Chemistry and Physics of Lipids 191 (2015) 25–37

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

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

# Studies of the physicochemical and structural properties of self-assembling cationic pyridine derivatives as gene delivery agents

Oksana Petrichenko[a,b], Martins Rucins[a], Aleksandra Vezane[c], Irena Timofejeva[c], Arkadij Sobolev[a], Brigita Cekavicus[a], Karlis Pajuste[a], Mara Plotniece[a], Marina Gosteva[a], Tatjana Kozlovska[c], Aiva Plotniece[a,*]

[a] Latvian Institute of Organic Synthesis, Aizkraukles str. 21, Riga LV-1006, Latvia
[b] Laboratory of Magnetic Soft Matter, Department of Physics, University of Latvia, Zellu str. 8, Riga LV-1002, Latvia
[c] Latvian Biomedical Research and Study centre, Ratsupites str. 1, Riga LV-1067, Latvia

ARTICLE INFO

*Article history:*
Received 18 June 2015
Received in revised form 5 August 2015
Accepted 6 August 2015
Available online 10 August 2015

*Keywords:*
Self-assembling pyridines
Synthetic lipids
Thermogravimetric analysis
Liposomes
Dynamic light scattering
DNA delivery

ABSTRACT

New amphiphilic pyridine derivatives containing dodecyloxycarbonyl substituents at positions 3 and 5 and cationic moieties at positions 2 and 6 have been designed and synthesised. Compounds of this type can be considered as synthetic lipids. The corresponding 1,4-dihydropyridine (1,4-DHP) derivatives have earlier been proposed as a promising tool for plasmid DNA (pDNA) delivery in vitro. In this work studies of the self-assembling properties of amphiphilic pyridine derivatives leading to the formation of liposomes, determination of particle size, zeta-potential and critical micelle concentration (CMC) with dynamic light scattering (DLS) measurements are described. Furthermore, thermal analysis of pyridine derivatives was performed using thermogravimetry analysis (TGA) and differential thermal analysis (DTA) as well as the ability to deliver the pEGFP-C1 plasmid DNA (that encodes GFP reporter) into the Baby hamster kidney-derived (BHK-21) cell line was used for evaluation of gene delivery properties. We have revealed that the new pyridine derivatives possessed self-assembling properties which were proved by formation of nanoparticles with the average size from 115 to 743 nm, the zeta-potentials in the range of 48–79 mV and CMC values in the range of 2–67 μM. DTA data showed that all processes were endothermic for all compounds. Additionally, we established that among the tested pyridines the representatives with *N*-methylpyrrolidinium or pyridinium moieties as cationic head-group at the positions 2 and 6 possessed higher pEGFP-C1 transfection activity into the BHK-21 cell line. Nevertheless, the obtained results indicated that correlation of the physicochemical, structural properties and gene delivery activities of the tested compounds were not completely elucidated yet. On the other hand, the synthesised pyridines as possible metabolites of promising delivery systems on the 1,4-DHP core possessed lower pDNA transfection activity than the corresponding 1,4-DHP amphiphiles.



## 1. Introduction

Gene therapy relies on successful delivery of therapeutic DNA into the nuclei of target cells. The first successful in vitro delivery of DNA by liposome-mediated gene transfer was demonstrated in 1980 (Fraley et al., 1980). Based on these initial findings, the search and studies for optimal delivery agents have been continued into various directions. However, understanding of structure–activity relationships (SAR) is limited despite the development of an important number of non-viral delivery systems varying in chemical functionalisation of molecules and compositions of reagents (van Gaal et al., 2011). Over the past decades, development of new non-viral vectors as gene delivery systems and liposomal drug delivery systems has resulted in elaboration of various nanopharmaceutical applications and become a competitive field for different research groups. Interest is growing towards the design of synthetic cationic lipid-like compounds as potential drug and gene delivery agents for transfer of genetic materials including high molecular weight DNA molecules into cells (Felgner et al., 1987) and for diagnostic applications (Marqués-Gallego and de Kroon, 2014). These lipid-like delivery systems are relatively non-toxic and non-immunogenic, moreover it is easy to use and produce them on a large scale (Al-Dosari and Gao, 2009; Guo and Huang, 2012; Lasic and Templeton, 1996; Margus et al., 2012; Rea et al., 2009).

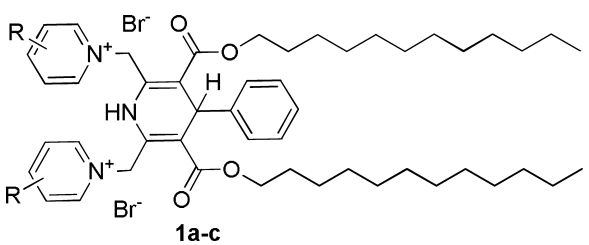
* Corresponding author.
*E-mail address:* aiva@osi.lv (A. Plotniece).

http://dx.doi.org/10.1016/j.chemphyslip.2015.08.005
0009-3084/

Lipoplexes formation of cationic non-viral vectors with negatively charged DNA is an important requirement for successful gene delivery. Molecular structure, the shape and thermal stability of liposome forming compounds, their zeta-potential, particle size, critical micelle concentration (CMC) have been found to be important factors influencing their self-assembling and DNA complexation properties, and subsequent gene delivery activity (Aravindan et al., 2009; Lv et al., 2006). Therefore, it is highly important to understand the correlations between structural, physicochemical and DNA transporting properties for different groups of non-viral vectors.

Previously, our group has developed and studied multiple cationic 1,4-dihydropyridine (1,4-DHP) amphiphiles (Fig. 1), which were capable of transfecting plasmid DNA (pDNA) into different cell lines in vitro (Hyvonen et al., 2000; Pajuste et al., 2013).

Variation of alkyl chain lengths at positions 3 and 5 and the amount of cationic moieties in the 1,4-DHP scaffold have been performed at the beginning of our studies. After which we have concluded that two cationic moieties at positions 2 and 6 as well as dodecyloxycarbonyl substituents at positions 3 and 5 were optimal for these kinds of synthetic lipid-like compounds. Thus, 1,1′-[(3,5-bis(dodecyloxycarbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl) dimethylen]-bispyridinium dibromide (1,4-DHP amphiphile **1a**, Fig. 1) was found to be more active among the tested 1,4-DHP amphiphiles (Hyvonen et al., 2000). The next step of these studies involved modification of the substituents at the cationic head-group of the amphiphilic 1,4-DHP molecule. After these studies it was established that the electronic nature of the substituent of the pyridinium moieties as the cationic head-group of the 1,4-DHP derivatives strongly affected the ability of these compounds to bind pDNA and transfer it into the cells. In this way, we have demonstrated that compounds with electron-donating substituents at para- or meta-position of the above mentioned groups (1,4-DHP amphiphiles **1b** and **c**, Fig. 1) showed high gene transfection efficacy (Pajuste et al., 2013). Obtained data showed that the novel 1,4-DHP amphiphiles **1a–c** appeared to be more active than commercially available cationic lipid DOTAP (*N*-(1-(2,3-dioleoyloxy) propyl)-*N*,*N*,*N*-trimethyl ammonium methylsulfate) and cationic polymer PEI 25 (polyethyleneimine of 25 kDa). For example, at the charge ratio 2 the transfection efficiency of 1,4-DHP amphiphile **1a** was about 5 times better than that of DOTAP and 45 times more effective than that of PEI 25 (Pajuste et al., 2013). Some basic structure–activity relationships have been already found for cationic 1,4-DHP derivatives as gene delivery systems and shown how the chemical structure affected self-assembling properties, pDNA binding ability and properties of formed 1,4-DHP amphiphile–pDNA complexes (Hyvonen et al., 2000; Pajuste et al., 2013). It would be beneficial for more profound SAR conclusions to continue studies on the influence of oxidised forms of cationic 1,4-DHP derivatives on the mentioned activities and characterisation of physicochemical properties of the corresponding cationic pyridine compounds.




**1a-c**

**1a**: R = H; **1b**: R = 3-$CH_3$; **1c**: R = 4-$N(CH_3)_2$

**Fig. 1.** Structures of cationic 1,4-dihydropyridine amphiphiles **1a–c** (Hyvonen et al., 2000; Pajuste et al., 2013).

Encouraged by these results, we have decided to obtain a novel group of delivery agents based on amphiphilic pyridine derivatives containing dodecyloxycarbonyl substituents at positions 3 and 5 and cationic moieties at positions 2 and 6, which would be structurally closely related to the most efficient 1,4-DHP derivative for pDNA delivery (1,4-DHP amphiphile **1a**, Fig. 1). Additionally, the self-assembling properties such as particle size, zeta-potential, critical micelle concentration of the synthesised pyridine derivatives were characterised by dynamic light scattering (DLS) measurements. The thermal analysis of liposome forming cationic compounds was performed using thermogravimetry analysis (TGA) and differential thermal analysis (DTA). The ability to deliver pEGFP-C1 plasmid DNA (that encodes GFP reporter) into the Baby hamster kidney-derived (BHK-21) cell line was used as a test for evaluation of gene delivery properties of lipid-like pyridine derivatives **6a–g**.

Characterisation of physicochemical properties of amphiphiles and understanding of the structure–activity relationships of these carriers in cells is essential for further improving design and functionality in order to develop new delivery systems using non-viral lipid-like compounds.

## 2. Materials and methods

### 2.1. General

$^{1}H$ and $^{13}C$ NMR spectra were recorded on a Varian Mercury BB (400 and 100.56 MHz, respectively) spectrometer. The chemical shifts of the atoms are reported in parts per million (ppm) relative to the residual signals of the solvent: DMSO-$d_6$ (δ 2.50 ppm) or $CDCl_3$ (δ 7.26 ppm) for $^{1}H$ NMR spectra and DMSO-$d_6$ (δ 39.5 ppm) or $CDCl_3$ (δ 77.2 ppm) for $^{13}C$ NMR spectra. Multiplicities are abbreviated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; br, broad. The coupling constants are expressed in Hertz. Mass spectra were obtained on a Waters Acquity UPLC system connected to Waters SQ Detector-2 operating in the ESI positive ion mode on Waters Acquity UPLC® BEH C18 column (1.7 μm, 2.1 × 50 mm) using a gradient elution with acetonitrile (0.01% $CF_3COOH$) in water (0.01% $CF_3COOH$) at a flow rate 0.5 mL/min and processed with Waters MassLynx 4.1 chromatography data system. Elemental analyses were determined on an Elemental Combustion System ECS 4010 (Costech Instruments). Melting points were determined on a SRS OptiMelt system (SRS Stanford Research Systems). The compounds were analysed by HPLC on a Waters Alliance 2695 system equipped with Waters 2485 UV–vis detector and Alltima CN column (5 μm, 4.6 × 150 mm, Grace) using gradient elution with acetonitrile (0.1% $H_3PO_4$) in water, at a flow rate of 1 mL/min. Peak areas were determined electronically with Waters Empower 2 chromatography data system. TLC was performed on Silica gel 60 $F_{254}$ aluminium sheets 20 × 20 cm (Merck), spots were visualised with UV light (254 and 365 nm).

All chemical reagents were purchased from Acros, AlfaAesar or Sigma–Aldrich and used without further purification.

### 2.2. Synthesis

#### 2.2.1. Synthesis of compounds ***2*** and ***5***

The 3,5-bis(dodecyloxycarbonyl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine (**2**) was obtained from acetoacetic acid dodecyl ester, benzaldehyde and ammonia via classical Hantzsch synthesis according to the already reported method (Hyvonen et al., 2000). The bromination of 2,6-methyl groups of compound **2** was performed with NBS according to the reported method giving 2,6-bis(bromomethyl)-3,5-bis(dodecyloxycarbonyl)-4-phenyl-1,4-dihydropyridine (**5**) (Pajuste et al., 2011). $^{1}H$ and $^{13}C$ NMR spectra