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Simplifying Progress

An Innovative Cationic Lipid Library for Efficient and Tunable mRNA-LNPs

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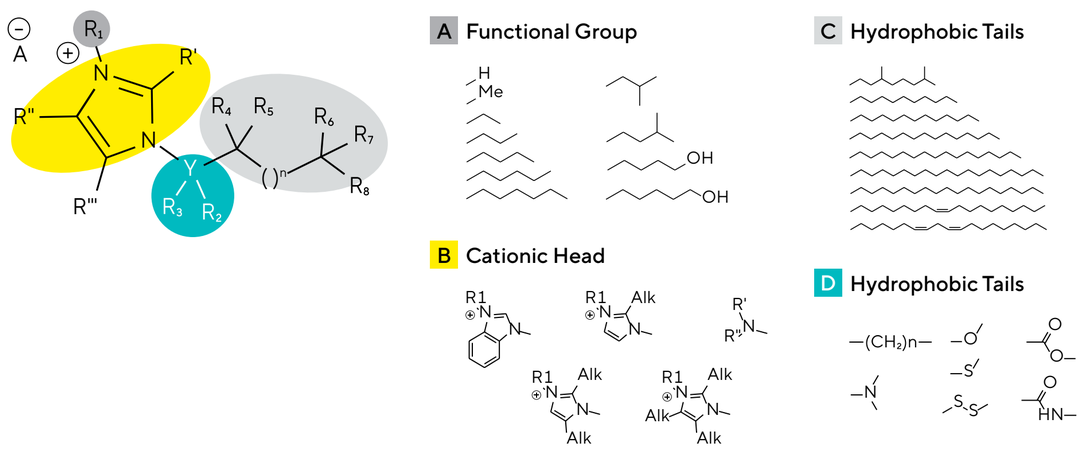
1. Introduction

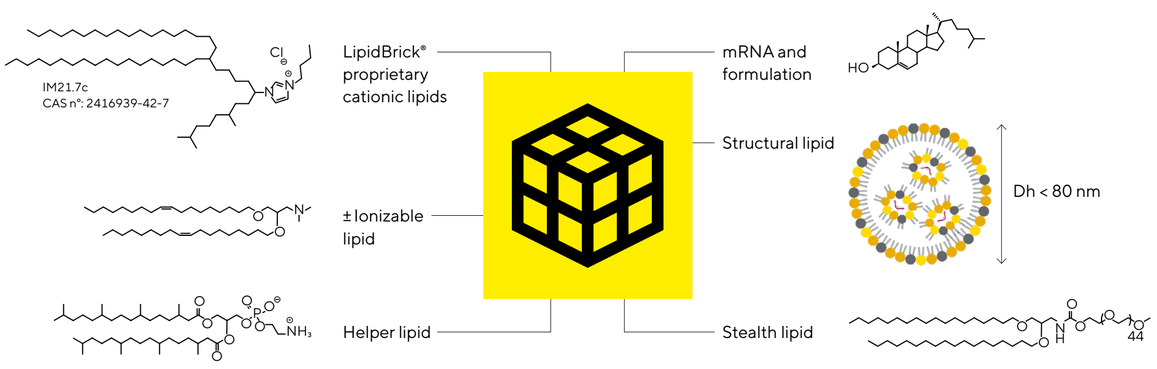
Lipid nanoparticles (LNP) have demonstrated high efficiency delivering RNA therapeutics in vivo. However, the properties of such nanoparticles obtained with conventional ionizable lipids are often hard to modulate, and especially their biodistribution profile. Such ionizable lipid-based nanoparticles often predominantly end up targeting the liver. One of the current challenges in the field consists of adjusting the particle chemical composition to the targeted application. Here, we have characterized a library of innovative imidazolium-based cationic lipids as key component of cationic LNPs (cLNP). We disclose their chemical structures and demonstrate their efficacy generating LNPs through characterization of their hydrodynamic diameters, Zeta potentials and encapsulation efficiencies. The resulting particles display high transfection efficiencies and have little to no impact on cell viability in vitro, on HEK293 and CaCo-2 cell lines. In vivo, the biodistribution of the cLNP highly depends on the cationic lipid chemical structures, targeting mainly lungs and spleen. Among this library, we have identified one cationic lipid as a potent additive in Moderna's Spikevax formulation.

2. Library of LipidBrick® Cationic Lipids



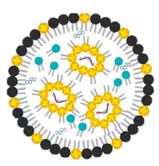
Note. Innovative cationic lipids have been synthetized following various modifications listed above. Several convergent synthetic routes have been optimized to generate lipids at multigram scale.

3. Used in 4 or 5-lipid LNP Compositions



4. Efficient LNPs In Vitro

Figure 1: *mRNA-LNPs Display a Size Below 100 nm, a Positive Zeta Potential, and Encaspulate mRNA Efficiently*

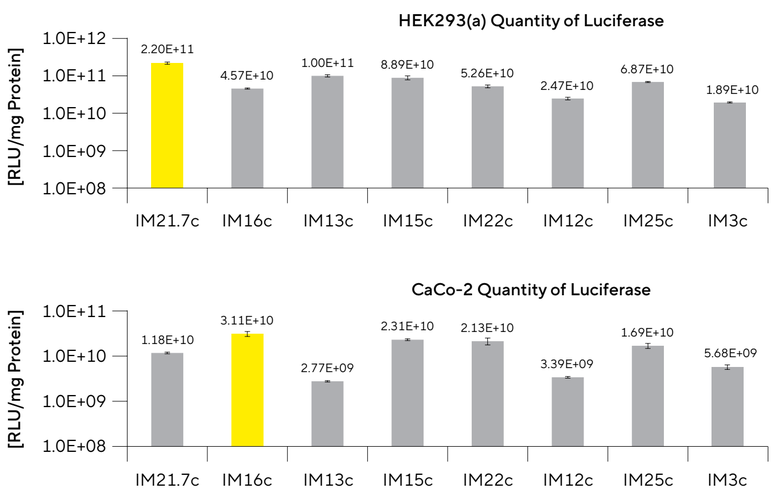


CleanCap® Fluc mRNA (50 µg/mL)

	IM21.7c	IM16c	IM13c	IM15c	IM22c	IM12c	IM25c	IM3c
Size [nm]	45±2	56±3	74±4	66±6	40±2	29±1	86±2	41±2
Zeta [mV]	+10	+13	+11	+9	+14	+12	+10	+16
PDI	0.158	0.132	0.090	0.113	0.140	0.120	0.053	0.104
EE (%)	99	99	99	99	99	99	99	99

Note. Size and Zeta potentials of the particles obtained after nanoprecipitation determined by DLS and ELS, respectively. Encapsulation efficiency was determined by Ribogreen assay.

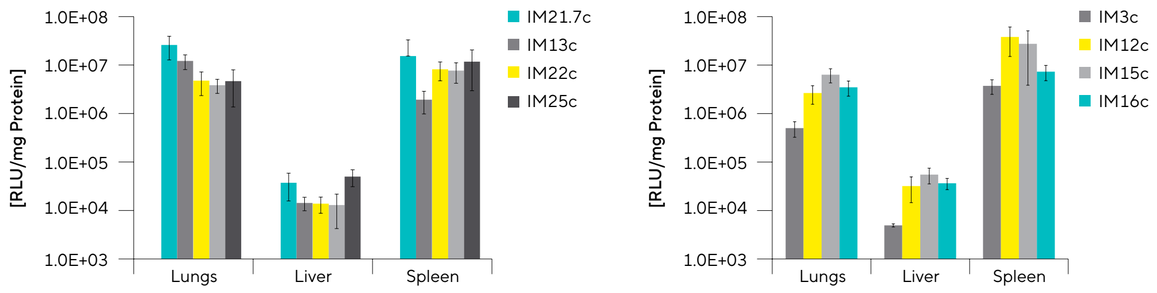
Figure 2: *LipidBrick® Library Offers Consistent LNP Efficacy & Flexibility in vitro*



Note. 500 ng of mRNA were added to 50,000 HEK293 cells or 40,000 CaCo-2 cells and luciferase expression was assessed 24 hours after transfection.

5. Adapt Tropism to Your Purposes with LipidBrick Library

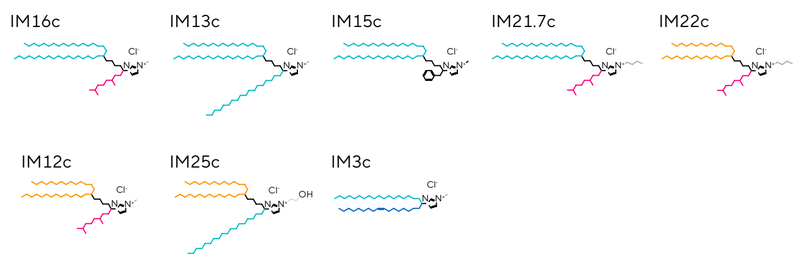
Figure 3: *Lipidbrick® Library of Lipids Can Efficiently Modulate the LNP Biodistribution*



Note. 10 µg of mRNA encoding FLuc were injected through retro-orbital (RO) injection. Luciferase expression was assessed 24 hours postinjection.

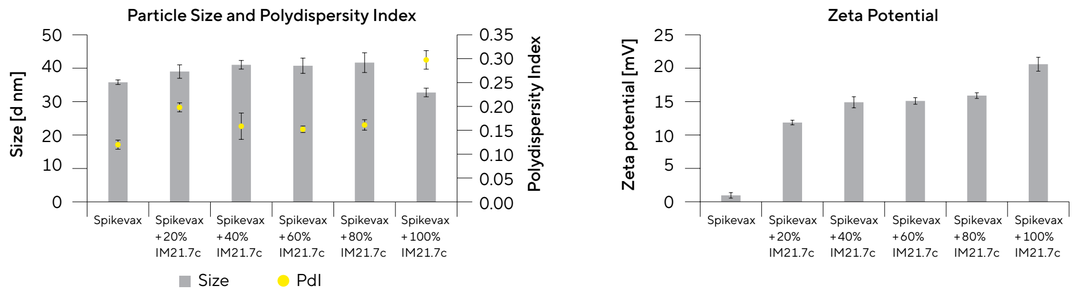
6. Straightforward Synthesis and Derivatization

Figure 4: *An efficient synthetic route has been developed to easily access a wide variety of cationic lipids at the multigram scale*



7. IM21.7c as an Efficient Plug-And-Play Cationic Lipid Additive

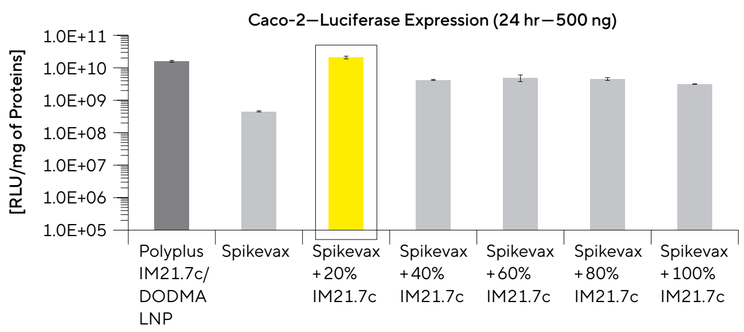
Figure 5: *Plug-And-Play addition of IM21.7c in Spikevax (SM-102 LNP) formulation is suitable and increases the zeta potential of LNPs*



Note. Size and Zeta potentials of the particles obtained after nanoprecipitation determined by DLS and ELS, respectively.

Figure 6: *Plug-And-Play addition of IM21.7c in Spikevax (SM-102 LNP) formulation leads to efficient LNPs in vitro*

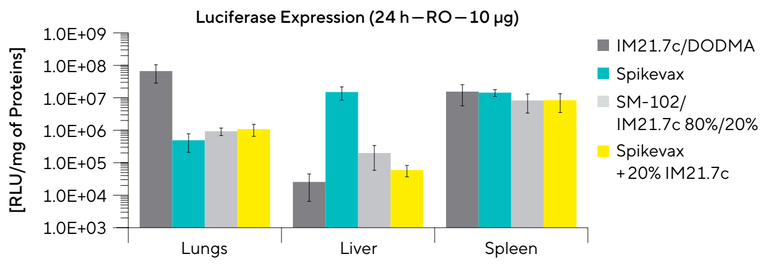
LNP Composition	Size [nm]	Zeta [mV]
IM21.7c/DODMA	63±2	+12
Spikevax	36±1	+1
+20% IM21.7c	39±2	+12
+40% IM21.7c	41±1	+15
+60% IM21.7c	41±2	+15
+80% IM21.7c	42±3	+16
+100% IM21.7c	33±1	+21



Note. Same increased transfection efficiency in vitro on HEK cells with addition of 20% of IM21.7c in Spikevax, Comirnaty and Onpattro's formulations. Similar results were also obtained with Comirnaty (ALC-0315 LNP) and Onpattro (MC3) formulations.

Figure 7: *Plug-And-Play addition or substitution of IM21.7c in Spikevax (SM-102 LNP) formulation Allows to Avoid the Liver while Maintaining High Tropism to the Spleen*

Cationic | Ionizable Lipid	Size [nm]	Zeta [mV]
IM21.7c/DODMA	57±4	+13
Spikevax	39±2	+1
SM-102/IM21.7c 80%/20% in Spikevax	37±3	+11
Spikevax + 20% IM21.7c	37±1	+11



Note. 10 µg of mRNA encoding FLuc were injected through retro-orbital (RO) injection. Luciferase expression was assessed 24 hours post-injection. LipidBrick® IM21.7c improves mRNA-LNP efficiency. LipidBrick® IM21.7c modulates LNP properties to adapt the biodistribution depending on the therapeutic purpose.

8. Conclusion

A novel family of proprietary cationic lipids solve a current limit of LNPs in being able to target different organs and cell types. The new cationic LNP formulation ensures same delivery efficacy as LNPs with ionizable lipids, while improving biodistribution to target organs other than liver.

Patent references: EP18306417; WO2024008967