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Double-tailed lipid modification as a promising candidate for oligonucleotide delivery in mammalian cells

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ABSTRACT

Background: The potential use of nucleic acids as therapeutic drugs has triggered the quest for oligonucleotide conjugates with enhanced cellular permeability. To this end, the biophysical aspects of previously reported potential lipid oligodeoxyribonucleotide conjugates were studied including its membrane-binding properties and cellular uptake.
Methods: These conjugates were fully characterized by MALDI-TOF mass spectrometry and HPLC chromatography. Their ability to insert into lipid model membrane systems was evaluated by Langmuir balance and confocal microscopy followed by the study of the internalization of a lipid oligodeoxyribonucleotide conjugate bearing a double-tail lipid modification (C_{28}) into different cell lines by confocal microscopy and flow cytometry. This compound was also compared with other lipid containing conjugates and with the classical lipoplex formulation using Transfectin as transfection reagent.
Results: This double-tail lipid modification showed better incorporation into both lipid model membranes and cell systems. Indeed, this lipid conjugation was capable of inserting the oligodeoxyribonucleotide into both liquid-disordered and liquid-ordered domains of model lipid bilayer systems and produced an enhancement of oligodeoxyribonucleotide uptake in cells, even better than the effect caused by lipoplexes. In addition, in β_2 integrin (CR3) expressing cells this receptor was directly involved in the enhanced internalization of this compound.
Conclusions: All these features confirm that the dual lipid modification (C_{28}) is an excellent modification for enhancing nucleic acid delivery without altering their binding properties.
General significance: Compared to the commercial lipoplex approach, oligodeoxyribonucleotide conjugation with C_{28} dual lipid modification seems to be promising to improve oligonucleotide delivery in mammalian cells.

1. Introduction

The ability of some small nucleic acid-based molecules such as DNA decoys, triple helix forming oligonucleotides, RNA decoys, antisense oligonucleotides, ribozymes, DNAzymes, siRNAs or aptamers to silence specific genes or to inhibit the biological activity of specific proteins has generated great interest for their use as research tools and therapeutic agents [1–3]. Unfortunately, biological applications of oligonucleotides meet a huge limitation namely cellular accessibility [4]. In fact, phospholipid bilayers represent a strong barrier for the highly negatively charged phosphodiester backbone of the oligonucleotides, leading to an extremely poor penetration across the cell membrane. Nevertheless, simple elimination of the anionic charge does not improve cellular uptake, as evidenced by chemically modified neutral backbone-containing oligonucleotides [5]. Therefore developing an appropriate delivery system in order to achieve their efficient cellular uptake remains an important challenge. In this regard, the use of lipid-based systems has long been considered as a possible method to solve cell permeation problems [6].

The introduction of both neutral and cationic lipids into oligonucleotides is one of the approaches to improve the delivery of these

Abbreviations: LOC, lipid oligonucleotide conjugate; TLC, thin-layer chromatography; ACN, acetonitrile; TEAA, triethylammonium acetate; TOF, time-of-flight; ODN, oligodeoxyribonucleotide; CPG, controlled-pore glass; PBS, phosphate-buffered saline; DOPC, 1,2-*dioleyl*-L-α-phosphatidylcholine; GUV, giant unilamellar vesicle; SPB, supported planar bilayer; eSM, egg sphingomyelin; Chol, cholesterol; L_d, liquid-disordered; L_o, liquid-ordered; MLV, multilamellar vesicle; SUV, small unilamellar vesicle; AFM, atomic force microscopy; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; GMFI, Geometric Mean Fluorescence Intensity; PFA, paraformaldehyde

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kinds of biomolecules, thereby favoring interaction with, or insertion into the plasma membrane along with improving their stability compared to unmodified oligonucleotides [7,8]. To date several lipophilic molecules, including cholesterol, phospholipids, α-tocopherol and alkyl chains, have been conjugated to oligonucleotides in order to obtain better clinically acceptable therapeutic drugs [9–14]. The main goal of these lipophilic compounds is to improve oligonucleotide delivery and to enhance their cellular uptake *via* receptor-mediated endocytosis by increasing the membrane permeability without altering the potential properties of the nucleic acid molecule, thereby achieving the desired gene expression inhibition [6,15–19].

Recently, our group reported the synthesis of several lipid oligonucleotide conjugates (LOCs) series containing neutral and cationic lipids at their 3′- and 5′-termini and their evaluation *in vitro* in RNA interference [20,21]. We observed that all lipid modifications did not disrupt the RNAi machinery obtaining in some cases promising results in inhibiting gene expression without using a transfection agent. Indeed, the siRNA carrying the C_{28} moiety at the 5′-end of the passenger strand showed the best inhibitory effects in the absence of the transfecting agent Oligofectamine [21]. It is well-known that cellular uptake and the intracellular distribution of nucleic acids may depend on both their surface properties and their interactions with the cell membrane. In the present work, we study such interactions induced by LOC conjugates, specially C_{28} moiety containing LOC (Fig. 1), using three model membrane systems (*i.e.*, monolayers, giant unilamellar vesicles and supported planar bilayers), and several

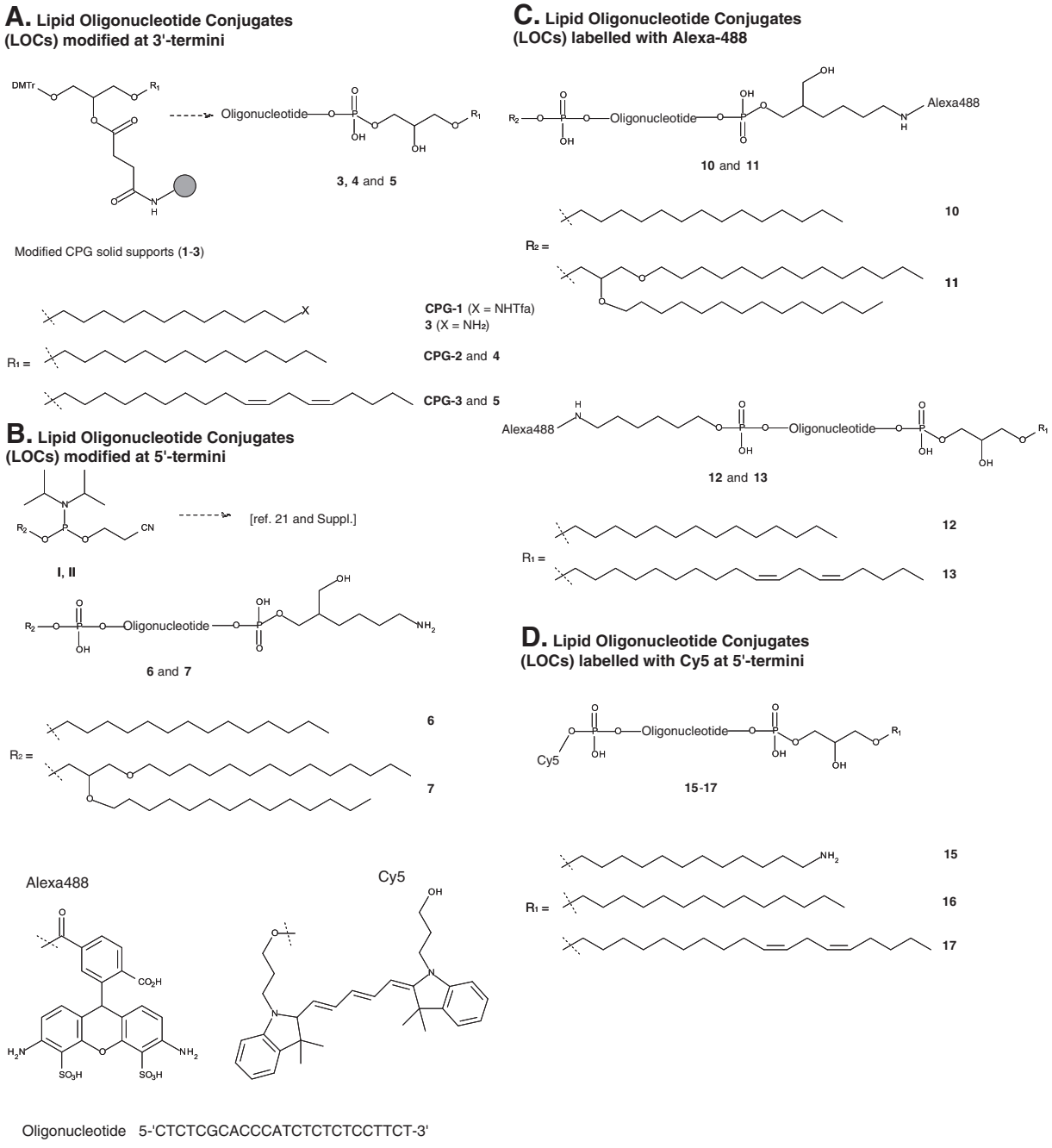

Fig. 1. Schematic representation of all modifications introduced into the GEM91 oligonucleotide. A) Selected lipids were covalently linked at 3′-termini (compounds **3**, **4** and **5**); B) Lipid phosphoramidites I and II were introduced at 5′-termini (compounds **6** and **7**); C) Alexa488 dye was introduced at both 5′-termini (compounds **10** and **11**) and 3′-termini (compounds **12** and **13**) and D) Cy5 dye was introduced at 5′-termini (compounds **15**, **16** and **17**).