectively. Coupling of this compound to **28** and **37** yielded **56** and **57**. The Fmoc groups were removed first with piperidine/DMF to give

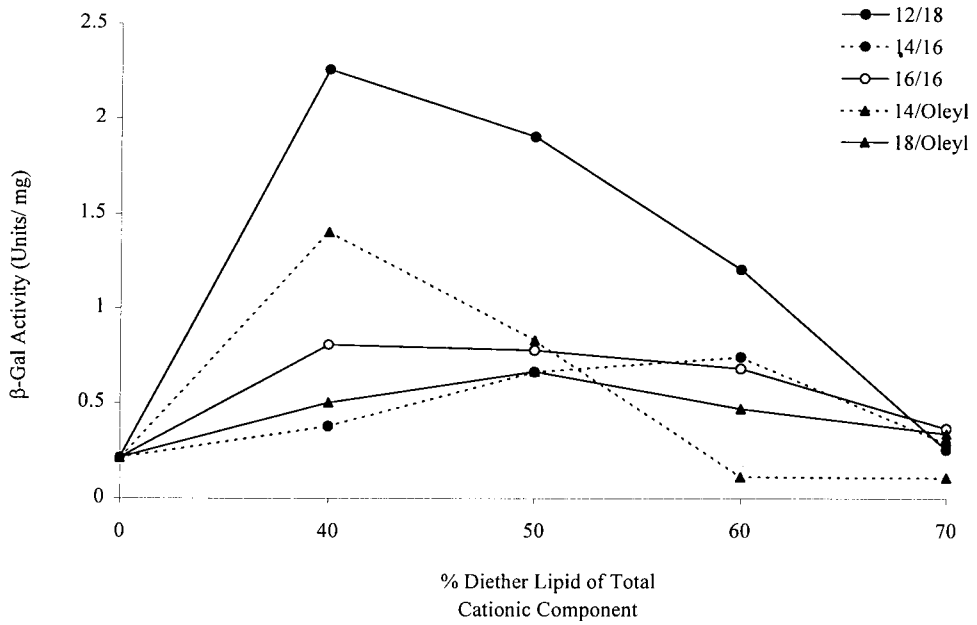

Figure 3. Graph showing the transfection efficiency of diether lipids at different formulation ratios with DC-Chol. DOPE concentration was constant for each formulation. For each point, the SEM was <10% of the mean value.

58 and **59** and, subsequently, the Mtr groups with TFA to give **60** and **61**. DC-Chol for use as a positive control was prepared following the method described by Gao and Huang.[13]

In Vitro Transfection Assay Results. All of the lipids synthesized were assayed in vitro for their ability to transfect cells with the pEFlacZ plasmid encoding the β-galactosidase (β-gal) enzyme. The V79 (Chinese hamster lung) cell line was used since it is robust, proliferates quickly, and is easy to transfect. The best lipids as determined from V79 assays were also tested in the HT29 human colon adenocarcinoma cell line. In addition to our interest in using a human cell line, we also wanted to assess the potency of the liposomes on a slower growing cell line, since it is known that transfection of such cells is more challenging. They were tested against positive control liposomes composed of DC-Chol and DOPE, since DC-Chol is a commercially available, well-established lipid that is easy and cheap to synthesize.[13]

Diether Lipids in the V79 Cell Line. The 15 diether lipids were each prepared as ternary liposome formulations with DC-Chol and DOPE at four ratios, as shown in Table 3. In each formulation, the DOPE concentration remained constant at 0.8 mM. Each formulation substituted an increasing amount of the diether lipid for DC-Chol, although the total cationic lipid concentration (diether lipid + DC-Chol) again remained constant at 1.2 mM. The controls are represented in Figures 3−6 by the points corresponding to 0 on the abscissa. Maximum transfection was shown to occur somewhere in this 40−70% interval of DC-Chol substitution (data not shown). The DC-Chol control formulation was used at a ± ratio of 5:1 when forming lipoplexes with plasmids and the other formulations at a ratio of 3:1, since these proved to be optimal (data not shown). The β-gal activity levels resulting from transfection with these 15 sets of formulations are shown in Figures 3−5. The experiments were performed four times, and the standard error of the mean (SEM) for each point was less than 10% of the mean value.

Table 3. Formulation Ratios of Diether Lipids Used in Transfection Experiments

	molar ratio of cationic lipid(s)	
liposome formulation	DC-Chol	diether lipid
control	1	0
1	0.6	0.4
2	0.5	0.5
3	0.4	0.6
4	0.3	0.7

Table 4. Formulation Ratios of Amino Acid Derivatives Used in Transfection Experiments

	molar ratio of cationic lipid(s)	
liposome formulation	DC-Chol	AA derivative
control	1	0
1	0.8	0.2
2	0.6	0.4
3	0.4	0.6
4	0.2	0.8
5	0	1

Alternative Head Group Lipids in the V79 Cell Line. The amino acid derivatives were assayed in a fashion similar to the diether lipids. The ratios of substitution were slightly different, as shown in Table 4.

Compounds **49** and **50** (Trp derivatives) aggregated when liposome formulation was attempted and so could not be assayed. Liposome formulations were found to demonstrate maximum transfection efficiency when mixed in a ± ratio of 5:1 with the plasmid DNA (data not shown). The experiment was performed four times, and resulting β-gal activity is shown in Figure 6. SEM values were all less than 10% of the value of the mean.

Transfection Assays in the HT29 Cell Line. Of the four formulations prepared, the first three contained either DC-Chol, **45**, or **60** in a 3:2 molar ratio with DOPE. The fourth contained a 50:50 mixture of **28** and DC-Chol as the cationic component, at an equivalent ratio with DOPE. The HT29 cells were cultured and transfected in an identical fashion to the V79 cells. The

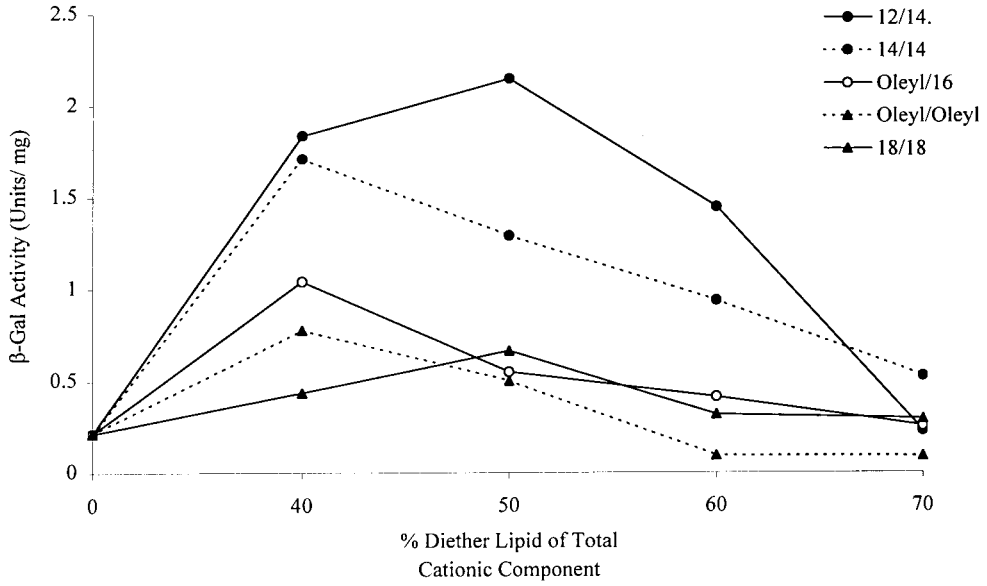

Figure 4. Graph showing the transfection efficiency of diether lipids at different formulation ratios with DC-Chol. DOPE concentration was constant for each formulation. For each point, the SEM was <10% of the mean value.

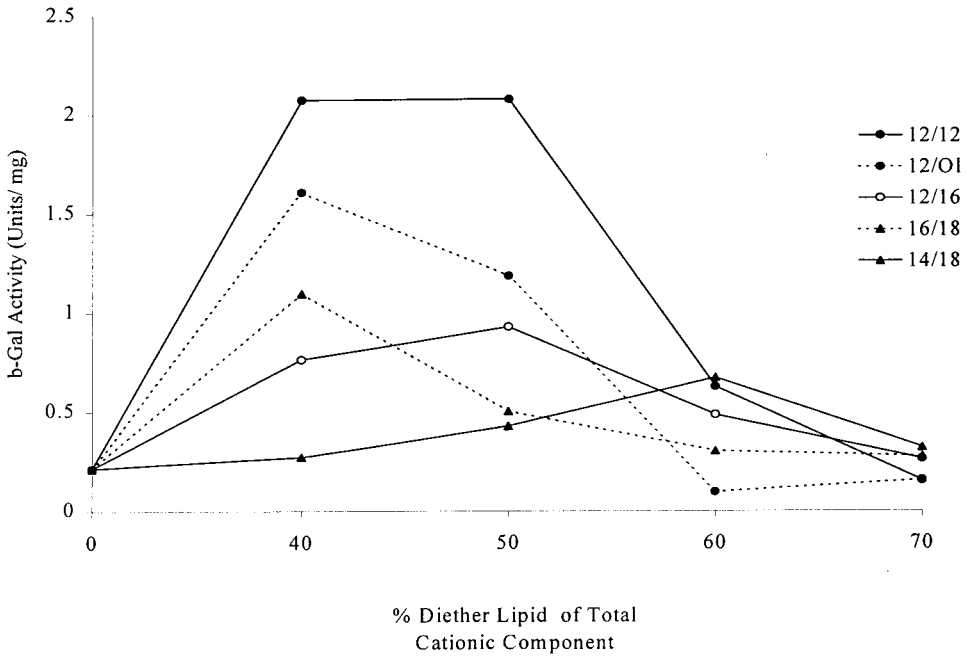

Figure 5. Graph showing the transfection efficiency of diether lipids at different formulation ratios with DC-Chol. DOPE concentration was constant for each formulation. For each point, the SEM was <10% of the mean value.

four liposome formulations were mixed at different ratios with pEFlacZ DNA, and β-gal expression was determined 18 h after transfection. The experiment was performed three times, and the results are displayed in Figure 7. SEM values were all less than 10% of the value of the mean.

It is observed that these three novel formulations are significantly better than DC-Chol. One notable property of the formulations is that the optimal DNA mixing ratio is different from the corresponding experiment when carried out on the V79 cells, as shown in Table 5.

Cytotoxicity Studies. A representative selection of formulations was assessed for cytotoxicity. The experiment was performed in quadruplicate, and the IC_{50} values are displayed in Table 6. Each formulation contained DOPE in a 2:3 molar ratio with the respective cationic lipid(s). The results show that the formulations containing **28**, **37**, and **38** are significantly less cytotoxic than the control, while that containing **45** is similarly cytotoxic and **60** slightly more so.

Discussion

This diether series of lipids focuses on the influence of the hydrophobic domain, with variation to both the carbon chain length and the degree of asymmetry. There are few extensive reports in the literature focusing on variation of alkyl chain length and still fewer with asymmetric lipids. Byk et al. synthesized a series of four lipids with double alkyl chain lengths of 12, 13, 14, and 18 carbon atoms.[23] They reported that the lipid with two C_{18} chains was the most efficient transfecting agent, when assayed in vitro on both HeLa and NIH3T3 cell lines. Balasubramaniam et al. synthesized a series that

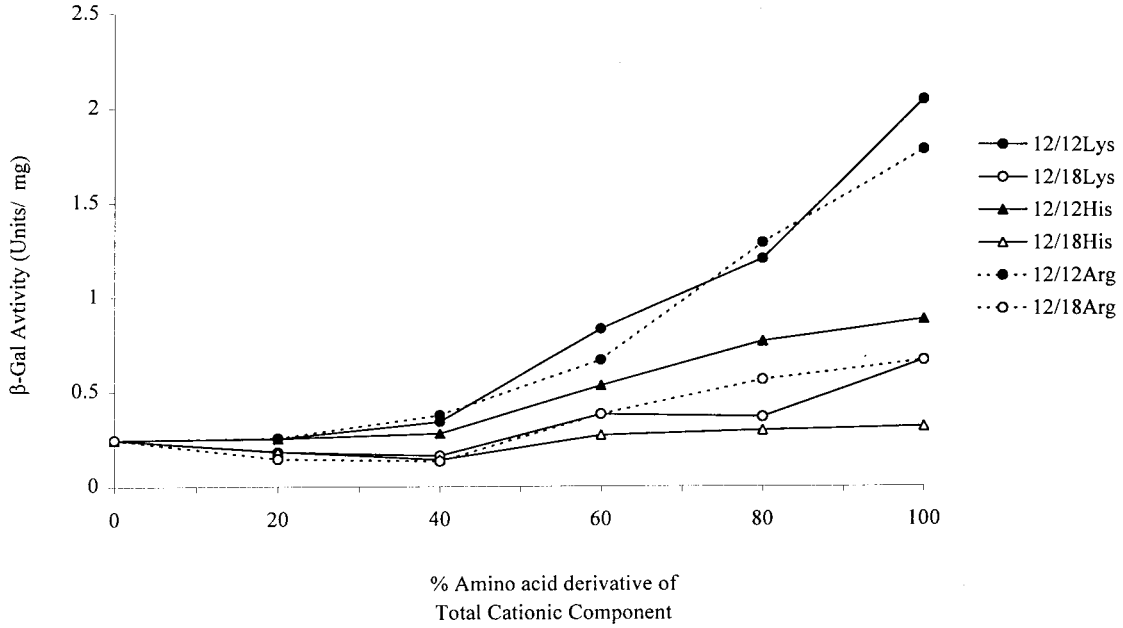

Figure 6. Graph showing the transfection efficiency of the amino acid derivatives at different formulation ratios with DC-Chol. DOPE concentration was constant for each formulation. For each point, the SEM was <10% of the mean value.

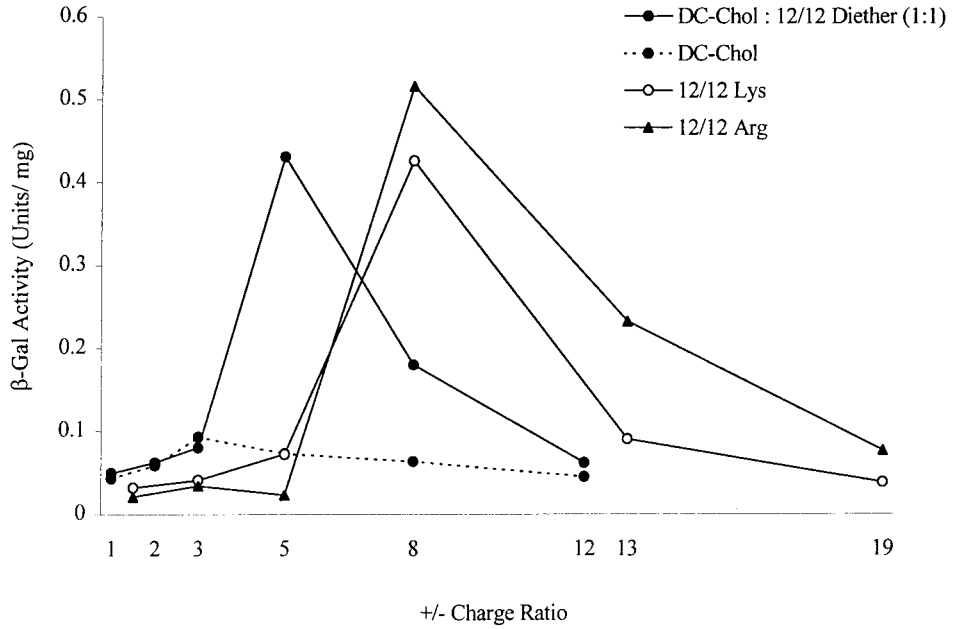

Figure 7. Graph showing the transfection efficiency of the formulations containing compounds **28**, **45**, and **60** as compared with the DC-Chol:DOPE control, at different ± charge ratio (liposome/DNA ratio). For each point, the SEM was <10% of the mean value.

Table 5. Comparison of ± Charge Ratio Required for Optimum Transfection for Cell Lines V79 vs HT29

cationic lipid component	peak ± ratio in V79 cells	peak ± ratio in HT29 cells
DC-Chol	5:1	3:1
DC-Chol:**33** (1:1)	3:1	5:1
12/12 Lys	5:1	8:1
12/12 Arg	5:1	8:1

included some asymmetric lipids.[21] The in vitro activities of asymmetric lipids were usually superior to the best symmetric analogues, the degree of improvement depending on the cell line. Other groups have reported homologous series where the highest in vitro transfection efficiency was obtained with lipids incorporating the shorter C_{14} chain.[18,24]

Table 6. IC_{50} Values of Some Representative Lipid Formulation

cationic lipid component	IC_{50} (μM) ± SEM
DC-Chol (control)	38 ± 10
28 (12/12 diether):DC-Chol (1:1)	75 ± 15
37 (12/18 diether):DC-Chol (1:1)	76 ± 19
38 (18/18 diether):DC-Chol (1:1)	67 ± 14
45 (12/12 Lys)	34 ± 12
60 (12/12 Arg)	20 ± 7

The results of the transfection assays indicate that rather than a formulation containing a single cationic lipid (either DC-Chol or diether lipid) as the most efficacious, ternary formulations containing both types of cationic lipid as well as DOPE are best. These formulations give transfection up to a 10-fold higher

than the DC-Chol/DOPE control. A possible reason for this interesting observation is that DC-Chol is based on cholesterol, a large, rigid tetracyclic molecule. It thus forms relatively strong intermolecular bonds, due to van der Waals forces, resulting in low bilayer fluidity. Research reported by Zabner et al. has demonstrated that lipoplexes are taken into cells via endocytosis and become entrapped in an endosome.[33] The bilayer of the lipoplex must break up for fusogenic events with the endosomal bilayer to take place, subsequently releasing the DNA into the cytoplasm before it is degraded. Strong, less fluid lipoplex bilayers may be inhibited from disintegrating quickly enough when inside the endosome, which in turn would result in less plasmid escaping enzymatic degradation. The diethers **28**−**42** have a much more flexible structure than DC-Chol, and including a proportion of them in the formulation would result in the bilayers also being less rigid. This could allow them, and thus the lipoplex, to fragment more easily, resulting in more plasmid escaping. Conversely, including too much of the flexible diether lipid could make the bilayer too unstable. This might render it susceptible to breakdown before it was required to do so, i.e., before it was inside the endosome. The observation of an optimal transfection efficiency somewhere between 100% DC-Chol and 100% diether lipid could, then, be explained by the required balance between rigidity and fluidity in the bilayer.

The second observation of note is that the shorter lipids such as 12/12 (**28**), 12/14 (**29**), and 14/14 (**31**) perform far better than the longer ones (18/18 (**38**), 18/oleyl (**39**), oleyl/oleyl (**39**)). Peak transfection levels with formulations containing the longer lipids represent a 3- or 4-fold improvement over the DC-Chol, as compared to up to 10-fold for the shorter lipids. This is in contrast to the findings of Byk et al.[23] Other groups, however, have found that shorter alkyl chain lengths lead to better in vitro transfection efficiency and hypothesized that this was due to the shorter chain facilitating more efficient intermembrane mixing.[18,21] Floch et al. found that the C_{14} lipid worked best in a series of symmetric dialkyl lipids ranging in length from C_{12} to C_{18} and C_{oleyl}.[24] Shorter lipids would also be expected to result in more fluid bilayers, and this may lend further substance to the hypothesis of the balance between rigidity and fluidity in the bilayer.

We also observed that the more asymmetric lipids (e.g., 12/18 (**37**) and 12/Oleyl (**40**)) performed relatively well. Balasubramaniam et al., who included four asymmetric lipids in their series, reported similar findings with their lipids.[21] They hypothesized that asymmetric lipids have a more fusogenic character, leading to their increased transfection efficacy. Another possibility is that the structure of the more asymmetric lipids allows a greater overlap of the alkyl chains within the lipid bilayer. This would give rise to greater van der Waals forces being created between them, meaning bilayers containing asymmetric lipids are subtly different in nature from those containing only symmetric lipids (see Figure 8). Either way, optimal transfection for **37** and **40** represents a 10- and 7-fold improvement over the DC-Chol/DOPE control, respectively.

For coupling to the amino acids, two of the most effective diether lipids were chosen—the short C_{12}/C_{12} lipid (**28**) and the particularly asymmetric C_{12}/C_{18} lipid (**37**). This generated eight compounds, although the Trp derivatives aggregated on formulation and would not form liposomes. Consequently, they could not be assayed. The transfection efficiencies obtained using these lipids are displayed in Figure 6. Two trends are observed. The first is that unlike the diethers, none of these amino acid compounds reach maximum transfection between 40 and 70% substitution. Instead, they carry on improving up to 100% substitution. Those containing **45** and **60** were particularly good and proved to be as efficient as the better diether lipids at optimal ratios, exhibiting approximately a 9- or 10-fold improvement over the DC-Chol/DOPE control. The increase using **54** was not so pronounced but still resulted in a 3-fold improvement over the control. The second observation is that the three compounds based on the 12/12 diether lipid **28** (compounds **45**, **54**, and **60**) are all more effective than their 12/18 analogues (compounds **46**, **55**, and **61**), at all comparative ratios of substitution. It is of interest that the most effective (**45** and **60**) of this series of compounds have headgroups similar in structure to some of the superior lipids previously reported.[6,12] Lipids such as GAP-DLRIE and CTAP have more flexible, multivalent headgroups with charges separated by several carbon units. This may be related to DNA-condensing properties, since spermidine and spermine (natural DNA-condensing agents) have similar motifs in their structure.

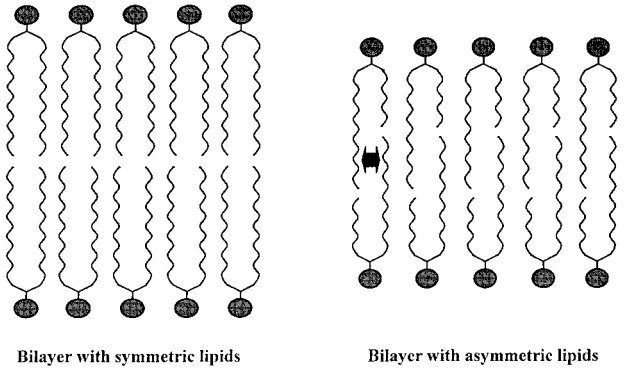

Figure 8. Schematic diagram showing how symmetric and asymmetric lipids may align within the bilayer. The structure of the asymmetric lipids could allow for much more overlap between molecules on opposite sides of the bilayer (shown by arrow).

Transfection results in the HT29 cell line confirmed the finding in V79 cells, in that the formulations containing the 12/12 diether, lysine-modified, and arginine-modified C12/12 achieve a maximum transfection efficiency 5−6-fold higher than the DC-Chol:DOPE control. The optimal liposome:DNA mixing ratio is different from the corresponding experiment when carried out on the V79 cells, demonstrating the need to carry out optimization experiments on both lipoplex and lipid formulations when changing cell lines. Another important observation is the significantly lower level of transfection in comparison with the V79 experiments, which can be attributed to the slower growth rate of HT29 cells.

Conclusions

In summary, two novel homologous series of lipids have been synthesized. The cationic headgroup and

hydrophobic domain have each been varied, so that the effect on transfection efficiency could be studied. This has led to an efficient route for the synthesis of asymmetric lipids and the production of an extensive range of candidates as nonviral vectors. The lipids that were prepared have been extensively tested, and several formulations have been shown to transfect different cell lines with up to 10 times the efficacy of the commonly used DC-Chol/DOPE formulation. The cytotoxicity assay shows that the IC_{50} values are at least comparable to the DC-Chol/DOPE control. The biological activity of the best diether lipids (**28** and **37**) and lipids with modified headgroup (**45** and **60**) in the optimal formulations determined in this study will be investigated further.

Experimental Section

Materials and Methods. All starting materials and reagents were purchased from Sigma-Aldrich Co. Ltd. unless otherwise stated. GPR solvents were purchased from BDH, apart from hexane (high-performance liquid chromatography grade), which was purchased from Laserchrom. TLC was carried out on aluminum sheets precoated with Kieselgel 60 F_{254} (Merck) and developed with either UV light or an ammonium molybdate dip as appropriate.

All compounds were characterized by ^{1}H NMR, high-resolution accurate mass spectrometry, and elemental combustion analysis. Where elemental analysis could not confirm product to an accuracy of within 0.4%, ^{13}C NMR was used to further substantiate characterization. ^{1}H NMR and ^{13}C NMR spectra were performed in $CDCl_3$ on a Bruker Avance 250 spectrometer (250 MHz). Melting points were determined on a Kofler hot-stage (Reichert-Thermovar) and are uncorrected. Elemental combustion analyses were carried out by C. H. N. Analysis Ltd., (Leicester, UK). It should be noted that numbering on diagrams refers to NMR characterization only. Accurate mass measurements were performed on a VG instruments ZAB-SE double focusing magnetic sector mass spectrometer at a resolution of 10 000.

1. Preparation of *N*-(2,3-Propenyl)phthalimide (1). Potassium phthalimide (2.59 g, 15 mmol) was added to a round-bottomed flask containing anhydrous DMF (100 mL). After dissolution, the contents were degassed under vacuum. The solution was then flushed with nitrogen, and allyl bromide (1.21 g, 0.86 mL, 10 mmol) was added. The reaction was stirred at 50 °C overnight. The majority of the DMF was then evaporated in vacuo, and CH_2Cl_2 (50 mL) was added to the remaining yellow liquid. The solution was then washed with water (2 × 50 mL) and brine (50 mL), dried over $MgSO_4$, and evaporated under reduced pressure. This yielded pale yellow crystals that were further purified by flash column chromatography (0−50% CH_2Cl_2−hexane) to give **1** as a white solid (1.50 g, 80.2%); R_f 0.5 (CH_2Cl_2); mp 66−67 °C. ^{1}H NMR (δ_H): 7.88 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.6 Hz, J_{meta} = 2.9 Hz), 7.74 (dd, 2H, $H_{arom4,5}$), 5.96−5.85 (m, 1H, NCH_2CH), 5.31−5.18 (m, 2H, $CH{=}CH_2$), 4.33−4.29 (m, 2H, NCH_2). ^{13}C NMR (δ_C): 167.72 (*C*ON*C*O), 133.83 ($C_{arom4,5}$), 131.98 ($C_{arom1,2}$), 131.44 (*C*H=CH$_2$), 123.14 ($C_{arom3,6}$), 117.58 (CH=*C*H$_2$), 39.89 (N*C*H$_2$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{11}H_{10}NO_2$, 188.0715; found, 188.0712.

2. Preparation of *N*-(2,3-Epoxypropyl)phthalimide (2). A solution of **1** (187 mg, 1 mmol) in CH_2Cl_2 (2 mL) was prepared under argon. In a separate flask, a solution of *m*-chloroperbenzoic acid (57−86%, 602 mg, 2 mmol minimum) in CH_2Cl_2 (3 mL) was prepared, also under argon, and then added to the solution of **1**. The solution was stirred for 3 days and was then evaporated under reduced pressure. The product, a white solid, was redissolved in THF (10 mL), and a solution of 4% sodium dithionite (6 mL) was added to reduce excess peracid to *m*-chlorobenzoic acid. The solution was stirred for 20 min, and then, EtOAc (50 mL) was added. It was then washed with water (40 mL), saturated aqueous $NaHCO_3$ (2 × 40 mL), water again (40 mL), and brine (40 mL) and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. This yielded **2** as a white solid (186 mg, 92%); R_f 0.4 (CH_2Cl_2); mp 98−100 °C. ^{1}H NMR (δ_H): 7.85 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.6 Hz, J_{meta} = 2.9 Hz), 7.72 (dd, 2H, $H_{arom4,5}$), 3.86 (ABX, 2H, $NCH_{2(AandB)}$, J_{AB} = 14.2 Hz, J_{AX} = 5.1 Hz, J_{BX} = 5.6 Hz), 3.30−3.20 (m, 1H, NCH_2CH), 3.84−2.65 (m, 2H, CH_2O). HRMS: (M + H)$^{\oplus}$ calcd for $C_{11}H_{10}NO_3$, 204.0661; found, 204.0663. Anal. Calcd for $C_{11}H_9NO_3$: C, H, N.

3. Preparation of *N*-(2-Hydroxy-3-lauryloxypropyl)-phthalimide (3). To a flask under argon containing **2** (20.1 g, 100 mmol) and lauryl alcohol (27.9 g, 150 mmol) was added CH_2Cl_2 (50 mL). After complete dissolution, boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) (1.42 g, 1.23 mL, 10 mmol) was added dropwise at room temperature with stirring. Almost immediately, opalescence was observed and the reaction was then stirred at 40 °C for 18 h. The mixture was then diluted with CH_2Cl_2 to a final volume of 300 mL and washed with saturated aqueous $NaHCO_3$ (150 mL), water (150 mL), and brine (150 mL), and the solvent was evaporated under reduced pressure to give a colorless oil. Flash column chromatography needed to be carried out twice, once with hexane/EtOAc (1:9) to remove excess lauryl alcohol and then with CH_2Cl_2 to remove unreacted epoxide. This yielded **3** as a white solid (27.60 g, 72.1%); R_f 0.35 (30% EtOAc−70% hexane); mp 72−74 °C. ^{1}H NMR (δ_H): 7.87 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.4 Hz, J_{meta} = 3.0 Hz), 7.73 (dd, 2H, $H_{arom4,5}$), 4.14−3.98 (m, 1H, C*H*OH), 3.85 (ABX, 2H, $NCH_{2(AandB)}$, J_{AB} = 14.0 Hz, J_{AX} = 7.3 Hz, J_{BX} = 4.4 Hz), 3.58−3.37 (m, 4H, OCH_2), 2.69 (d, 1H, O*H*, J = 6.2 Hz), 1.63−1.45 (m, 4H, OCH_2CH_2), 1.37−1.17 (m, 18H, $CH_{2(lauryl)}$), 0.88 (t, 3H, CH_3, J = 6.6 Hz). HRMS: (M + H)$^{\oplus}$ calcd for $C_{23}H_{36}NO_4$, 390.2644; found, 390.2620. Anal. Calcd for $C_{23}H_{35}NO_4$: C, H, N.

4. Preparation of *N*-(2-Hydroxy-3-myristyloxypropyl)-phthalimide (4). Compound **4** was prepared analogously to **3** on a 100 mmol scale to give after 2 step chromatography (hexane/EtOAc (1:9), CH_2Cl_2) **4** as a white solid (29.05 g, 70.7%); R_f 0.35 (30% EtOAc−70% hexane); mp 78−79 °C. ^{1}H NMR (δ_H): 7.87 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.4 Hz, J_{meta} = 3.0 Hz), 7.73 (dd, 2H, $H_{arom4,5}$), 4.15−3.98 (m, 1H, C*H*OH), 3.86 (ABX, 2H, $NCH_{2(AandB)}$, J_{AB} = 14.0 Hz, J_{AX} = 7.3 Hz, J_{BX} = 4.4 Hz), 3.58−3.36 (m, 4H, OCH_2), 2.69 (d, 1H, O*H*, J = 6.1 Hz), 1.62−1.44 (m, 4H, OCH_2CH_2), 1.37−1.13 (m, 22H, $CH_{2(myristyl)}$), 0.88 (t, 3H, CH_3, J = 6.6 Hz). HRMS: (M + H)$^{\oplus}$ calcd for $C_{25}H_{40}NO_4$, 418.2957; found, 418.2940. Anal. Calcd for $C_{25}H_{39}NO_4$: C, H, N.

5. Preparation of *N*-(2-Hydroxy-3-palmityloxypropyl)-phthalimide (5). Compound **5** was prepared analogously to **3** on a 50 mmol scale to give on purification the product **5** as a white solid (16.26 g, 74.1%); R_f 0.35 (30% EtOAc−70% hexane); mp 87−88 °C. ^{1}H NMR (δ_H): 7.87 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.5 Hz, J_{meta} = 3.0 Hz), 7.73 (dd, 2H, $H_{arom4,5}$), 4.14−3.99 (m, 1H, C*H*OH), 3.85 (ABX, 2H, $NCH_{2(AandB)}$, J_{AB} = 14.0 Hz, J_{AX} = 7.3 Hz, J_{BX} = 4.4 Hz), 3.59−3.39 (m, 4H, OCH_2), 2.70 (d, 1H, O*H*, J = 6.2 Hz), 1.62−1.45 (m, 4H, OCH_2CH_2), 1.37−1.12 (m, 26H, $CH_{2(palmityl)}$), 0.88 (t, 3H, CH_3, J = 6.6 Hz). HRMS: (M + H)$^{\oplus}$ calcd for $C_{27}H_{44}NO_4$, 446.3270; found, 446.3255. Anal. Calcd for $C_{27}H_{43}NO_4$: C, H, N.

6. Preparation of *N*-(2-Hydroxy-3-stearyloxypropyl)-phthalimide (6). Compound **6** was prepared analogously to **3** on a 50 mmol scale to give after chromatography (hexane/EtOAc (1:9), CH_2Cl_2, **6** as a white solid (17.59 g, 75.2%); R_f 0.35 (30% EtOAc−70% hexane); mp 88−89 °C. ^{1}H NMR (δ_H): 7.86 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.4 Hz, J_{meta} = 3.0 Hz), 7.73 (dd, 2H, $H_{arom4,5}$), 4.13−3.99 (m, 1H, C*H*OH), 3.85 (ABX, 2H, $NCH_{2(AandB)}$, J_{AB} = 14.1 Hz, J_{AX} = 7.3 Hz, J_{BX} = 4.4 Hz), 3.59−3.40 (m, 4H, OCH_2), 2.74 (d, 1H, O*H*, J = 6.0 Hz), 1.62−1.46 (m, 4H, OCH_2CH_2), 1.37−1.16 (m, 30H, $CH_{2(stearyl)}$), 0.88 (t, 3H, CH_3, J = 6.5 Hz). HRMS: (M + Na)$^{\oplus}$ calcd for $C_{29}H_{47}NO_4Na$, 496.3403; found, 496.3420. Anal. Calcd for $C_{29}H_{47}NO_4$: C, H, N.

7. Preparation of *N*-(2-Hydroxy-3-oleyloxypropyl)-phthalimide (7). Compound **7** was prepared analogously to **3** on a 100 mmol scale. Following purification by chromatography (hexane/EtOAc (1:9), the compound **7** was yielded as a colorless low melting point solid (31.20 g, 67%); R_f 0.35 (30%

EtOAc−70% hexane); mp ∼25 °C. ^{1}H NMR (δ_H): 7.83 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.6 Hz, J_{meta} = 3.1 Hz), 7.73 (dd, 2H, $H_{arom4,5}$), 5.41−5.24 (m, 2H, C*H*=C*H*), 4.13−3.98 (m, 1H, C*H*OH), 3.82 (ABX, 2H, NC$H_{2(AandB)}$, J_{AB} = 14.0 Hz, J_{AX} = 7.3 Hz, J_{BX} = 4.5 Hz), 3.55−3.35 (m, 4H, OCH_2), 2.89 (d, 1H, O*H*, J = 6.0 Hz), 2.07−1.91 (m, 4H, CH_2CH=CHCH_2), 1.60−1.45 (m, 2H, OCH$_2$CH_2), 1.41−1.18 (m, 30H, C$H_{2(oleyl)}$), 0.86 (t, 3H, CH_3, J = 6.5 Hz). HRMS: (M + H)$^\oplus$ calcd for $C_{29}H_{45}NO_4$, 472.3427; found, 472.3400. Anal. Calcd for $C_{29}H_{47}NO_4$: C, H, N.

8. Preparation of Lauryl Triflate (8). To an ice-cooled flask containing dried, distilled CH_2Cl_2 (100 mL) under argon was added trifluoromethanesulfonic anhydride (18.33 g, 10.94 mL, 65 mmol), followed by anhydrous pyridine (5.14 g, 5.26 mL, 65 mmol). Fuming was observed, and a white precipitate formed. The cooling bath was removed, and a solution of lauryl alcohol (9.32 g, 50 mmol) in distilled CH_2Cl_2 (40 mL) was added dropwise over a period of 10 min with stirring. The solution was stirred for 2 h at room temperature, and then water (20 mL) was added to quench the reaction. CH_2Cl_2 (200 mL) was added, and the solution was washed twice with water (2 × 100 mL). The aqueous layers were backwashed with CH_2Cl_2 (20 mL), and the organic layers were combined, washed with brine (100 mL), dried over $MgSO_4$, and evaporated under reduced pressure to yield a pale brown oil. The oil was dissolved in hexane (20 mL) and loaded onto a 1 in. bed of silica. The product was eluted with hexane and diethyl ether, taking care not to coelute the more polar, colored byproducts. The hexane was removed under reduced pressure to yield **8** as a colorless oil (15.26 g, 96.0%); R_f 0.7 (hexane/ether 1:1). ^{1}H NMR (δ_H): 4.56 (t, 2H, OCH_2, J = 6.5 Hz), 1.91−1.79 (m, 2H, OCH$_2$CH_2), 1.53−1.20 (m, 18H, C$H_{2(lauryl)}$), 0.91 (t, 3H, CH_3, J = 6.6 Hz). Instability of the product prevented further characterization.

9. Preparation of Myristyl Triflate (9). This was prepared analogously to **8** on a 50 mmol scale. Compound **9** was yielded as a colorless oil (15.53 g, 90%); R_f 0.7 (hexane/ether 1:1). ^{1}H NMR (δ_H): 4.54 (t, 2H, OCH_2, J = 6.5 Hz), 1.89−1.77 (m, 2H, OCH$_2$CH_2), 1.50−1.20 (m, 22H, C$H_{2(myristyl)}$), 0.89 (t, 3H, CH_3, J = 6.5 Hz).

10. Preparation of Palmityl Triflate (10). This was prepared analogously to **8** on a 75 mmol scale. Compound **10** was yielded as a white, waxy solid (20.95 g, 81.4%); R_f 0.7 (hexane/ether 1:1). ^{1}H NMR (δ_H): 4.55 (t, 2H, OCH_2, J = 6.5 Hz), 1.90−1.69 (m, 2H, OCH$_2$CH_2), 1.54−1.12 (m, 26H, C$H_{2(palmityl)}$), 0.89 (t, 3H, CH_3, J = 6.5 Hz).

11. Preparation of Stearyl Triflate (11). This was prepared in an almost identical way to **13** on a 100 mmol scale. Because stearyl alcohol is particularly unreactive, slightly larger equivalents of trifluoromethanesulfonic anhydride and pyridine were used (125 mmol). Also, because of its lower solubility, the stearyl alcohol was added in solid form under an argon blanket. Compound **11** was yielded as a waxy, white solid (29.45 g, 73.6%); R_f 0.7 (hexane/ether 1:1). ^{1}H NMR (δ_H): 4.55 (t, 2H, OCH_2, J = 6.5 Hz), 1.91−1.68 (m, 2H, H$_2$), 1.5−1.1 (m, 30H, C$H_{2(stearyl)}$), 0.87 (t, 3H, CH_3, J = 6.5 Hz).

12. Preparation of Oleyl Triflate (12). Compound **12** was prepared analogously to **8** on a 77 mmol scale. Oleyl alcohol was first distilled under vacuum (bp 182−184 °C/1.5 mm). Compound **12** was yielded as a colorless liquid (22.20 g, 72.1%); R_f 0.7 (hexane/ether 1:1). ^{1}H NMR (δ_H): 5.42−5.33 (m, 2H, C*H*=C*H*), 4.54 (t, 2H, OCH_2, J = 6.5 Hz), 2.13−1.93 (m, 4H, CH_2CH=CHCH_2), 1.90−1.69 (m, 2H, OCH$_2$CH_2), 1.54−1.12 (m, 26H, C$H_{2(oleyl)}$), 0.89 (t, 3H, CH_3, J = 6.5 Hz).

13. Preparation of *N*-(2,3-Dilauryloxypropyl)phthalimide (13). To a mixture of **3** (3.40 g, 8.7 mmol), **8** (5.00 g, 15.7 mmol) and 1,8-bis(dimethylamino)naphthalene (proton sponge −3.36 g, 15.7 mmol) under argon was added anhydrous distilled CH_2Cl_2 (30 mL). The yellow solution was refluxed under argon for 78 h. TLC (50% ether, 50% hexane) suggested that some starting materials were still present, but no further reaction was taking place. During this time, the reaction had turned dark orange/brown, and a precipitate had formed. CH_2Cl_2 was then removed under reduced pressure to give a dark orange oil, and hexane was added (100 mL). The mixture was sonicated to ensure dissolution of product and filtered through a 1 in. bed of Celite 521, and the filtrate was evaporated under reduced pressure to yield an orange oil. Flash column chromatography (1−5% ether/hexane) gave **13** as a colorless solid (3.79 g, 77.9%); R_f 0.5 (hexane/ether 1:1); mp 38−39 °C. ^{1}H NMR (δ_H): 7.84 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.5 Hz, J_{meta} = 3.3 Hz), 7.70 (dd, 2H, $H_{arom4,5}$), 3.90−3.70 (m, 3H, NCH_2C*H*), 3.65−3.30 (m, 6H, OCH_2), 1.60−1.36 (m, 4H, OCH$_2$CH_2), 1.35−1.05 (m, 36H, C$H_{2(lauryl)}$), 0.89 (t, 6H, CH_3, J = 6.3 Hz). HRMS: (M + H)$^\oplus$ calcd for $C_{35}H_{60}NO_4$, 558.4522; found, 558.4541. Anal. Calcd for $C_{35}H_{59}NO_4$: C, H, N.

14. Preparation of *N*-(2-Myristyloxy-3-lauryloxypropyl)phthalimide (14). Compound **14** was prepared analogously to **13** on a 5 mmol scale to give after chromatography (1−5% ether/hexane) **14** as colorless solid (2.10 g, 71.7%); R_f (hexane/ether 1:1); mp 39−40 °C. ^{1}H NMR (δ_H): 7.85 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.4 Hz, J_{meta} = 3.1 Hz), 7.73 (dd, 2H, $H_{arom4,5}$), 3.90−3.70 (m, 3H, NCH_2C*H*), 3.65−3.35 (m, 6H, OCH_2), 1.55−1.36 (m, 4H, OCH$_2$CH_2), 1.35−1.10 (m, 40H, C$H_{2(lauryl/myristyl)}$), 0.89 (t, 6H, CH_3, J = 6.2 Hz). HRMS: (M + H)$^\oplus$ calcd for $C_{37}H_{64}NO_4$, 586.4835; found, 586.4811. Anal. Calcd for $C_{37}H_{63}NO_4$: C, H, N.

15. Preparation of *N*-(2-Palmityloxy-3-lauryloxypropyl)phthalimide (15). Compound **15** was prepared analogously to **13** on a 6 mmol scale to give after chromatography (1−5% ether/hexane) **15** as a colorless solid (2.65 g, 72.0%); R_f 0.5 (hexane/ether 1:1); mp 46−47 °C. ^{1}H NMR (δ_H): 7.86 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.5 Hz, J_{meta} = 3.2 Hz), 7.73 (dd, 2H, $H_{arom4,5}$), 3.93−3.73 (m, 3H, NCH_2C*H*), 3.67−3.35 (m, 6H, OCH_2), 1.60−1.37 (m, 4H, OCH$_2$CH_2), 1.36−1.10 (m, 44H, C$H_{2(lauryl/palmityl)}$), 0.89 (t, 6H, CH_3, J = 6.5 Hz). HRMS: (M + H)$^\oplus$ calcd for $C_{39}H_{68}NO_4$, 614.5148; found, 614.5192. Anal. Calcd for $C_{39}H_{67}NO_4$: C, H, N.

16. Preparation of *N*-(2,3-Dimyristyloxypropyl)phthalimide (16). Compound **16** was prepared analogously to **13** on a 21 mmol scale to give after chromatography (1−5% ether/hexane), **16** as a colorless solid (9.87 g, 76.6%); R_f 0.5 (hexane/ether 1:1); mp 47−48 °C. ^{1}H NMR (δ_H): 7.85 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.5 Hz, J_{meta} = 3.3 Hz), 7.73 (dd, 2H, $H_{arom4,5}$), 3.95−3.65 (m, 3H, NCH_2C*H*), 3.65−3.35 (m, 6H, OCH_2), 1.60−1.37 (m, 4H OCH$_2$CH_2), 1.36−1.05 (m, 44H, C$H_{2(myristyl)}$), 0.88 (t, 6H, CH_3, J = 6.5 Hz). HRMS: (M + H)$^\oplus$ calcd for $C_{39}H_{68}NO_4$, 614.5148; found, 614.5136. Anal. Calcd for $C_{39}H_{67}NO_4$: C, H, N.

17. Preparation of *N*-(2-Palmityloxy-3-myristyloxypropyl)phthalimide (17). Compound **17** was prepared analogously to **13** on a 6 mmol scale to give after chromatography (1−5% ether/hexane) **17** as a colorless solid (2.97 g, 77.2%); R_f 0.5 (hexane/ether 1:1); mp 50 °C. ^{1}H NMR (δ_H): 7.85 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.6 Hz, J_{meta} = 3.1 Hz), 7.71 (dd, 2H, $H_{arom4,5}$), 3.95−3.65 (m, 3H, NCH_2C*H*), 3.65−3.35 (m, 6H, OCH_2), 1.60−1.37 (m, 4H, OCH$_2$CH_2), 1.36−1.05 (m, 48H, C$H_{2(myristyl/palmityl)}$), 0.88 (t, 6H, CH_3, J = 6.5 Hz). HRMS: (M + H)$^\oplus$ calcd for $C_{41}H_{72}NO_4$, 642.5461; found, 642.5434. Anal. Calcd for $C_{41}H_{71}NO_4$: C, H, N.

18. Preparation of *N*-(2-Stearyloxy-3-myristyloxypropyl)phthalimide (18). Compound **18** was prepared analogously to **13** on a 4 mmol scale to give after chromatography (1−5% ether/hexane) **18** as a colorless solid (1.80 g, 67.0%); R_f 0.5 (hexane/ether 1:1); mp 54−55 °C. ^{1}H NMR (δ_H): 7.85 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.6 Hz, J_{meta} = 3.1 Hz), 7.71 (dd, 2H, $H_{arom4,5}$), 3.95−3.70 (m, 3H, NCH_2C*H*), 3.70−3.35 (m, 6H, OCH_2), 1.60−1.36 (m, 4H, OCH$_2$CH_2), 1.35−1.08 (m, 52H, C$H_{2(myristyl/stearyl)}$), 0.88 (t, 6H, CH_3, J = 6.6 Hz). HRMS: (M + H)$^\oplus$ calcd for $C_{43}H_{76}NO_4$, 670.5774; found, 670.5787. Anal. Calcd for $C_{43}H_{75}NO_4$: C, H, N.

19. Preparation of *N*-(2-Oleyloxy-3-myristyloxypropyl)phthalimide (19). Compound **19** was prepared analogously to **13** on a 4 mmol scale to give after chromatography (1−5% ether/hexane) **19** as a colorless waxy solid (2.40 g, 64.8%); R_f 0.5 (hexane/ether 1:1); mp 34−36 °C. ^{1}H NMR (δ_H): 7.86 (dd, 2H, $H_{arom3,6}$, J_{ortho} = 5.4 Hz, J_{meta} = 3.0 Hz), 7.72 (dd, 2H, $H_{arom4,5}$), 5.43−5.25 (m, 2H, C*H*=C*H*), 3.90−3.70 (m, 3H, NCH_2C*H*), 3.70−3.35 (m, 6H, OCH_2), 2.10−1.90 (m, 4H, CH_2-

CH=CHCH_2), 1.60−1.41 (m, 4H, OCH$_2$CH_2), 1.40−1.12 (m, 44H, C$H_{2(\text{myristyl/oleyl})}$), 0.88 (t, 6H, C$H_3$, J = 6.6 Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{43}H_{74}NO_4$, 668.5618; found, 668.5592. Anal. Calcd for $C_{43}H_{73}NO_4$: C, H, N.

20. Preparation of *N*-(2,3-Dipalmityloxypropyl)phthalimide (20). Compound **20** was prepared analogously to **13** on a 5 mmol scale to give after chromatography (1−5% ether/hexane) **20** as a colorless solid (2.63 g, 78.6%); R_f 0.5 (hexane/ether 1:1), ether 50%; mp 61 °C. 1H NMR (δ_H): 7.86 (dd, 2H, $H_{\text{arom3,6}}$, J_{ortho} = 5.6 Hz, J_{meta} = 3.1 Hz), 7.71 (dd, 2H, $H_{\text{arom4,5}}$), 3.95−3.73 (m, 3H, NCH_2CH), 3.66−3.38 (m, 6H, OCH_2), 1.60−1.40 (m, 4H, OCH$_2$CH_2), 1.38−1.10 (m, 52H, C$H_{2(\text{palmityl})}$), 0.88 (t, 6H, CH_3, J = 6.6 Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{43}H_{76}NO_4$, 670.5774; found, 670.5787. Anal. Calcd for $C_{43}H_{75}NO_4$: C, H, N.

21. Preparation of *N*-(2-Stearyloxy-3-palmityloxypropyl)phthalimide (21). Compound **21** was prepared analogously to **13** on a 4 mmol scale to give after chromatography (1−5% ether/hexane) **21** as a colorless solid (1.77 g, 63.4%); R_f 0.5 (hexane/ether 1:1); mp 62 °C. 1H NMR (δ_H): 7.86 (dd, 2H, $H_{\text{arom3,6}}$, J_{ortho} = 5.6 Hz, J_{meta} = 3.1 Hz), 7.71 (dd, 2H, $H_{\text{arom4,5}}$), 3.95−3.73 (m, 3H, NCH_2CH), 3.65−3.38 (m, 6H, OCH_2), 1.60−1.39 (m, 4H, OCH$_2$CH_2), 1.38−1.10 (m, 56H, C$H_{2(\text{palmityl/stearyl})}$), 0.89 (t, 6H, C$H_3$, J = 6.5 Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{45}H_{80}NO_4$, 698.6087; found, 698.6113. Anal. Calcd for $C_{45}H_{79}NO_4$: C, H, N.

22. Preparation of *N*-(2-Lauryloxy-3-stearyloxypropyl)phthalimide (22). Compound **22** was prepared analogously to **13** on a 8.7 mmol scale to give after chromatography (1−5% ether/hexane) **22** as a colorless solid (3.87 g, 79.8%); R_f 0.5 (hexane/ether 1:1); mp 51−53 °C. 1H NMR (δ_H): 7.86 (dd, 2H, $H_{\text{arom3,6}}$, J_{ortho} = 5.5 Hz, J_{meta} = 3.0 Hz), 7.71 (dd, 2H, $H_{\text{arom4,5}}$), 3.94−3.72 (m, 3H, NCH_2CH), 3.66−3.36 (m, 6H, OCH_2), 1.57−1.37 (m, 4H, OCH$_2$CH_2), 1.35−1.10 (m, 48H, C$H_{2(\text{stearyl/lauryl})}$), 0.89 (t, 6H, C$H_3$, J = 6.5 Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{41}H_{72}NO_4$, 642.5461; found, 642.5434. Anal. Calcd for $C_{41}H_{71}NO_4$: C, H, N.

23. Preparation of *N*-(2,3-Distearyloxypropyl)phthalimide (23). Compound **23** was prepared analogously to **13** on a 4 mmol scale to give after chromatography (1−5% ether/hexane) **23** as a colorless solid (2.29 g, 789.8%); R_f 0.5 (hexane/ether 1:1); mp 68 °C. 1H NMR (δ_H): 7.86 (dd, 2H, $H_{\text{arom3,6}}$, J_{ortho} = 5.3 Hz, J_{meta} = 3.1 Hz), 7.71 (dd, 2H, $H_{\text{arom4,5}}$), 3.94−3.70 (m, 3H, NCH_2CH), 3.67−3.35 (m, 6H, OCH_2), 1.57−1.37 (m, 4H, OCH$_2$CH_2), 1.36−1.10 (m, 60H, C$H_{2(\text{stearyl})}$), 0.83 (t, 6H, CH_3, J = 6.5 Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{47}H_{84}NO_4$, 726.6400; found, 726.6383. Anal. Calcd for $C_{47}H_{83}NO_4$: C, H, N.

24. Preparation of *N*-(2-Oleyloxy-3-stearyloxypropyl)-phthalimide (24). Compound **24** was prepared analogously to **13** on a 3 mmol scale to give after chromatography (1−5% ether/hexane) **24** as a colorless waxy solid (1.47 g, 68.9%); R_f 0.5 (hexane/ether 1:1); mp 39−40 °C. 1H NMR (δ_H): 7.86 (dd, 2H, $H_{\text{arom3,6}}$, J_{ortho} = 5.4 Hz, J_{meta} = 3.2 Hz), 7.72 (dd, 2H, $H_{\text{arom4,5}}$), 5.46−5.26 (m, 2H, CH=CH), 3.93−3.68 (m, 3H, NCH_2CH), 3.66−3.33 (m, 6H, OCH_2), 2.10−1.85 (m, 4H, CH_2-CH=CHCH_2), 1.57−1.38 (m, 4H, OCH$_2$CH_2), 1.38−1.05 (m, 52H, C$H_{2(\text{stearyl/oleyl})}$), 0.88 (t, 6H, C$H_3$, J = 6.5 Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{47}H_{82}NO_4$, 724.6244; found, 724.6270. Anal. Calcd for $C_{45}H_{77}NO_4$: C, H, N.

25. Preparation of *N*-(2-Lauryloxy-3-oleyloxypropyl)-phthalimide (25). Compound **25** was prepared analogously to **13** on an 8.7 mmol scale to give after chromatography (1−5% ether/hexane) **25** as a colorless oil (4.25 g, 76.2%); R_f 0.5 (hexane/ether 1:1). 1H NMR (δ_H): 7.86 (dd, 2H, $H_{\text{arom3,6}}$, J_{ortho} = 5.6 Hz, J_{meta} = 3.1 Hz), 7.71 (dd, 2H, $H_{\text{arom4,5}}$), 5.43.5.25 (m, 2H, CH=CH), 3.95−3.66 (m, 3H, NCH_2CH), 3.66−3.33 (m, 6H, OCH_2), 2.10−1.85 (m, 4H, CH_2CH=CHCH_2), 1.60−1.38 (m, 4H, OCH$_2$CH_2), 1.38−1.00 (m, 40H, C$H_{2(\text{oleyl/lauryl})}$), 0.88 (t, 6H, C$H_3$, J = 6.1 Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{41}H_{70}NO_4$, 640.5305; found, 640.5320. Anal. Calcd for $C_{41}H_{69}NO_4$: C, H, N.

26. Preparation of *N*-(2-Oleyloxy-3-palmityloxypropyl)phthalimide (26). Compound **26** was prepared analogously to **13** on a 5 mmol scale to give after chromatography (1−5% ether/hexane) **26** as a colorless waxy solid (2.44 g, 70.1%); R_f 0.5 (hexane/ether 1:1); mp 37−40 °C. 1H NMR (δ_H): 7.86 (dd, 2H, $H_{\text{arom3,6}}$, J_{ortho} = 5.4 Hz, J_{meta} = 3.1 Hz), 7.72 (dd, 2H, $H_{\text{arom4,5}}$), 5.43−5.27 (m, 2H, CH=CH), 3.95−3.70 (m, 3H, NCH_2CH), 3.68−3.35 (m, 6H, OCH_2), 2.10−1.85 (m, 4H, CH_2-CH=CHCH_2), 1.56−1.39 (m, 4H, OCH$_2$CH_2), 1.39−1.07 (m, 48H, C$H_{2(\text{palmityl/oleyl})}$), 0.88 (t, 6H, C$H_3$, J = 6.5 Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{45}H_{78}NO_4$, 696.5931; found, 696.5890. Anal. Calcd for $C_{45}H_{77}NO_4$: C, H, N.

27. Preparation of *N*-(2,3-Dioleyloxypropyl)phthalimide (27). Compound **27** was prepared analogously to **13** on an 10 mmol scale to give after chromatography (1−5% ether/hexane) **27** as a colorless oil (5.95 g, 82.5%); R_f 0.5 (hexane/ether 1:1). 1H NMR (δ_H): 7.82 (dd, 2H, $H_{\text{arom3,6}}$, J_{ortho} = 5.5 Hz, J_{meta} = 3.0 Hz), 7.68 (dd, 2H, $H_{\text{arom4,5}}$), 5.39.5.22 (m, 4H, CH=CH), 3.91−3.65 (m, 3H, NCH_2CH), 3.65−3.31 (m, 6H, OCH_2), 2.09−1.86 (m, 8H, CH_2CH=CHCH_2), 1.55−1.36 (m, 4H, OCH$_2$CH_2), 1.36−1.03 (m, 44H, C$H_{2(\text{oleyl})}$), 0.85 (t, 6H, CH_3, J = 6.6 Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{47}H_{80}NO_4$, 722.6087; found, 722.6102. Anal. Calcd for $C_{47}H_{79}NO_4$: C, H, N.

28. Preparation of 2,3-Dilauryloxypropylamine (28). To a stirred, warmed (∼40 °C) solution of **13** (4.0 g, 7.17 mmol) in ethanol (75 mL) was added hydrazine monohydrate (8.97 g, 8.70 mL, 179 mmol,). The solution was refluxed for 18 h, during which time a colorless precipitate developed. The suspension was filtered, and the filtrate evaporated under reduced pressure. This gave a yellow oil, which was purified by flash column chromatography (0.5−5% MeOH/CH_2Cl_2) to give **28** as a colorless oil (2.73 g, 89.0%); R_f 0.3 (10% MeOH, 90% CH_2Cl_2). 1H NMR (δ_H): 3.65−3.58 (m, 1H, NCH$_2$CH), 3.55−3.35 (m, 6H, OCH_2), 2.89 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.3 Hz, J_{AX} = 3.5 Hz, J_{BX} = 6.4 Hz), 1.85−1.72 (m, 2H, NH$_2$), 1.72−1.55 (m, 4H, OCH$_2$CH_2), 1.55−1.20 (m, 36H, C$H_{2(\text{lauryl})}$), 0.90 (t, 6H, CH_3, J = 6.5 Hz). 13C NMR (δ_C): 80.20 (NCH$_2$CH), 72.07 (CHCH$_2$O), 71.61 (CHCH$_2$OCH$_2$), 70.73 (CHOCH$_2$), 43.90 (NCH$_2$), 32.30 (CH$_2$CH$_2$CH$_3$), 30.56−29.72 (CH$_{2(\text{lauryl})}$), 26.54 (OCH$_2$CH$_2$CH$_2$), 23.05 (CH$_2$CH$_3$), 14.46 (CH$_3$). HRMS: $(M + H)^{\oplus}$ calcd for $C_{27}H_{58}NO_2$, 428.4485; found, 428.4468.

29. Preparation of 2-Myristyloxy-3-lauryloxypropylamine (29). Compound **29** was prepared analogously to **28** on a 3.2 mmol scale to give after chromatography (0.5−5% MeOH/CH_2Cl_2) **29** as a pale yellow oil (1.19 g, 82.6%); R_f 0.3 (10% MeOH, 90% CH_2Cl_2). 1H NMR (δ_H): 3.70−3.55 (m, 1H, NCH$_2$CH), 3.55−3.30 (m, 6H, OCH_2), 2.80 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.2 Hz, J_{AX} = 3.6 Hz, J_{BX} = 6.4 Hz), 1.65−1.45 (m, 6H, OCH$_2$CH_2, NH$_2$), 1.45−1.15 (m, 40H, C$H_{2(\text{lauryl/myristyl})}$), 0.88 (t, 6H, C$H_3$, J = 6.6 Hz). 13C NMR (δ_C): 80.15 (NCH$_2$CH), 72.10 (CHCH$_2$O), 71.61 (CHCH$_2$OCH$_2$), 70.76 (CHOCH$_2$), 43.88 (NCH$_2$), 32.31 (CH$_2$CH$_2$CH$_3$), 30.57−29.75 (CH$_{2(\text{lauryl/myristyl})}$), 26.55 (OCH$_2CH_2CH_2$), 23.07 (CH$_2CH_3$), 14.48 (CH$_3$). HRMS: $(M + H)^{\oplus}$ calcd for $C_{29}H_{62}NO_2$, 456.4781; found, 456.4766.

30. Preparation of 2-Palmityloxy-3-lauryloxypropylamine (30). Compound **30** was prepared analogously to **28** on a 3.2 mmol scale to give after chromatography (0.5−5% MeOH/CH_2Cl_2) **30** as a pale yellow oil/wax (1.30 g, 84.1%); R_f 0.3 (10% MeOH, 90% CH_2Cl_2). 1H NMR (δ_H): 3.67−3.55 (m, 1H, NCH$_2$CH), 3.55−3.30 (m, 6H, OCH_2), 2.79 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.3 Hz, J_{AX} = 3.6 Hz, J_{BX} = 6.3 Hz), 1.65−1.47 (m, 4H, OCH$_2$CH_2), 1.47−1.38 (m, 2H, NH$_2$), 1.38−1.15 (m, 44H, C$H_{2(\text{lauryl/palmityl})}$), 0.88 (t, 6H, C$H_3$, J = 6.5 Hz). 13C NMR (δ_C): 80.05 (NCH$_2$CH), 72.10 (CHCH$_2$O), 71.62 (CHCH$_2$-OCH$_2$), 70.75 (CHOCH$_2$), 43.83 (NCH$_2$), 32.31 (CH$_2$CH$_2$CH$_3$), 30.57−29.75 (CH$_{2(\text{lauryl/palmityl})}$), 26.55 (OCH$_2CH_2CH_2$), 23.07 (CH$_2CH_3$), 14.46 (CH$_3$). HRMS: $(M + H)^{\oplus}$ calcd for $C_{31}H_{66}NO_2$, 484.5085; found, 484.5094.

31. Preparation of 2,3-Dimyristyloxypropylamine (31). Compound **31** was prepared analogously to **28** on a 7.3 mmol scale to give after chromatography (0.5−5% MeOH/CH_2Cl_2) **31** as a pale yellow wax (3.12 g, 88.2%); R_f 0.3 (10% MeOH, 90% CH_2Cl_2). 1H NMR (δ_H): 3.61−3.55 (m, 1H, NCH$_2$CH), 3.55−3.32 (m, 6H, OCH_2), 2.77 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.2 Hz, J_{AX} = 3.6 Hz, J_{BX} = 6.4 Hz), 1.62−1.40 (m, 6H, OCH$_2$CH_2,

NH_2), 1.40−1.10 (m, 44H, C$H_{2(\text{myristyl})}$), 0.86 (t, 6H, CH_3, J = 6.6 Hz). ^{13}C NMR (δ_C): 80.33 (NCH$_2$*C*H), 72.10 (CH*C*H$_2$O), 71.62 (CHCH$_2$O*C*H$_2$), 70.77 (CHO*C*H$_2$), 43.95 (N*C*H$_2$), 32.33 (*C*H$_2$CH$_2$CH$_3$), 30.58−29.77 (*C*H$_{2(\text{myristyl})}$), 26.57 (OCH$_2$CH$_2$*C*H$_2$), 23.10 (*C*H$_2$CH$_3$), 14.52 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{31}H_{66}NO_2$, 484.5086; found, 484.5094.

32. Preparation of 2-Palmityloxy-3-myristyloxypropylamine (32). Compound **32** was prepared analogously to **28** on a 3.84 mmol scale to give after chromatography (0.5−5% MeOH/CH$_2$Cl$_2$) **32** as a pale yellow wax (1.81 g, 92.1%); R_f 0.3 (10% MeOH, 90% CH$_2$Cl$_2$). ^{1}H NMR (δ_H): 3.67−3.51 (m, 1H, NCH$_2$C*H*), 3.51−3.33 (m, 6H, OCH_2), 2.80 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.2 Hz, J_{AX} = 3.5 Hz, J_{BX} = 6.3 Hz), 1.65−1.44 (m, 6H, OCH$_2$CH_2, NH$_2$), 1.44−1.18 (m, 48H, C$H_{2(\text{myristyl/palmityl})}$), 0.89 (t, 6H, C$H_3$, J = 6.5 Hz). ^{13}C NMR (δ_C): 80.17 (NCH$_2$*C*H), 72.09 (CH*C*H$_2$O), 71.62 (CHCH$_2$O*C*H$_2$), 70.75 (CHO*C*H$_2$), 43.88 (N*C*H$_2$), 32.32 (*C*H$_2$CH$_2$CH$_3$), 30.57−29.75 (*C*H$_{2(\text{myristyl/palmityl})}$), 26.56 (OCH$_2CH_2$*C*H$_2$), 23.07 (*C*H$_2CH_3$), 14.48 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{33}H_{70}NO_2$, 512.5425; found, 512.5407.

33. Preparation of 2-Stearyloxy-3-myristyloxypropylamine (33). Compound **33** was prepared analogously to **28** on a 2.43 mmol scale to give after chromatography (0.5−5% MeOH/CH$_2$Cl$_2$) **33** as a pale yellow wax (0.98 g, 74.7%); R_f 0.3 (10% MeOH, 90% CH$_2$Cl$_2$). ^{1}H NMR (δ_H): 3.60−3.50 (m, 1H, NCH$_2$C*H*), 3.50−3.30 (m, 6H, OCH_2), 2.79 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 12.9 Hz, J_{AX} = 2.1 Hz, J_{BX} = 5.6 Hz), 2.40−2.25 (m, 2H, NH$_2$), 1.60−1.35 (m, 4H, OCH$_2$CH_2), 1.35−1.10 (m, 52H, C$H_{2(\text{myristyl/stearyl})}$), 0.89 (t, 6H, C$H_3$, J = 6.5 Hz). ^{13}C NMR (δ_C): 79.75 (NCH$_2$*C*H), 72.10 (CH*C*H$_2$O), 71.52 (CHCH$_2$-O*C*H$_2$), 70.74 (CHO*C*H$_2$), 43.70 (N*C*H$_2$), 32.33 (*C*H$_2$CH$_2$CH$_3$), 30.55−29.77 (*C*H$_{2(\text{myristyl/stearyl})}$), 26.55 (OCH$_2CH_2$*C*H$_2$), 23.08 (*C*H$_2CH_3$), 14.49 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{35}H_{74}NO_2$, 540.5720; found, 540.5735.

34. Preparation of 2-Oleyloxy-3-myristyloxypropylamine (34). Compound **34** was prepared analogously to **28** on a 1.5 mmol scale to give after chromatography (0.5−5% MeOH/CH$_2$Cl$_2$) **34** as a pale yellow oil/wax (0.657 g, 81.4%); R_f 0.3 (10% MeOH, 90% CH$_2$Cl$_2$). ^{1}H NMR (δ_H): 5.43−5.28 (m, 2H, C*H*═C*H*), 3.70−3.55 (m, 1H, NCH$_2$C*H*), 3.50−3.33 (m, 6H, OCH_2), 2.80 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.2 Hz, J_{AX} = 3.6 Hz, J_{BX} = 6.3 Hz), 2.11−1.89 (m, 4H, CH_2CH═CHCH_2), 1.66−1.46 (m, 6H, OCH$_2$CH_2, NH$_2$), 1.46−1.18 (m, 44H, C$H_{2(\text{myristyl/oleyl})}$), 0.88 (t, 6H, C$H_3$, J = 6.5 Hz). ^{13}C NMR (δ_C): 130.31, 130.20 (*C*H═*C*H), 80.23 (NCH$_2$*C*H), 72.11 (CH*C*H$_2$O), 71.62 (CHCH$_2$O*C*H$_2$), 70.75 (CHO*C*H$_2$), 43.93 (N*C*H$_2$), 32.32 (*C*H$_2$CH$_2$CH$_3$), 30.58−29.67, 27.60 (*C*H$_{2(\text{myristyl/oleyl})}$), 26.56 (OCH$_2$-CH$_2$*C*H$_2$), 23.08 (*C*H$_2CH_3$), 14.49 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{35}H_{72}NO_2$, 538.5563; found, 538.5540.

35. Preparation of 2,3-Dipalmityloxypropylamine (35). Compound **35** was prepared analogously to **28** on a 3.15 mmol scale to give after chromatography (0.5−5% MeOH/CH$_2$Cl$_2$) **35** as a pale yellow wax (1.36 g, 79.9%); R_f 0.3 (10% MeOH, 90% CH$_2$Cl$_2$). ^{1}H NMR (δ_H): 3.67−3.56 (m, 1H, NCH$_2$C*H*), 3.56−3.34 (m, 6H, OCH_2), 2.81 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.3 Hz, J_{AX} = 3.5 Hz, J_{BX} = 6.4 Hz), 1.67−1.43 (m, 6H, OCH$_2$CH_2, NH$_2$), 1.41−1.15 (m, 52H, C$H_{2(\text{palmityl})}$), 0.89 (t, 6H, CH_3, J = 6.5 Hz). ^{13}C NMR (δ_C): 80.14 (NCH$_2$*C*H), 72.10 (CH*C*H$_2$O), 71.62 (CHCH$_2$O*C*H$_2$), 70.76 (CHO*C*H$_2$), 43.90 (N*C*H$_2$), 32.32 (*C*H$_2$CH$_2$CH$_3$), 30.58−29.76 (*C*H$_{2(\text{palmityl})}$), 26.56 (OCH$_2$CH$_2$*C*H$_2$), 23.08 (*C*H$_2$CH$_3$), 14.48 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{35}H_{74}NO_2$, 540.5720; found, 540.5730.

36. Preparation of 2-Stearyloxy-3-palmityloxypropylamine (36). Compound **36** was prepared analogously to **28** on a 2.30 mmol scale to give after chromatography (0.5−5% MeOH/CH$_2$Cl$_2$) **36** as a pale yellow wax (0.98 g, 76.4%); R_f 0.3 (10% MeOH, 90% CH$_2$Cl$_2$). ^{1}H NMR (δ_H): 3.66−3.52 (m, 1H, NCH$_2$C*H*), 3.52−3.32 (m, 6H, OCH_2), 2.80 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.1 Hz, J_{AX} = 2.9 Hz, J_{BX} = 5.8 Hz), 2.22−2.08 (m, 2H, NH$_2$), 1.62−1.41 (m, 4H, OCH$_2$CH_2), 1.41−1.10 (m, 56H C$H_{2(\text{palmityl/stearyl})}$), 0.86 (t, 6H, C$H_3$, J = 6.5 Hz). ^{13}C NMR (δ_C): 79.84 (NCH$_2$*C*H), 72.13 (CH*C*H$_2$O), 71.56 (CHCH$_2$-O*C*H$_2$), 70.77 (CHO*C*H$_2$), 43.78 (N*C*H$_2$), 32.34 (*C*H$_2$CH$_2$CH$_3$), 30.56−29.78 (C$H_{2(\text{palmityl/stearyl})}$), 26.56 (OCH$_2CH_2$*C*H$_2$), 23.10 (*C*H$_2CH_3$), 14.52 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{37}H_{78}NO_2$, 568.6033; found, 568.6055.

37. Preparation of 2-Lauryloxy-3-stearyloxypropylamine (37). Compound **37** was prepared analogously to **28** on a 4.58 mmol scale to give after chromatography (0.5−5% MeOH/CH$_2$Cl$_2$) **37** as a pale yellow wax (2.15 g, 91.8%); R_f 0.3 (10% MeOH, 90% CH$_2$Cl$_2$). ^{1}H NMR (δ_H): 3.68−3.56 (m, 1H, NCH$_2$C*H*), 3.56−3.32 (m, 6H, OCH_2), 2.80 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.2 Hz, J_{AX} = 3.7 Hz, J_{BX} = 6.4 Hz), 1.67−1.44 (m, 6H, OCH$_2$CH_2, NH$_2$), 1.38−1.15 (m, 48H, C$H_{2(\text{stearyl/lauryl})}$), 0.88 (t, 6H, C$H_3$, J = 6.6 Hz). ^{13}C NMR (δ_C): 80.11 (NCH$_2$*C*H), 72.11 (CH*C*H$_2$O), 71.61 (CHCH$_2$O*C*H$_2$), 70.76 (CHO*C*H$_2$), 43.89 (N*C*H$_2$), 32.32 (*C*H$_2$CH$_2$CH$_3$), 30.57−29.75 (*C*H$_{2(\text{stearyl/lauryl})}$), 26.56 (OCH$_2CH_2$*C*H$_2$), 23.07 (*C*H$_2CH_3$), 14.49 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{33}H_{70}NO_2$, 512.5422; found, 512.5407.

38. Preparation of 2,3-Distearyloxypropylamine (38). Compound **38** was prepared analogously to **28** on a 1.37 mmol scale to give after chromatography (0.5−5% MeOH/CH$_2$Cl$_2$) **38** as a pale yellow wax (0.65 g, 79.6%); R_f 0.3 (10% MeOH, 90% CH$_2$Cl$_2$). ^{1}H NMR (δ_H): 3.65−3.52 (m, 1H, NCH$_2$C*H*), 3.52−3.32 (m, 6H, OCH_2), 2.78 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.2 Hz, J_{AX} = 3.5 Hz, J_{BX} = 6.4 Hz), 1.70−1.60 (m, 2H, NH$_2$) 1.60−1.40 (m, 4H, OCH$_2$CH_2), 1.40−1.10 (m, 60H, C$H_{2(\text{stearyl})}$), 0.86 (t, 6H, CH_3, J = 6.4 Hz). ^{13}C NMR (δ_C): 80.24 (NCH$_2$*C*H), 72.10 (CH*C*H$_2$O), 71.61 (CHCH$_2$O*C*H$_2$), 70.77 (CHO*C*H$_2$), 43.92 (N*C*H$_2$), 32.34 (*C*H$_2$CH$_2$CH$_3$), 30.58−29.78 (*C*H$_{2(\text{stearyl})}$), 26.57 (OCH$_2$CH$_2$*C*H$_2$), 23.10 (*C*H$_2$CH$_3$), 14.52 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{39}H_{82}NO_2$, 596.6346; found, 596.6330.

39. Preparation of 2-Oleyloxy-3-stearyloxypropylamine (39). Compound **39** was prepared analogously to **28** on a 0.83 mmol scale to give after chromatography (0.5−5% MeOH/CH$_2$-Cl$_2$) **39** as a colorless wax (0.454 g, 92.1%); R_f 0.3 (10% MeOH, 90% CH$_2$Cl$_2$). ^{1}H NMR (δ_H): 5.41−5.28 (m, 2H, C*H*═C*H*), 3.68−3.55 (m, 1H, NCH$_2$C*H*), 3.55−3.33 (m, 6H, OCH_2), 2.79 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.2 Hz, J_{AX} = 3.5 Hz, J_{BX} = 6.3 Hz), 2.07−1.80 (m, 6H, CH_2CH═CHCH_2, NH$_2$), 1.63−1.45 (m, 4H, OCH$_2$CH_2), 1.40−1.13 (m, 52H, C$H_{2(\text{stearyl/oleyl})}$), 0.86 (t, 6H, C$H_3$, J = 6.5 Hz). ^{13}C NMR (δ_C): 130.30, 130.20 (*C*H═*C*H), 80.11 (NCH$_2$*C*H), 72.12 (CH*C*H$_2$O), 71.59 (CHCH$_2$O*C*H$_2$), 70.78 (CHO*C*H$_2$), 43.87 (N*C*H$_2$), 32.34 (*C*H$_2$CH$_2$CH$_3$), 30.58−29.78, 27.61 (*C*H$_{2(\text{stearyl/oleyl})}$), 26.53 (OCH$_2CH_2$*C*H$_2$), 23.10 (*C*H$_2CH_3$), 14.52 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{39}H_{80}NO_2$, 594.6189; found, 594.6173.

40. Preparation of 2-Lauryloxy-3-oleyloxypropylamine (40). Compound **40** was prepared analogously to **28** on a 3.89 mmol scale to give after chromatography (0.5−5% MeOH/CH$_2$-Cl$_2$) **40** as a pale yellow oil (1.99 g, 90.5%); R_f 0.3 (10% MeOH, 90% CH$_2$Cl$_2$). ^{1}H NMR (δ_H): 5.37−5.23 (m, 2H, C*H*═C*H*), 3.65−3.50 (m, 1H, NCH$_2$C*H*), 3.50−3.30 (m, 6H, OCH_2), 2.76 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.2 Hz, J_{AX} = 3.4 Hz, J_{BX} = 6.4 Hz), 2.02−1.87 (m, 6H, CH_2CH═CHCH_2, NH$_2$), 1.85−1.75 (m, 2H, NH$_2$), 1.60−1.45 (m, 4H, OCH$_2$CH_2), 1.35−1.11 (m, 40H, C$H_{2(\text{oleyl/lauryl})}$), 0.89 (t, 6H, C$H_3$, J = 6.5 Hz). ^{13}C NMR (δ_C): 130.28, 130.18 (*C*H═*C*H), 80.14 (NCH$_2$*C*H), 72.07 (CH*C*H$_2$O), 71.58 (CHCH$_2$O*C*H$_2$), 70.74 (CHO*C*H$_2$), 43.86 (NCH$_2$), 32.32 (*C*H$_2$CH$_2$CH$_3$), 30.56−29.51, 27.59 (*C*H$_{2(\text{oleyl/lauryl})}$), 26.56 (OCH$_2$-CH$_2$*C*H$_2$), 23.08 (*C*H$_2CH_3$), 14.49 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{33}H_{68}NO_2$, 510.5235; found, 510.5250.

41. Preparation of 2-Palmityloxy-3-oleyloxypropylamine (41). Compound **41** was prepared analogously to **28** on a 3.0 mmol scale to give after chromatography (0.5−5% MeOH/CH$_2$Cl$_2$) **41** as a pale yellow wax (1.26 g, 74.1%); R_f 0.3 (10% MeOH, 90% CH$_2$Cl$_2$). ^{1}H NMR (δ_H): 5.41−5.30 (m, 2H, C*H*═C*H*), 3.69−3.56 (m, 1H, NCH$_2$C*H*), 3.56−3.35 (m, 6H, OCH_2), 2.82 (ABX, 2H, NC$H_{2(\text{AandB})}$, J_{AB} = 13.2 Hz, J_{AX} = 3.7 Hz, J_{BX} = 6.2 Hz), 2.12−1.93 (m, 4H, CH_2CH═CHCH_2), 1.78−1.64 (m, 2H, NH$_2$), 1.64−1.49 (m, 4H, OCH$_2$CH_2), 1.38−1.17 (m, 48H, C$H_{2(\text{palmityl/oleyl})}$), 0.89 (t, 6H, C$H_3$, J = 6.5 Hz). ^{13}C NMR (δ_C): 130.31, 130.21 (*C*H═*C*H), 79.77 (NCH$_2$*C*H), 72.13 (CH*C*H$_2$O), 71.58 (CHCH$_2$O*C*H$_2$), 70.77 (CHO*C*H$_2$), 43.76 (N*C*H$_2$), 32.32 (*C*H$_2$CH$_2$CH$_3$), 30.56−29.72, 27.61 (*C*H$_{2(\text{palmityl/oleyl})}$), 26.56 (OCH$_2CH_2$*C*H$_2$), 23.08 (*C*H$_2CH_3$), 14.49 (*C*H$_3$). HRMS: (M + H)$^{\oplus}$ calcd for $C_{37}H_{76}NO_2$, 566.5876; found, 566.5861.

42. Preparation of 2,3-Dioleyloxypropylamine (42). Compound **42** was prepared analogously to **28** on a 1.5 mmol scale to give after chromatography (0.5−5% MeOH/CH_2Cl_2) **42** as a pale yellow oil (1.92 g, 87.5%); R_f 0.3 (10% MeOH, 90% CH_2Cl_2). ^{1}H NMR (δ_H): 5.44−5.24 (m, 4H, C*H*=C*H*), 3.68−3.54 (m, 1H, NCH$_2$C*H*), 3.54−3.33 (m, 6H, OC*H*$_2$), 2.80 (ABX, 2H, NC*H*$_{2(AandB)}$, J_{AB} = 13.2 Hz, J_{AX} = 3.5 Hz, J_{BX} = 6.4 Hz), 2.12−1.88 (m, 4H, C*H*$_2$CH=CHC*H*$_2$), 1.61−1.45 (m, 4H, OCH$_2$C*H*$_2$), 1.45−1.38 (m, 2H, NH$_2$), 1.38−1.20 (m, 48H, C*H*$_{2(oleyl)}$), 0.88 (t, 6H, C*H*$_3$, J = 6.6 Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{39}H_{78}NO_2$, 592.6033; found, 592.6010. Anal. Calcd for $C_{47}H_{79}NO_4$: C, H, N.

43. Preparation of *N*-α,ϵ-Di-*tert*-butyloxycarbonyl-L-lysine(2,3-dilauryloxy)propylamide (43). A flask containing *N*-α,ϵ-di-*tert*-butyloxycarbonyl-L-lysine *N*-hydroxysuccinimide ester (399 mg, 0.9 mmol) and **28** (257 mg, 0.6 mmol) was flushed with argon. Anhydrous THF (10 mL) was added, and the reaction was refluxed at 85 °C overnight. The solvent was then removed, and CH_2Cl_2 (30 mL) was added. The solution was washed with $NaHCO_3$ (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL), and each of the aqueous layers was backwashed with CH_2Cl_2 (10 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow solid. The solid was purified by column chromatography (10−30% ethyl acetate/hexane) to give **43** as a white solid (420 mg, 92.6%); R_f 0.35 (30% ethyl acetate/hexane); mp 58.60 °C. ^{1}H NMR (δ_H): 6.47−6.35 (m, 1H, N*H*$_{(amide)}$), 5.16−5.02 (m, 1H, α-N*H*$_{(carbamate)}$), 4.67−4.51 (m, 1H, ϵ-N*H*$_{(carbamate)}$), 4.10−3.93 (m, 1H, C*H*NH), 3.62−3.19 (m, 9H, C*H*$_2$C*H*(OC*H*$_2$)C*H*$_2$OC*H*$_2$), 3.15−3.01 (m, 2H, CH$_2$C*H*$_2$NH), 1.90−1.48 (m, 10H, CH(C*H*$_2$)$_3$CH$_2$NH, OCH$_2$C*H*$_2$), 1.43 (s, 18H, C(C*H*$_3$)), 1.36−1.16 (m, 36H, C*H*$_{2(lauryl)}$), 0.86 (t, 6H, CH$_2$C*H*$_3$ J = 6.6 Hz). ^{13}C NMR (δ_C): 172.30 (*C*O$_{(amide)}$), 156.52 (*C*ONH(CH)), 156.00 (*C*ONH(CH$_2$)), 80.33 (*C*HCH$_2$O), 79.59 ((*C*(CH$_3$)$_3$), 72.26 (CH*C*H$_2$O), 71.83 (CHCH$_2$O*C*H$_2$), 70.71 (CHO*C*H$_2$), 54.98 (*C*HNH), 41.11 (N*C*H$_2$CH), 40.53 (CH$_2$*C*H$_2$NH), 32.76 (*C*H$_2$-CH$_2$*C*H$_2$CH$_2$NH), 32.32 (*C*H$_2$CH$_2$CH$_3$), 30.45−29.75 (*C*H$_{2(lauryl)}$), 28.84, 28.74 (C(*C*H$_3$)$_3$), 26.51 (OCH$_2$CH$_2$*C*H$_2$), 23.08 (*C*H$_2$CH$_3$), 22.99 (*C*H$_2$(CH$_2$)$_2$NH), 14.50 (CH$_2$*C*H$_3$). HRMS: $(M + H)^{\oplus}$ calcd for $C_{43}H_{86}NO_7$, 756.6486; found, 756.6466.

44. Preparation of *N*-α,ϵ-Di-*tert*-butyloxycarbonyl-L-lysine(2-lauryloxy-3-stearyloxy)propylamide (44). Compound **44** was prepared analogously to **43** on a 0.4 mmol scale to give, after chromatography (10−30% ethyl acetate/hexane), **44** as a white solid (297 mg, 88.5%); R_f 0.35 (30% ethyl acetate/hexane); mp 62−64 °C. ^{1}H NMR (δ_H): 6.47−6.36 (m, 1H, N*H*$_{(amide)}$), 5.15−5.03 (m, 1H, α-N*H*$_{(carbamate)}$), 4.65−4.51 (m, 1H, ϵ-N*H*$_{(carbamate)}$), 4.09−3.94 (m, 1H, C*H*NH), 3.61−3.20 (m, 9H, C*H*$_2$C*H*(OC*H*$_2$)C*H*$_2$OC*H*$_2$), 3.16−3.01 (m, 2H, CH$_2$C*H*$_2$NH), 1.90−1.49 (m, 10H, CH(C*H*$_2$)$_3$CH$_2$NH, OCH$_2$C*H*$_2$), 1.43 (s, 18H, (C(C*H*$_3$)), 1.36−1.16 (m, 48H, C*H*$_{2(lauryl/stearyl)}$), 0.87 (t, 6H, CH$_2$C*H*$_3$, J = 6.6 Hz). ^{13}C NMR (δ_C): 172.30 (*C*O$_{(amide)}$), 156.51 (*C*ONH(CH)), 156.01 (*C*ONH(CH$_2$)), 80.35 (*C*HCH$_2$O), 79.58 (*C*(CH$_3$)$_3$), 72.26 (CH*C*H$_2$O), 71.84 (CHCH$_2$O*C*H$_2$), 70.71 (CHO*C*H$_2$), 54.97 (*C*HNH), 41.13 (N*C*H$_2$CH), 40.51 (CH$_2$*C*H$_2$-NH), 32.78 (CH$_2$CH$_2$*C*H$_2$CH$_2$NH), 32.32 (*C*H$_2$CH$_2$CH$_3$), 30.46−29.76 (*C*H$_{2(lauryl/stearyl)}$), 28.84, 28.74 (C(*C*H$_3$)$_3$), 26.51 (OCH$_2$-CH$_2$*C*H$_2$), 23.06 (*C*H$_2$CH$_3$), 23.01 (*C*H$_2$(CH$_2$)$_2$NH), 14.51 (CH$_2$*C*H$_3$). HRMS: $(M + H)^{\oplus}$ calcd for $C_{49}H_{98}NO_7$, 840.7396; found, 840.7405.

45. Preparation of L-Lysine(2,3-dilauryloxy)propylamide (45). To a flask containing 95% TFA (20 mL) was added **43** (420 mg, 0.56 mmol), and the solution was stirred for 1.5 h. The solvent was then removed under reduced pressure, and the resulting white solid was purified by column chromatography (CH_2Cl_2−CH_2Cl_2:MeOH:NH_3 96.5:3:0.5) to give **45** as a white solid (273 mg, 88.1%); R_f 0.30 (CH_2Cl_2:MeOH:NH_3 92:7:1); mp 53 °C. ^{1}H NMR (δ_H): 7.60−7.50 (m, 1H, N*H*$_{(amide)}$), 3.69−3.20 (m, 10H, C*H*NH$_2$, C*H*$_2$C*H*(OC*H*$_2$)C*H*$_2$OC*H*$_2$), 2.77−2.65 (m, 2H, C*H*$_2$NH$_2$), 1.71−1.39 (m, 14H, CH(C*H*$_2$)$_3$CH$_2$NH$_2$, OCH$_2$C*H*$_2$, 2N*H*$_2$), 1.39−1.16 (m, 36H, C*H*$_{2(lauryl)}$), 0.88 (t, 6H, CH$_2$C*H*$_3$, J = 6.5 Hz). ^{13}C NMR (δ_C): 175.54 (*C*O$_{(amide)}$), 72.23 (CH*C*H$_2$O), 71.91 (CHCH$_2$O*C*H$_2$), 70.68 (CHO*C*H$_2$), 55.38 (*C*HNH$_2$), 41.33 (N*C*H$_2$CH), 40.79 (*C*H$_2$NH$_2$), 35.11 (*C*H$_2$-CHNH$_2$), 32.32 (*C*H$_2$CH$_2$CH$_3$), 31.53 (*C*H$_2$CH$_2$NH$_2$), 30.48−29.76 (*C*H$_{2(lauryl)}$), 26.52 (OCH$_2$CH$_2$*C*H$_2$), 23.28 (*C*H$_2$(CH$_2$)$_2$NH$_2$), 23.08 (*C*H$_2$CH$_3$), 14.50 (CH$_2$*C*H$_3$). HRMS: $(M + H)^{\oplus}$ calcd for $C_{33}H_{70}N_3O_3$, 556.5422; found, 556.5417.

46. Preparation of L-Lysine(2-lauryloxy-3-stearyloxy)-propylamide (46). Compound **46** was prepared analogously to **45** on a 0.35 mmol scale to give, after chromatography (CH_2Cl_2−CH_2Cl_2:MeOH:NH_3 96.5:3:0.5), **46** as a white solid (170 mg, 75.9%); R_f 0.30 (CH_2Cl_2:MeOH:NH_3 92:7:1); mp 56 °C. ^{1}H NMR (δ_H): 7.60 (t, 1H, N*H*$_{(amide)}$ J = 5.1 Hz), 3.63−3.17 (m, 10H, C*H*NH$_2$, C*H*$_2$C*H*(OC*H*$_2$)C*H*$_2$OC*H*$_2$), 3.17−3.04 (m, 4H, 2N*H*$_2$), 2.84−2.72 (m, 2H, C*H*$_2$NH$_2$), 1.63−1.36 (m, 14H, CH-(C*H*$_2$)$_3$CH$_2$NH$_2$, OCH$_2$C*H*$_2$, 2N*H*$_2$), 1.36−1.17 (m, 48H, C*H*$_{2(lauryl/stearyl)}$), 0.86 (t, 6H, CH$_2$C*H*$_3$, J = 6.6 Hz). ^{13}C NMR (δ_C): 175.55 (*C*O$_{(amide)}$), 72.24 (CH*C*H$_2$O), 71.93 (CHCH$_2$O*C*H$_2$), 70.67 (CHO*C*H$_2$), 55.48 (*C*HNH$_2$), 41.56 (N*C*H$_2$CH), 40.79 (*C*H$_2$NH$_2$), 35.21 (*C*H$_2$CHNH$_2$), 32.32 (*C*H$_2$CH$_2$CH$_3$), 31.99 (*C*H$_2$CH$_2$NH$_2$), 30.48−29.76 (*C*H$_{2(lauryl/stearyl)}$), 26.52 (OCH$_2$-CH$_2$*C*H$_2$), 23.31 (*C*H$_2$(CH$_2$)$_2$NH$_2$), 23.08 (*C*H$_2$CH$_3$), 14.50 (CH$_2$*C*H$_3$). HRMS: $(M + H)^{\oplus}$ calcd for $C_{39}H_{82}N_3O_3$, 640.6363; found, 640.6356.

47. Preparation of *N*-α-Fluorenylmethoxycarbonyl-L-tryptophan(2,3-dilauryloxy)propylamide (47). A flask containing *N*-α-fluorenylmethoxycarbonyl-L-tryptophan pentafluorophenyl ester (889 mg, 1.5 mmol) and **28** (428 mg, 1.0 mmol) was flushed with argon. Anhydrous THF (15 mL) was added, and the reaction was refluxed at 85 °C for 1 h. The solvent was then removed, and CH_2Cl_2 (30 mL) was added. The solution was washed with $NaHCO_3$ (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL), and each of the aqueous layers was backwashed with CH_2Cl_2 (10 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow solid. The solid was purified by column chromatography (CH_2Cl_2−0.5% MeOH/CH_2Cl_2) to give **47** as a pale yellow oil (598 mg, 71.7%); R_f 0.50 (2% MeOH/CH_2Cl_2). ^{1}H NMR (δ_H): 8.11−8.03 (m, 1H, N*H*$_{(indole)}$), 7.76 (d, 2H, *H*$_{(arom,Fmoc)}$, J = 7.4 Hz), 7.74−7.63 (m, 1H, *H*$_{(arom,indole)}$), 7.59−7.49 (m, 3H, N*H*$_{(amide)}$, *H*$_{(arom,Fmoc)}$), 7.42−7.00 (m, 8H, *H*$_{(arom,indole)}$, *H*$_{(arom,Fmoc)}$), 5.58−5.45 (m, 1H, N*H*$_{(carbamate)}$), 4.56−4.25 (m, 3H, C*H*(CO)NH, CHC*H*$_{2(Fmoc)}$), 4.19 (t, 1H, C*H*CH$_{2(Fmoc)}$, J = 7.0 Hz), 3.50−3.07 (m, 11H, C*H*$_2$CHNH, C*H*$_2$C*H*(OC*H*$_2$)C*H*$_2$-OC*H*$_2$), 1.55−1.30 (m, 4H, OCH$_2$C*H*$_2$), 1.30−1.09 (m, 36H, C*H*$_{2(lauryl)}$), 0.88 (t, 6H, CH$_2$C*H*$_3$, J = 6.5 Hz). ^{13}C NMR (δ_C): 171.59 (*C*O$_{(amide)}$), 156.33 (CO$_{(carbamate)}$), 144.23 (*C*$_{(arom,Fmoc)}$), 141.70 (*C*$_{(arom,Fmoc)}$), 136.69 (*C*$_{(arom,indole)}$), 128.13 (*C*$_{(arom,Fmoc)}$), 127.85 (*C*$_{(arom,indole)}$), 127.50 (*C*$_{(arom,Fmoc)}$), 125.51 (*C*$_{(arom,Fmoc)}$), 123.50 (*C*$_{(arom,indole)}$), 122.80 (*C*$_{(arom,indole)}$), 120.39, 120.350 (*C*$_{(arom,indole)}$, *C*$_{(arom,Fmoc)}$), 119.24 (*C*$_{(arom,indole)}$), 111.68 (*C*$_{(arom,indole)}$), 111.06 (*C*$_{(arom,indole)}$), 72.17 (CH*C*H$_2$O), 71.57 (CHCH$_2$O*C*H$_2$), 70.59 (CHO*C*H$_2$), 67.56 (*C*HCH$_{2(Fmoc)}$), 56.37 (*C*HNH), 47.57 (CH*C*H$_{2(Fmoc)}$), 41.25 (N*C*H$_2$CH), 32.34 (*C*H$_2$CH$_2$CH$_3$), 30.38−29.78 (*C*H$_{2(lauryl)}$), 29.27 (*C*H$_2$CHNH), 26.44 (OCH$_2$CH$_2$*C*H$_2$), 23.10 (*C*H$_2$CH$_3$), 14.53 (CH$_2$*C*H$_3$). HRMS: $(M + H)^{\oplus}$ calcd for $C_{54}H_{81}N_3O_4Na$, 858.6122; found, 858.6125.

48. Preparation of *N*-α-Fluorenylmethoxycarbonyl-L-tryptophan(2-lauryloxy-3-stearyloxy)propylamide (48). Compound **48** was prepared analogously to **47** on a 1 mmol scale to give, after chromatography (CH_2Cl_2−0.5% MeOH/CH_2Cl_2), **48** as a yellow wax (750 mg, 81.7%); R_f 0.50 (2% MeOH/CH_2Cl_2). ^{1}H NMR (δ_H): 8.11−8.03 (m, 1H, N*H*$_{(indole)}$), 7.76 (d, 2H, *H*$_{(arom,Fmoc)}$, J = 7.3 Hz), 7.73−7.63 (m, 1H, *H*$_{(arom,indole)}$), 7.59−7.49 (m, 2H, *H*$_{(arom,Fmoc)}$), 7.43−7.00 (m, 9H, N*H*$_{(amide)}$, *H*$_{(arom,indole)}$, *H*$_{(arom,Fmoc)}$), 5.59−5.45 (m, 1H, N*H*$_{(carbamate)}$), 4.53−4.25 (m, 3H, C*H*(CO)NH, CHC*H*$_{2(Fmoc)}$), 4.20 (t, 1H, C*H*CH$_{2(Fmoc)}$, J = 7.1 Hz), 3.52−3.04 (m, 11H, C*H*$_2$CHNH, C*H*$_2$C*H*(OC*H*$_2$)C*H*$_2$-OC*H*$_2$), 1.53−1.30 (m, 4H, OCH$_2$C*H*$_2$), 1.30−1.11 (m, 48H, C*H*$_{2(lauryl/stearyl)}$), 0.88 (t, 6H, CH$_2$C*H*$_3$, J = 6.6 Hz). ^{13}C NMR (δ_C): 171.46 (*C*O$_{(amide)}$), 156.30 (CO$_{(carbamate)}$), 144.23 (*C*$_{(arom,Fmoc)}$), 141.70 (*C*$_{(arom,Fmoc)}$), 136.66 (*C*$_{(arom,indole)}$), 128.12 (*C*$_{(arom,Fmoc)}$), 127.85 (*C*$_{(arom,indole)}$), 127.45 (*C*$_{(arom,Fmoc)}$), 125.51 (*C*$_{(arom,Fmoc)}$), 123.50 (*C*$_{(arom,indole)}$), 122.83 (*C*$_{(arom,indole)}$), 120.38, (*C*$_{(arom,indole)}$, *C*$_{(arom,Fmoc)}$), 119.28 (*C*$_{(arom,indole)}$), 111.62 (*C*$_{(arom,indole)}$), 111.10 (*C*$_{(arom,indole)}$), 72.17 (CH*C*H$_2$O), 71.58 (CHCH$_2$O*C*H$_2$), 70.58 (CHO*C*H$_2$), 67.53 (*C*HCH$_{2(Fmoc)}$), 56.33 (*C*HNH), 47.58

($CHCH_{2(Fmoc)}$), 41.22 (NCH_2CH), 32.33 ($CH_2CH_2CH_3$), 30.37−29.77 ($CH_{2(lauryl/stearyl)}$), 29.30 (CH_2CHNH), 26.48 ($OCH_2CH_2CH_2$), 23.10 (CH_2CH_3), 14.52 (CH_2CH_3). HRMS: $(M + H)^{\oplus}$ calcd for $C_{60}H_{94}N_3O_4$, 920.7196; found, 920.7244.

49. Preparation of L-Tryptophan(2,3-dilauryloxy)-propylamide (49). To a solution of **47** (598 mg, 0.97 mmol) in DMF (8 mL) was added piperidine (2 mL), and the reaction was stirred for 45 min. The solvent was then removed under high vacuum, and the resulting yellow oil was purified by column chromatography (50% EtOAc/hexane−100% EtOAc). Freeze-thawing under vacuum was used to obtain **49** as a pale yellow wax (462 mg, 80.8%); R_f 0.20 (EtOAc). ^{1}H NMR (δ_H): 8.22−8.12 (m, 1H, $NH_{(indole)}$), 7.67 (d, 1H, $H_{(arom,indole)}$, $J = 7.5$ Hz), 7.56−7.44 (m, 1H, $NH_{(amide)}$), 7.36 (d, 1H, $H_{(arom,indole)}$, $J = 7.7$ Hz), 7.23−7.06 (m, 3H, $H_{(arom,indole)}$), 3.79−3.70 (m, 1H, $CHNH_2$), 3.58−3.18 (m, 9H, $CH_2CH(OCH_2)CH_2OCH_2$), 3.01−2.88, (m, 2H, CH_2CHNH_2), 1.97−1.70 (m, 2H, NH_2), 1.60−1.41 (m, 4H, OCH_2CH_2), 1.36−1.12 (m, 36H, $CH_{2(lauryl)}$), 0.88 (t, 6H, CH_2CH_3, $J = 6.5$ Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{38}H_{68}N_3O_3$, 614.5253; found, 614.5261. Anal. Calcd for $C_{38}H_{67}N_3O_3$: C, H, N.

50. Preparation of L-Tryptophan(2-lauryloxy-3-stearyloxy)propylamide (50). Compound **50** was prepared analogously to **49** on a 0.74 mmol scale to give, after chromatography (50% EtOAc/hexane−100% EtOAc), **50** as a yellow wax (310 mg, 77.3%); R_f 0.20 (EtOAc). ^{1}H NMR (δ_H): 8.28−8.18 (m, 1H, $NH_{(indole)}$), 7.68 (d, 1H, $H_{(arom,indole)}$, $J = 7.7$ Hz), 7.61−7.49 (m, 1H, $NH_{(amide)}$), 7.36 (d, 1H, $H_{(arom,indole)}$, $J = 7.5$ Hz), 7.23−7.03 (m, 3H, $H_{(arom,indole)}$), 3.77−3.67 (m, 1H, $CHNH_2$), 3.61−3.20 (m, 9H, $CH_2CH(OCH_2)CH_2OCH_2$), 3.00−2.84, (m, 2H, CH_2CHNH_2), 1.87−1.62 (m, 2H, NH_2), 1.62−1.40 (m, 4H, OCH_2CH_2), 1.40−1.12 (m, 48H, $CH_{2(lauryl/stearyl)}$), 0.88 (t, 6H, CH_2CH_3, $J = 6.6$ Hz). HRMS: $(M + H)^{\oplus}$ calcd for $C_{44}H_{80}N_3O_3$, 698.5422; found, 698.6200. Anal. Calcd for $C_{44}H_{79}N_3O_3$: C, H, N.

51. Preparation of *N*-α-*t*-Butyloxycarbonyl-*N*-im-trityl-L-histidine Pentafluorophenyl Ester (51). To a flask containing pentafluorophenol (5 mmol, 0.92 g) and *N*-α-*tert*-butyloxycarbonyl-*N*-im-trityl-L-histidine (4 mmol, 1.99 g) under nitrogen was added CH_2Cl_2 (20 mL). A solution of EDC (5 mmol, 0.96 g) in CH_2Cl_2 (20 mL) was added dropwise, and the solution was stirred for 2 h at room temperature. It was then washed with $NaHCO_3$ (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The product was purified by column chromatography (5−20% ethyl acetate/hexane) to give **51** (1.42 g, 53.5%) as a white foam. R_f 0.85 (40% EtOAc/hexane). The product was found to be unstable by 2D TLC and was used immediately in the next step.

52. Preparation of *N*-α-Butyloxycarbonyl-*N*-im-trityl-L-histidine(2,3-dilauryloxy)propylamide (52). A flask containing **51** (400 mg, 0.63 mmol) and **28** (193 mg, 0.45 mmol) was flushed with argon. Anhydrous THF (5 mL) was added, and the reaction was refluxed at 85 °C overnight. The solvent was then removed, and CH_2Cl_2 (20 mL) was added. The solution was washed with $NaHCO_3$ (1 × 10 mL), water (1 × 10 mL), and brine (1 × 10 mL), and each of the aqueous layers was backwashed with CH_2Cl_2 (5 mL). The organic layers were combined, dried ($MgSO_4$), and evaporated under reduced pressure to give a colorless oil. The solid was purified by column chromatography (10−50% ether/hexane) to give **52** as a pale yellow oil (309 mg, 75.7%); R_f 0.25 (70% ether/hexane). ^{1}H NMR (δ_H): 7.41−7.36 (m, 1H, N=C*H*N), 7.46−7.28 (m, 9H, $H_{(arom,Trt)}$), 7.14−7.06 (m, 6H, $H_{(arom,Trt)}$), 7.06−6.95 (m, 1H, $NH_{(amide)}$), 6.63 (s, 1H, C=C*H*N), 6.44−6.30 (m, 1H, $NH_{(carbamate)}$), 4.48−4.34 (m, 1H, C*H*NH), 3.60−2.90 (m, 11H, CH_2CHNH, $CH_2CH(OCH_2)CH_2OCH_2$), 1.61−1.47 (m, 4H, OCH_2CH_2), 1.42 (s, 9H, $C(CH_3)$), 1.35−1.12 (m, 36H, $CH_{2(lauryl)}$), 0.88 (t, 6H, CH_2CH_3, $J = 6.6$ Hz). ^{13}C NMR (δ_C): 171.98 ($CO_{(amide)}$), 156.02 ($C(CH_3)_3$), 142.70 ($C_{(arom,Trt)}$), 138.74 (N=*C*HN), 137.32, 137.25 (*C*=CHN), 130.15 ($C_{(arom,Trt)}$), 128.48 ($C_{(arom,Trt)}$), 120.04 (C=*C*HN), 80.04 (*C*(CH_3)), 72.22 ($CHCH_2O$), 71.69, 71.63 ($CHCH_2OCH_2$), 70.93, 70.78 ($CHOCH_2$), 55.37 (*C*HNH), 41.17, 40.90 (NCH_2CH), 32.32 ($CH_2CH_2CH_3$), 30.72 (*C*H_2CHNH), 30.52−29.76 ($CH_{2(lauryl)}$), 28.79 ($C(CH_3)_3$), 26.51, 26.48 ($OCH_2CH_2CH_2$), 23.08 (CH_2CH_3), 14.51 (CH_2CH_3). HRMS: $(M + Na)^{\oplus}$ calcd for $C_{57}H_{86}N_4O_5Na$, 929.6513; found, 929.6496.

53. Preparation of *N*-α-Butyloxycarbonyl-*N*-im-trityl-L-histidine(2-lauryloxy-3-stearyloxy)propylamide (53). Compound **53** was prepared analogously to **52** on a 0.52 mmol scale to give, after chromatography (10−50% ether/hexane), **53** as a pale yellow oil (396 mg, 76.9%); R_f 0.25 (70% ether/hexane). ^{1}H NMR (δ_H): 7.49−7.39 (m, 1H, N=C*H*N), 7.39−7.29 (m, 9H, $H_{(arom,Trt)}$), 7.17−7.07 (m, 6H, $H_{(arom,Trt)}$), 7.07−6.98 (m, 1H, $NH_{(amide)}$), 6.65 (s, 1H, C=C*H*N), 6.37−6.26 (m, 1H, $NH_{(carbamate)}$), 4.48−4.35 (m, 1H, C*H*NH), 3.58−2.92 (m, 11H, CH_2CHNH, $CH_2CH(OCH_2)CH_2OCH_2$), 1.61−1.47 (m, 4H, OCH_2CH_2), 1.42 (s, 9H, $C(CH_3)$), 1.37−1.13 (m, 48H, $CH_{2(lauryl/stearyl)}$), 0.88 (t, 6H, CH_2CH_3, $J = 6.6$ Hz). ^{13}C NMR (δ_C): 171.72 ($CO_{(amide)}$), 156.13 ($C(CH_3)_3$), 142.14 ($C_{(arom,Trt)}$), 138.45 (N=*C*HN), 137.01, 136.88 (*C*=CHN), 130.10 ($C_{(arom,Trt)}$), 128.75, 128.64 ($C_{(arom,Trt)}$), 120.20 (C=*C*HN), 80.05 (*C*(CH_3)), 72.19 ($CHCH_2O$), 71.70 ($CHCH_2OCH_2$), 70.92, 70.80 ($CHOCH_2$), 55.62, 55.41 (*C*HNH), 41.15, 40.98 (NCH_2CH), 32.33 ($CH_2CH_2CH_3$), 30.72, 30.51 (*C*H_2CHNH), 30.51−29.76 ($CH_{2(lauryl/stearyl)}$), 28.76 ($C(CH_3)_3$), 26.51, 26.48 ($OCH_2CH_2CH_2$), 23.09 (CH_2CH_3), 14.52 (CH_2CH_3). HRMS: $(M + Na)^{\oplus}$ calcd for $C_{63}H_{98}N_4O_5Na$, 1013.7412; found, 1013.7435.

54. Preparation of L-Histidine(2,3-dilauryloxy)propylamide (54). To a flask containing TFA:TIS:water (95:2.5:2.5, 20 mL) was added **52** (300 mg, 0.33 mmol), and the solution was stirred for 1.5 h. The solvent was then removed under reduced pressure, and the resulting white solid was purified by column chromatography (CH_2Cl_2−CH_2Cl_2:MeOH:NH_3 92:7:1) to give **54** as a colorless glassy solid (141 mg, 75.8%); R_f 0.50 (CH_2Cl_2:MeOH:NH_3 92:7:1); mp 55−56 °C. ^{1}H NMR (δ_H): 7.82−7.67 (m, 1H, $NH_{(amide)}$), 7.56−7.51 (m, 1H, N=C*H*NH), 6.85 (s, 1H, C=C*H*NH), 3.71−2.90 (m, 14H, CH_2CHNH_2, $CH_2CH(OCH_2)CH_2OCH_2$, NH_2), 1.61−1.45 (m, 4H, OCH_2CH_2), 1.37−1.14 (m, 36H, $CH_{2(lauryl)}$), 0.87 (t, 6H, $H_{12''}$, $H_{12'''}$, $J = 6.6$ Hz). ^{13}C NMR (δ_C): 175.20, 175.12 ($CO_{(amide)}$), 135.68, 135.56 (N=*C*HNH), 132.64, 131.84 (*C*=CHNH), 121.26, 120.84 (C=*C*HNH), 77.22 (*C*HCH_2O), 72.25 (CH*C*H_2O), 71.65 ($CHCH_2OCH_2$), 70.80, 70.66 ($CHOCH_2$), 55.57, 55.21 ($CHNH_2$), 40.82, 40.56 (NCH_2CH), 32.30 ($CH_2CH_2CH_3$), 32.15 (CH_2CHNH_2), 30.43−29.74 ($CH_{2(lauryl)}$), 26.49 ($OCH_2CH_2CH_2$), 23.07 (CH_2CH_3), 14.49 (CH_2CH_3). HRMS: $(M + H)^{\oplus}$ calcd for $C_{33}H_{65}N_4O_3$, 565.5042; found, 565.5057.

55. Preparation of L-Histidine(2-lauryloxy-3-stearyloxy)propylamide (55). Compound **55** was prepared analogously to **54** on a 0.36 mmol scale to give, after chromatography (CH_2Cl_2−CH_2Cl_2:MeOH:NH_3 92:7:1), **55** as a white solid (207 mg, 88.9%); R_f 0.50 (CH_2Cl_2:MeOH:NH_3 92:7:1); mp 56−58 °C. ^{1}H NMR (δ_H): 7.82−7.68 (m, 1H, $NH_{(amide)}$), 7.55−7.50 (m, 1H, N=C*H*NH), 6.85 (s, 1H, C=C*H*NH), 3.72−2.90 (m, 14H, CH_2CHNH_2, $CH_2CH(OCH_2)CH_2OCH_2$, NH_2), 1.60−1.45 (m, 4H, OCH_2CH_2), 1.36−1.13 (m, 48H, $CH_{2(lauryl/stearyl)}$), 0.87 (t, 6H, CH_2CH_3, $J = 6.6$ Hz). ^{13}C NMR (δ_C): 175.11, 175.01 ($CO_{(amide)}$), 135.67, 135.51 (N=*C*HNH), 131.29, 130.39 (*C*=CHNH), 121.85, 120.81 (C=*C*HNH), 77.20 (*C*HCH_2O), 72.25 (CH*C*H_2O), 71.65 ($CHCH_2OCH_2$), 70.81, 70.67 ($CHOCH_2$), 55.47, 55.07 ($CHNH_2$), 40.85, 40.51 (NCH_2CH), 32.32 ($CH_2CH_2CH_3$), 32.18, 32.00 (CH_2CHNH_2), 30.44−29.75 ($CH_{2(lauryl/stearyl)}$), 26.51 ($OCH_2CH_2CH_2$), 23.08 (CH_2CH_3), 14.50 (CH_2CH_3). HRMS: $(M + H)^{\oplus}$ calcd for $C_{39}H_{77}N_4O_3$, 649.5983; found, 649.5996.

56. Preparation of *N*-α-Fluorenylmethoxycarbonyl-N^G-4-methoxy-2,3,6-trimethylbenzene Sulfonyl-L-arginine(2,3-dilauryloxy)propylamide (56). A flask containing *N*-α-fluorenylmethoxycarbonyl-N^G-4-methoxy-2,3,6-trimethylbenzene sulfonyl-L-arginine pentafluorophenyl ester (1.162 g, 1.5 mmol) and **28** (428 mg, 1.0 mmol) was flushed with argon. Anhydrous THF (15 mL) was added, and the reaction was refluxed at 85 °C for 1 h. The solvent was then removed, and CH_2Cl_2 (30 mL) was added. The solution was washed with $NaHCO_3$ (1 × 20 mL), water (1 × 20 mL), and brine (1 × 20 mL), and each of the aqueous layers was backwashed with CH_2Cl_2 (10 mL). The organic layers were combined, dried

($MgSO_4$), and evaporated under reduced pressure to give a colorless oil, which was purified by column chromatography (CH_2Cl_2−1% MeOH/CH_2Cl_2). This gave **56** as a pale yellow oil (750 mg, 74.0%); R_f 0.30 (3% MeOH/CH_2Cl_2). ^{1}H NMR (δ_H): 7.74 (d, 2H, $H_{(arom,Fmoc)}$, J = 7.6 Hz), 7.56 (d, 2H, $H_{(arom,Fmoc)}$, J = 7.2 Hz), 7.37 (t, 2H, $H_{(arom,Fmoc)}$, J = 7.5 Hz), 7.27 (d, 2H, $H_{(arom,Fmoc)}$, J = 7.4 Hz), 6.94−6.82 (m, 1H, N$H_{(amide)}$), 6.49 (s, 1H, $H_{(arom,Mtr)}$), 6.23−5.90 (m, 3H, N$H_{(guanidine)}$), 5.86 (d, 1H, N$H_{(carbamate)}$), 4.35 (t, 2H, CHC$H_{2(Fmoc)}$, J = 7.0 Hz), 4.25−4.10 (m, 2H, CHNH, CHCH$_{2(Fmoc)}$), 3.78 (s, 3H, OC$H_{3(Mtr)}$), 3.54−3.30 (m, 9H, CH_2CH(OCH_2)CH_2OCH_2), 3.30−3.10 (m, 2H, NHCH_2CH$_2$), 2.69, 2.61 (2s, 6H, 2C$H_{3(2,6-Mtr)}$), 2.10 (s, 3H, C$H_{3(3-Mtr)}$), 1.78−1.40 (m, 8H, NHCHCH_2CH_2, OCH$_2$CH_2), 1.30−1.10 (m, 36H, C$H_{2(lauryl)}$), 0.87 (t, 6H, CH$_2$CH_3, J = 6.6 Hz). ^{13}C NMR (δ_C): 172.05 (C$O_{(amide)}$), 158.99 ($C_{(arom,Mtr)}$), 156.54 (NHC(=NH)NH), 144.16 ($C_{(arom,Fmoc)}$), 141.69 ($C_{(arom,Fmoc)}$), 139.04 ($C_{(arom,Mtr)}$), 137.16 ($C_{(arom,Mtr)}$), 133.73 ($C_{(arom,Mtr)}$), 128.15 ($C_{(arom,Fmoc)}$), 127.50 ($C_{(arom,Fmoc)}$), 125.46 ($C_{(arom,Fmoc)}$), 125.19 ($C_{(arom,Mtr)}$), 120.34 ($C_{(arom,Fmoc)}$), 112.12 ($C_{(arom,Mtr)}$), 72.23 (CHCH$_2$O), 71.69 (CHCH$_2$OCH$_2$), 70.68 (CHOCH$_2$), 67.54 (CHCH$_{2(Fmoc)}$), 55.81 (OC$H_{3(Mtr)}$), 54.64 (CHNH), 47.53 (CHCH$_{2(Fmoc)}$), 41.00 (NHCH$_2$CH$_2$), 32.33 (CH$_2$CH$_2$CH$_3$), 30.81−29.77 (C$H_{2(lauryl)}$), 26.50 (OCH$_2$CH$_2$CH$_2$), 25.51 (NH$_2$CHCH$_2$), 25.24 (NHCH$_2$CH$_2$), 24.52 (C$H_{3(6-Mtr)}$), 23.10 (CH$_2$CH$_3$), 18.74 (C$H_{3(2-Mtr)}$), 14.52 (CH$_2$CH$_3$), 12.35 (C$H_{3(3-Mtr)}$). HRMS: (M + H)$^⊕$ calcd for $C_{59}H_{94}N_5O_7S$, 1016.6835; found, 1016.6874.

57. Preparation of *N*-α-Fluorenylmethoxycarbonyl-*N*^{G}-4-methoxy-2,3,6-trimethylbenzene Sulfonyl-L-arginine(2-lauryloxy-3-stearyl-oxy)propylamide (57). Compound **57** was prepared analogously to **56** on a 1 mmol scale to give, after chromatography (CH_2Cl_2−1% MeOH/CH_2Cl_2), **57** as a pale yellow oil (809 mg, 73.5%); R_f 0.3 (3% MeOH/CH_2Cl_2). ^{1}H NMR (δ_H): 7.73 (d, 2H, $H_{(arom,Fmoc)}$, J = 7.5 Hz), 7.56 (d, 2H, $H_{(arom,Fmoc)}$, J = 7.2 Hz), 7.37 (t, 2H, $H_{(arom,Fmoc)}$, J = 7.7 Hz), 7.26 (d, 2H, $H_{(arom,Fmoc)}$, J = 6.8 Hz), 6.96−6.82 (m, 1H, N$H_{(amide)}$), 6.49 (s, 1H, $H_{(arom,Mtr)}$), 6.25−5.96 (m, 3H, N$H_{(guanidine)}$), 5.90 (d, 1H, NH$_{(carbamate)}$, J = 6.8 Hz), 4.35 (t, 2H, CHC$H_{2(Fmoc)}$, J = 7.0 Hz), 4.25−4.10 (m, 2H, CHNH, CHCH$_{2(Fmoc)}$), 3.79 (s, 3H, OC$H_{3(Mtr)}$), 3.54−3.30 (m, 9H, CH_2CH(OCH_2)CH_2OCH_2), 3.30−3.11 (m, 2H, NHCH_2CH$_2$), 2.69, 2.62 (2s, 6H, 2C$H_{3(2,6-Mtr)}$), 2.10 (s, 3H, C$H_{3(3-Mtr)}$), 1.79−1.40 (m, 8H, NHCHCH_2CH_2, OCH$_2$CH_2), 1.30−1.10 (m, 48H, C$H_{2(lauryl/stearyl)}$), 0.87 (t, 6H, CH$_2$CH_3, J = 6.6 Hz). ^{13}C NMR (δ_C): 172.33 (C$O_{(amide)}$), 158.96 ($C_{(arom,Mtr)}$), 156.63 (NHC(=NH)NH), 144.17 ($C_{(arom,Fmoc)}$), 141.65 ($C_{(arom,Fmoc)}$), 139.01 ($C_{(arom,Mtr)}$), 137.10 ($C_{(arom,Mtr)}$), 133.63 ($C_{(arom,Mtr)}$), 128.13 ($C_{(arom,Fmoc)}$), 127.50 ($C_{(arom,Fmoc)}$), 125.50 ($C_{(arom,Fmoc)}$), 125.24 ($C_{(arom,Mtr)}$), 120.36 ($C_{(arom,Fmoc)}$), 112.13 ($C_{(arom,Mtr)}$), 72.18 (CHCH$_2$O), 71.66 (CHCH$_2$OCH$_2$), 70.75 (CHOCH$_2$), 67.56 (CHCH$_{2(Fmoc)}$), 55.80 (OC$H_{3(Mtr)}$), 54.79 (CHNH), 47.51 (CHCH$_{2(Fmoc)}$), 40.98 (NHCH$_2$CH$_2$), 32.33 (CH$_2$CH$_2$CH$_3$), 30.56−29.77 (C$H_{2(lauryl/stearyl)}$), 26.50 (OCH$_2$CH$_2$CH$_2$), 25.70 (NH$_2$CHCH$_2$), 25.44 (NHCH$_2$CH$_2$), 24.54 (C$H_{3(6-Mtr)}$), 23.10 (CH$_2$CH$_3$), 18.75 (C$H_{3(2-Mtr)}$), 14.52 (CH$_2$CH$_3$), 12.35 (C$H_{3(3-Mtr)}$). HRMS: (M + Na)$^⊕$ calcd for $C_{65}H_{105}N_5O_7SNa$, 1122.7653; found, 1122.7632.

58. Preparation of *N*^{G}-4-Methoxy-2,3,6-trimethylbenzenesulfonyl-L-arginine(2,3-dilauryloxy)propylamide (58). Compound **58** was prepared analogously to **49** on a 0.7 mmol scale to give, after chromatography (CH_2Cl_2−CH_2Cl_2:MeOH:NH_3 92:7:1), **58** as a colorless oil (501 mg, 89.9%); R_f 0.6 (CH_2Cl_2:MeOH:NH_3 92:7:1). ^{1}H NMR (δ_H): 7.71−7.61 (m, 1H, N$H_{(amide)}$), 6.52 (s, 1H, $H_{(arom,Mtr)}$), 6.42−6.22 (m, 3H, N$H_{(guanidine)}$), 3.82 (s, 3H, OC$H_{3(Mtr)}$), 3.63−3.33 (m, 9H, CH_2CH(OCH_2)CH_2OCH_2), 3.33−3.10 (m, 3H, CHNH$_2$, NHCH_2CH$_2$), 2.69, 2.61 (2s, 6H, 2C$H_{3(2,6-Mtr)}$), 2.17 (s, 3H, C$H_{3(3-Mtr)}$), 1.94−1.81 (m, 2H, NH_2), 1.69−1.40 (m, 8H, NH$_2$CHCH_2CH_2, OCH$_2$CH_2), 1.40−1.15 (m, 36H, C$H_{2(lauryl)}$), 0.87 (t, 6H, CH$_2$CH_3, J = 6.6 Hz). HRMS: (M + H)$^⊕$ calcd for $C_{43}H_{82}N_5O_6S$, 796.5986; found, 796.6028. Anal. Calcd for $C_{43}H_{81}N_5O_6S$: C, H, N.

59. Preparation of *N*^{G}-4-Methoxy-2,3,6-trimethylbenzenesulfonyl-L-arginine(2-lauryloxy-3-stearyloxy)propylamide (59). Compound **59** was prepared analogously to **49** on a 0.7 mmol scale to give, after chromatography (CH_2Cl_2−CH_2Cl_2:MeOH:NH_3 92:7:1), **59** as a white solid (559 mg, 90.7%); R_f 0.6 (CH_2Cl_2:MeOH:NH_3 92:7:1); mp 75−77 °C. ^{1}H NMR (δ_H): 7.72−7.60 (m, 1H, N$H_{(amide)}$), 6.52 (s, 1H, $H_{(arom,Mtr)}$), 6.42−6.19 (m, 3H, N$H_{(guanidine)}$), 3.82 (s, 3H, OC$H_{3(Mtr)}$), 3.62−3.35 (m, 9H, CH_2CH(OCH_2)CH_2OCH_2), 3.35−3.11 (m, 3H, CHNH$_2$, NHCH_2CH$_2$), 2.69, 2.62 (2s, 6H, 2C$H_{3(2,6-Mtr)}$), 2.17 (s, 3H, C$H_{3(3-Mtr)}$), 2.09−1.89 (m, 2H, NH_2), 1.73−1.41 (m, 8H, NH$_2$CHCH_2CH_2, OCH$_2$CH_2), 1.38−1.11 (m, 48H, C$H_{2(lauryl/stearyl)}$), 0.88 (t, 6H, CH$_2$CH_3, J = 6.4 Hz). HRMS: (M + H)$^⊕$ calcd for $C_{49}H_{94}N_5O_6S$, 880.6937; found, 880.6925. Anal. Calcd for $C_{49}H_{93}N_5O_6S$: C, H, N.

60. Preparation of L-Arginine(2,3-dilauryloxy)propylamide (60). To a flask containing a mixture of TFA, phenol, water, 1,2-ethane-dithiol, thioanisole, and TIS (81:5:5:5:2.5:1) (20 mL) was added **58** (199 mg, 0.25 mmol), and the solution was stirred for 8 h. The solvent was then removed under reduced pressure, and the product was purified by column chromatography (CH_2Cl_2−CH_2Cl_2:MeOH:NH_3 92:7:1) to give **60** as a colorless oil (120 mg, 82.2%); R_f 0.1 (CH_2Cl_2:MeOH:NH_3 92:7:1). ^{1}H NMR (δ_H): 8.01−7.88 (m, 2H, N$H_{(amide)}$, CH$_2$-CH$_2$NH), 7.18−6.92 (m, 3H, C(NH_2)NH), 3.86−3.63 (m, 3H, CHNH_2), 3.63−3.10 (m, 11H, NHCH_2CH$_2$, CH_2CH(OCH_2)CH_2-OCH_2), 1.86−1.40 (m, 8H, NH$_2$CHCH_2CH_2, OCH$_2$CH_2), 1.40−1.17 (m, 36H, C$H_{2(lauryl)}$), 0.86 (t, 6H, CH$_2$CH_3, J = 6.6 Hz). ^{13}C NMR (δ_C): 173.60 (C$O_{(amide)}$), 157.83 (NH$_2$C(=NH)NH), 72.23 (CHCH$_2$O), 71.38 (CHCH$_2$OCH$_2$), 70.71 (CHOCH$_2$), 54.04 (CHNH$_2$), 41.09 (NCH$_2$CH, NHCH$_2$CH$_2$), 32.33 (CH$_2$CH$_2$CH$_3$), 31.07 (NH$_2$CHCH$_2$), 30.39−29.78 (C$H_{2(lauryl)}$), 26.47 (OCH$_2$-CH$_2$CH$_2$), 24.97 (NHCH$_2$CH$_2$), 23.08 (CH$_2$CH$_3$), 14.49 (CH$_2$CH$_3$). HRMS: (M + H)$^⊕$ calcd for $C_{33}H_{70}N_5O_3$, 584.5490; found, 584.5479.

61. Preparation of L-Arginine(2-lauryloxy-3-stearyloxy)propylamide (61). Compound **61** was prepared analogously to **60** on a 0.25 mmol scale to give, after chromatography (CH_2Cl_2−CH_2Cl_2:MeOH:NH_3 92:7:1), **61** as a colorless oil (140 mg, 83.8%); R_f 0.1 (CH_2Cl_2:MeOH:NH_3 92:7:1). ^{1}H NMR (δ_H): 8.08−7.90 (m, 2H, N$H_{(amide)}$, CH$_2$CH$_2$NH), 7.16−6.99 (m, 3H, C(NH_2)NH), 3.73−3.62 (m, 1H, CHNH$_2$), 3.62−3.12 (m, 11H, NHCH_2CH$_2$, CH_2CH(OCH_2)CH_2OCH_2), 2.70−2.58 (m, 2H, NH_2), 1.83−1.41 (m, 8H, NH$_2$CHCH_2CH_2, OCH$_2$CH_2), 1.34−1.15 (m, 48H, C$H_{2(lauryl/stearyl)}$), 0.88 (t, 6H, CH$_2$CH_3, J = 6.6 Hz). ^{13}C NMR (δ_C): 173.63 (C$O_{(amide)}$), 157.90 (NH$_2$C(=NH)-NH), 72.22 (CHCH$_2$O), 71.42 (CHCH$_2$OCH$_2$), 70.71 (CHOCH$_2$), 54.03 (CHNH$_2$), 41.07 (NCH$_2$CH, NHCH$_2$CH$_2$), 32.33 CH$_2$CH$_2$-CH$_3$), 31.07 (NH$_2$CHCH$_2$), 30.41−29.77 (C$H_{2(lauryl/stearyl)}$), 26.48 (OCH$_2$CH$_2$CH$_2$), 25.03 (NHCH$_2$CH$_2$), 23.09 (CH$_2$CH$_3$), 14.50 (CH$_2$CH$_3$). HRMS: (M + H)$^⊕$ calcd for $C_{39}H_{82}N_5O_3$, 668.6426; found, 668.6418.

Liposome Formulation and Lipoplex Formation. DOPE (48 μL of a 12.5 mg mL^{-1} $CHCl_3$ solution, i.e., 0.6 mg, 0.8 μmol) was added to a solution containing cationic lipid component (0.6 mg, 1.2 μmol) in $CHCl_3$ (0.5 mL) in a flask under argon. One milliliter of sterilized HEPES buffer (20 mM, pH 7.8) was added to the lipid solution, and the mixture was bath-sonicated for 5 min until it became opalescent. The organic solvent was removed in vacuo, and the liposome formulation was sonicated for a further 5 min. Liposomes were then extruded through a filter with a porosity of 200 nm using a LiposoFast Basic manual extruder (Avestin Inc., Ottawa, Canada) at 37 °C.

To form the lipoplexes for transfection, plasmid DNA containing the *lac*Z gene under the control of the Elongation Factor promoter (pEFlacZ) was diluted to a concentration of 0.1 mg mL^{-1} in phosphate buffered saline (PBS). DNA solution (4 μL) was then dispensed into a 500 μL Eppendorf tube for each well of cells to be transfected. The corresponding amount of liposome formulation to be used in the experiment (typically 3−5 μL, at a total lipid concentration of 1.2 mg mL^{-1}) was then added to the DNA, and the total volume was made up to 32 μL with PBS. Each tube was then vortexed briefly and left to stand for 15−20 min.

Cell Culture and Transfection. All tissue culture media and reagents were obtained from Life Technologies, Inc. (Paisley, U.K.) unless otherwise stated. V79 (chinese hamster lung) and HT29 (human colon adenocarcinoma) cell lines were obtained from the European Collection of Cell Cultures (Sal-

isbury, Wiltshire, U.K.). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 0.5% Ciproxin Infusion (Bayer, Newbury, U.K.). Cells were incubated at 37 °C in an atmosphere with 60% relative humidity and 5% CO_2 in a TC2323 incubator (BoroLabs, Aldermaston, U.K.). Cells were dissociated and disaggregated by washing with PBS, followed by incubation with 0.25% trypsin and 1 mmol ethylenediaminetetraacetic acid in Hank's Balanced Salt Solution.

Twenty-four hours before transfection, cells were seeded into 6-well plates (35 mm well diameter). Each well received 1.5×10^5 cells and 2 mL of DMEM, and cells were then incubated for 24 h. Before transfection, wells were rinsed twice with serum free (SF) DMEM (2×0.8 mL) before receiving a final aliquot (0.8 mL) of SF DMEM. The lipoplex formulations were then taken up in 168 μL of SF DMEM, added to the cells, and incubated for a period of 6 h. SF DMEM was then replaced with 2 mL of fully supplemented DMEM, and cells were incubated for a further 18 h.

***β*-Galactosidase Assay.** For cell harvest, cells were first washed with cold PBS (2×0.5 mL), and then, 200 μL of extraction buffer (250 mM Tris-HCl, 10% v/v glycerol and 1% v/v Triton X-100, pH 7.5) was added. The cells were lysed in situ (5 min at room temperature), and the extracts were collected and clarified by centrifugation in a microfuge (5 min at 14 000 rpm). To determine the quantity of β-gal present, 80 μL of the cellular extract was mixed with 520 μL of *lac*Z buffer (1 M Na_2HPO_4 (60 mL), 1 M NaH_2PO_4 (40 mL), 1 M KCl (10 mL), 1 M $MgSO_4$ (1 mL), 1 M Tris/HCl pH 7.8 (20 mL), distilled H_2O (869 mL), ONPG (905 mg), and β-mercaptoethanol (2.5 mL)), and the mixture was left for 20−60 min.

The reaction was then stopped by addition of 1 M Na_2CO_3 (250 μL), and the intensity of the yellow color that developed was measured at a wavelength of 420 nm using a Uvikon 922 spectrophotometer (Kontron Instruments, Milan, Italy). The overall protein content of the cellular extract was determined by the Bio-Rad Protein Assay Reagent (Bio-Rad Laboratories Ltd., Munich, Germany).

Cytotoxicity Assay. Twenty-four hours before dosing with liposomes, V79 cells were dissociated and disaggregated and dispensed into 96-well plates using a Multidrop 8 channel peristaltic pump (Thermo Labsystems, Helsinki, Finland). Each of the wells received approximately 500 cells in 100 μL of medium. Cells were then incubated for 24 h, and then, 150 μL of liposome formulation was dispensed into the first well of a row. The medium and liposomes were gently agitated using a pipet, before 150 μL of the resulting mixture was removed and added to the next well. This was repeated across the row to give a dilution series. Each well was supplemented with 100 μL of medium, and the plates were then incubated for a period of 72 h. After this time, the medium was removed and the cells were fixed with a 10% solution of trichloroacetic acid. The plates were then rinsed in water, and 50 μL of dye solution (0.1% sulforhodamine B in 1% acetic acid) was dispensed per well. Excess dye agent was then washed off under a stream of 1% acetic acid, and the plates were left to dry. A total of 150 μL of 10 mM Tris/HCl (pH 8) was dispensed per well, plates were shaken for 10 min, and absorption was measured at 540 nm using a Multiskan plate reader (Thermo Labsystems, Helsinki, Finland).

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References

(1) Niculescu-Duvaz, I.; Spooner, R.; Marais, R.; Springer, C. J. Gene-Directed Enzyme Prodrug Therapy. *Bioconjugate Chem.* **1998**, *9*, 4−22.

(2) Crystal, R. G. Transfer of Genes to Humans—Early Lessons and Obstacles to Success. *Science* **1995**, *270*, 404−410.

(3) MacLachlan, I.; Cullis, P.; Graham, R. W. Progress towards a Synthetic Virus for Systemic Gene Therapy. *Curr. Opin. Mol. Ther.* **1999**, *1*, 252−259.

(4) Felgner, P. L.; Gadek, T. R.; Holm, M.; Roman, R.; Chan, H. W.; Wenz, M.; Northrop, J. P.; Ringold, G. M.; Danielsen, M. Lipofection—A Highly Efficient, Lipid-Mediated DNA-Transfection Procedure. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 7413−7417.

(5) Stamatatos, L.; Leventis, R.; Zuckermann, M. J.; Silvius, J. R. Interactions of Cationic Lipid Vesicles With Negatively Charged Phospholipid-Vesicles and Biological-Membranes. *Biochemistry* **1988**, *27*, 3917−3925.

(6) Wheeler, C. J.; Felgner, P. L.; Tsai, Y. J.; Marshall, J.; Sukhu, L.; Doh, S. G.; Hartikka, J.; Nietupski, J.; Manthorpe, M.; Nichols, M.; Plewe, M.; Liang, X. W.; Norman, J.; Smith, A.; Cheng, S. H. A Novel Cationic Lipid Greatly Enhances Plasmid DNA Delivery and Expression in Mouse Lung. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11454−11459.

(7) Ren, T.; Song, Y. K.; Zhang, G.; Liu, D. Structural Basis of DOTMA for its High Intravenous Transfection Activity in Mouse. *Gene Ther.* **2000**, *7*, 764−768.

(8) Obika, S.; Yu, W.; Shimoyama, A.; Uneda, T.; Miyashita, K.; Doi, T.; Imanishi, T. A Symmetrical and Biodegradable Cationic Lipid: Synthesis and Application for Efficient Gene Transfection. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1817−1820.

(9) Behr, J. P.; Demeneix, B.; Loeffler, J. P.; Mutul, J. P. Efficient Gene-Transfer Into Mammalian Primary Endocrine-Cells With Lipopolyamine-Coated DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 6982−6986.

(10) Solodin, I.; Brown, C. S.; Bruno, M. S.; Chow, C. Y.; Jang, E. H.; Debs, R. J.; Heath, T. D. A Novel Series of Amphiphilic Imidazolinium Compounds For in-Vitro and in-Vivo Gene Delivery. *Biochemistry* **1995**, *34*, 13537−13544.

(11) Vigneron, J. P.; Oudrhiri, N.; Fauquet, M.; Vergely, L.; Bradley, J. C.; Basseville, M.; Lehn, P.; Lehn, J. M. Guanidinium-Cholesterol Cationic Lipids: Efficient Vectors for the Transfection of Eukaryotic Cells. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9682−9686.

(12) Cooper, R. G.; Etheridge, C. J.; Stewart, L.; Marshall, J.; Rudginsky, S.; Cheng, S. H.; Miller, A. D. Polyamine Analogues of 3 β-[*N*-(*N*′,*N*′- dimethylaminoethane)carbomoyl]cholesterol (DC-Chol) as Agents for Gene Delivery. *Chem.−Eur. J.* **1998**, *4*, 137−151.

(13) Gao, X.; Huang, L. A Novel Cationic Liposome Reagent For Efficient Transfection of Mammalian-Cells. *Biochem. Biophys. Res. Commun.* **1991**, *179*, 280−285.

(14) Moradpour, D.; Schauer, J. I.; Zurawski, V. R.; Wands, J. R.; Boutin, R. H. Efficient Gene Transfer into Mammalian Cells with Cholesteryl- Spermidine. *Biochem. Biophys. Res. Commun.* **1996**, *221*, 82−88.

(15) Tang, F. X.; Hughes, J. A. Introduction of a Disulfide Bond into a Cationic Lipid Enhances Transgene Expression of Plasmid DNA. *Biochem. Biophys. Res. Commun.* **1998**, *242*, 141−145.

(16) Tang, F. X.; Hughes, J. A. Synthesis of a Single-Tailed Cationic Lipid and Investigation of its Transfection. *J. Controlled Release* **1999**, *62*, 345−358.

(17) Tang, F. X.; Hughes, J. A. Use of Dithiodiglycolic Acid as a Tether for Cationic Lipids Decreases the Cytotoxicity and Increases Transgene Expression of Plasmid DNA in vitro. *Bioconjugate Chem.* **1999**, *10*, 791−796.

(18) Felgner, J. H.; Kumar, R.; Sridhar, C. N.; Wheeler, C. J.; Tsai, Y. J.; Border, R.; Ramsey, P.; Martin, M.; Felgner, P. L. Enhanced Gene Delivery and Mechanism Studies With a Novel Series of Cationic Lipid Formulations. *J. Biol. Chem.* **1994**, *269*, 2550−2561.

(19) Floch, V.; Loisel, S.; Guenin, E.; Herve, A. C.; Clement, J. C.; Yaouanc, J. J.; des Abbayes, H.; Ferec, C. Cation Substitution in Cationic Phosphonolipids: A New Concept to Improve Transfection Activity and Decrease Cellular Toxicity. *J. Med. Chem.* **2000**, *43*, 4617−4628.

(20) Song, Y. K.; Liu, F.; Chu, S. Y.; Liu, D. X. Characterization of Cationic Liposome-Mediated Gene Transfer in vivo by Intravenous Administration. *Hum. Gene Ther.* **1997**, *8*, 1585−1594.

(21) Balasubramaniam, R. P.; Bennett, M. J.; Aberle, A. M.; Malone, J. G.; Nantz, M. H.; Malone, R. W. Structural and Functional Analysis of Cationic Transfection Lipids: The Hydrophobic Domain. *Gene Ther.* **1996**, *3*, 163−172.

(22) Byk, G.; Wetzer, B.; Frederic, M.; Dubertret, C.; Pitard, B.; Jaslin, G.; Scherman, D. Reduction-Sensitive Lipopolyamines as a Novel Nonviral Gene Delivery System for Modulated Release of DNA with Improved Transgene Expression. *J. Med. Chem.* **2000**, *43*, 4377−4387.

(23) Byk, G.; Dubertret, C.; Escriou, V.; Frederic, M.; Jaslin, G.; Rangara, R.; Pitard, B.; Crouzet, J.; Wils, P.; Schwartz, B.; Scherman, D. Synthesis, Activity, and Structure−Activity Relationship Studies of Novel Cationic Lipids for DNA Transfer. *J. Med. Chem.* **1998**, *41*, 224−235.

(24) Floch, V.; Audrezet, M. P.; Guillaume, C.; Gobin, E.; Le Bolch, G.; Clement, J. C.; Yaouanc, J. J.; Des Abbayes, H.; Mercier, B.; Leroy, J. P.; Abgrall, J. F.; Ferec, C. Transgene Expression Kinetics after Transfection with Cationic Phosphonolipids in Hematopoietic Nonadherent Cells. *Biochim. Biophys. Acta-Biomembr.* **1998**, *1371*, 53−70.

(25) Carruthers, N. I.; Spitler, J. M.; Wong, S. C.; Blythin, D. J.; Chen, X.; Shue, H. J.; She, H. S.; Lee, J. F.; Rizzo, C.; Ting, P. C.; West, R. E. Synthesis of a Series of Sulfinic Acid Analogues of GABA and Evaluation of Their GABA(A) Receptor Affinities. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3059−3064.

(26) Guivisdalsky, P. N.; Bittman, R. Regiospecific Opening of Glycidyl Derivatives Mediated By Boron-Trifluoride−Asymmetric-Synthesis of Ether-Linked Phospholipids. *J. Org. Chem.* **1989**, *54*, 4637−4642.

(27) Aoki, T.; Poulter, C. D. Archaebacterial Isoprenoids−Synthesis of 2,3-Di-O-Phytanyl-Sn-Glycerol and Its 1,2-Isomer. *J. Org. Chem.* **1985**, *50*, 5634−5636.

(28) Thompson, D. H.; Svendsen, C. B.; Dimeglio, C.; Anderson, V. C. Synthesis of Chiral Diether and Tetraether Phospholipids−Regiospecific Ring-Opening of Epoxy Alcohol Intermediates Derived From Asymmetric Epoxidation. *J. Org. Chem.* **1994**, *59*, 2945−2955.

(29) Ing, H. R.; Manske, R. H. F. A Modification of the Gabriel Synthesis of Amines. *J. Chem. Soc.* **1926**, 2348−2351.

(30) Kisfaludy, L.; Schon, I. Preparation and Applications of Pentafluorophenyl Esters of 9-Fluorenylmethyloxycarbonyl Amino-Acids For Peptide-Synthesis. *Synthesis* **1983**, 325−327.

(31) Sheehan, J. C.; Cruickshank, P. A.; Boshart, G. L. A Convenient Synthesis of Water-Soluble Carbodiimdes. *J. Org. Chem.* **1961**, *26*, 2525−2528.

(32) Pearson, D. A.; Blanchette, M.; Baker, M. L.; Guindon, C. A. Trialkylsilanes as Scavengers for the Trifluoroacetic-Acid Deblocking of Protecting Groups in Peptide-Synthesis. *Tetrahedron Lett.* **1989**, *30*, 2739−2742.

(33) Zabner, J.; Fasbender, A. J.; Moninger, T.; Poellinger, K. A.; Welsh, M. J. Cellular and Molecular Barriers to Gene-Transfer by a Cationic Lipid. *J. Biol. Chem.* **1995**, *270*, 18997−19007.

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