Lipid Nanoparticles for mRNA Delivery

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Introduction to Ionizable Lipids

Lipid Nanoparticle (Delivery Vehicle)

 pegylated lipid
 ionizable lipid
 phospholipid
 cholesterol
 RNA

SM-102
Moderna

ALC-0315
Pfizer-BioNTech

MC3
Properties of Ionizable Lipids

- 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**

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Wt lipid per dose (30 µg mRNA/dose)</th>
<th>Wt lipid 1B doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionizable lipid</td>
<td>0.35 mg</td>
<td>351 kg</td>
</tr>
<tr>
<td>PEG-lipid</td>
<td>0.034 mg</td>
<td>34.4 kg</td>
</tr>
<tr>
<td>DSPC</td>
<td>0.072 mg</td>
<td>72 kg</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.14 mg</td>
<td>142 kg</td>
</tr>
</tbody>
</table>

PEG

DSPC

largest quantity by far
Potential Compounds from Cashew Nut Shell Liquid as building blocks for Ionizable Lipids?

Major components

- Cardanols
- Anacardic acids

Minor components

- Cardols

Cashew nut shell liquid
Waste product
- Extracted from inedible shells
Precedence for aromatic ionizable lipids

Single Component lipid:

Drew Weissman: Intravenous injection (IV)
Our work: Intramuscular injection (IM)
C-15 Compounds from Cashew Nut Shell Liquid for Ionizable Lipids

- Mainly hydrogenated cardanol
- Simple extraction, distillation/separation and hydrogenation
- 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

- 17 ionizable lipids

Raw materials available in Africa
Roughly 1 g of ionizable lipid per shell
Convert all nine compounds into one?

**Anacardic acids**

**Cardanols**

**Cardols (minor)**

**Decarboxylation**

**Hydrogenation**

**Meta hydroxylation reaction**

Separate cardanols from cardols:

(Aq. MeOH/\text{NH}_3, Hexane)

Cardanols

Hydrogenation

Hydrogenated Cardol
Cardanol-Derived Ionizable Lipids

Cardanol

Cardanol-derived Ionizable Lipid:

Weissman lipid (162):

Functional equivalence?

[Chemical structures and reactions]

- Cardanol (14)
  - MsO
  - DMF, 100°C, 16h.
  - quant.
- Cardanol-derived Ionizable Lipid:
  - [Ir(COD)(OMe)]2 (1-2 mol%), dtbbpy (3% mol%) B2Pin₂
  - neat, 110°C, 2h.
  - quant.
  - 92% yield
- Weissman lipid (162):
  - O₂, K₂CO₃
  - EtOH, rt, 2h.
  - quant.
- RK-003 (63% yield)
Importance of Ionizable group: *In vitro* formulations single component

**Firefly luciferase (Fluc) assay**

**No Luminescence:**

- **RK-001**
- **RK-002**
- **RK-003**
- **RK-004**
- **RK-005**

**MC3**
Examples

In vitro formulations single component

Firefly luciferase (Fluc) assay

Precipitation - Too large

Modern lipid (4 component)
Other examples of 1,3,5-ionizable lipids

- CS-001: Aryl linker
- CS-005: Precipitation - Linker too large
- CS-006: Alkyne linker
- CS-007: Alkyne linker, Branched chain

All compounds are under 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)

![Chemical structures of RK003 and 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

RK-008
Current Research: Making and testing Weismann-162 as a standard for IM injections

Percec and Weissman method:

Reagents and Conditions: i) BnCl, K₂CO₃, KI, DMF, 80 °C, 5 h; ii) RBr, K₂CO₃, DMF, 120 °C, 2 h; iii) H₂, Pd/C, DCM, MeOH, 12 h; iv) LiAlH₄, THF, 0–23 °C, 1 h; v) 4-Bromobutyric acid, SOCl₂, DMF (cat.), DCM, 23 °C, 1 h, then DMAP, NEt₃, DCM, 0–23 °C, 2 h; vi) DCC, DPTS, 12 h; vii) K₂CO₃, MeCN, 95 °C, 3 h.

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Current Research: Modifying the cardanol C-15 chain

Cardanol

R =

\[ \text{Lipid tail} \]

\[ \text{Linker group} \]

\[ \text{Ionizable group} \]
Modification of the C-15 Chain

\[
\text{AcO}_2, \text{NEt}_3, \text{neat, 16h.} \quad \xrightarrow{\text{quant.}} \quad \text{OH} \quad \xrightarrow{\text{i.} \; \text{O}_3, \text{MeCN:H}_2\text{O, rt, 20 min.}} \quad \xrightarrow{\text{ii.} \; \text{NaClO}_3, \text{rt, 16h.}} \quad \xrightarrow{90\%} \\
\text{OAc} \quad \xrightarrow{\text{NaOH, EtOH, rt, 1h.}} \quad \xrightarrow{\text{quant.}} \quad \xrightarrow{\text{DMF, 100°C, 16h.}} \quad 84\%
\]

\[
\text{76\%} \quad \xrightarrow{\text{i.} \; [\text{Ir(COD)(OMe)}_2]_2 (1-2 \text{ mol\%), dtbbpy (3\% mol\%) B_3\text{Pin}_2; neat, 110 \text{ °C, 2h.}} \quad \xrightarrow{\text{ii.} \; \text{H}_2\text{O}_2, \text{EtOH, rt, 2h.}}
\]

\[
\text{OAc} \quad \xrightarrow{\text{DMF, 120°C, 2h.}} \quad \text{quant.}
\]

\[
\text{Cl}^- \quad \text{NEt}_3 \quad \xrightarrow{\text{DCM, rt, 1h.}} \quad \xrightarrow{\text{quant.}} \quad \text{OH}
\]

\[
\text{Cl}^- \quad \text{Kl, K}_2\text{CO}_3 \quad \xrightarrow{\text{MeCN, reflux, 16h.}} \quad 77\%
\]

\[
\text{RK-010}
\]
**Cashew nut shell oil**

- Extracted from inedible shells

**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
- C-5 cardanol
- C-5 Cardol (olivetol)

<table>
<thead>
<tr>
<th>Pack Size</th>
<th>Quantity</th>
<th>Price (USD)</th>
<th>Sub Total</th>
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<tbody>
<tr>
<td>10mg</td>
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<tr>
<td>100mg</td>
<td>-</td>
<td>$426.00</td>
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<tr>
<td>500mg</td>
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<td>$1740.00</td>
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<tr>
<td>1g</td>
<td>-</td>
<td>$3244.00</td>
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- C-5 Cardol (olivetol)
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

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World Health Organization
Medicines Patent Pool
mRNA Vaccine Technology Transfer Hub
National Institute of Communicable Diseases