Bioorganic Chemistry 79 (2018) 334–340

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

[12]aneN$_3$-based lipid with naphthalimide moiety for enhanced gene transfection efficiency

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ARTICLE INFO

Keywords:
Non-viral vectors
Cationic lipids
Transfection efficiency
[12]aneN3
Naphthalimide

ABSTRACT

Three cationic lipids derived from [12]aneN$_3$ modified with naphthalimide (**1a**), oleic acid (**1b**) and octadecylamine (**1c**) were designed and synthesized. In vitro transfection showed that all these liposomes can deliver plasmid DNA into the tested cell lines. Among these liposomes, **1a** gave the best transfection efficiency (TE) in A549 cells, which was higher than that of lipofectamine 2000. More importantly, the TE of **1a** was dramatically increased in the presence of 10% serum. These results suggested that **1a** might be a promising non-viral gene vector, and also give further insight for developing novel high performance gene delivery agents.

1. Introduction

Gene therapy is a promising strategy in the treatment of genetic diseases and has attracted significant interest in clinical trials over the past two decades [1–8]. However, it is greatly limited due to the lack of nontoxic and high efficient gene delivery vectors [9]. Previously, viral vectors such as adenoviruses, lentiviruses and retroviruses have been successfully applied in several clinical applications because of their effective gene delivery ability [10–14], but their inherent drawbacks such as carcinogenicity, immunogenicity and difficulty of industrial production restrained them from therapeutic applications in human [15]. In contrast, non-viral vectors such as cationic lipids, cationic polymers and nanoparticles have received increased attention due to their low immunogenicity, good biocompatibility and easy production as well [16–26]. Among these non-viral vectors, cationic polymers show high transfection efficiency. However the usage of cationic polymers in applications in gene delivery is generally limited because of their high toxicity and non-degradable nature. In comparison, cationic lipids hold great potential for clinical gene therapy.

Cationic lipids as a type of important and potential non-viral gene vectors possess more advantages such as biodegradability, low cytotoxicity, structure variety and easy production, etc. [27,28]. Though these advantages of cationic lipids appear attractive, still low transfection efficiency and serum stability cannot satisfy the requirements of gene therapy. To improve the performance of cationic lipids, different kinds of aliphatic chains were used as hydrophobic domain of cationic lipids. The structure-activity correlations of cationic lipids were also studied. But these studies usually focus on changing the length and number of aliphatic chains [29]. The effects of big rigid aromatic units and flexible aliphatic chains on transfection efficiency have been seldom studied.

Recently, we developed a series of [12]aneN$_3$ cationic lipids modified with different rigid units such as naphthalimide, tetraphenylethene and coumarin [30–33]. It was found that the transfection efficiency was related to the number of rigid moieties and macrocyclic polyamine [12]aneN$_3$ units [31,33], as well as to the distance between naphthalimide moiety and [12]aneN$_3$ units [32]. Moreover, rigid naphthalimide unit, possessing strong green fluorescence and exceptional photo-stability, can be applied for monitoring the process of cellular uptake, DNA translocation and release [30]. However, these works have just focused on rigid units, while the flexible aliphatic chains had been ignored. To compare the difference between long flexible aliphatic chains and big rigid aromatic unit, we designed and synthesized three cationic lipids containing rigid naphthalimide unit (**1a**), unsaturated aliphatic chain (**1b**) and saturated aliphatic chain (**1c**). Due to good fluorescence properties of **1a**, the distribution of **1a**/

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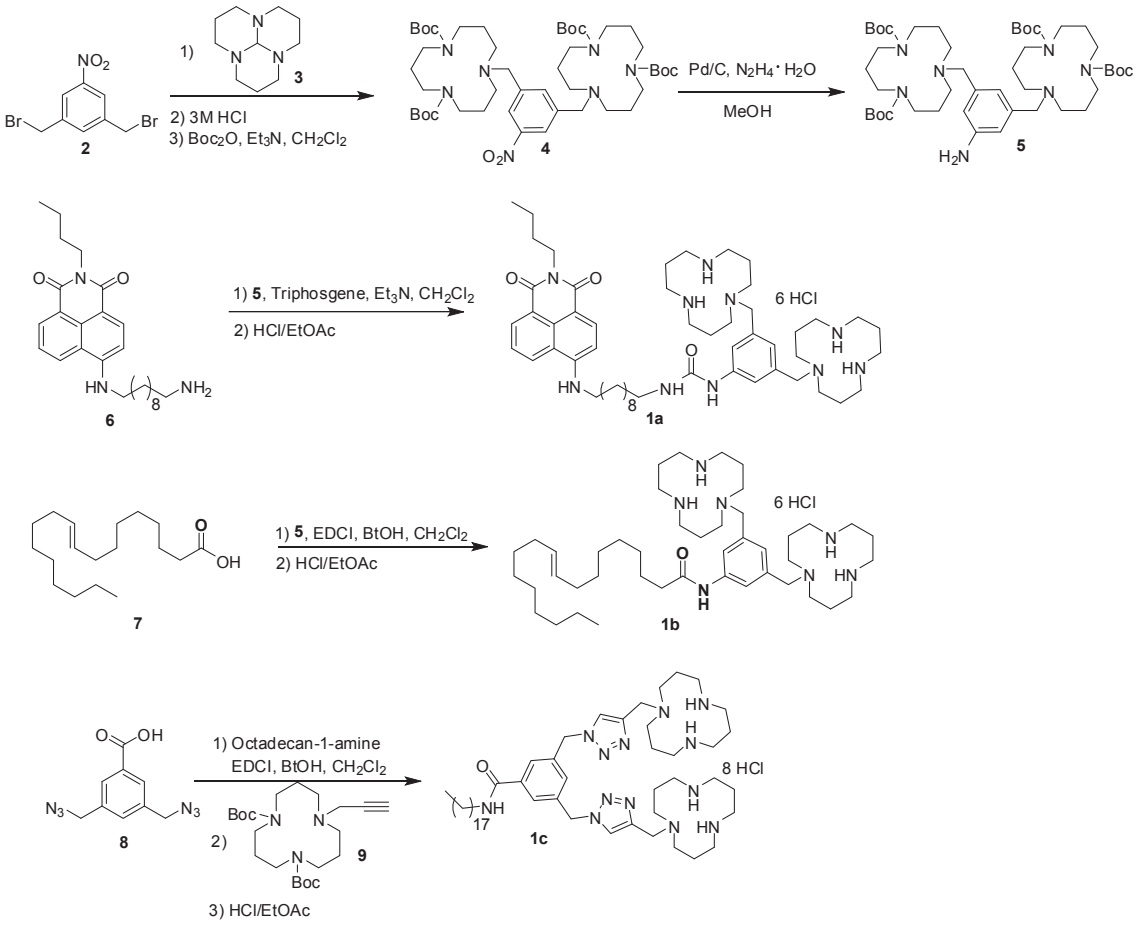
https://doi.org/10.1016/j.bioorg.2018.04.018
Received 6 March 2018; Received in revised form 16 April 2018; Accepted 24 April 2018
Available online 03 May 2018
0045-2068/



Scheme 1. The synthesis of cationic lipids **1a**, **1b** and **1c.**

DNA complexes and cellular uptake mechanism studies can be easily carried out through fluorescence microscopy. On the other hand, non-fluorescent compounds **1b** and **1c** can be applied in green fluorescent protein (eGFP) experiments. Among these lipids, **1a** gave the best transfection efficiency (TE) in A549 cells and exhibited good serum-tolerance ability, indicating that **1a** may serve as a promising non-viral gene delivery vector.

2. Results and discussion

2.1. Synthesis of target cationic lipids *1a-1c*

Target lipids **1a**, **1b** and **1c** were synthesized as shown in Scheme 1. The key intermediate Boc-protected [12]aneN$_3$ **5** was obtained through two steps. 1,3-bis(bromomethyl)-5-nitrobenzene **2** [34] was reacted with pre-[12]aneN$_3$ **3** in anhydrous acetonitrile, followed by acidification with 3 M HCl solution and protection with Boc_2O to give compound **4**. Subsequently, the reaction of **4** with hydrazine hydrate in presence of 10% Pd/C yielded the key intermediate **5**. Compounds **5** and **6** were easily linked by triphosgene under basic conditions and further de-protection of Boc under acidic conditions resulted target compound **1a**. Compound **1b** was obtained by reaction of oleic acid **7** with compound **5** in the presence of (1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride) (EDC·HCl) and *N*-hydroxybenzotriazole (HOBt). 3,5-bis(azidomethyl)benzoic acid **8** [30] was reacted with octadecylamine in dichloromethane to produce the key intermediate *N*-(3,5-bis(azidomethyl)phenyl)stearamide. Subsequently, the click reaction of the key intermediate with propargyl [12]aneN$_3$ **9** [35] and de-protection of Boc under acidic conditions resulted in target cationic lipid **1c**. All new compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, IR and MS.

2.2. Gel retardation assay

Cationic liposomes were formed from the combination of title lipids with 1,2-dioleoyl-*sn-glycero*-3-phosphoethanolamine (DOPE) in the molar ratio of 1:2. To investigate the interaction between liposomes and DNA, agarose gel electrophoresis assays were carried out in HEPES buffer (50 mM, pH 7.4) at 37 °C (Fig. 1). The results indicated that liposomes **1a** (containing rigid naphthalimide unit) and **1c** had stronger DNA retardation activity than **1b**, with complete retardation observed at concentrations of 20 μM, 20 μM and 50 μM, respectively. The weaker retardation activity of **1b** can be attributed to the presence of electron-rich unsaturated alkyl chain, which could shield the positive charge of the liposome [36].

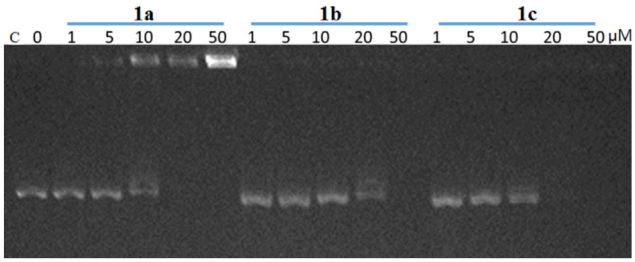


Fig. 1. Agarose gel electrophoresis of liposomes **1a–1c** complexed with DNA to form lipoplexes at different concentrations; Condition: pUC18 DNA (9 μg/mL), HEPES (50 mM, pH 7.4), 37 °C, 5 min.