

Lipid Nanoparticles for mRNA Delivery

School of Chemistry, University of the Witwatersrand

Charles de Koning

Robin Klintworth

Connor Stockley

Aneesa Gani

Kennedy Ngwira

Songeziwe Ntsimango

Together with

Antiviral Gene Therapy Research Unit,
University of the Witwatersrand

Patrick Arbuthnot

Abdullah Ely

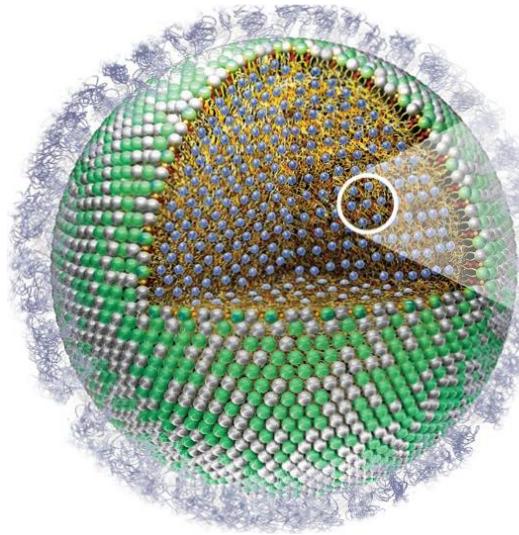
Kirstie Bloom

Dylan Kairuz



Introduction to Ionizable Lipids

Lipid Nanoparticle
(Delivery Vehicle)



pegylated lipid



ionizable lipid



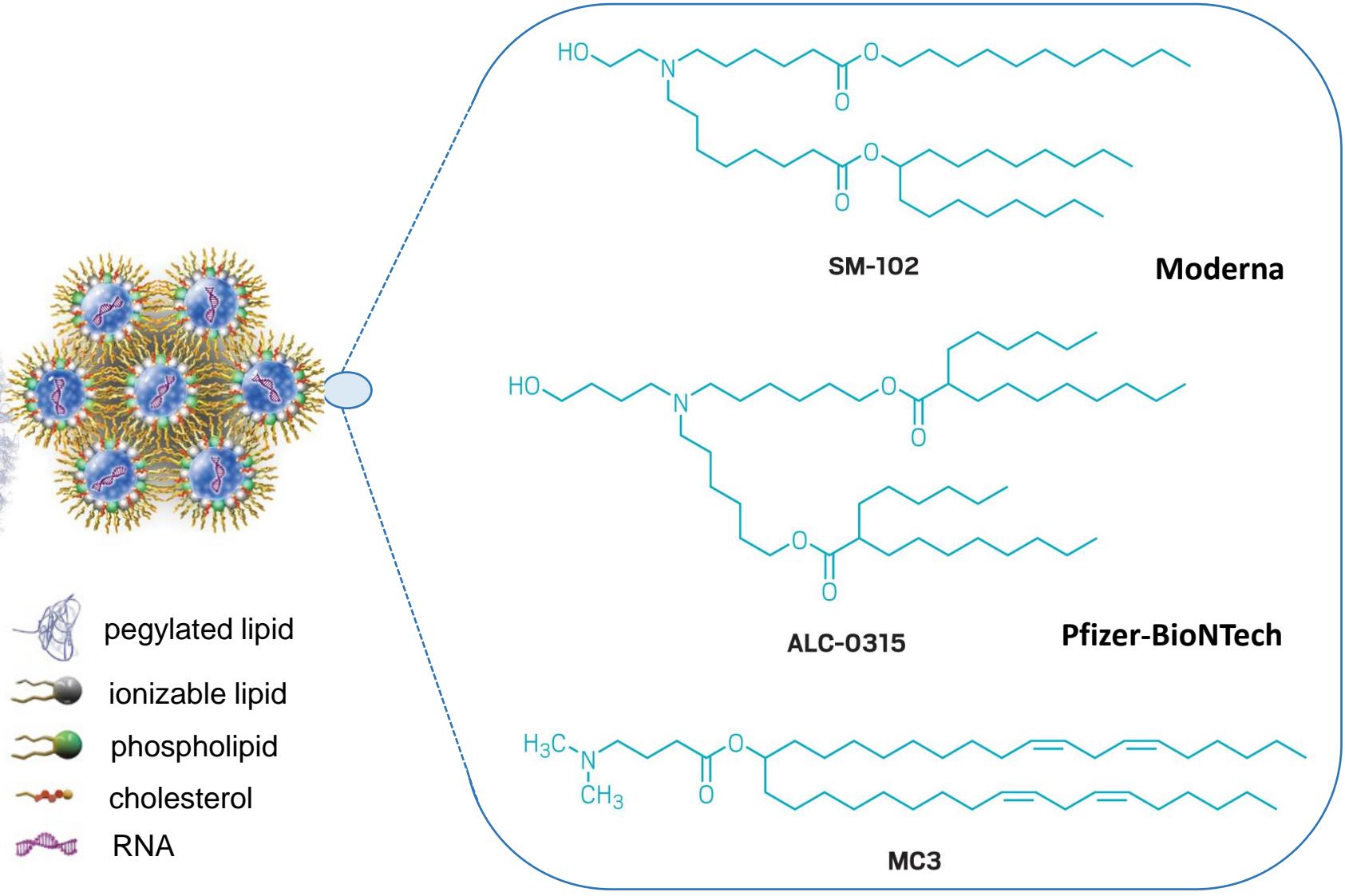
phospholipid



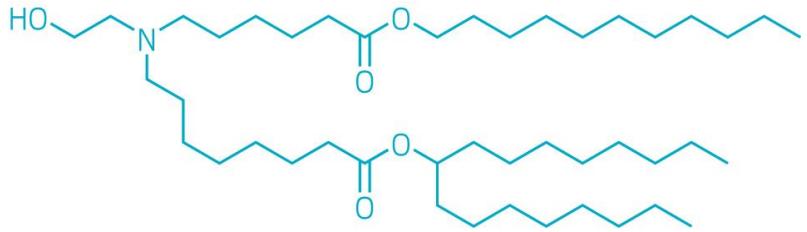
cholesterol



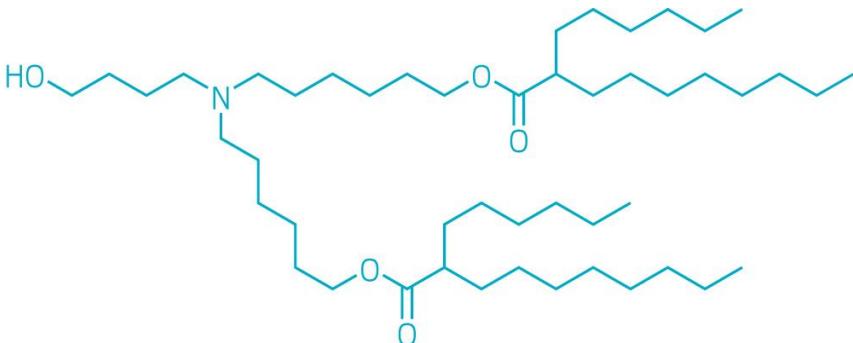
RNA



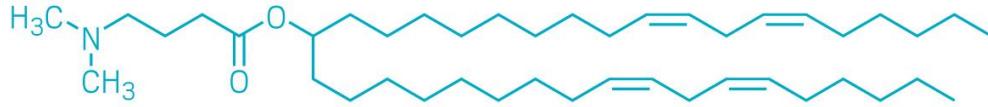
Properties of Ionizable Lipids



SM-102



ALC-0315

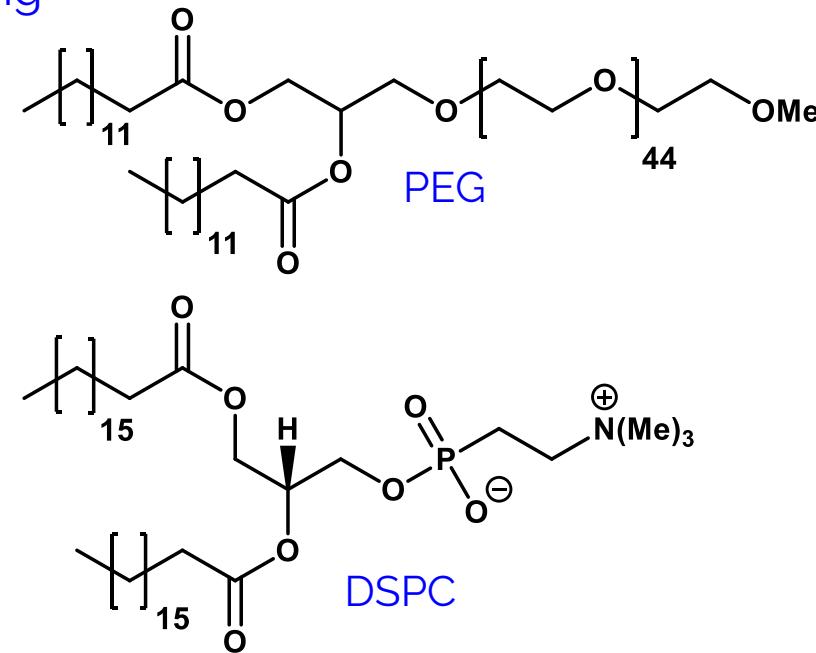


MC3

- Aliphatic chains, often branched
- Ionizable nitrogen, often alcohol chain attached
- Ester group can aid with biodegradability

Each dose of the Moderna COVID-19 Vaccine contains the following ingredients: a total lipid content of 1.93 mg

- SM-102, ionizable lipid
- polyethylene glycol [PEG] 2000 dimyristoyl glycerol [DMG],
- cholesterol, and
- 1,2-distearoyl-sn-glycero-3-phosphocholine [DSPC],
- 0.31 mg tromethamine,
- 1.18 mg tromethamine hydrochloride,
- 0.043 mg acetic acid,
- 0.20 mg sodium acetate trihydrate, and
- 43.5 mg sucrose.

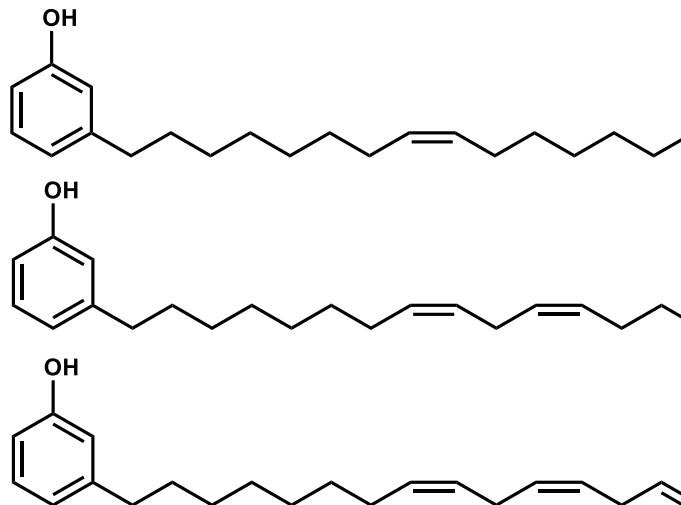


Pfizer/BioNTech COVID-19 Vaccine

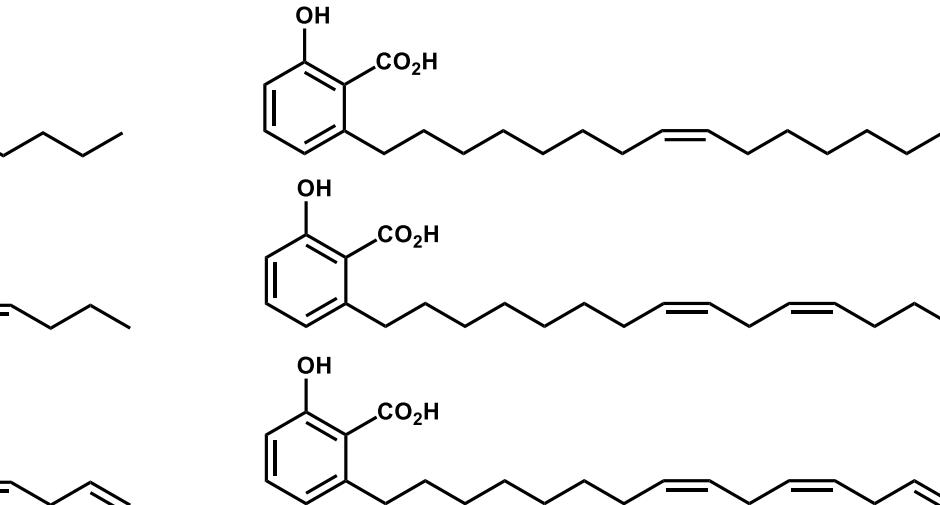
Lipid	Wt lipid per dose (30 µg mRNA/dose)	Wt lipid 1B doses
Ionizable lipid	0.35 mg	351 kg
PEG-lipid	0.034 mg	34.4 kg
DSPC	0.072 mg	72 kg
Cholesterol	0.14 mg	142 kg

largest quantity
by far

Potential Compounds from Cashew Nut Shell Liquid as building blocks for Ionizable Lipids?

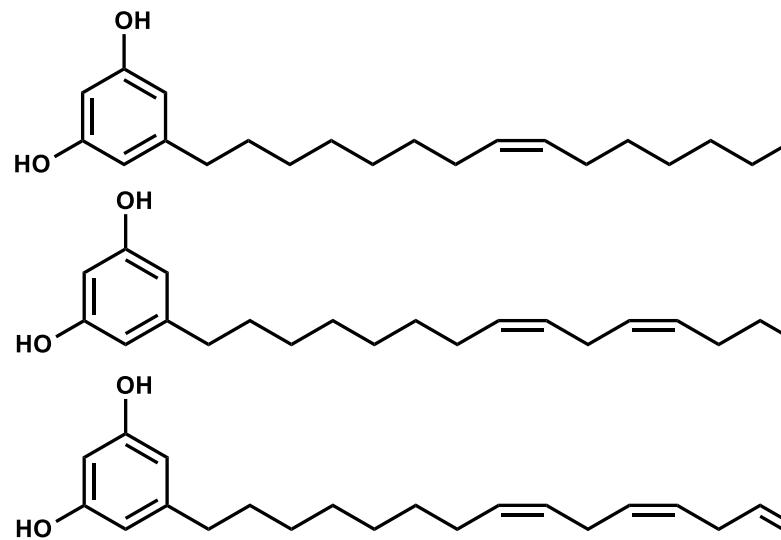


Cardanols



Major components

Anacardic acids



Cardols

Minor components



Cashew nut shell liquid

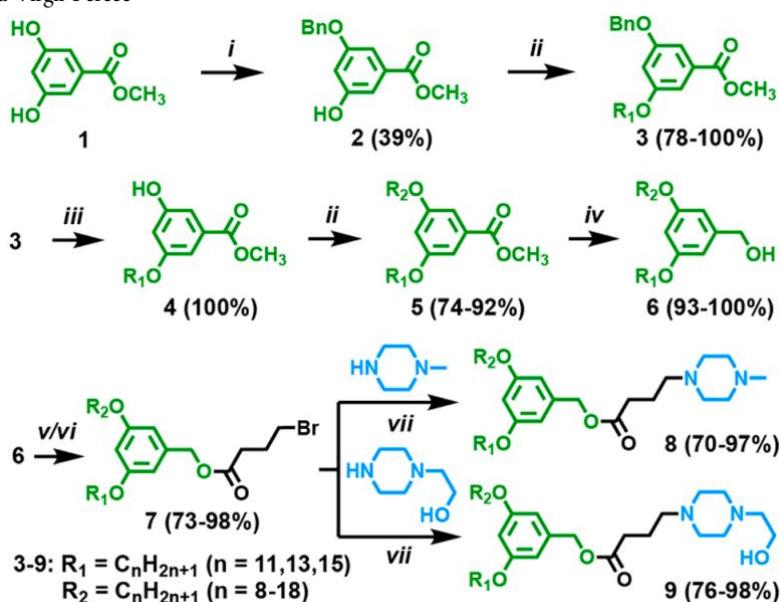
Waste product

- Extracted from inedible shells

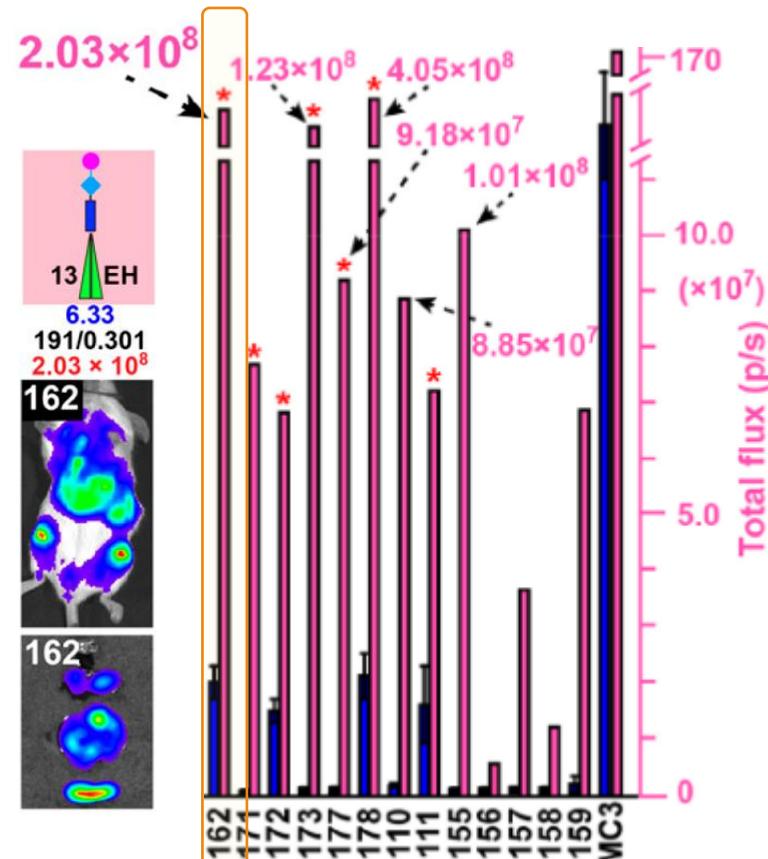
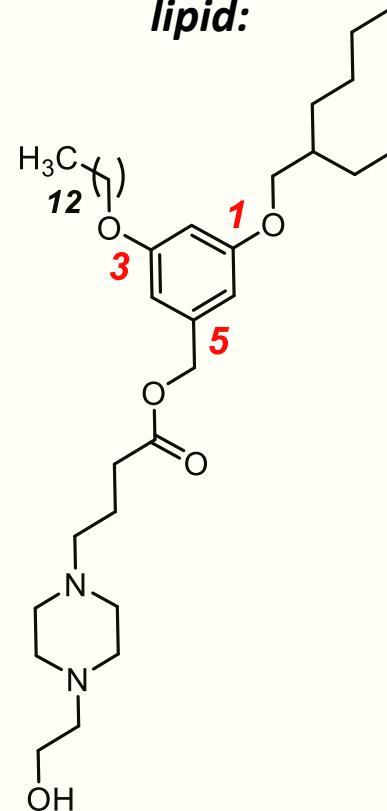
Precedence for aromatic ionizable lipids

The Unexpected Importance of the Primary Structure of the Hydrophobic Part of One-Component Ionizable Amphiphilic Janus Dendrimers in Targeted mRNA Delivery Activity

Dapeng Zhang,[§] Elena N. Atochina-Vasserman,[§] Juncheng Lu, Devendra S. Maurya, Qi Xiao, Matthew Liu, Jasper Adamson, Nathan Ona, Erin K. Reagan, Houping Ni, Drew Weissman,* and Virgil Percec*



Single Component lipid:



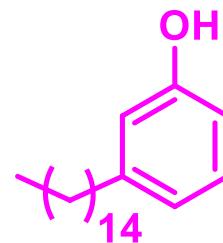
Drew Weissman: Intravenous injection (IV)
Our work: Intramuscular injection (IM)

Luminescence *in vitro*
Luminescence *in vivo*

C-15 Compounds from Cashew Nut Shell Liquid for Ionizable Lipids

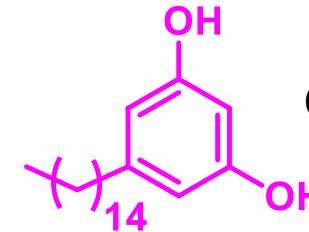


Simple extraction,
distillation/separation
and hydrogenation



Mainly hydrogenated
cardanol

meta hydroxylation
reaction

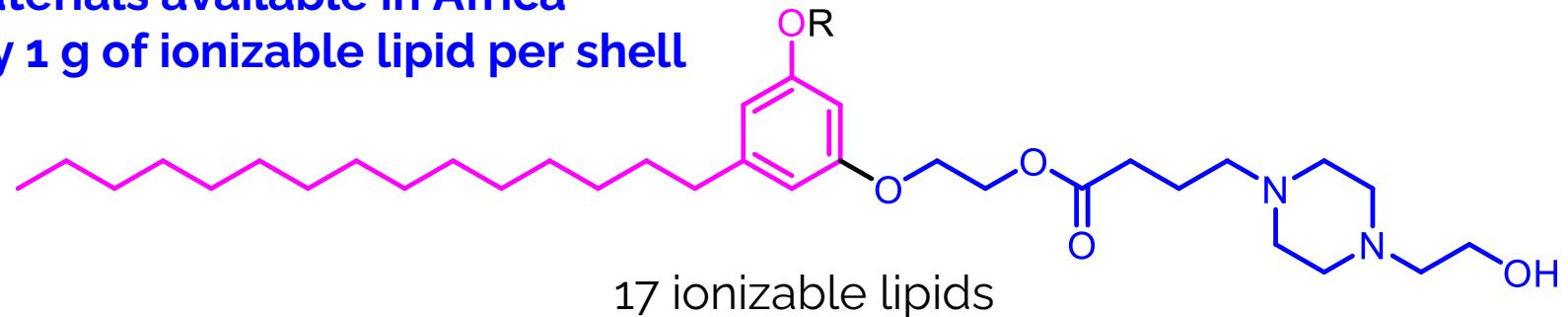


hydrogenated
Cardol (1,3,5-trisubstituted
benzene)

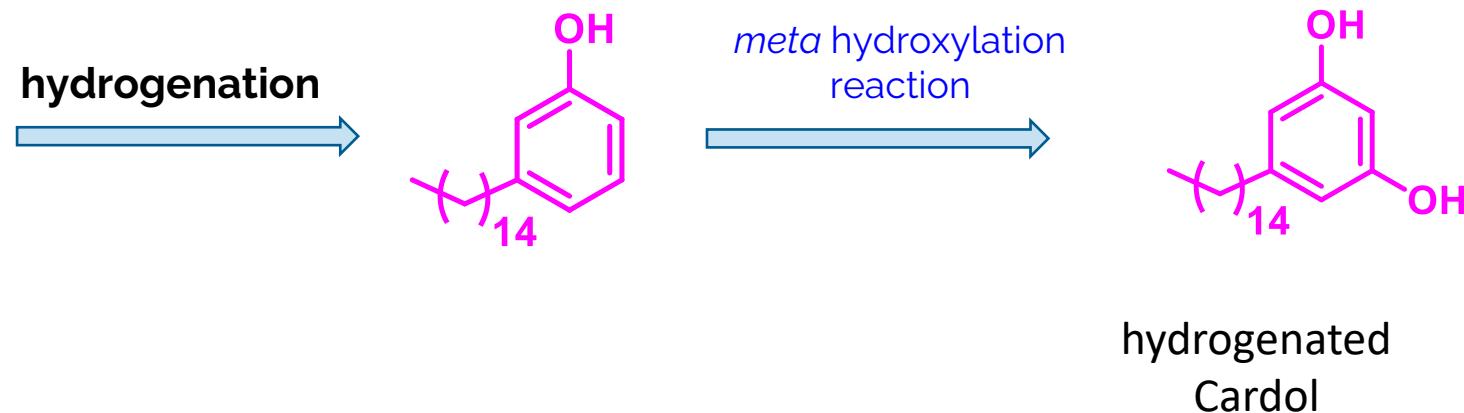
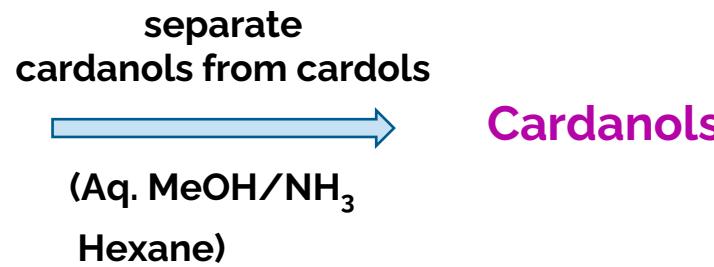
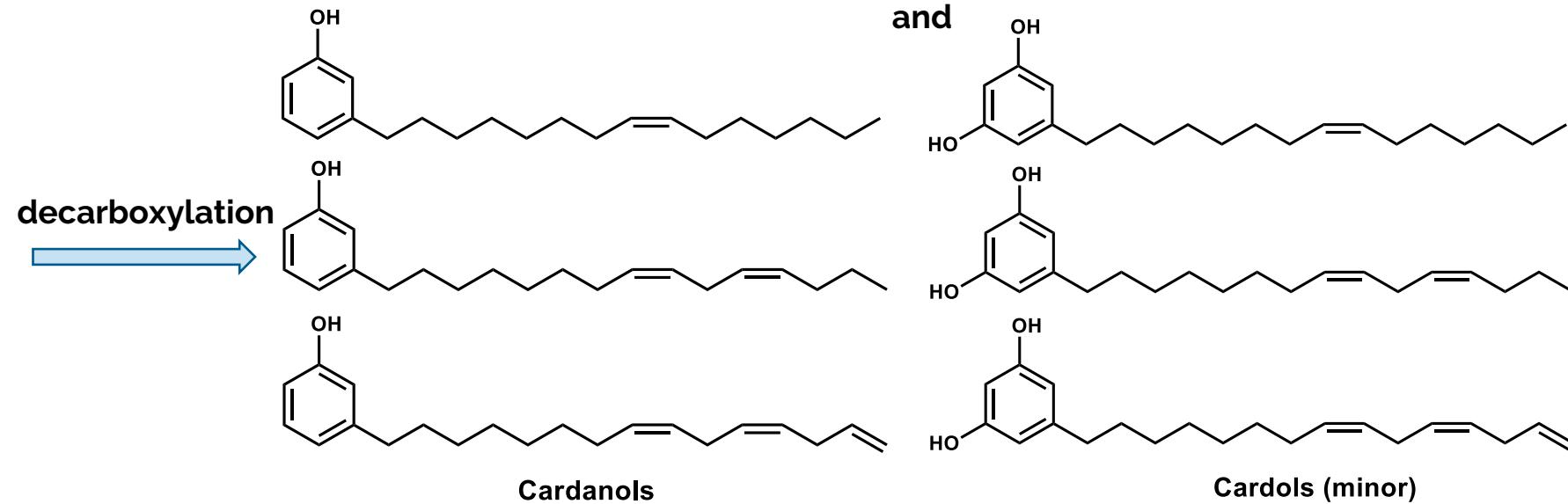
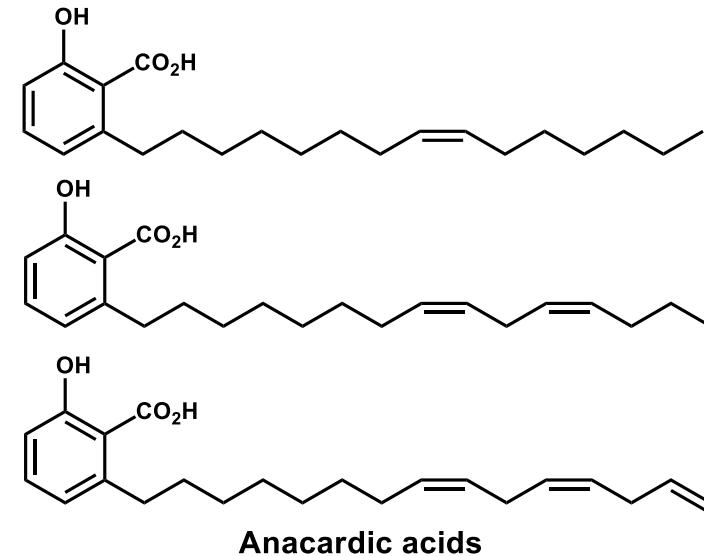
500mg \$1897.00

5 synthetic steps

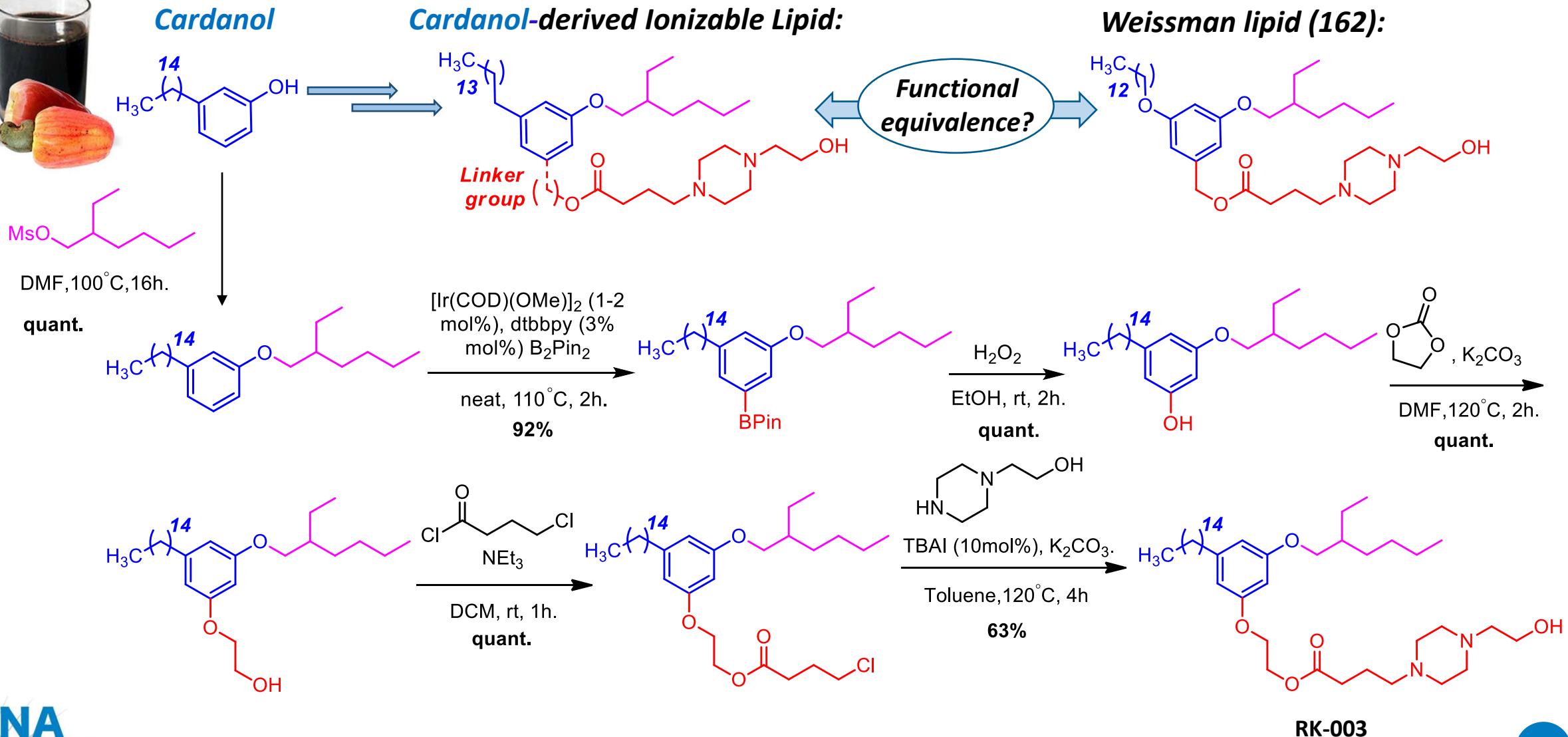
- Green Chemistry, Scalable
- Only last step requires chromatography
- All steps >90% yield
- **Raw materials available in Africa**
- **Roughly 1 g of ionizable lipid per shell**



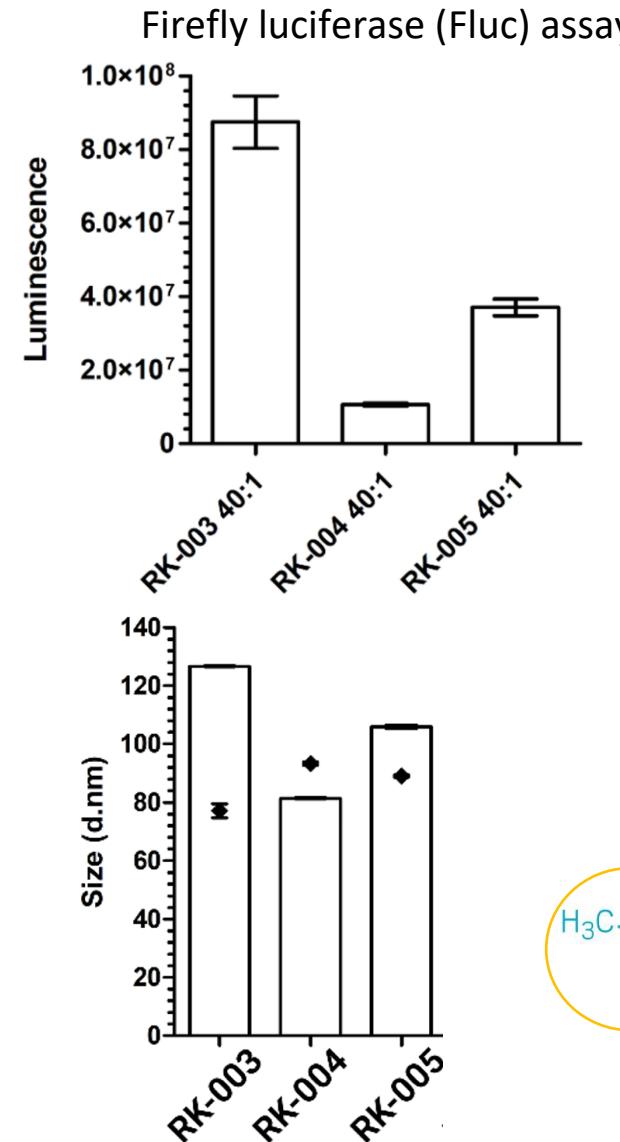
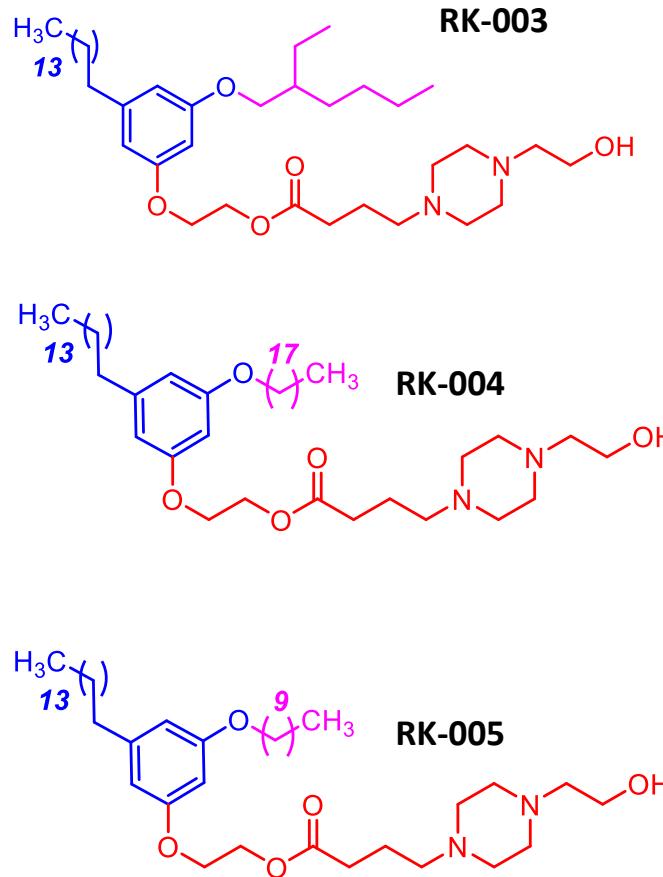
Convert all nine compounds into one?



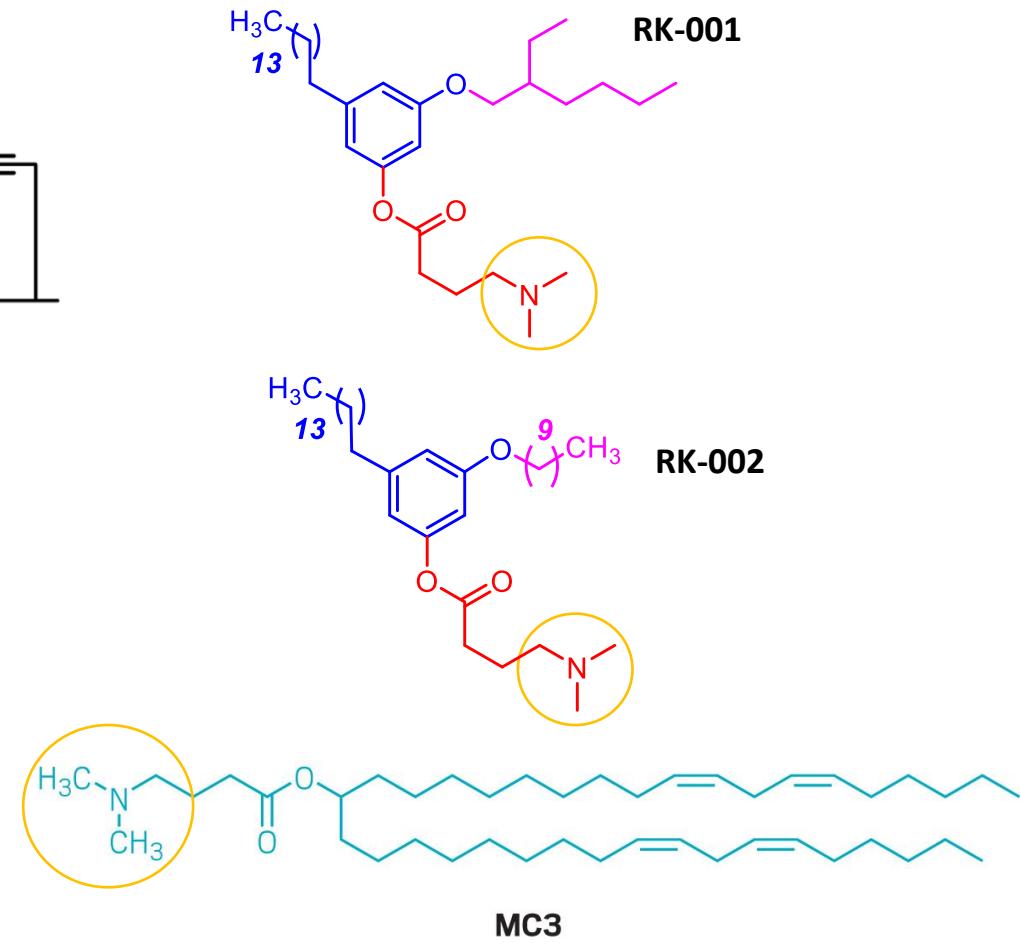
Cardanol-Derived Ionizable Lipids



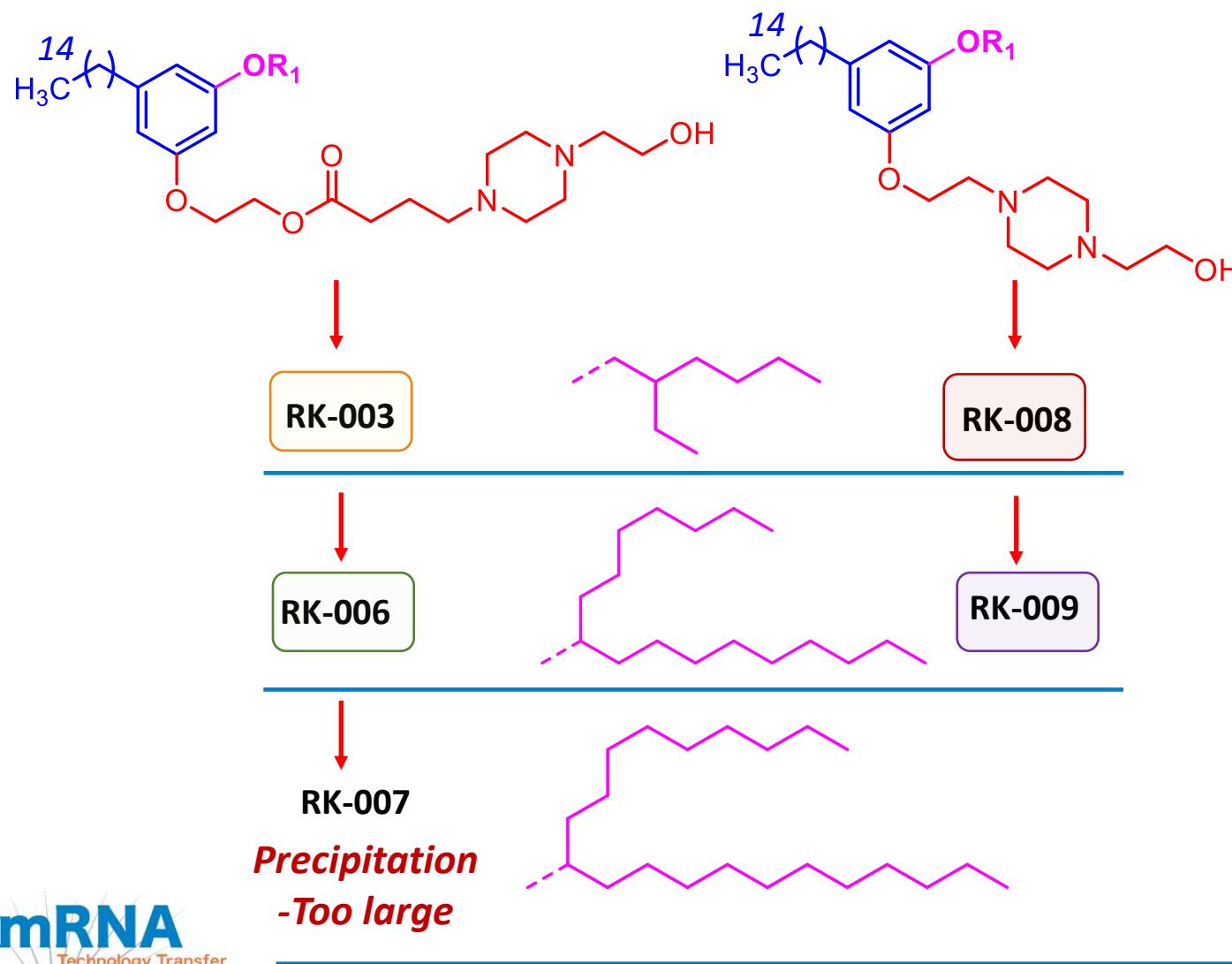
Importance of Ionizable group: *In vitro* formulations single component



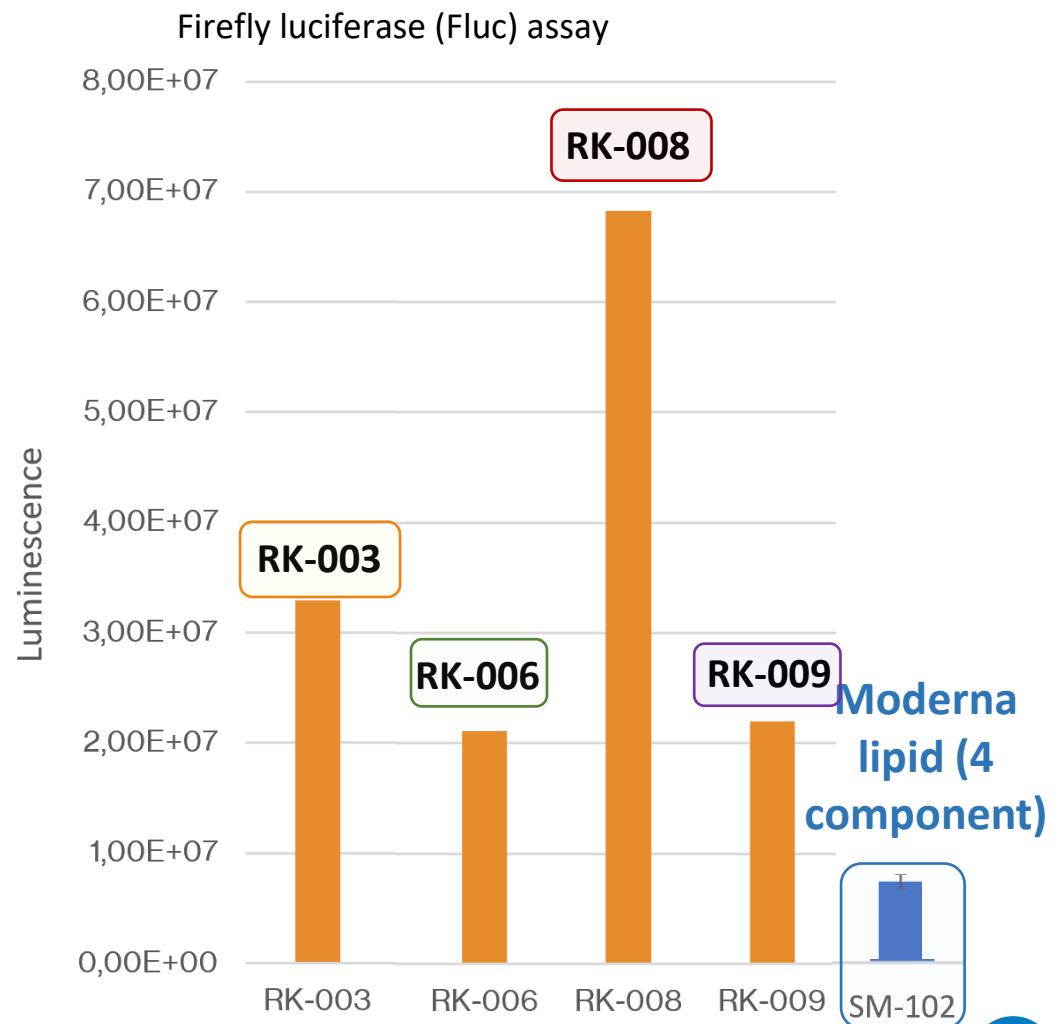
No Luminescence:



Examples

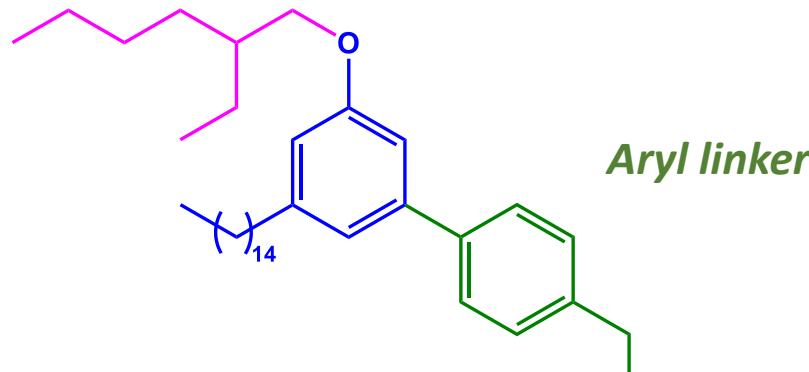


In vitro formulations single component



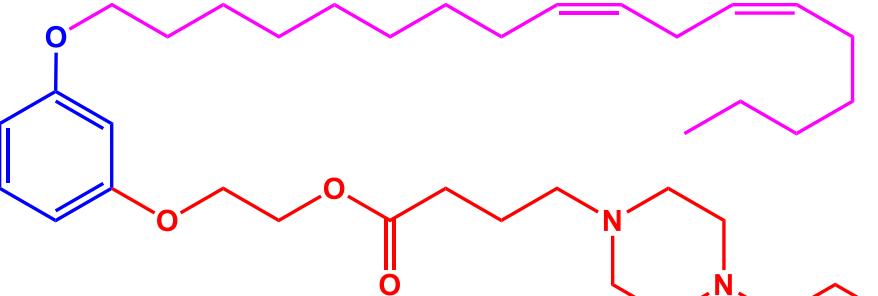
Other examples of 1,3,5-ionizable lipids

CS-001



Aryl linker

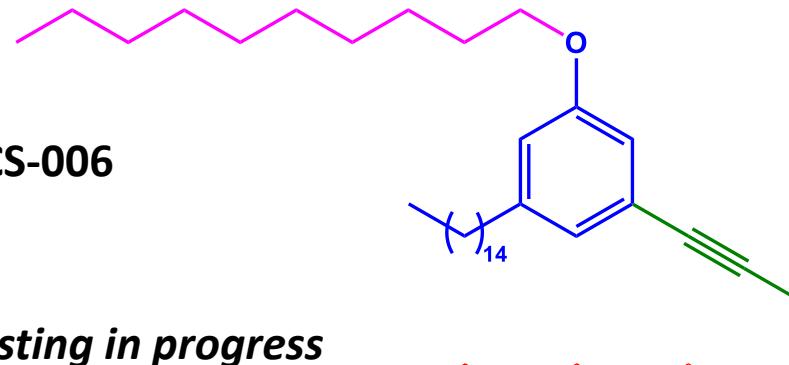
*Precipitation
-Linker too large*



CS-005

Testing in progress

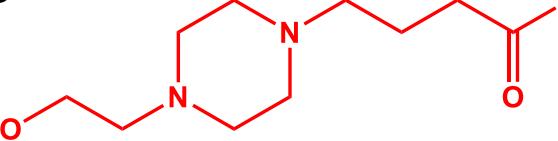
CS-006



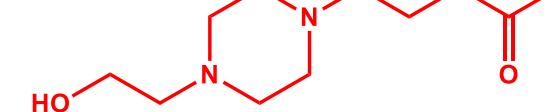
Alkyne linker

Testing in progress

CS-007

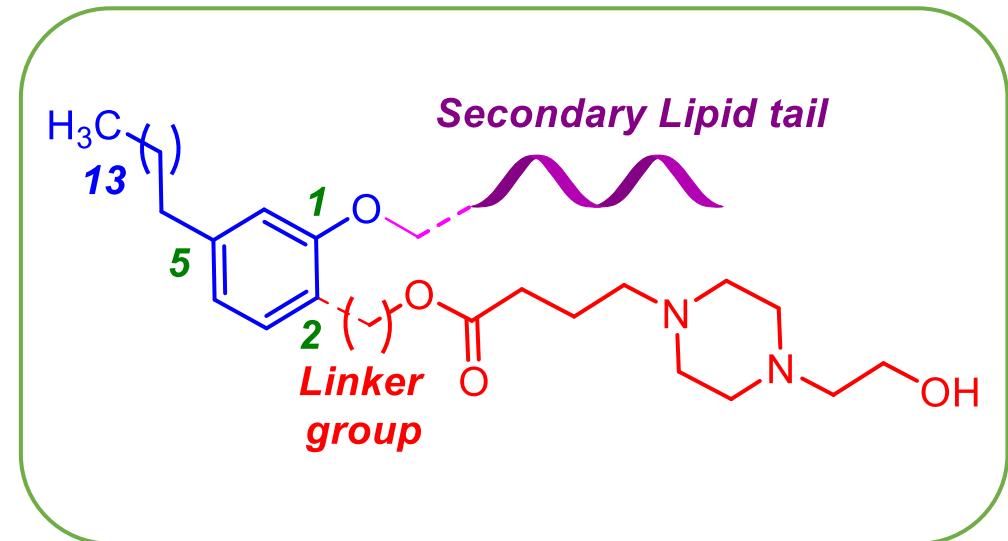
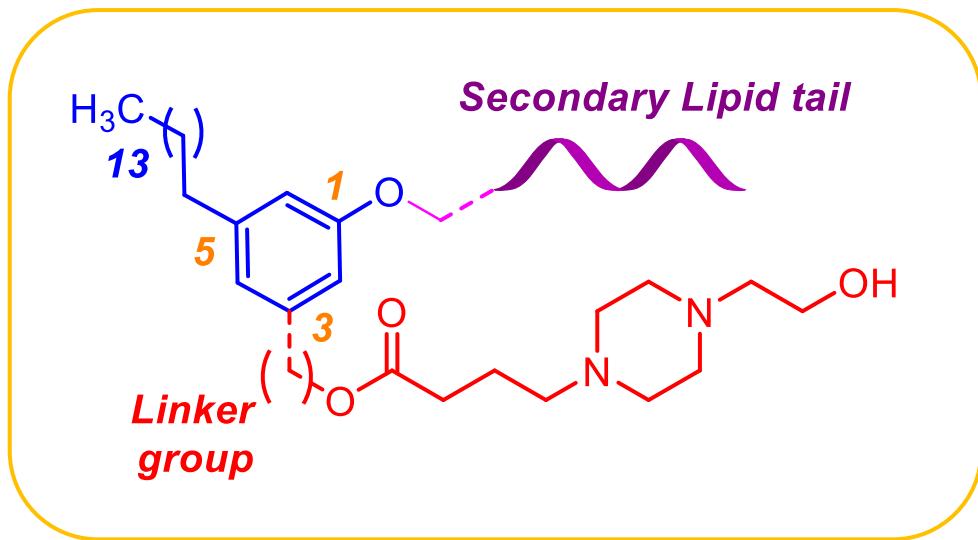
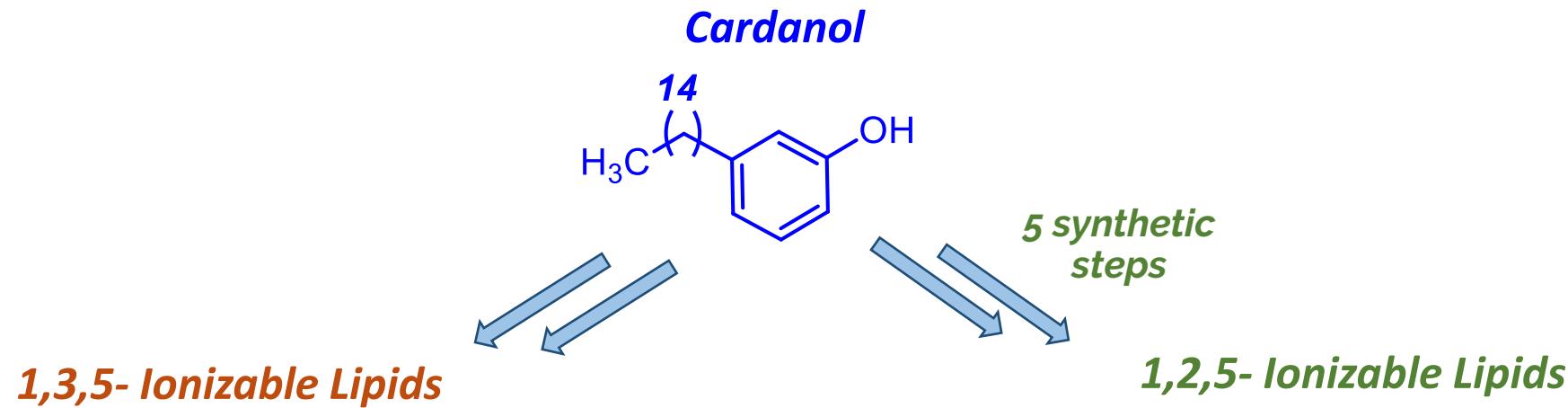


*Alkyne linker
Branched
chain*

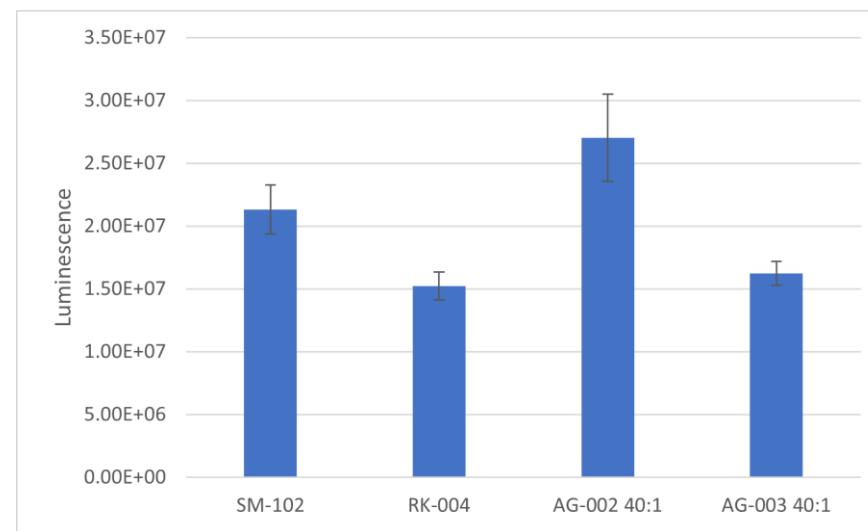
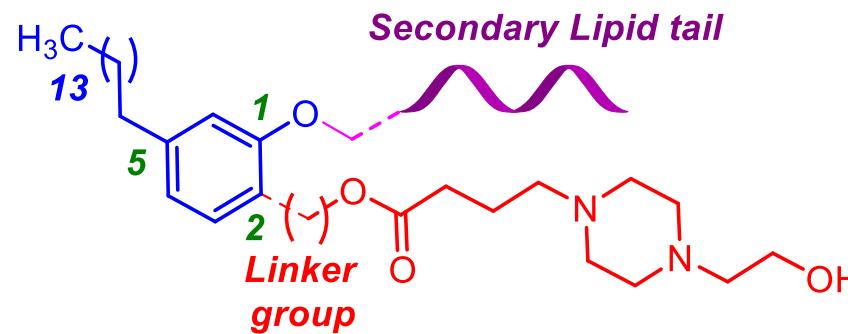


Testing in progress

1,2,5-trisubstituted ionizable lipids from cardanol

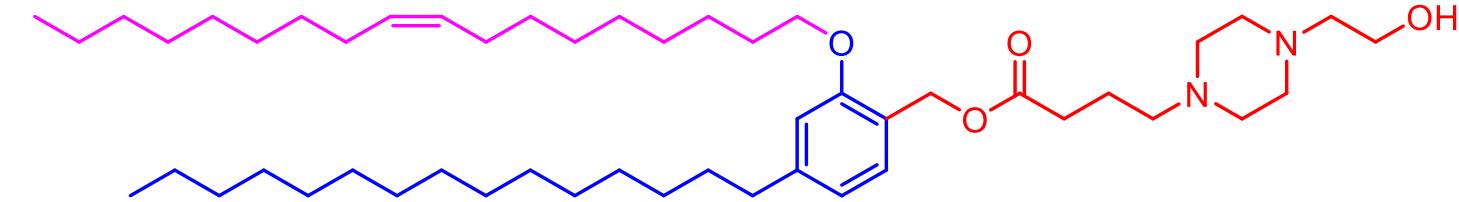


Synthesized 1,2,5 ionizable lipids



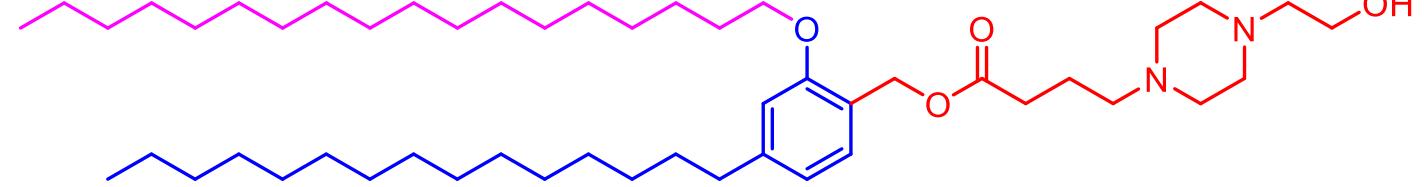
Unsaturated chains

AG-002

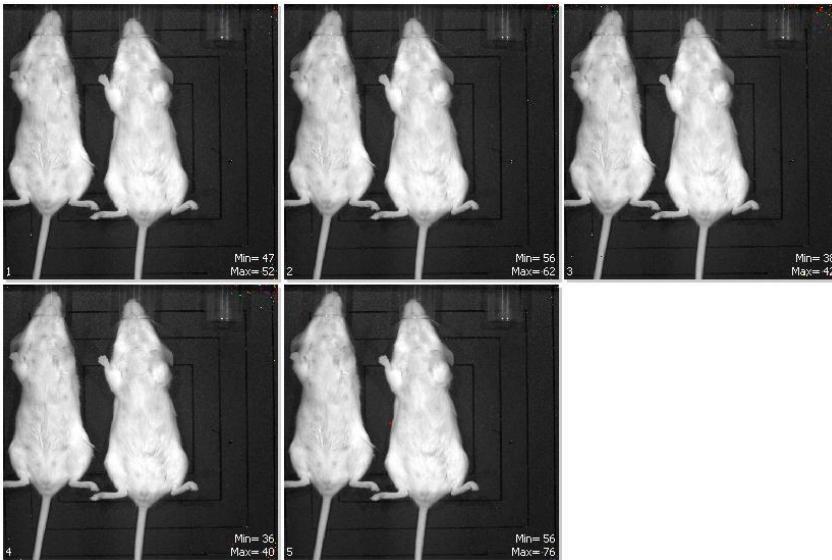


Saturated chains

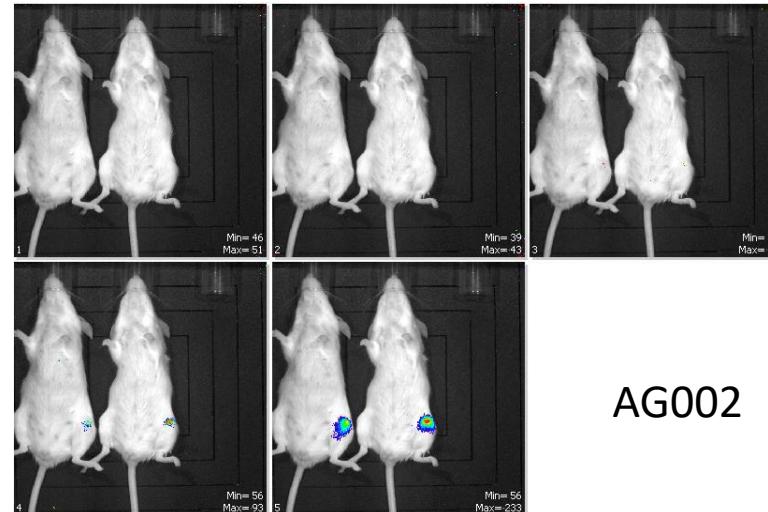
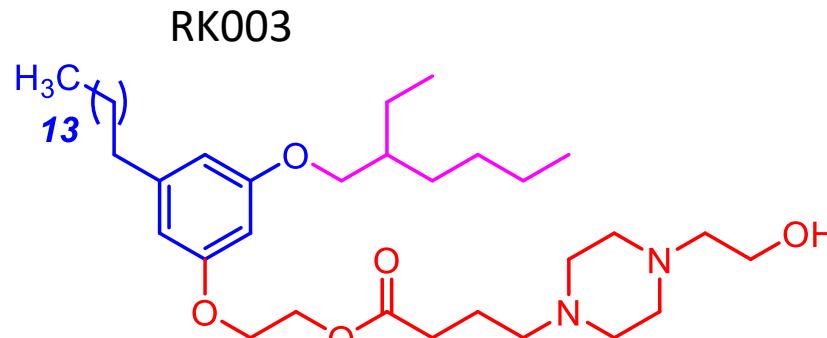
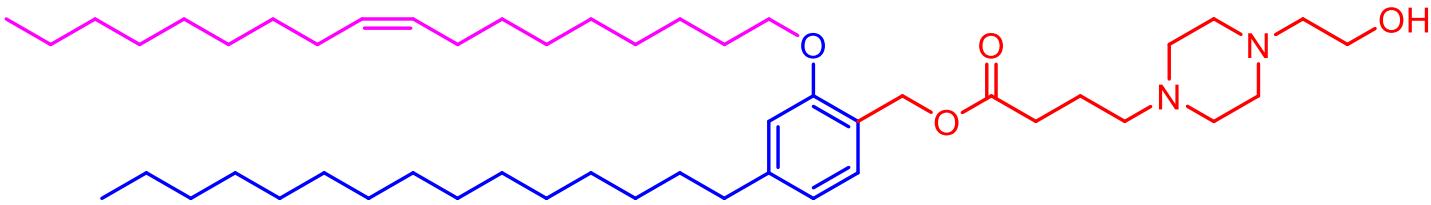
AG-003



Delivery using new ionizable lipids in mice

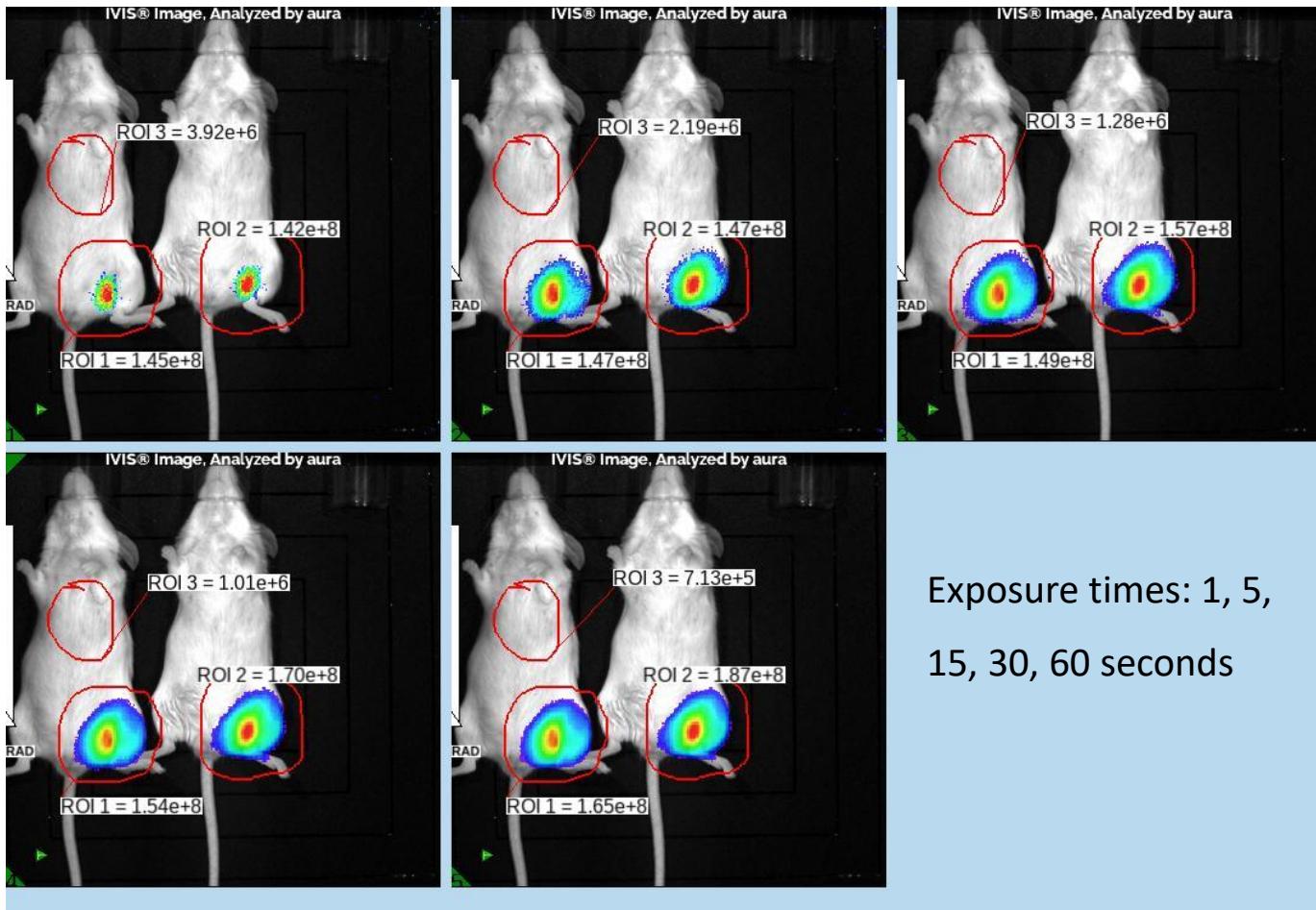


- Single component lipid nanoparticle (LNP)
- Intramuscular injection (IM)

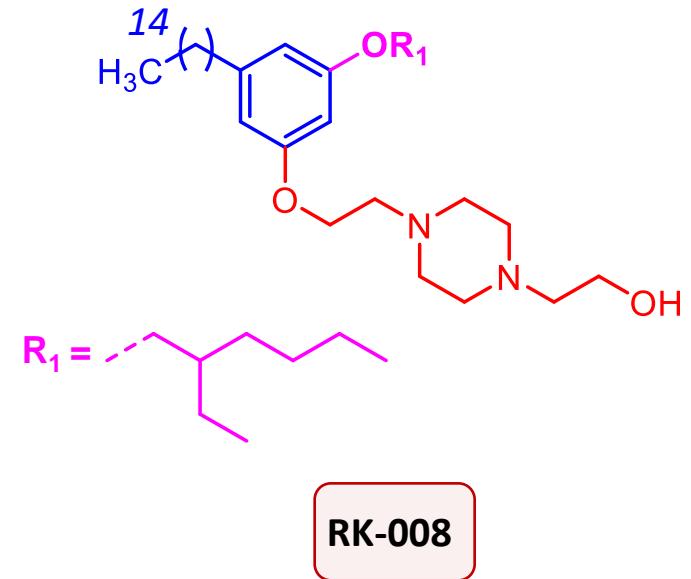


AG002

Four component formulation using microfluidics

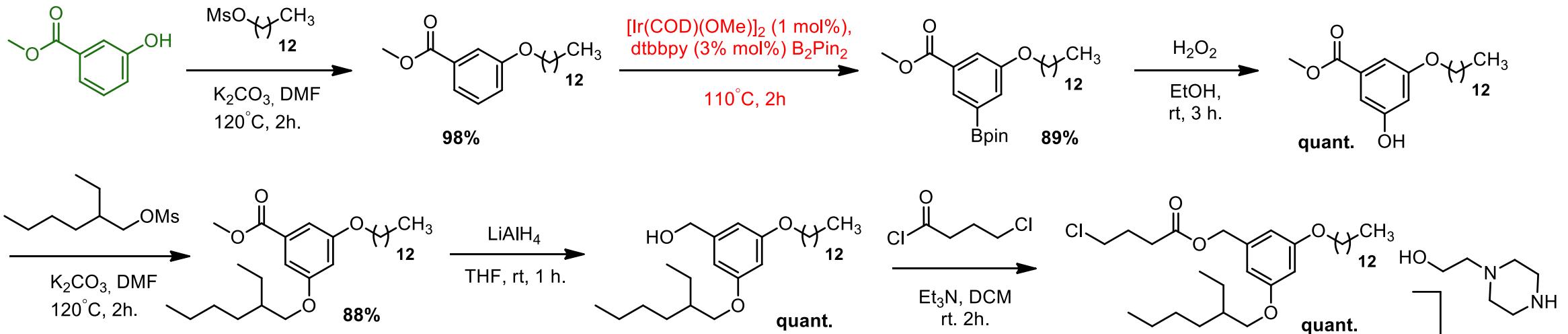


Exposure times: 1, 5,
15, 30, 60 seconds

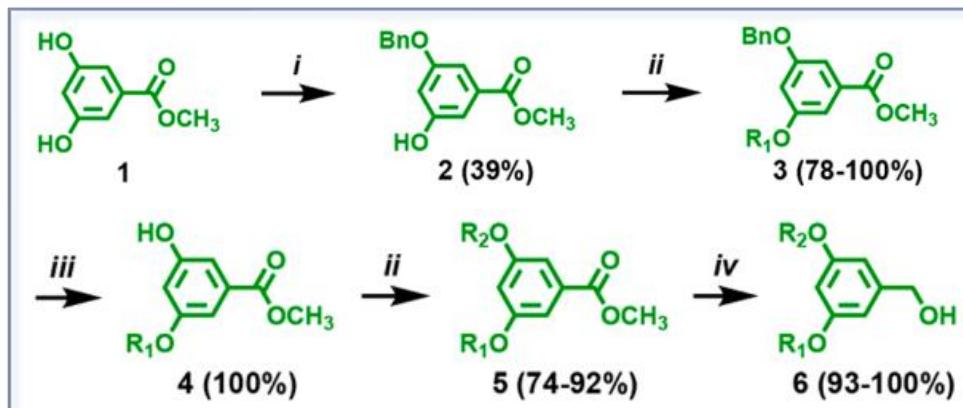


Cholesterol: Distearoylphosphatidylcholine (DSPC): Dimyristoyl glycerol (DMG) –polyethyleneglycol (PEG) 2000
40:1 w:w lipid:mRNA

Current Research: Making and testing Weismann-162 as a standard for IM injections



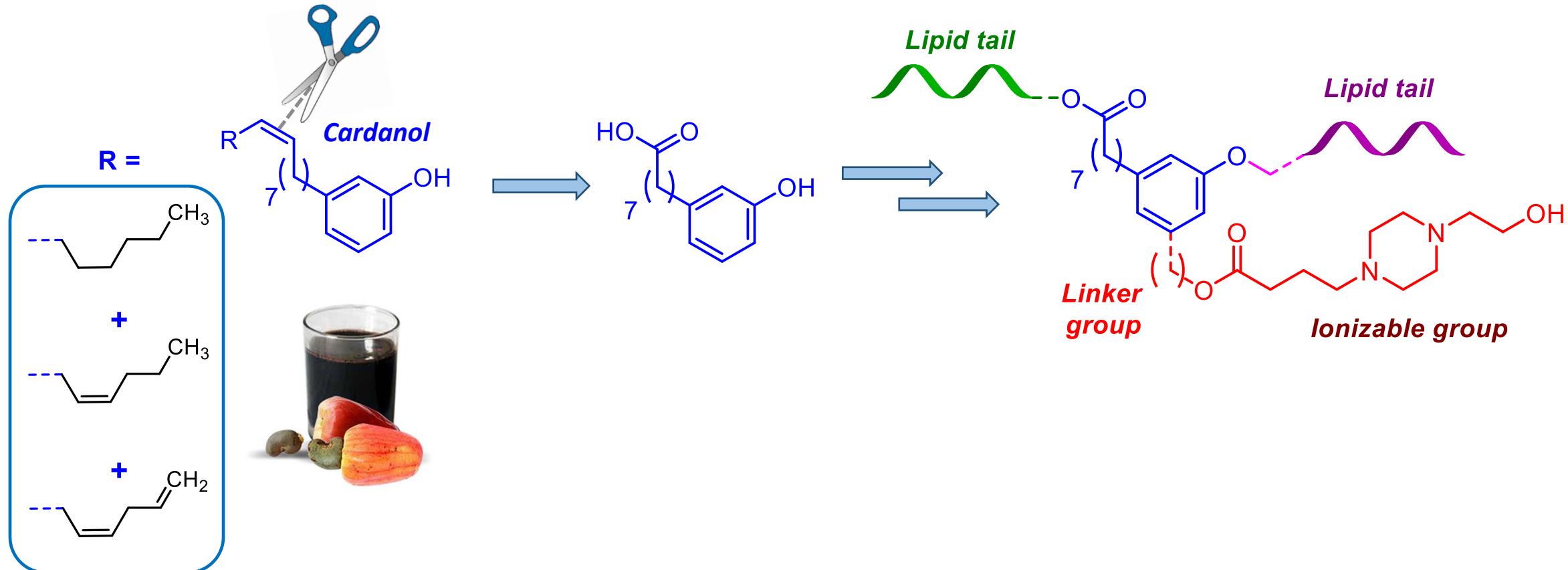
Percec and Weissman method:



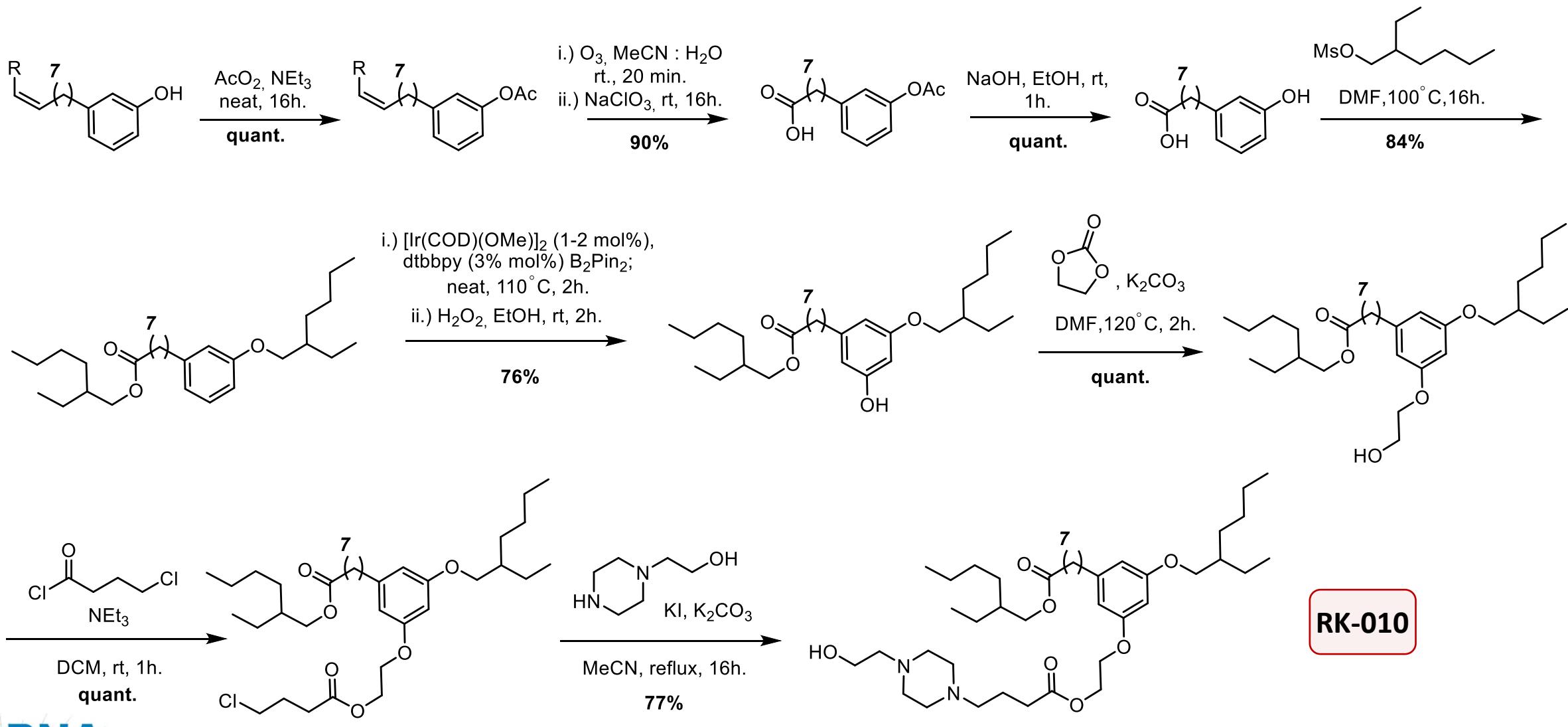
Reagents and Conditions: i) BnCl , K_2CO_3 , KI , DMF, 80°C , 5 h; ii) RBr , K_2CO_3 , DMF, 120°C , 2 h; iii) H_2 , Pd/C , DCM, MeOH, 12 h; iv) LiAlH_4 , THF, $0-23^\circ\text{C}$, 1 h; v) 4-Bromobutyric acid, SOCl_2 , DMF (cat.), DCM, 23°C , 1 h, then DMAP, NET_3 , DCM, $0-23^\circ\text{C}$, 2 h; vi) DCC, DPTS, 12 h; vii) K_2CO_3 , MeCN, 95°C , 3 h.

J. Am. Chem. Soc. 2022, 144, 4746–4753

Current Research: Modifying the cardanol C-15 chain



Modification of the C-15 Chain

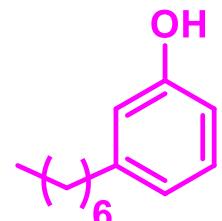


C-7 and C-5 Compounds from Cashew Nut Shell Liquid

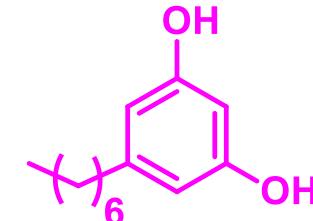


Simple extraction,
distillation and
ozonolysis reaction

Plus other steps



C-7
cardanol



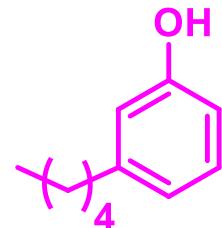
C-7
Cardol

Pack Size	Quantity	Price (USD)	Sub Total
10mg	- 0 +	\$ 129.00	0.0
100mg	- 0 +	\$ 426.00	0.0
500mg	- 0 +	\$ 1740.00	0.0
1g	- 0 +	\$ 3244.00	0.0

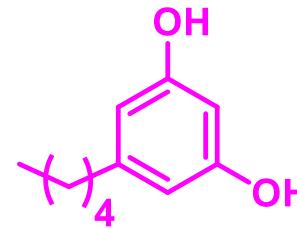
Cashew nut shell oil

Waste product

- Extracted from inedible shells



C-5
cardanol



C-5
Cardol (olivetol)

Pack Size	Quantity	Price (USD)	Sub Total
1g	- 0 +	\$ 58.00	0.0
2.5g	- 0 +	\$ 68.00	0.0
5g	- 0 +	\$ 104.00	0.0
10g	- 0 +	\$ 148.00	0.0
25g	- 0 +	\$ 287.00	0.0
100g	- 0 +	\$ 1030.00	0.0

Intellectual Property

Provisional Patent filed on 18 September in South Africa ZA2023/08807

- The use of ionizable lipids derived from cashew nut shell liquid and their formulation into single or multicomponent lipid nanoparticles (LNPs) and their ability to deliver mRNA
- The synthesis of the hydrogenated cardol and the C-7 and C-5 analogs (olivetol) from cashew nut shell liquid

Acknowledgements

Robin Klintworth
Connor Stockley
Aneesa Gani
Kennedy Ngwira
Songeziwe Ntsimango

Antiviral Gene Therapy Research Unit,
University of the Witwatersrand, Patrick Arbuthnot



South African Medical Research Council
University of the Witwatersrand
World Health Organization
Medicines Patent Pool
mRNA Vaccine Technology Transfer Hub
National Institute of Communicable Diseases

