Chemical synthesis of a DNA strand modified by a DEB-FAPy-dG adduct of 1,3-butadiene

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Abstract

1,3-Butadiene (BD) is a known mammalian carcinogen and is found abundantly in cigarette smoke, automobile exhaust, and polymer industry settings. Upon entering the human body, BD is metabolized by cytochrome P450 monooxygenases CYP2E1 and CYP2A6 to multiple reactive electrophiles, including 1,2:3,4-diepoxybutane (DEB). DEB has the potential to alkylate the N7 position of guanine to form a formamidopyrimidine (FAPy) adduct, termed DEB-FAPy-dG. This adduct has been implicated in disease pathways and is known to be a player in carcinogenesis, but the specific mechanisms by which it acts are largely unknown. In this work, a novel synthetic pathway to chemically synthesize a phosphoramidite containing the DEB-FAPy-dG adduct is reported, which will allow for selective incorporation of the adduct into synthetic DNA. Analysis of this synthetic DNA strand will reveal how the noncanonical nucleobase may impact chain extension, base pairing, and polymerase activity, which will contribute important knowledge to the understanding of how BD and its metabolites contribute to disease. The new synthetic pathway sets the foundation for future work in this field, including the creation and analysis of a DNA strand modified by DEB-FAPy-dG and the syntheses of DNA adducts formed by other metabolites of BD, both of which will contribute to the understanding of the effects of DNA adducts and how the effects of these adducts may be mitigated.

Goals

1. Synthesize a phosphoramidite containing a DEB-FAPy-dG adduct to insert into an oligodeoxynucleotide
2. Synthesize a nucleoside triphosphate containing a DEB-FAPy-dG adduct to incorporate into DNA using DNA polymerase
3. Determine the specific mechanism by which the DNA adduct impacts DNA replication, causes mutations, and leads to disease

Metabolism of 1,3-Butadiene to Form DNA Adducts

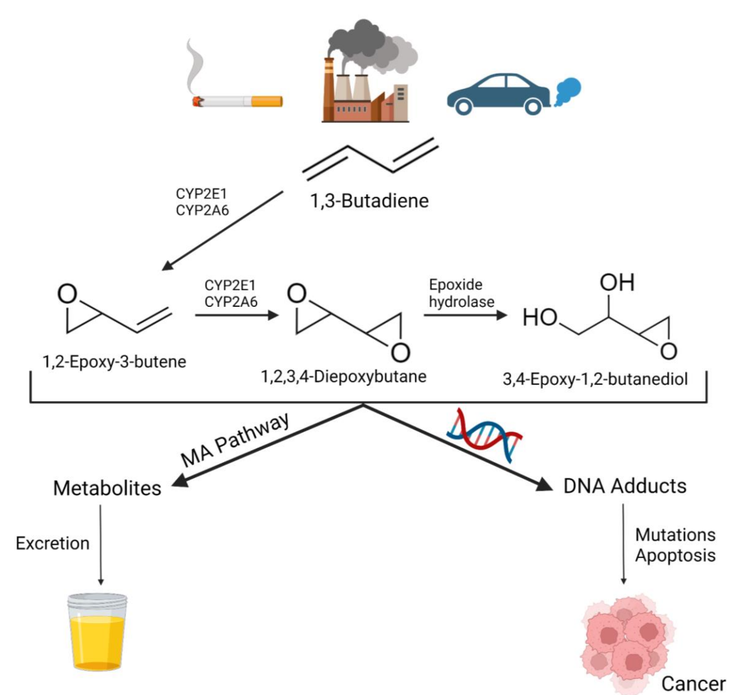

Figure 1: Metabolic pathway of 1,3-butadiene.[1,2]

Synthetic Pathway of DEB-FAPy-dG Phosphoramidite and Oligodeoxynucleotide Synthesis

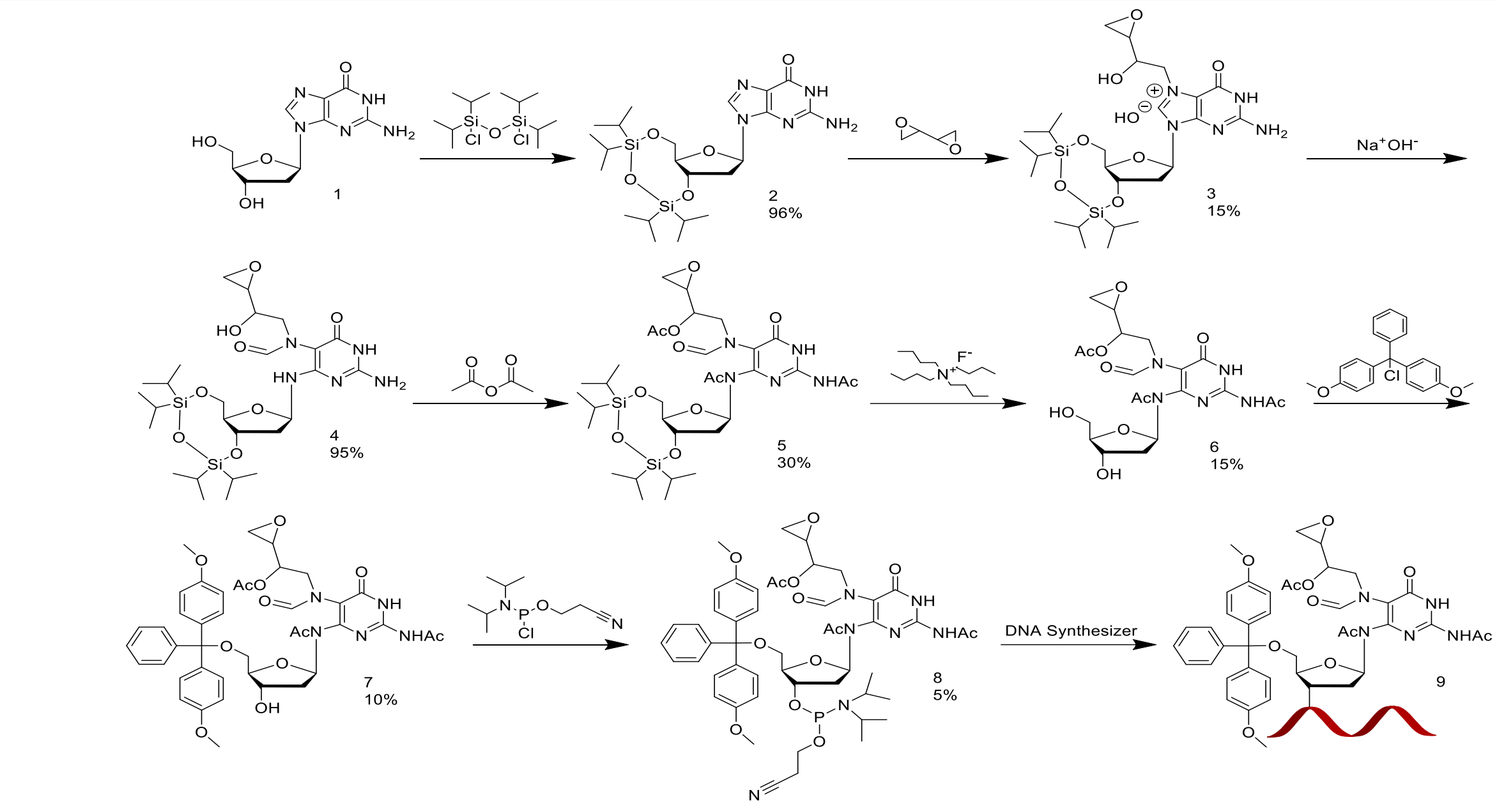

Figure 2: Nine step synthesis of an oligonucleotide containing a DEB-FAPy-dG adduct.

Synthesis of DEB-FAPy-dG Triphosphate

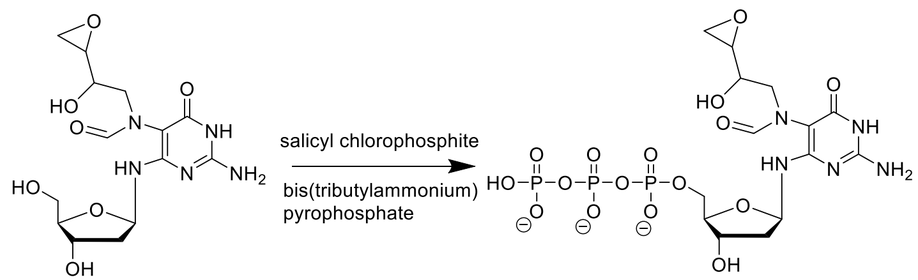

Figure 3: Synthesis of a triphosphate containing the DEB-FAPy-dG adduct.[3,4]

Confirmation of the Synthesis of a DEB-FAPy-dG Adduct

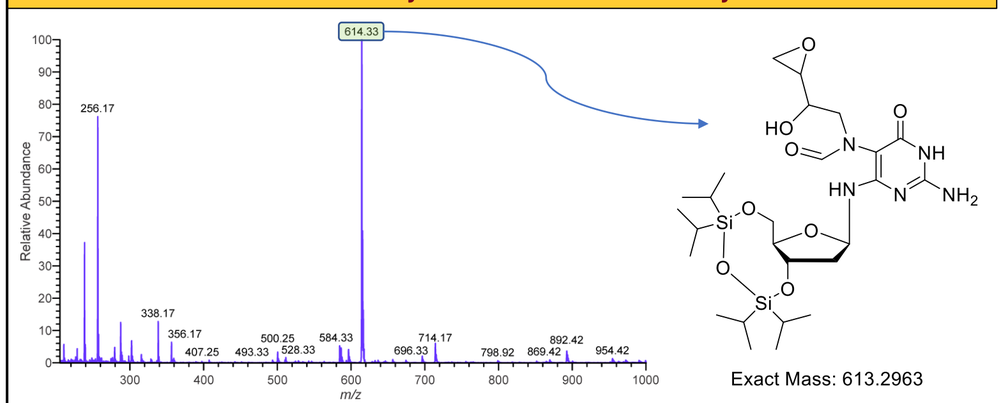

Figure 4: Mass spectrum of the DEB-FAPy-dG adduct, one of the adducts that forms upon exposure to 1,3-butadiene (Figure 1)

Future Directions

- Optimize the final steps of the synthetic pathways to synthesize the phosphoramidite, DNA strand, and triphosphate
- Study adduct-induced DNA damage in cells by inserting synthetic DNA into a plasmid
- Perform primer extension assays using the triphosphate and DNA polymerase to analyze the specific effects the adduct has on DNA base pairing and replication

References

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Acknowledgements

- Department of Chemistry, University of Minnesota
- Tretyakova Lab
- Bob Carlson, Program Specialist, University of Minnesota
- Figures created on BioRender.com