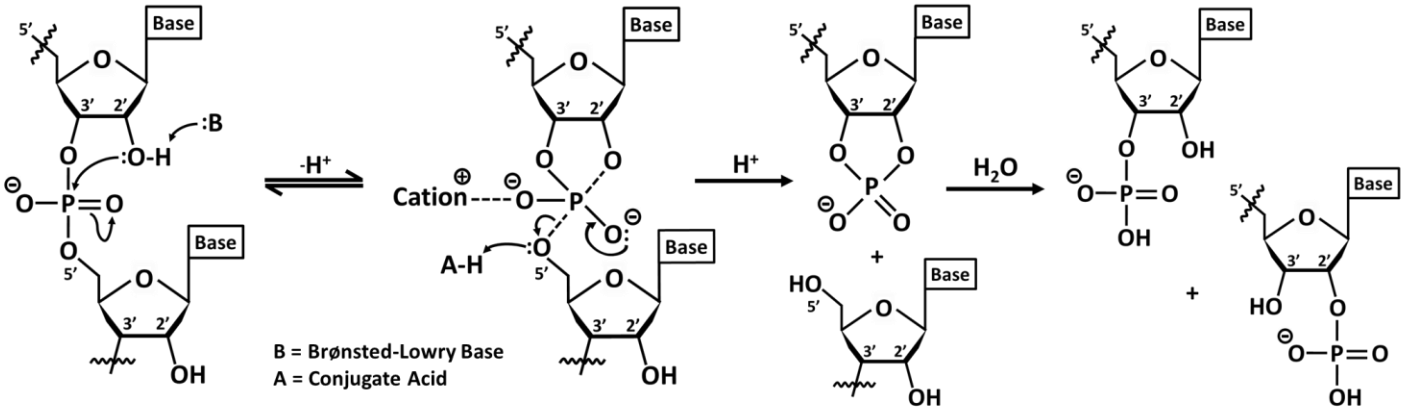
Potential Causes of mRNA Vaccine and Therapeutic Instability

1) RNA Hydrolysis – RNA is Sensitive to Hydrolysis when Stored in Aqueous Solutions

Spontaneous or Auto-Hydrolysis; pH Induced; Enzymatic Cleavage; Metal Catalyzed

Example Mechanism of RNA Hydrolysis: Brønsted–Lowry Acid–Base Catalysis[1-4]

2′ Proton Abstraction Nucleophilic Attack on Adjacent Phosphate	Formation of Pentacoordinated Phosphorane Acid Donates H^+ to 5′ O Resulting in Strand Cleavage	2′,3′-Cyclic Phosphate Results in Mixture of 2′ and 3′ Terminal Phosphate

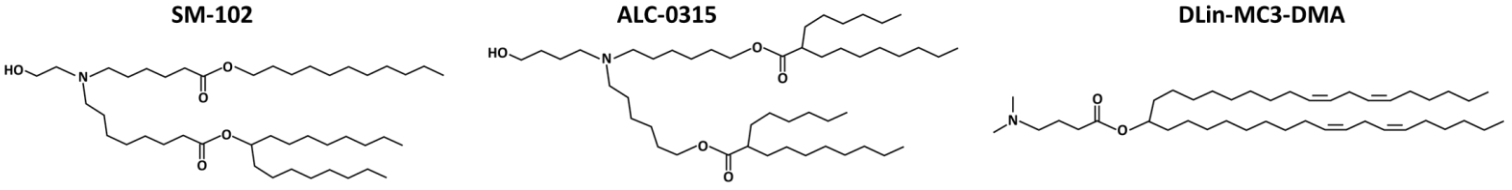


1. Oivanen, M. *et al. Chem. Rev.* 98, 961–990 (1998)
2. Perrin, C. L. *J. Org. Chem.* 60, 1239–1243 (1995)
3. Li, Y. & Breaker, R. R. *J. Am. Chem. Soc.* 121, 5364–5372 (1999)
4. Perreault, D. M. *et al. Angew. Chem. Int. Ed. Engl.* 36, 432–450 (1997)

2) RNA Base Adducts – Ionizable Lipids can Form Aldehydes that React with RNA Bases

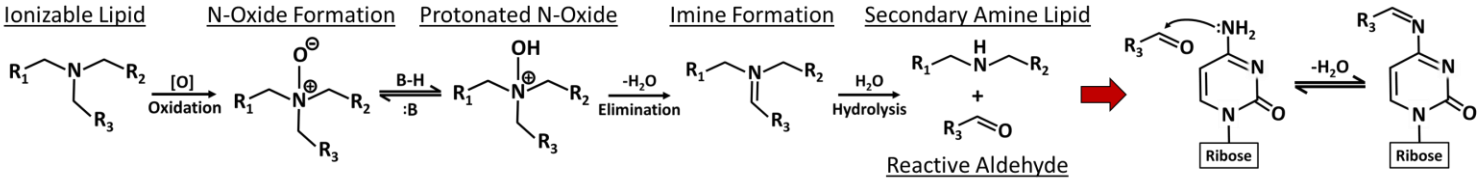
Example Ionizable Lipids and Mechanism of Adduct Formation[5,6]

SM-102 | ALC-0315 | DLin-MC3-DMA



Formation of Reactive Aldehydes through Oxidation of Ionizable Lipid

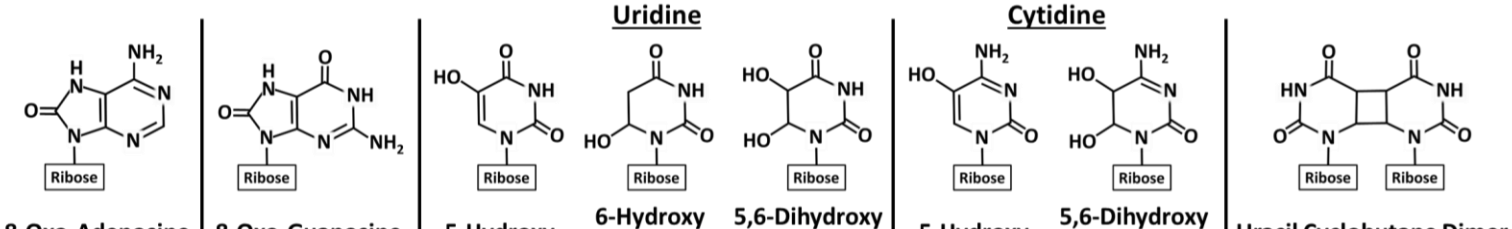
RNA Base Adduct Formation
Aldehyde Reacts with Primary Amines



5. Packer, M., *et al. Nat. Commun.* 12, 6777 (2021)
6. Blenke, E. O. *et al. J. Pharm. Sci.* 112, 386–403 (2023)

3) RNA Base Oxidation – Oxidation Induced Modifications can Alter RNA Bases[7-10]

Auto-Oxidation; UV/light; Metal Catalyzed; Peroxides (via lipid or PEG oxidation)



8-Oxo-Adenosine | 8-Oxo-Guanosine | 5-Hydroxy | 6-Hydroxy 5,6-Dihydro | 5,6-Dihydroxy 5,6-Dihydro | 5-Hydroxy | 5,6-Dihydroxy 5,6-Dihydro | Uracil Cyclobutane Dimer

7. Kamiya, M. *et al. Pharmaceutics* 14, 2357 (2022)
8. Tanaka, M. & Chock, P. B. *Front. Mol. Biosci.* 8, (2021)
9. Velema, W. A. & Lu, Z. *JACS Au* 3, 316–332 (2023)
10. Wurtmann, E. J. *et al. Crit. Rev. Biochem. Mol. Biol.* 44, 34–49 (2009)

Kyle P. Heim, PhD Alpine Medical Technology, LLC Kyle@AlpineMed.Tech 2024