Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Lipoic acid functionalized amino acids cationic lipids as gene vectors

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ARTICLE INFO

Article history:
Received 15 May 2016
Revised 28 July 2016
Accepted 18 August 2016
Available online xxxx

Keywords:
Lipoic acid
Amino acids
Non-viral vector
Cationic lipid
Serum

ABSTRACT

A series of reducible cationic lipids **4a**–**4f** with different amino acid polar-head groups were prepared. The novel lipid contains a hydrophobic lipoic acid (LA) moiety, which can be reduced under reductive conditions to release of the encapsulated plasmid DNA. The particle size, zeta potential and cellular uptake of lipoplexes formed with DNA, as well as the transfection efficacy (TE) were characterized. The TE of the cationic lipid based on arginine was especially high, and was 2.5 times higher than that of a branched polyethylenimine in the presence of 10% serum.

Efficient delivery of nucleic acids for therapeutic purposes requires delivery systems able to compact the genetic material, maintain its structural integrity while overcoming various extra- and intracellular delivery barriers, and unload the genetic cargo to target cells/tissues.[1] Both viral and non-viral delivery systems have been used for gene delivery.[2] Although virus-based vectors are more efficient than non-viral delivery systems, their immunogenicity and toxicity have impeded their clinic use.[3] Among physical- and chemical-based non-viral vectors, cationic lipid is an ideal platform to carry therapeutic agent.[4] Generally, cationic lipids had a better outlook in clinic use because of its uniform properties, less immunity and low cytotoxicity. Due to their specific structure, hydrophilic head, hydrophobic tail and linker between these two domains,[5] cationic lipids can delivery both hydrophilic molecules and hydrophobic substances in their relevant construction.[4] However, the main flaw of cationic lipids is their low TE compared with viral carriers.[6]

To overcome the drawbacks of cationic lipids, numbers of methods such as polyethylene glycol (PEG) coating,[7] intra physiological conditions triggering[8] and extra corresponding[9] were established. In recent years, reduction-bioresponsive nanoparticles have appeared as one of the most promising systems to achieve targeted cytosolic drug release. As compared to other intracellular sensitive means, such as pH, enzyme and temperature, reduction-trigger have several unique advantages like fast degradation in intracellular reducing environment, and triggering cargo release in cancer cells for its higher glutathione tripeptide (GSH) concentration (100–1000 times higher than normal cells).[10] As regards the synthesis of reduction sensitive cationic lipids for gene delivery, the main strategy consisted of including a single linear disulfide bond into the molecular structure of the vector.[11] In addition, the use of a ferrocenyl unit as a reduction sensitive group was also reported.[12]

In our efforts to find novel cationic lipids with high TEs and good biocompatibility, six amino acids based cationic lipids, **4a**–**4e** (Scheme 1), containing a lipoic acid (LA) moiety at the terminus of the acyl chain were synthesized. LA is produced naturally in the human body and is common used for the treatment of various diseases including Alzheimer's diseases and diabetes.[13] We recently found that amino acid based cationic lipids functionalized with linear disulfide linkage could efficiently deliver and release DNA into cells owing to prompt degradation of disulfide bond under the intracellular reductive environment.[14] Here, we hypothesized that LA functionalized amino acids cationic lipids would facilitate DNA condensation and cellular uptake while rapid conversion of lipoyl group (hydrophobic) into dihydrolipoyl group (hydrophilic) would results in 'active' intracellular release of DNA (Scheme 1). Moreover, as opposed to linear disulfides responsible carriers, sulfocompound LA which have high ring tension may improve the cellular uptake.[15] The interaction between DNA and liposomes formed from these lipids was investigated. The results show that these materials have the potential to be efficient gene vectors.

The title lipids **4** were synthesized according to the route shown in Scheme 2. Compound **2** was synthesized by coupling *N*-Boc-1,6-diaminohexane (Boc = *tert*-butyloxycarbonyl) **1** and lipoic acid. Subsequently, precursors **3** were synthesized by coupling the deprotected product of **2** with Boc-protected amino acid

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http://dx.doi.org/10.1016/j.bmcl.2016.08.050
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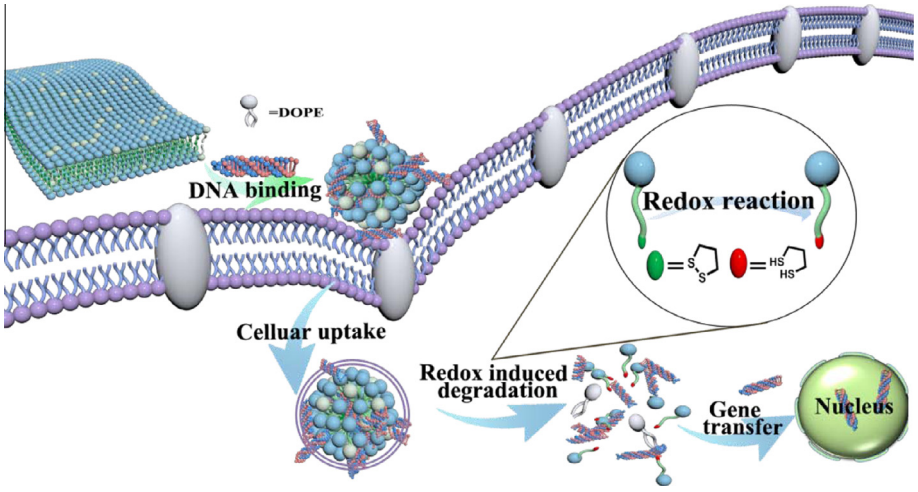
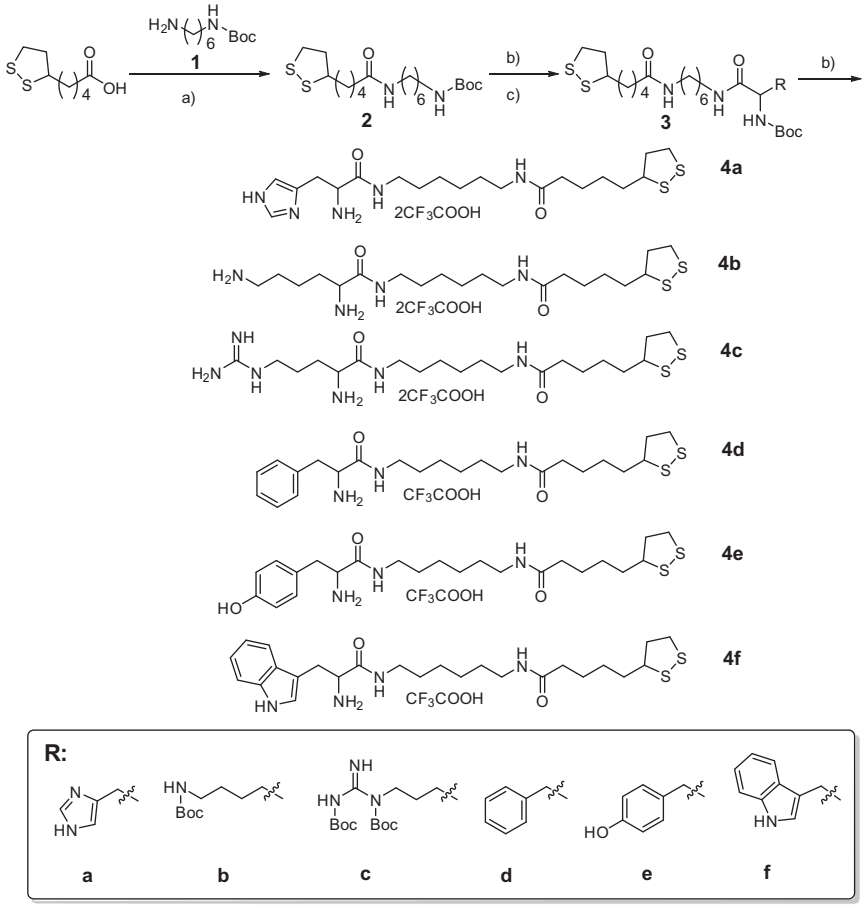


Scheme 1. Schematic diagram illustrating lipoic acid functionalized amino acid cationic lipids for pDNA encapsulation and intracellular redox induced pDNA release.



Scheme 2. Synthesis route of title lipids **4**. Reagents and conditions: (a) EDC·HCl, HOBt, *N*,*N*-diisopropylethylamine (DIEA) (b) CF_3COOH, CH_2Cl_2; (c) N^{α}-Boc-L-histidine, *N*-Boc-L-tryptophan, N^{α},N^{ε}-di-Boc-L-lysine, *N*-Boc-L-tyrosine, *N*-Boc-L-phenylalanine, or N^{α}-N^{ω}-$N'^{\omega'}$-tri-Boc-L-arginine, EDC·HCl, HOBt, DIEA.

(N^{α}-Boc-L-histidine, N^{α},N^{ε}-di-Boc-L-lysine, N^{α}-N^{ω}-$N'^{\omega'}$-tri-Boc-L-arginine, *N*-Boc-L-phenylalanine, *N*-Boc-L-tyrosine or *N*-Boc-L-tryptophan) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt). Finally, target lipids were obtained by removing the Boc groups with trifluoroacetic acid in anhydrous CH_2Cl_2. All of the lipidic compounds were structurally characterized by using NMR spectroscopy and HRMS.

Cationic liposomes are formed from either cationic lipids alone or, more frequently, from a combination of a cationic lipid and a neutral co-lipid such as 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), a lipid/DOPE ratio of 1:1 was used throughout this study. Agarose-gel retardation and ethidium bromide (EB) dye displacement assays were used to evaluate the plasmid DNA (pDNA)-binding abilities of the cationic lipids.[11b] The results presented in Figure 1 show that **4** can effectively bind to DNA