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Charge-switching amino acids-based cationic lipids for efficient gene delivery

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ABSTRACT

A series of charge-switching amino acids-based cationic lipids **4a**–**4e** bearing a benzyl ester at the terminus of the acyl chain, but differing in the polar-head group were prepared. The physicochemical properties of these lipids, including size, zeta potential and cellular uptake of the lipoplexes formed from with DNA, as well as the transfection efficiency (TE), were investigated. The results showed that the chemical structure of the cationic head-group clearly affects the physicochemical parameters of the amino acid-based lipids and especially the TE. The selected lipid, **4c** gave 2.1 times higher TE than bPEI 25k in the presence of 10% serum in HeLa cells, with little toxicity.

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Gene therapy has been receiving much attention due to its promising to treat a gamut of diseases.[1] Successful gene therapy relies on the development of safe and efficient delivery vectors.[2] Viral vectors are the most effective vehicles for gene therapy, but their potential clinic applications were impeded owing to their immunogenicity and toxicity.[3] Therefore, the use of non-viral vectors, such as cationic lipid-based vectors that offer advantages over viral vectors in these aspects, has attraction broad attention.[4,5]

Cationic lipids are amphiphilic organic molecules and generally consist of three functional units: a hydrophilic head group, a hydrophobic tail and a linker between above two parts.[6] The positively charged headgroups play a crucial role in gene delivery in that they can bind and complex with nucleic acids, leading to DNA condensation.[7] This complexation between cationic amphiphiles and nucleic acids is mainly governed by electrostatic interactions between the positively charged amphiphiles and the negatively charged phosphate backbone of the nucleic acid. In biology, the recognition of nucleic acids by proteins involves more than electrostatic interactions.[8,9] Examination of these protein nucleic acid recognition motifs typically reveals structures rich in basic and aromatic amino acids that provide important electrostatic and stacking contributions to binding.[10–12] In addition, the utilization of amino acids to construct cationic amphiphiles has attracted increasing attentions for their good biological compatibility and biodegradability.[13,14]

Good DNA condensation is a prerequisite for efficient gene delivery. Besides the release of the DNA in cell would also largely affects the gene transfection efficacy.[9] Stimulus-responsive or functional cationic nanocarriers have attracted increasing interest for gene delivery application in recent years because they can greatly enhance intracellular release of genetic payload and usually exhibit lower cytotoxicity.[15] Up to date, many functional delivery systems with pH,[16,17] reduction,[5,18,19] enzyme[20] responsive capabilities have been developed and utilized to prepare advanced gene/drug delivery carriers. Grinstaff group reported a series of charge-reversal lipids which possesses benzyl esters at the end of alkyl chains. These lipids transform from cationic to anionic lipids intracellularly via esterase-induced hydrolysis showed high transfection activity.[9,21]

With these inspirations in mind, herein we describe a series of charge-switching amino acids-based cationic lipids. It is anticipated that these lipids could bind pDNA, and would trigger the responsive release of pDNA payload upon the interaction with intracellular esterase illustrated in Scheme 1. The physio-chemical properties, cytotoxicity, and in vitro gene transfection activity of these lipids were investigated and the results showed that these materials were potentially efficient gene vectors with relatively low cytotoxicity.

The title lipids **4** were synthesized according to the route shown in Scheme 2. Compound **2** was synthesized by conventional dicyclohexylcarbodiimide (DCC) coupling between 12-(Boc-amino)do-

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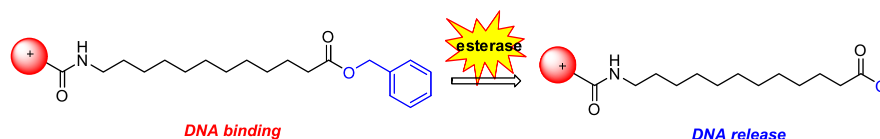
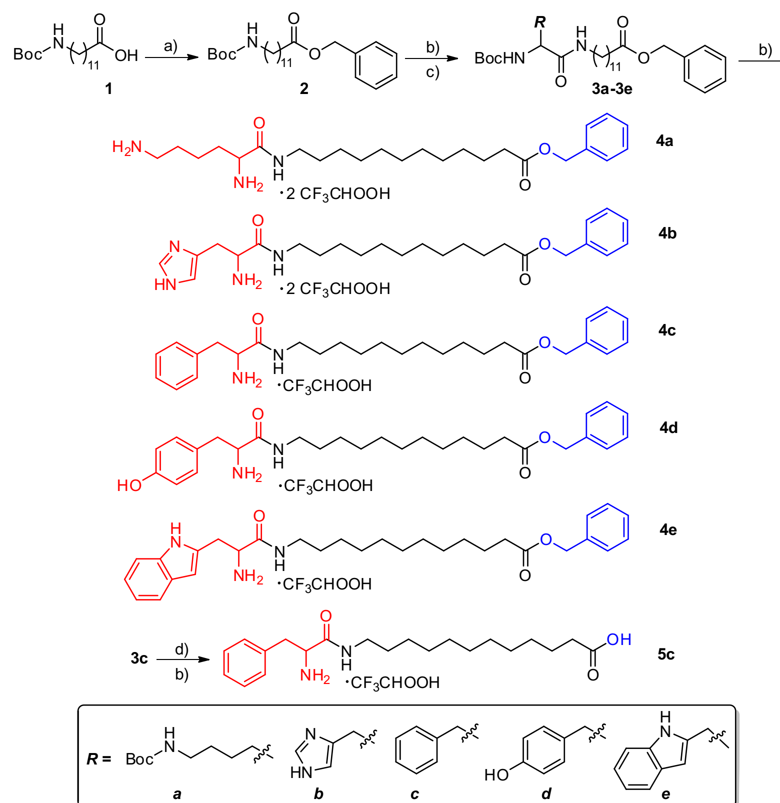


Scheme 1. Schematic diagram illustrating charge-switching cationic lipids for pDNA encapsulation and the esterase-responsive pDNA release.



Scheme 2. Synthesis route of title lipids **4a**–**e**. Reagents and conditions: (a) phenylmethanol, DCC, 4-dimethylaminopyridine (DMAP); (b) CF_3COOH, CH_2Cl_2; (c) N^{α}-Boc-L-histidine, N^{α},N^{ε}-di-Boc-L-lysine, *N*-Boc-L-phenylalanine, *N*-Boc-L-tyrosine or *N*-Boc-L-tryptophan, EDC·HCl, HOBt, *N*,*N*-diisopropylethylamine (DIEA); (d) 2 N NaOH.

decanoic acid (Boc = *tert*-butoxycarbonyl) **1** and phenylmethanol. Subsequently, precursor **3a**–**e** were synthesized by coupling the deprotection product of compound **2** with various Boc-protected amino acids in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt). Finally, target lipids **4a**–**e** were obtained by removing the Boc groups with trifluoroacetic acid in anhydrous CH_2Cl_2. To make a lipid with the same headgroup but with no benzyl ester as positive control, compound **5c** through **3c** was also synthesized (Scheme 2). All the structures of novel lipidic compounds were characterized by NMR and HR-MS.

Cationic liposomes are formed from either individual cationic lipid or more frequently from a combination of cationic lipid and neutral colipids such as DOPE, and the lipid/DOPE ratio of 1:1 was used herein. Agarose-gel retardant assays and ethidium bromide (EB) dye replacement were employed to evaluate their plasmid DNA-binding abilities.[17] Figure 1A shows **4a**–**4e** could effectively bind to DNA and retard its electrophoretic mobility. Complete DNA mobility retardation could be achieved at N/P ratio of 4 for **4a**, **4c** and 6 for **4b**, **4d** and **4e** for the liposomes formed from these lipids, respectively. In addition, ethidium bromide dye replacement for **4a**–**e** and **5c** was also employed to evaluate their plasmid DNA-binding abilities (Fig. 1B). It was shown that the fluorescent intensities of **4a**–**e** were significantly decreased with the rise of N/P ratio (from 0 to 6), indicating that EB was replaced by the act of the cationic lipid binding. However, the fluorescent intensity of **5c** was hardly changed. For basic amino acids-based lipids **4a**–**4b**, liposome formed from **4b** which utilized (L)-histidine as cationic headgroup exhibited a weaker fluorescence quenching effect (lower pDNA binding affinity) may due to the weakly basic character of imidazole moiety.[14,22] For aromatic amino acids containing lipids **4c**–**4e**, Phenylalanine-based lipid **4c** show the highest DNA binding ability. This result was consistent with agarose-gel retardant assays (Fig. 1A). Further, to determine whether the esterase would release the DNA, **4c**/DOPE/pDNA/EtBr solution (N/P = 4) was incubated with a porcine liver esterase at pH 7.4 (10 mM HEPES buffer, 150 units/mL). The intensity of fluorescence, which reflects the dissociation of the liposome from the DNA as a consequence of the hydrolysis of the terminal benzyl ester, increased slightly (Fig. 1C). DNA was released from the **4c** complex with around 60% fluorescence recovered in 2 h in the presence of esterase, while almost no fluorescence recovery was observed for **4c** complexes in the absence of esterase (Fig. 1C).

Proper size and zeta potential are important factors for liposome/DNA complexes (lipoplexes) used as gene vectors. The particle sizes of different lipoplexes were measured by dynamic light scattering (DLS) assays, and the results are shown in Figure 2A. The lipoplex sizes of **4a**, **4c** and **4d** were observed in a range of

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