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Novel imidazole-functionalized cyclen cationic lipids: Synthesis and application as non-viral gene vectors

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ABSTRACT

A series of novel 1,4,7,10-tetraazacyclododecanes (cyclen)-based cationic lipids bearing histidine imidazole group **10a**–**10e** were synthesized. These amphiphilic molecules have different hydrophobic tails (long chain, cholesterol or α-tocopherol) and various type of linking groups (ether, carbamate or ester). These molecules were used as non-viral gene delivery vectors, and their structure–activity relationships were investigated. As expected, the imidazole group could largely improve the buffering capabilities comparing to cyclen. The liposomes formed from **10** and dioleoylphosphatidyl ethanolamine (DOPE) could bind and condense plasmid DNA into nanoparticles with proper size and zeta-potentials. Comparing with Lipofectamine 2000, the formed lipoplexes gave lower transfected cells proportion, but higher fluorescence intensity, indicating their good intracellular delivering ability. Furthermore, results indicate that transfection efficiency of the cationic lipids is influenced by not only the hydrophobic tails but also the linking group. The cyclen-based cationic lipid with α-tocopherol hydrophobic tail and an ester linkage could give the highest transfection efficiency in the presence of serum.

1. Introduction

Gene therapy has gained significant attention over the past two decades as a potential method for survival against many diseases, such as cancer, diabetes, cystic fibrosis, AIDS and cardiovascular ailments, etc.[1,2] The therapy involves the delivery of a specific gene (DNA) to targeted cells to combat the disease at the level of its origin. Research efforts are currently focused on designing safe and effective gene delivery system that compact and protect oligonucleotides for gene therapy.[3] Traditionally, gene delivery systems are broadly based on either viral or non-viral mediated vectors. Viral vectors are significantly more efficient in delivering the gene, however, fundamental problems including toxicity, immunogenicity, limitations with respect to targeting of specific cell types, and potential for mutagenesis limited their potential clinic use.[4] Among physical and chemical based non-viral vectors, liposomes have particularly excellent potential for gene delivery applications.[3]

Lipids are amphiphilic organic molecules that contain a hydrophilic head and a hydrophobic tail bridged by a linkage.[5] The hydrophilic head group which may bind to the negatively charged phosphate group of nucleic acid generally has one (guanidinium,[6] amine,[7–9] pyridinium moieties[10] or imidazolium[10,11]) or more (polyamines[12–14] or amino acids[15–17]) cationic groups. The hydrophobic tail, which may be long hydrocarbon chain or steroids, represents a non-polar part that could form bilayer aggregates and interact with cellular membrane.[7] The linkage usually contains a biodegradable chemical bond (ester,[12,16,17] amide[13,17,18] or carbamoyl[15]) or a non-degradable ether bond.[8,9] The structure of cationic lipids is significant for the transfection efficiency of cationic liposomes.[3,5] Since the first report from Felgner and co-workers,[19] various systematic modifications have been performed at the hydrophobic parts, linkage regions and the head groups to optimize the DNA delivery to various mammalian cell lines. However, the available lipids were still far from the requirement of in vivo application because of their potential toxicity and relative low transfection efficiency. Consequently, the development of novel non-toxic cationic lipid gene vectors is of great importance.

In our ongoing research of designing efficient novel cationic transfection vectors, we recently demonstrated the potential of novel 1,4,7,10-tetraazacyclododecane (cyclen)-based gene delivery systems.[20] The use of cyclen as hydrophilic head group is based on its unique structural characteristics. Cyclen has four amine groups with different pK_a values (pK_a = 10.51, 9.49, 1.6 and 0.8, respectively).[21] The amines with strong basicity (pK_a = 10.51 and 9.49) can be highly protonated in neutral pH range, leading to its good cationic property and subsequent binding ability toward

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DNA. Meanwhile, the cyclic backbone which is hard to self-fold can also retain it high DNA binding affinity.[20b,22] However, the other amines on cyclen have too weak basicity to be protonated in the more acidic endosome environment (pH 5.0–6.5). In other words, cyclen lacks the amino groups having the so-called 'proton sponge effect', which might benefit the endosome escape.[23] Thus, incorporating pH sensitive group whose pK_a value is in the endosomal pH range into the cyclen-based lipid might promote endosome escape, leading to better gene transfection. Considering the appropriate pK_a of imidazole (~6),[24–26] in this report, we designed and synthesized a series of cyclen–imidazole based cationic lipids which might have good buffering ability, and their structures are shown in Figure 1. Some hydrophobic moieties with different functions were attached to the cyclen–imidazole structure, and their interactions with plasmid DNA were studied.

2. Results and discussion

2.1. Synthesis of the cyclen–imidazole based amphiphilic lipids

To explore the important relationship between molecular structure and biological function, structure-diversity oriented synthesis has been focused in current chemical biology.[3,5] As shown in Scheme 1, a series of novel cationic lipids with protonated cyclen head group and different hydrophobic tails bridged by a histidine moiety were designed and synthesized. The hydrophobic moieties **1**–**5** bearing an amino group were firstly prepared through different methods. Severel typical hydrophobic structures including long hydrocarbon chain, cholesteryl and tocopheryl which may have special properties were introduced via amide (**1**), ether (**2**, **3**), carbamate (**4**) or ester (**5**) bonds. On the other hand, cyclen–imidazole moiety **8** was prepared from tri-Boc-cyclen-acetic acid **6** and L-histidine methyl ester **7** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl), *N*,*N*-diisopropylethylamine (DIEA) and *N*-hydroxybenzotriazole (HOBt). Subsequent coupling between compound **8** and amines **1**–**5** gave the precursors **9a**–**9e**, respectively. Target lipids **10a**–**10e** were obtained by removing the Boc groups using trifluoroacetic acid in anhydrous CH_2Cl_2. For the five target molecules, **10a**–**10c** (prepared from **1** to **3**) have different hydrophobic groups, while **10c**–**10e** (prepared from **3** to **5**) are differed from their linking groups. All new compounds were characterized by NMR and HRMS.

2.2. Buffering capability

As the golden standard for the transfection efficiency of non-viral gene vectors, PEI was known as its good buffering ability and the consequent 'proton-sponge effect'.[27] To determine the buffering abilities of the synthesized lipids **10a–10e** containing imidazole moiety, acid–base titration studies were conducted over a pH range of 2–10. As shown in Figure 2, comparing with cyclen, the imidazole-attached molecules showed large improved buffering abilities which were close to PEI. For the different hydrophobic groups, long hydrocarbon chain (**10a**) might have the most

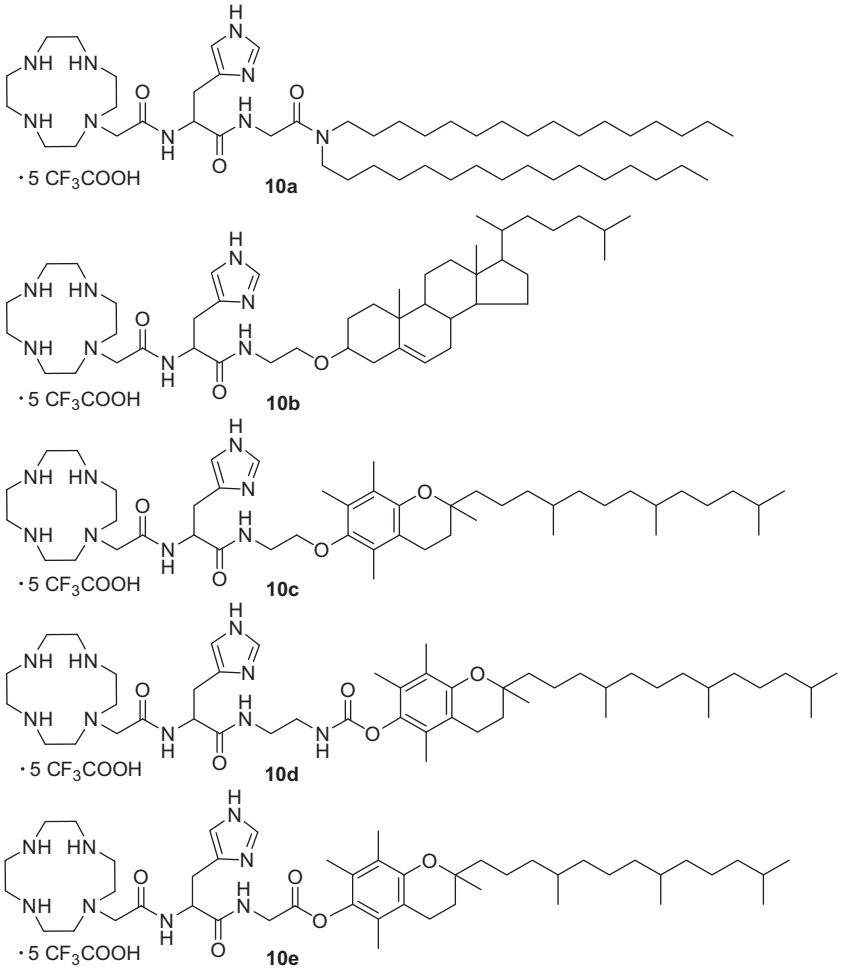

Figure 1. Structures of the title lipids **10a**–**10e**.