Organic & Biomolecular Chemistry

RSC Publishing

PAPER

Tuning molecular interactions in lipid-oligonucleotides assemblies *via* locked nucleic acid (LNA)-based lipids†

Cite this: *Org. Biomol. Chem.*, 2013, **11**, 7108

Amit Patwa,[a,b] Gilmar Salgado,[b,c] François Dole,[d,e] Laurence Navailles[d,e] and Philippe Barthélémy*[a,b]

Received 25th June 2013,
Accepted 27th August 2013

DOI: 10.1039/c3ob41707g

www.rsc.org/obc

Hybrid nucleotide-lipids containing locked nucleic acid (LNA) show enhanced hybridization properties with complementary single strand RNAs compared to DNA lipid analogues. The LNA adenosine lipid features unique binding properties with a high binding affinity for poly-uridine and the entropically driven formation of a stable complex ($K_d \approx 43$ nM). Enhanced hybridization properties of LNA-based lipids should be applicable for the development of oligonucleotide (ON) delivery systems or as small molecule binders to RNA for novel therapeutic strategies.

Locked nucleic acids (LNA) have been developed in order to increase the binding affinity of modified oligonucleotides for their complementary target, enhance their nuclease resistance,[1] and modulate the biological,[2] and physico-chemical[3–6] properties of oligonucleotides. These characteristics, which are due to structurally rigid modification, render LNA an interesting synthetic material for the design of hybrid amphiphiles possessing a nucleoside as a lipid head group and lipophilic alkyl chains; nucleolipids (NLs).[7–9] Extensive studies carried out in the past decade clearly show that the supramolecular properties of NLs are strongly influenced by the nucleoside structures and conformations.[10,11] One particular interest in developing locked NLs is to modulate their binding properties *via* the restriction of the nucleoside moiety in the 3′-endo (north) conformation. From a biomedical applications viewpoint, supramolecular complexes constructed *via* non-coulombic interactions[12,13] (*i.e.* base-pairing principles) are of interest for nucleic acid delivery. The thermodynamics of these interactions have been studied previously in different oligonucleotide complexes including duplexes, triplexes[14,15] and quadruplexes.[16–19] Interestingly, LNA nucleotides have been inserted into the ON sequences in order to enhance complex stability.[20,21] However, to our knowledge the formation of complexes involving ON associated with hybrid LNA based lipids has not been investigated so far. Creating LNA based NLs that form stable complexes with ONs could serve as a novel tool for nucleic acid formulations and/or for the regulation of gene expression similarly as ONs (antisense, triplex-forming oligonucleotide[22] *etc.*).

In the present study, to further investigate the influence of the conformational flexibility of the nucleotide-lipids ribose on their interactions with oligonucleotide partners, we synthesized novel locked nucleic acid (LNA)-based lipids, having a dialkylphosphate chain attached to the 3′-secondary hydroxyl of LNA. The four nucleotide-based lipids (two LNA and two DNA based amphiphiles) with their preferred conformation are shown in Fig. 1. To the best of our knowledge, there are no previous examples of a double-alkyl chain with locked nucleoside-3′-monophosphate amphiphiles. Here, we report that the LNA modification of NLs results in enhanced hybridization

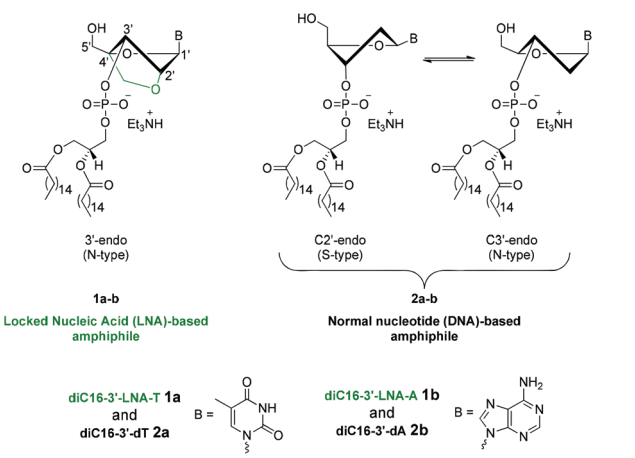

Fig. 1 LNA (left) and DNA (right) based nucleotide lipids investigated in this study.

[a]*Univ. Bordeaux, ARNA laboratory, F-33076 Bordeaux, France. E-mail: philippe.barthelemy@inserm.fr; Fax: +33 5 5757 1015; Tel: +33 5 5757 4853*
[b]*INSERM, U869, ARNA laboratory, F-33000 Bordeaux, France*
[c]*Univ. Bordeaux, IECB laboratoire ARNA, 33076 Bordeaux, France*
[d]*CNRS, CRPP, UPR 8641, F-33600 Pessac, France*
[e]*Univ. Bordeaux, CRPP, UPR 8641, F-33600 Pessac, France*

†Electronic supplementary information (ESI) available: Experimental procedures, NMR, MS, TEM, DLS, FTIR and ITC data. See DOI: 10.1039/c3ob41707g