Bioconjugate Chemistry

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Combinatorial Synthesis and High-Throughput Screening of Alkyl Amines for Nonviral Gene Delivery

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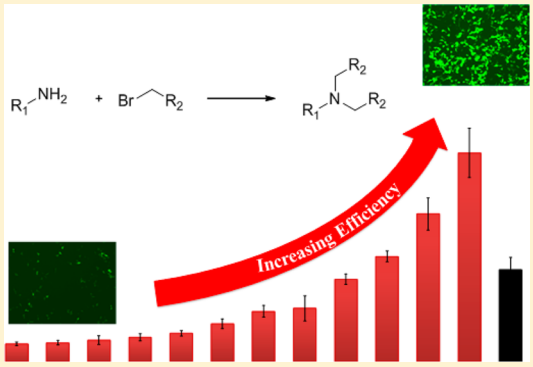
Supporting Information

ABSTRACT: Efficient delivery of plasmid DNA and siRNA into cells is essential for biological and biomedical research. Although significant efforts have been made to develop efficient nonviral vectors, such as cationic lipids and polymers, most of the vectors require multistep synthesis, which complicates both fast structural optimizations and combinatorial synthesis of such vectors. Here, we present a facile, single-step method based on an alkylation of amines, allowing for the fast parallel synthesis of libraries of cationic lipid-like molecules (lipidoids). We exploited the method to synthesize 200 lipidoids, which were screened for their transfection efficiency in HEK293T cells. The screen resulted in about 2% of new lipidoids capable of efficient cell transfection similar or higher than the efficiency of Lipofectamine 2000. In addition, we observed an enhancement of cellular transfection by combining single- with double-chain lipidoids, which was attributed to the different roles of the single- and double-tailed lipids in the mixed liposomes.



INTRODUCTION

Chemical reagents that efficiently deliver nucleic acids into cells have revolutionized biological research since the calcium phosphate transfection process was first described nearly 40 years ago.[1] Chemically based transfection reagents include cationic lipids,[2−6] cationic linear and branched polymers,[7−13] dendrimers,[14−18] cationic proteins/peptides,[19−22] and inorganic nanoparticles.[23−28] Another commonly used nucleic acid delivery system is based on viral vectors.[29−34] The types of nucleic acids most commonly used for cell transfections are plasmid DNA molecules harboring a gene of interest or small double-stranded RNA molecules, termed small interfering RNAs (siRNAs), that specifically interact with and degrade the complementary cellular mRNA. Cellular transfection is therefore used for both gain-of-function as well as loss-of-function genetic experimentation, allowing valuable insights to gene function. *In vivo* cellular gene delivery via specially designed vectors also shows high potential for therapeutic applications.

Although viral vectors are the most common gene delivery reagents in clinical trials,[35] lipid-based vectors are most often used for *in vitro* delivery of nucleic acids into cells. Several commercialized cationic lipids are widely used as transfection reagents, for example, *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA) (in Lipofectin)[36,37] and *N*,*N*-dimethyl-*N*-[2-(sperminecarboxamido)ethyl]-2,3-bis-(dioleyloxy)-1-propaniminium pentahydrochloride (DOSPA) (in Lipofectamine).[2] Such cationic lipids are usually synthesized via multistep synthesis routes, requiring protecting groups and excessive purifications,[38] thus limiting fast structural optimizations. Simple one- or two-step synthetic methods leading to lipid-like molecules could significantly increase the rate of discovery of novel efficient gene-delivery systems. So far only few such methods permitting combinatorial synthesis of lipids have been reported. For example, Anderson et al. described two methods based on aza-Michael addition and amine addition to epoxides to synthesize lipid-like molecules for siRNA delivery.[39−43] Recently, we described a two-step method based on thiol-yne chemistry for the combinatorial synthesis of cationic lipids for gene delivery applications.[44]

Here we demonstrate that simple alkylation of amines with long alkyl halides can be used to create lipid-like molecules capable of cell transfection. We show an application of this method for a fast combinatorial synthesis of 200 lipid-like molecules. The produced library was used in a high-throughput cell transfection screen, which resulted in identification of about 2% of the synthesized lipidoids possessing transfection efficiencies similar to or higher than that of Lipofectamine 2000 (L2K).

EXPERIMENTAL PROCEDURES

Materials. Ethanol (99%), 1-(2-aminoethyl)pyrrolidine (98%), 1-(2-aminoethyl)pyrrolidine (99%), *N*,*N*-diethylethylenediamine (99%), 3-(diethylamino)propylamine (99%), 3,3′-

Received: March 28, 2013
Revised: July 23, 2013
Published: August 5, 2013

diamino-*N*-methyldipropylamine (96%), 1-bromtetradecane (98%), 1-ioddodecane (98%), 1-iodhexadecane (98%), 1-bromoctadecane (98%), 1-bromononane (99%), and 1-iodooctane (98%) were purchased from Sigma-Aldrich (Germany). 3-(Dimethylamino)-1-propylamine (98%) was purchased from Fluka (Germany). Tris(2-aminoethyl)amine (97%), 1,4-bis(3-aminopropyl)piperazine (98%), and phenethylamine (99%) were purchased from Alfa Aesar (Germany). 1,3-Diaminopropane (99%), diethylenetriamine (98%), ethylenediamine (99%), 3-(methylamino)propylamine (99%), 1,8-diamino-3,6-dioxaoctane (99%), ethanolamine (99%), 1-amino-2-methoxyethane (98%), *N*-(2-hydroxyethyl)-1,3-diaminopropane (99%), 2-(2-aminoethylamino)ethanol (98%), 2-piperazin-1-ylethylamine (98%), 1,2-diaminocyclohexane (99%), 1-iododecane (99%), 1-bromoundecane (97%), 1-iodohexane (99%), and 1-iodoheptane (97%) were purchased from Merck (Germany). Plasmid DNA mixture consisting of 67.5 ng of pCS2+β-galactosidase and 7.5 ng of pEGFP-1 (Clontech, Germany) was used per well of a 96-well plate. 1,2-Di(9*Z*-octadecenoyl)-*sn*-glycero-3-phosphoethanolamine (DOPE) was bought from Gorden Pharma Switzerland LLC (Liestal, Switzerland). Lipofectamine (L2K) was purchased from Life Technologies (Germany).

Library Synthesis. Cs_2CO_3 (1.5*n* equiv, where *n* is the number of NH bonds present in the corresponding amine) in THF (2 mL) was placed in a 20-mL vial. An amine (1 equiv, 0.25 mmol) and alkyl halide (*n* equiv) were added to the vial. The resulting mixture was shaken (180 rpm) at 40 °C for 20 h. After cooling, the solvent was removed under reduced pressure, followed by addition of 1 mL of *n*-hexane to the residue. The resulting mixture was transferred to a 1-mL Eppendorf tube and centrifuged at 13,000 rpm. Supernatant was transferred to a new 20-mL vial. The solvent was again removed under reduced pressure to give crude product as a mixture of mono- and multialkylated amines that were further used without additional purification.

ESI-MS and HR-MS Characterization. Electrospray ionization mass spectrometry (ESI-MS) was performed using an API 4000 Quadrupol mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo V electrospray ion source (TurboIonSpray probe, Applied Biosystems) operating in positive ion mode at a source temperature of 400 °C. Nitrogen was used as nebulizer, curtain, collision, and auxiliary gas. Instrument controlling and data acquisition were carried out using the Analyst Software V 1.4 (Applied Biosystems, Foster City, CA, USA). All parameter settings were optimized by flow injection experiments with standard solutions infused into the mass spectrometer using a syringe pump (Harvard Apparatus Inc., South Natick, USA) at an infusion flow rate of 10 μL/min. Lipidoids were dissolved in methanol and tested in ESI-MS at 5 μg/mL concentration. High-resolution mass spectra (HR-MS) were recorded using the Bruker ICR APEX-QE, Vacuum Generators ZAB-2F, Finnigan MAT TSQ 700 and JEOL JMS-700.

NMR Characterization. ^{1}H NMR spectra were recorded at room temperature on the Bruker DRX-500 (500 MHz). Unless stated otherwise, all spectra were recorded in deuterochloroform purchased from Sigma-Aldrich. All chemical shifts are given in ppm (δ) units relative to tetramethylsilane (singlet δ_H = 0.00). Calibration was achieved using the residual solvent signal of chloroform at δ_H = 7.27. Analyses followed first order and the following abbreviations were used throughout the text: s = singlet, br. s = broad singlet, d = doublet, t = triplet, q = quartet, quin = quintet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet. Coupling constants (J) are given in hertz (Hz).[13]C NMR spectra were recorded as ^{1}H-decoupled spectra at room temperature on the Bruker DRX-500 (125 MHz). Spectra were calibrated to the signal of $CDCl_3$ (central line of triplet δ_C = 77.00).

Preparation of Cationic Liposomes in the Initial Screen. A 200 μL solution of DOPE (0.0067 mol/L) and 100 μL of a solution of lipidoid (0.0175 mol/L) in ethanol were mixed and vortexed for 10 s. Then, 700 μL of a 200 mM sodium acetate buffer (pH = 5) was added, and the solution was vortexed for 30 s.

Preparation of Cationic Liposomes for the Optimization of Hits. Stock solutions of DOPE (7.44 mg/mL, 0.01 mol/L) and lipidoid (0.01 mol/L) were made in absolute ethanol. Stock solutions of DOPE and lipidoids were mixed to produce 300 μL solutions with three different lipid/DOPE mole ratios (2:1, 1:1, and 1:2) in Eppendorf tubes, followed by vortexing for 10 s and mixing with 700 μL of 200 mM sodium acetate buffer (pH = 5). The solution was then vortexed again for 10 s.

Preparation of Cationic Liposomes To Study Synergistic Effects. Stock solutions of DOPE (7.44 mg/mL, 0.01 mol/L) and lipidoids 1C16-D (0.01 mol/L) and 1C16-S (0.01 mol/L) were made in absolute ethanol. 1C16-D and 1C16-S were first mixed at four different mole ratios (3:1, 2:1, 1:1, 1:2) to produce solutions of combined lipidoids. The final molar concentration of the lipidoids and DOPE were the same as the concentration of cationic liposomes for the optimization of hits. Stock solutions of DOPE and combined lipidoids were further mixed in Eppendorf tubes to produce 300 μL of solutions containing lipidoids/DOPE mixtures of 1:1 mole ratios, followed by vortexing for 10 s and mixing with 700 μL of 200 mM sodium acetate buffer (pH = 5). The solution was then vortexed again for 10 s before using as a transfection reagent.

Dynamic Light Scattering. Dynamic light scattering (DLS) measurement was performed using the Malvern Zetasizer Nano ZS (Malvern, Germany). Cationic liposome samples were prepared by diluting 32 μL of liposomes with 768 μL of 50 mM NaOAc/HOAc buffer (pH = 5). Lipoplex samples with different DNA amounts (75 ng, 150 ng, and 200 ng DNA per well of a 96-well plate) were prepared as follows: (a) 16 μL of liposome solutions was diluted with 384 μL of 50 mM NaOAc/HOAc buffer (pH = 5), and 30 μL of DNA (GFP/LacZ = 1:10; 0.1 μg/μL) was diluted with 370 μL of 50 mM NaOAc/HOAc pH = 5 buffer; (b) 16 μL of liposomes was diluted with 384 μL of 50 mM NaOAc/HOAc pH = 5 buffer, and 60 μL of DNA (GFP/LacZ = 1:10; 0.1 μg/μL) was diluted with 340 μL of 50 mM NaOAc/HOAc pH = 5 buffer; (c) 16 μL of liposomes was diluted with 384 μL of 50 mM NaOAc/HOAc pH = 5 buffer, and 80 μL of DNA (GFP/LacZ = 1:10; 0.1 μg/μL) was diluted with 320 μL of 50 mM NaOAc/HOAc pH = 5 buffer. Diluted liposomes and DNA solutions were mixed to form 800 μL of lipoplex solutions. Particle size (hydrodynamic diameter) and surface charge (ζ-potential) were measured sequentially by using a standard operating procedure. The particle size was presented as the z-average diameter, which is the intensity-weighted mean hydrodynamic size of particles measured by DLS.

***In Vitro* Transfection.** For the HEK293 cell transfection screen, 0.4 μL of liposomal reagent, 75 ng of plasmid DNA (67.5 ng pCS-LacZ and 7.5 ng pCMV-EGFP), and 4 × 10^4 cells

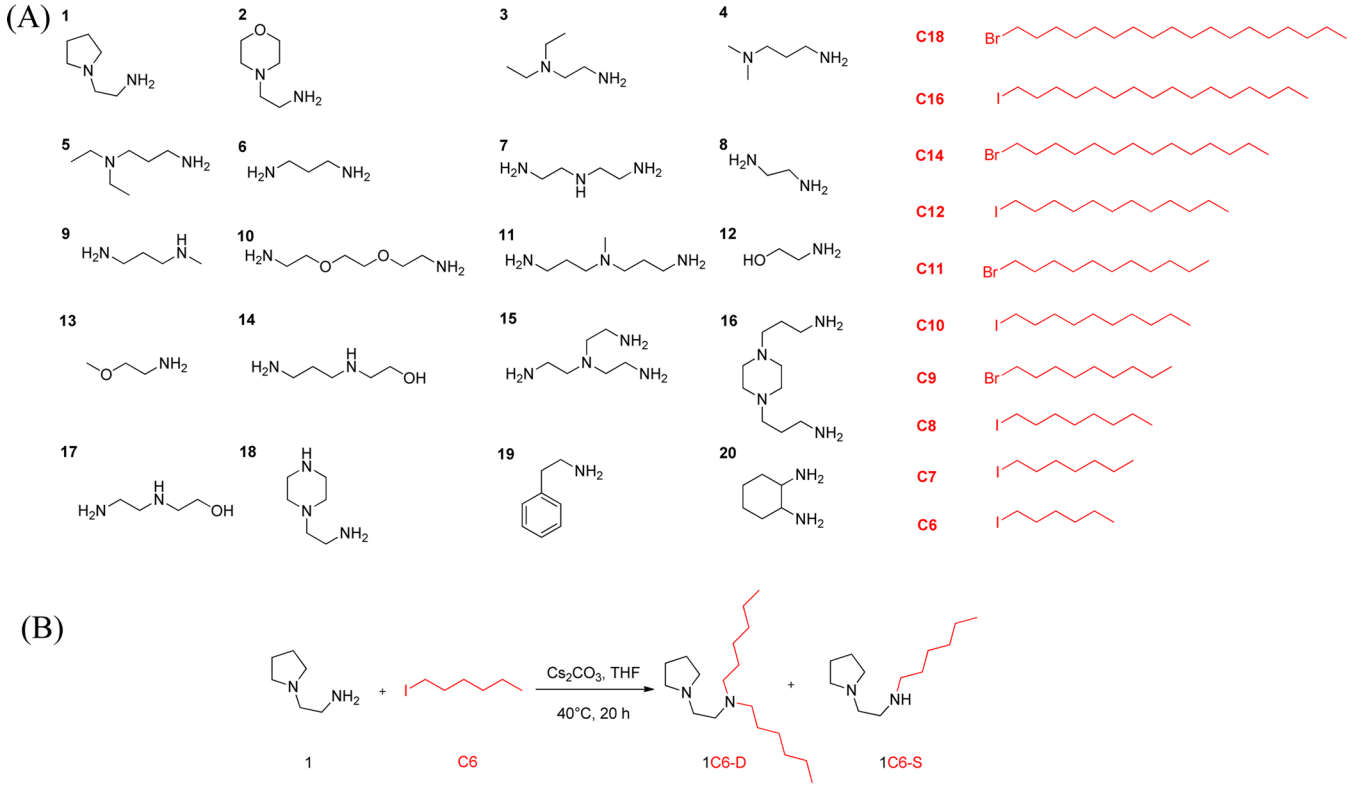


Figure 1. Combinatorial synthesis of lipidoids via alkylation of amines. (A) Twenty amines and 10 alkylhalides (C6−C12, C14, C16, and C18) were used in the synthesis. (B) Scheme of the synthesis of one typical lipidoid from the library.

were used per well in 96-well plates using a one-step protocol that combines cell plating and transfection. Liposomal reagent and DNA were each diluted in a final volume of 10 μL of 50 mM sodium acetate buffer (pH 5.0), combined, and mixed with pipet action to give 20 μL of lipoplex solution. After lipoplex incubation at room temperature for 30 min, 80 μL of a freshly trypsinized cell suspension in DMEM supplemented with 10% FCS was added, mixed gently with a pipet, and transferred to a 96-well. After 16 h of incubation at 37 °C in 5% CO_2, Hoechst 33342 was added to a final concentration of 1 μg/mL by pipetting 10 μL of a 10 μg/mL solution. After a further 30 min of incubation to allow staining of DNA within nuclei, cells were imaged on an Olympus IX81 automated fluorescent microscope. Images for bright field, Hoechst, and GFP were acquired. Cell transfection efficiencies were estimated using CellProfiler[45] after counting the number of GFP positive cells and dividing by the total number of cells (nuclei). Relative transfection efficiency was determined by dividing the transfection efficiencies of lipidoids with that of L2K.

RESULTS AND DISCUSSION

Synthesis of Alkylamine Lipidoids. In order to produce a library of lipidoids, parallel synthesis was performed using 20 different amines and 10 alkyl halides (C6−C12, C14, C16, C18) (Figure 1A). Direct alkylation was performed in the presence of Cs_2CO_3 to trap the acid produced during alkylation (Figure 1B). After 20 h of alkylation reaction, the alkylamines were isolated by extraction with hexane. Using this one-step method, a library of 200 lipidoid mixtures was synthesized in a single day. Since each lipidoid mixture contained both single and double as well as in some cases multialkylated amines, the overall number of individual lipidoids in the library came to approximately 500 molecules. All lipidoid mixtures were analyzed for their ability to delivery DNA into cells. After efficient lipidoid mixtures were identified, selected mixtures could be purified to isolate individual lipidoids possessing high transfection efficiencies.

Initial Screen. Lipidoids were screened for transfection activity by delivering eGFP plasmid into HEK293T cells in culture. A helper lipid, phosphatidylethanolamine (DOPE), was admixed with individual lipidoids in order to aid fusion with cellular membranes and enhance endosomal escape of DNA within cells.[46−48] Lipidoids were mixed with DOPE at molar ratio of 1:1 in ethanol then diluted in NaOAc/HOAc pH = 5.0 buffer to yield liposomal suspensions containing 30% ethanol and approximately 2 mg/mL total lipid. The acidic buffer was used in order to protonate the amino head groups of the lipidoids. We used two plasmids because there is a requirement to have a fixed amount of total DNA for the transfection protocol; however, this amount (75 ng per 96-well) is too much for the EGFP expression construct alone and would result in the overexpression of toxic levels of the GFP protein in cells. For this reason we "make up" to the total amount of DNA using an inert expression construct that is not toxic for cells (the pCS-LacZ, encoding the β-galactosidase gene). Cell transfection was performed using a one-step method, where freshly trypsinized and resuspended cells were added directly to the lipoplexes, followed by transfer to culture wells. The following day the nuclei of adherent cells were stained with Hoechst, and imaged automatically with a fluorescent microscope. In this assay, Hoechst staining was used to count the total cell number and also indicate cell toxicity. CellProfiler software[45] was used to calculate transfection efficiency by quantifying the ratio of GFP transfected to total cell number. The average transfection efficiencies were calculated and are

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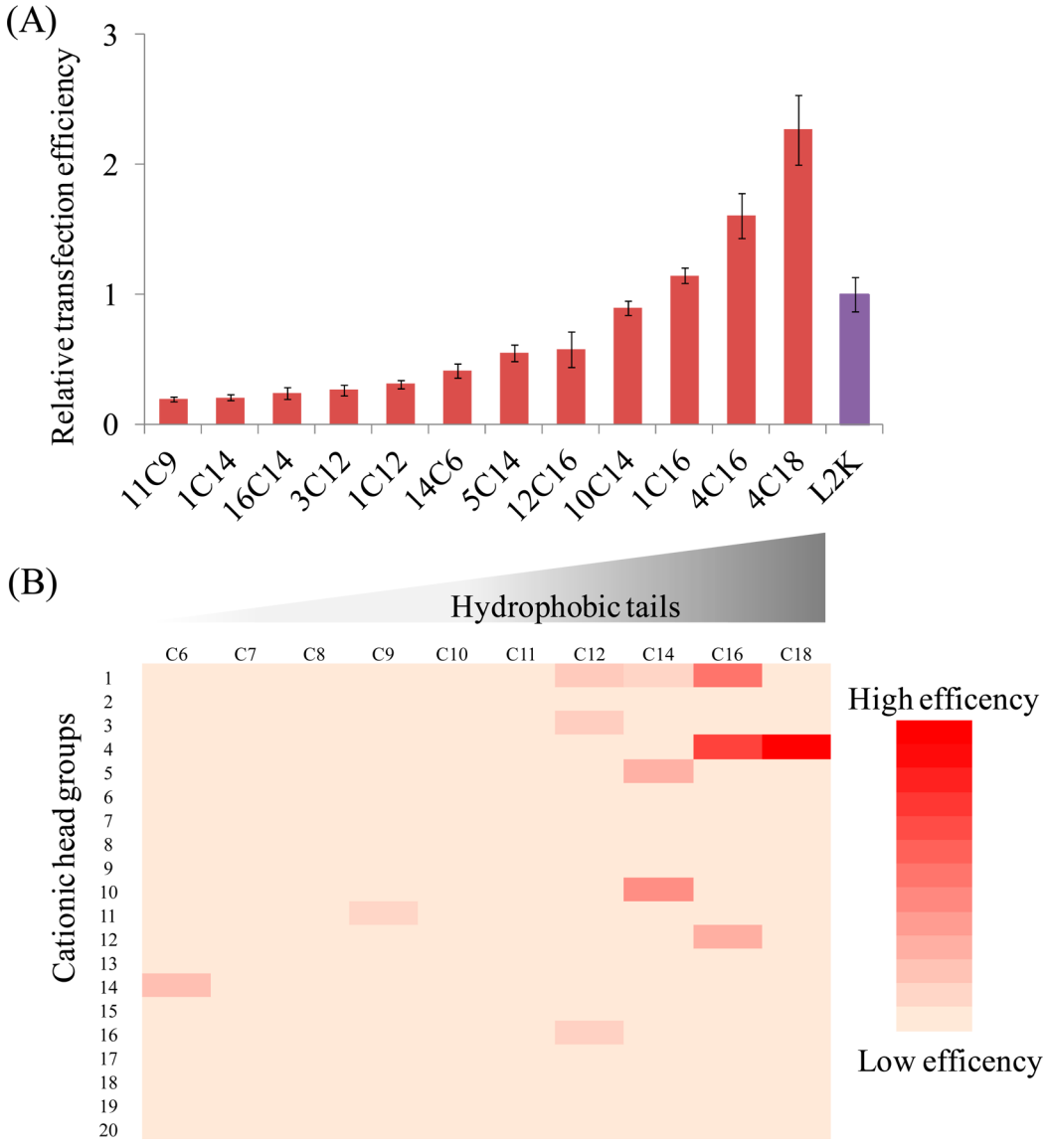

Figure 2. Results of *in vitro* cell transfection screening of the lipidoid library. (A) Graph showing relative transfection efficiency with respect to Lipofectamine 2000 of selected lipidoids with transfection efficiencies above 20%. Lipidoids/DOPE (1:1) mixtures were screened by delivering eGFP plasmid into HEK293T cells in 96-well plates. (B) Graph showing correlations between the nature of cationic head groups, length of hydrophobic tails, and the efficiency of cell transfection.

shown in Supplementary Tables S3–S6. Lipofectamine 2000 (L2K) was used as a positive control. Relative transfection efficiency was determined by dividing the average transfection efficiencies of lipidoids with that of L2K.

In this screen, 12 lipidoid mixtures showed transfection activity in HEK293T cells, 4 of which had an efficiency equal to or greater than that of L2K (Figure 2A). Notably, for these top 4 hits, increasing the length of hydrophobic tails from C14 up to C18 resulted in a corresponding increase in transfection efficiency (Figure 2B), which is in accordance with our previous study showing a particular importance of the length of hydrophobic tails for transfection efficiency.[44] With respect to the amine head groups, those lipidoids containing either 1-(2-aminoethyl)pyrrolidine (1) or 3-(dimethylamino)-1-propylamine (4) (Figure 1A) showed the highest activity. It is notable that the dimethylamine headgroup has been already used in several nonviral gene delivery systems, including both lipid-[44] and polymer-based[49] vectors, where it has shown superior performance in cell transfection. These results indicate that both the nature of cationic head groups and the length of hydrophobic tails are important determinants in transfection efficiency.

Cell Transfection Optimization. To further optimize and analyze the performance of the identified lipidoids in cell transfection, we repeated synthesis of the 12 hits identified in the initial screen. These lipids were used to prepare liposomal transfection reagents using three different lipidoid/DOPE mole ratios (2:1, 1:1, 1:2). Figure 3 shows that although 1C12, 1C16, 4C16, 4C18, and 10C14 maintain high transfection efficiency, several hits identified during the initial screen show a decreased efficiency. A difference in the ratio of mono- and disubstituted amines produced by the two synthesis reactions likely explains the variation. This was confirmed by testing the efficiency of mono- and disubstituted lipidoids mixed at different ratios (see below). Interestingly, the hits with the longest hydrophobic chains (C16 and C18) were more effective when combined

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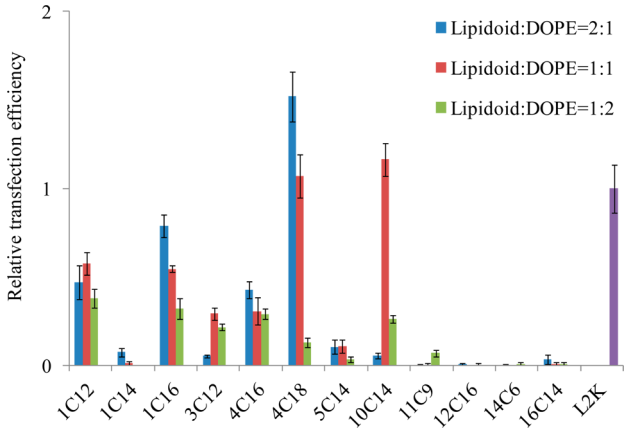

Figure 3. Relative transfection efficiencies in HEK293T cells (with respect to Lipofectamine 2000) of selected hits of three different lipidoid/DOPE molar ratios (2:1, 1:1, 1:2). Amount of DNA is 75 ng for 0.4 μL of liposome solution (400 ng total lipid), which is equivalent to a lipidoid/DNA mass ratio of 5.3:1.

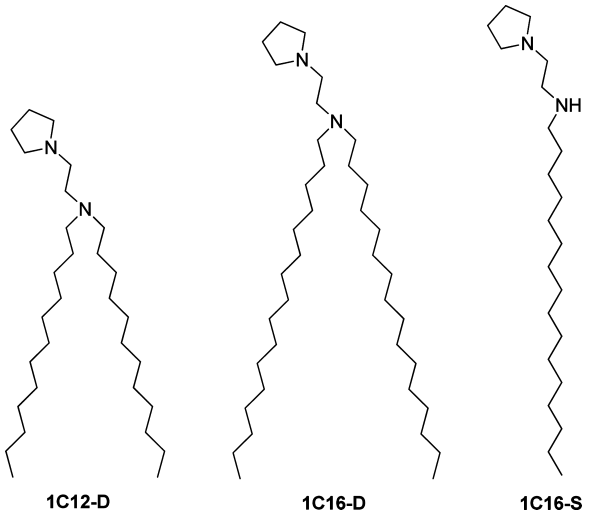

Figure 4. Structures of 1C12-D, 1C16-D, and 1C16-S lipidoids.

with a lower ratio of DOPE, which itself has two long C18 tails (blue bars, Figure 3).

To further investigate the performance of the novel alkylamines as gene delivery vectors, two lipidoids from the hits (1C12, 1C16) were purified. 1C12 was shown to contain

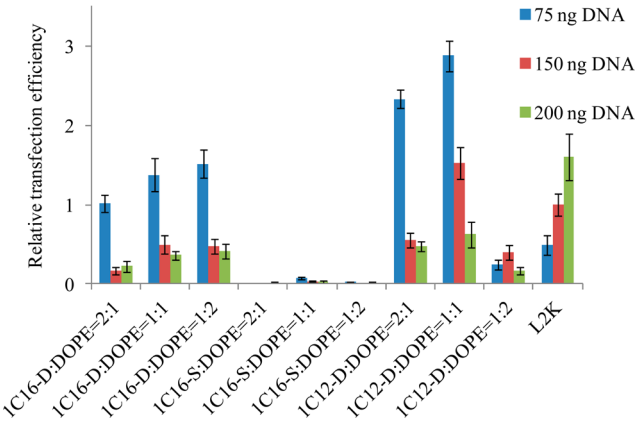

Figure 6. Optimization of *in vitro* DNA delivery into HEK293T cells by selected hits at three different lipidoid/DOPE molar ratios.

mainly the double-chain lipid (1C12-D). Purification of the 1C16 sample led to the isolation of lipidoids bearing both single (1C16-S) and double (1C16-D) alkyl chains (Figure 4). HR-MS and NMR confirmed the structures (see Supporting Information).

In order to optimize the transfection efficiency of 1C12-D, 1C16-D, and 1C16-S, three different lipidoid/DOPE ratios (2:1, 1:1, 1:2), as well as three different DNA/liposome ratios (75, 150, or 200 ng DNA per 800 ng liposome reagent, equivalent to lipidoid/DNA mass ratios of 10.7:1, 5.3:1, and 4:1, respectively) were tested and compared. Fluorescent micrographs of the transfection results are shown in Figure 5. 1C12-D and 1C16-D with two hydrophobic tails maintained significant transfection efficiency. For both 1C12-D and 1C16-D the transfection efficiency decreased with increasing the amount of DNA added to liposomes. The optimal lipidoid/DNA mass ratio was found to be 10.7:1, (75 ng plasmid DNA per 0.4 μL liposome solution per 96-well). Interestingly, 1C16-S with only one hydrophobic tail showed very low efficiency (Figure 6), suggesting that hydrophobicity of a single alkyl tail was not enough to facilitate cellular transfection. In addition, although the particle size of 1C16-D lipoplexes (liposome–DNA complexes) stabilized at 450 nm within the first 30 min after mixing the liposomes with DNA, the size of 1C16-S lipoplexes continued to increase above 1 μm (Supplementary Figure S1).

Size and Surface Charge of Lipid Particles. Liposome's size and surface charge (ζ-potential) are known to have a major

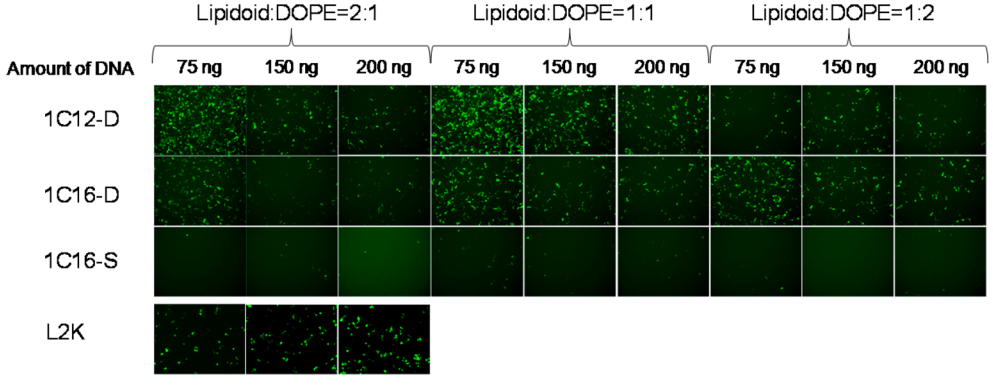

Figure 5. Fluorescent microscope images of *in vitro* DNA delivery into HEK293T cells by purified 1C12-D, 1C16-D, and 1C16-S at three different lipidoid/DOPE molar ratios.

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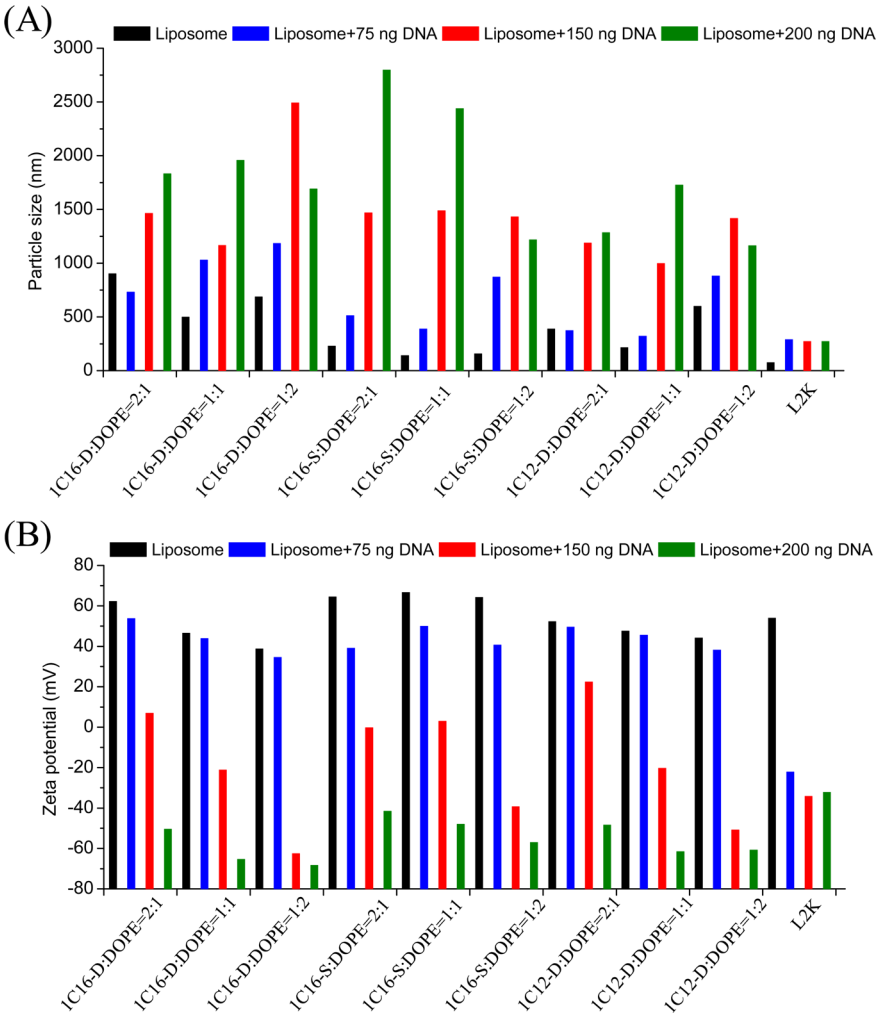

Figure 7. Particle size (A) and ζ-potential (B) of liposomes and lipoplexes formed by 1C12-D, 1C16-D, and 1C16-S at three different lipidoid/DOPE ratios (2:1, 1:1, 1:2) and different liposome/DNA ratios. The particle size was presented as the z-average diameter, which is the intensity weighted mean hydrodynamic size of particles measured by DLS.

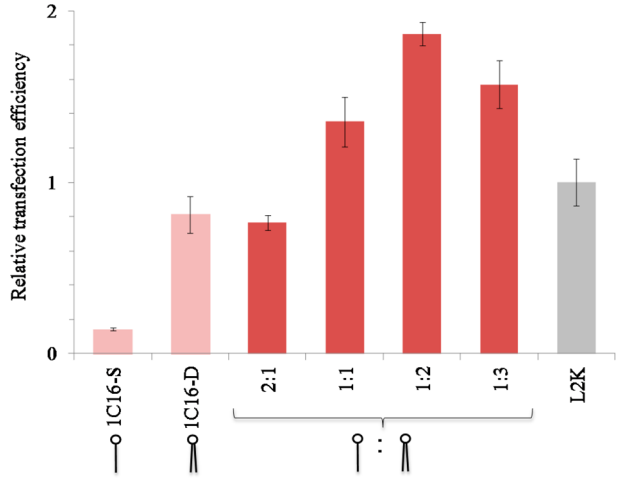

Figure 8. Synergistic effect of the combination of lipidoids with single (1C16-S) and double (1C16-D) hydrophobic tails on cell transfection efficiency. HEK293T cells in 96-well plate were transfected with 75 ng total DNA (eGFP/LacZ = 1:10) and 0.4 μL of liposome solution (lipidoid/DOPE = 1:1). This is equivalent to a lipidoid/DNA mass ratio of 5.3:1. Ratios shown below graph indicate 1C16-S/1C16-D. L2K = lipofectamine 2000.

effect on transfection. To investigate why the transfection efficiency decreased with the increase of DNA amount as well as to understand better the reason for decreased activity of the single-tailed lipid, we next analyzed the particle size and surface charge of lipid particles before (liposomes) and after the complexation with DNA (lipoplexes). The experiment was done using DLS. Figure 7A shows that the size of lipoplexes increases from 200 to 600 nm to more than 2 μm with increasing amounts of plasmid DNA. With respect to surface charge, the ζ-potentials of lipoplexes decrease from +60 mV to −60 mV upon increasing the amount of plasmid DNA (Figure 7B). Both results indicate that small particle size and high positive surface charge are two key parameters to maintain high transfection efficiency. Liposomes composed of single chained lipids were marginally smaller than those composed of double-chained lipids (black bars, Figure 7A); however, overall the size and surface charge of lipoplexes composed of the single-tailed lipidoid did not significantly differ from that of the double-tailed lipids. This means that the observed decrease in efficiency is likely not due to the size and surface charge of liposomes or lipoplexes. A reduced stability of the lipoplexes composed of the single-tailed lipids could be a possible explanation. Another conclusion from this study is that the amount of DOPE in liposomes has only minor effect on the size and surface charge

dx.doi.org/10.1021/bc400158w | Bioconjugate Chem. 2013, 24, 1543−1551

of the lipoplexes, which is also reflected in the transfection efficiencies (Figure 6).

Synergistic Increase of Transfection Efficiency. In the initial screen the lipidoid 1C16 showed higher transfection efficiency compared with that of 1C12 (Figure 2); however, subsequent analysis of the purified double-chained lipidoid species showed the reverse (Figure 6). Interestingly, the synergistic siRNA delivery was observed by combining lipid-like materials.[50] We therefore reasoned that the separation of the single- and double-alkylated 1C16 lipidoids (Figure 4) may account for the observed decrease in transfection efficiency. To test this hypothesis and to investigate whether a combination of 1C16 lipidoids with different number of hydrophobic tails has a positive synergistic effect on cell transfection, we recombined 1C16-D and 1C16-S at four different 1C16-D/1C16-S molar ratios (3:1, 2:1, 1:1, 1:2) and, for each, prepared liposomes at 1:1 lipidoid/DOPE ratio. The final molar concentration of lipidoids and DOPE was the same as for the samples prepared during optimization of the cell transfection. The results of cell transfection with these mixtures are shown in Figure 8. Interestingly, and as hypothesized, the addition of the single-tailed lipid 1C16-S to the double-chained 1C16-D significantly enhances the transfection efficiency in HEK293T cells. We next monitored the time-dependent change of the particle size (Supporting Figure S1) and ζ-potential (Supporting Figure S2) of lipoplexes during the first 30 min after mixing liposomes with plasmid DNA. The ζ-potential of liposomes formed by single-tailed lipid 1C16-S was much higher than that of double-tailed lipid 1C16-D. After adding the single-tailed lipid 1C16-S to the double-tailed lipid 1C16-D, the ζ-potential of liposome increased from 46 to 63 mV. The particle size of lipoplexes formed by 1C16-D is more stable and smaller than 1C16-S. Increasing the proportion of 1C16-D in the 1C16-S liposomes results in lipoplexes that are more stable and the final particle size of lipoplexes decreases. These results indicate that the single and double-tailed lipids play different roles in the formation of lipoplexes. The single-tailed lipid 1C16-S increases the surface charge of the liposomes and lipoplexes, while the double-tailed lipid 1C16-D forms smaller and more stable lipoplexes. The different roles of lipids also give us a hint that we may miss some hits in the initial screen.

CONCLUSIONS

We have presented a facile one-step method applicable for parallel combinatorial synthesis of new vectors for gene delivery applications. The method was used to synthesize 200 alkylamine lipidoids that were screened for their transfection efficiency leading to the identification of 12 hits showing efficiencies comparable or higher than that of Lipofectamine 2000. The hits were further optimized and characterized by measuring the size and ζ-potential of liposomes and lipoplexes. In addition, we have found that combining lipidoids with one and two hydrophobic tails has a positive synergistic effect on cellular transfection efficiency. This effect was attributed to the different roles of the lipids in the mixed liposomes: the single-tailed lipid was shown to increase the positive surface charge, and the double-tailed lipid improved the stability of the vesicles. We believe that the developed cost-effective method for combinatorial synthesis of cationic lipid-like molecules and the new insights about the effect of a liposome composition on transfection efficiency will help in the development of more efficient nonviral gene delivery systems.

ASSOCIATED CONTENT

Supporting Information

Experimental details of chemical synthesis, ^{1}H and ^{13}C NMR spectra of purified compounds, and the results of dynamic light scattering. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Peravali (ITG) for his assistance with the Olympus IX81 automated fluorescent imaging microscope, Dr. Brenner-Weiß (IFG) and his group, especially Michael Nusser, for their help on API 4000 Quadruple mass spectrometer, and Sven Stahl (INT) for his help with NMR Bruker AMX 500 spectrometer. This work was supported by funding from the Deutsche Forschungsgemeindchaft (DFG) as part of the Wnt Forschergruppe, FOR 1036 (Davidson Lab) and Helmholtz Association's Initiative and Networking Fund (grant VH-NG-621) (Levkin Lab).

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