



Synthesis, properties, and reactions of vitamin K analogs

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Bradley P. Klemm, Jeremiah J. Stromich, Enkhtuul Tsoygtbaatar and Philip M. Doyle

Department of Chemistry, University of Wisconsin-Eau Claire, Eau Claire, WI 54702, U.S.A.

(Faculty Mentors: David E. Lewis, UWEC; Michael D. Caldwell, M.D., Ph.D., Marshfield Clinic)

Goals

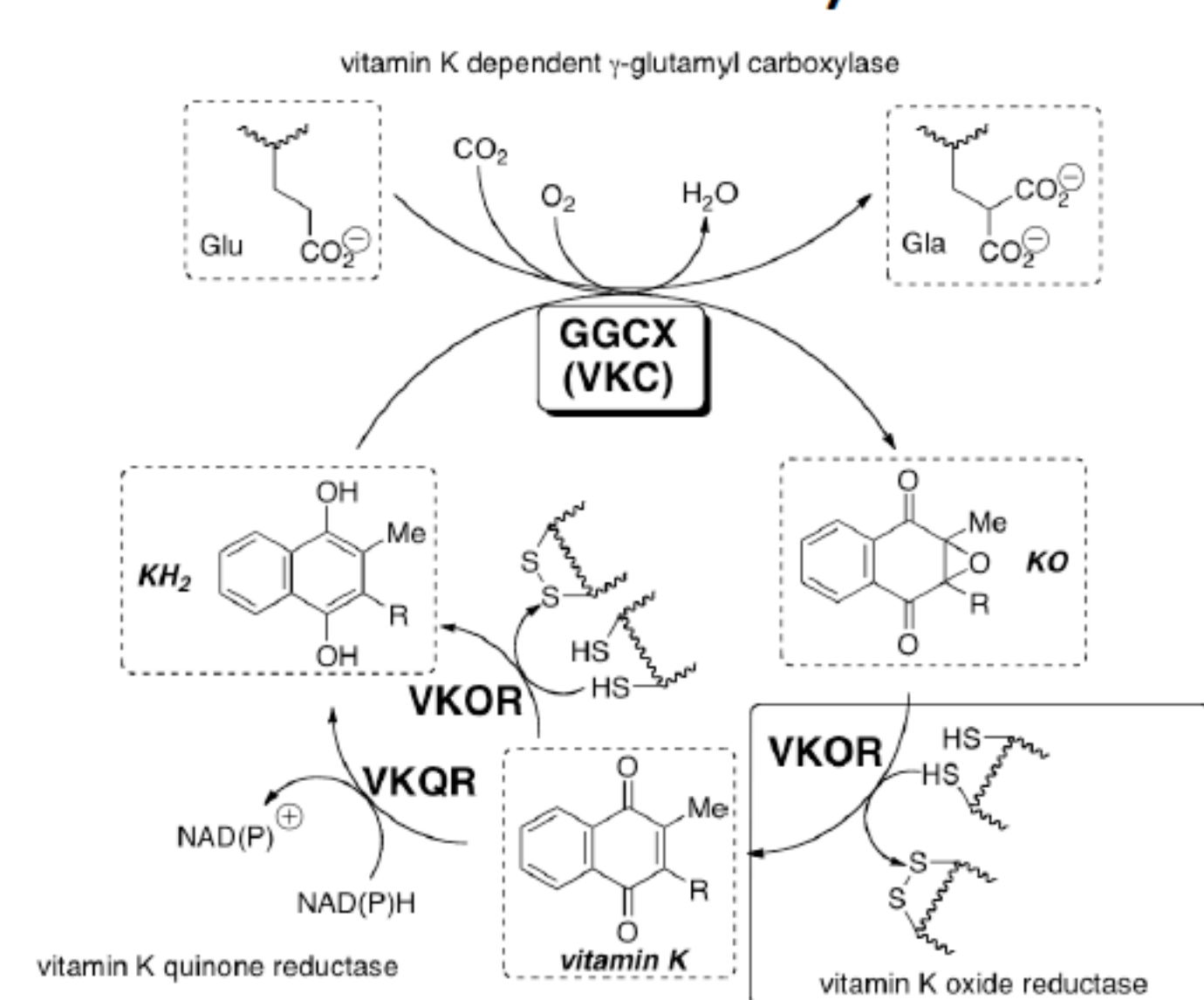
- Conjugate warfarin with Vitamin K analogues for testing in rats
- Characterize any new anti-coagulating compounds, including stereochemistry
- Determine the mechanistic details of warfarin conjugate interaction with VKOR enzyme

Introduction

Vitamin K is an important dietary supplement which is utilized as a indispensable cofactor in the clotting process. It is converted from reduced vitamin K (KH₂) to the epoxide (KO) in this process.

The active form (KH₂) is regenerated through a cycle using the enzyme, VKORC1. The conversion of KO to vitamin K is unique in the pathway in that it is achieved *only* by the VKOR enzyme, whereas other steps can be catalyzed by more than one enzyme.

The Vitamin K Cycle



We have discovered new compounds that enhances the anticoagulant activity of warfarin in rats, without being hepatotoxic or nephrotoxic.

We are attempting to generate analogues of vitamin KO which compete with KO in VKOR. If the analogues do compete with KO in this manner, we may be able to develop new oral anticoagulants that are safer than warfarin. These products are hypothesized to strongly mirror warfarin activity *in vivo*.

Acknowledgments

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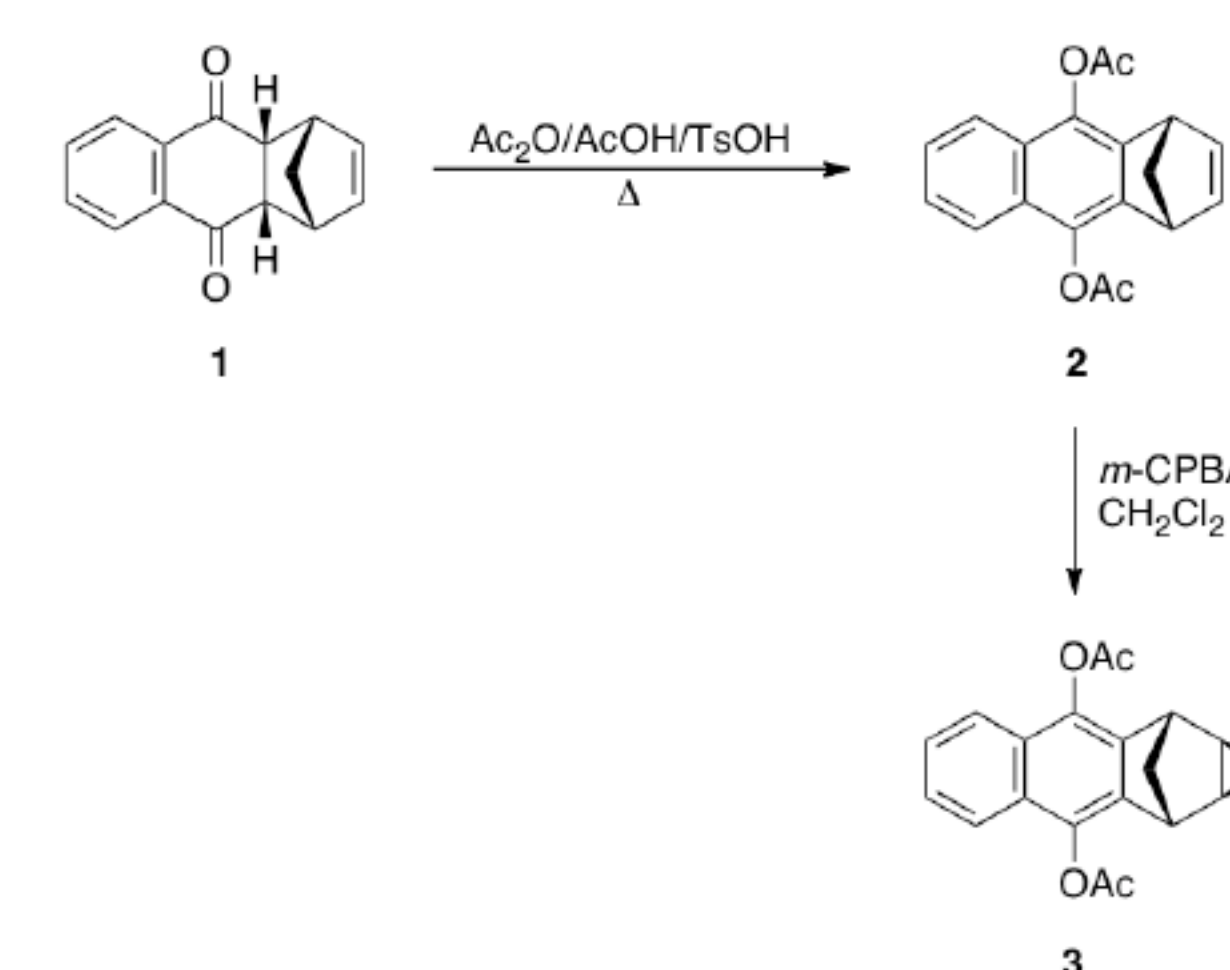
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- University of Wisconsin-Eau Claire Office of Research and Sponsored Programs
- Marshfield Clinic Research Foundation

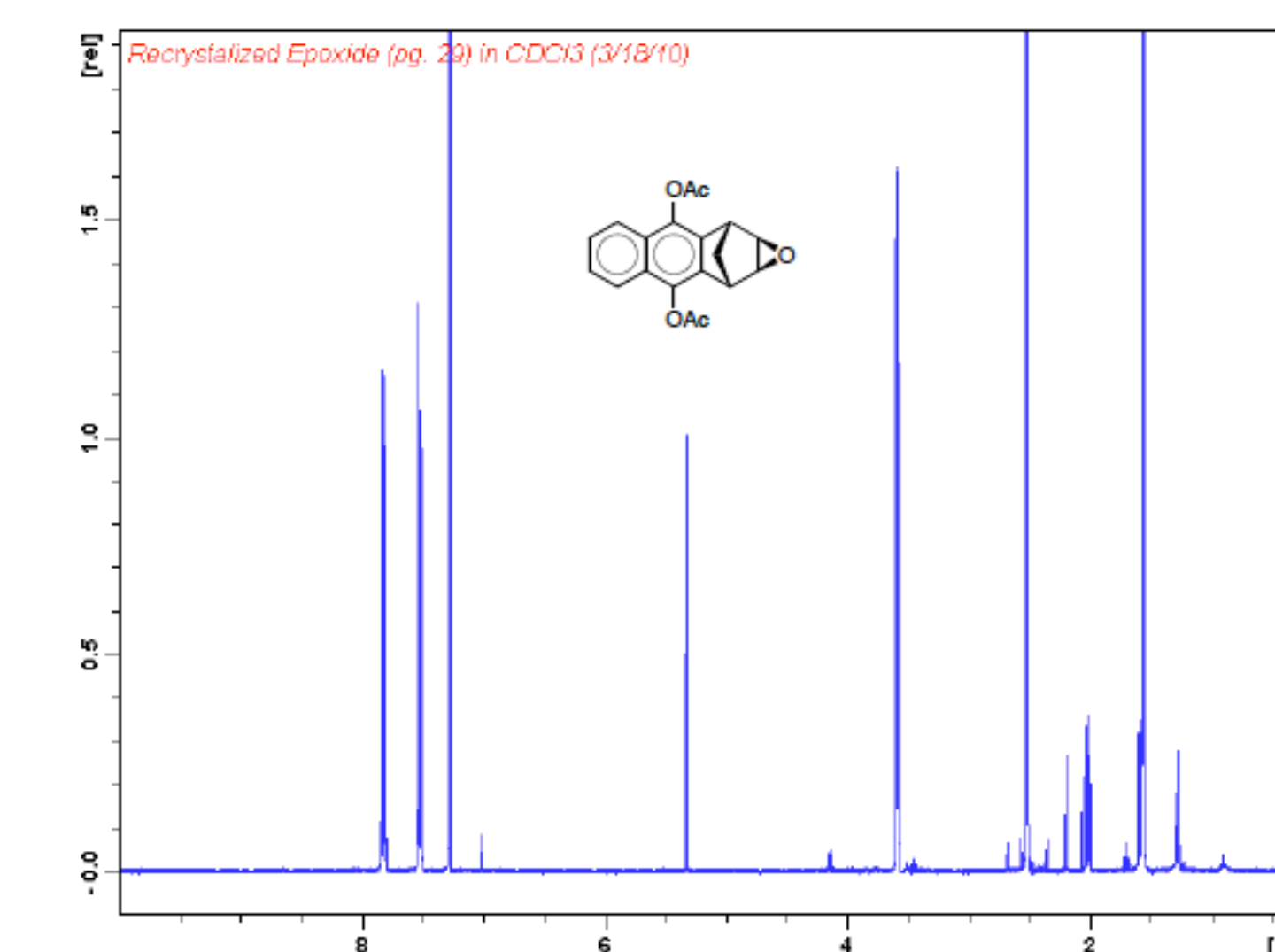
Synthesis of Epoxide Precursor

- The diacetate was dissolved in a minimum amount of dichloromethane
- One equivalent of oxidizing agent, *m*-chloro-perbenzoic acid, was added portion-wise at half-hour intervals
- The solution was vigorously stirred for 24 hours, and the remaining acid was neutralized with calcium hydroxide
- The product was recrystallized from ethyl acetate-hexane, yielding a rust-colored solid

Synthesis of Precursor Molecules



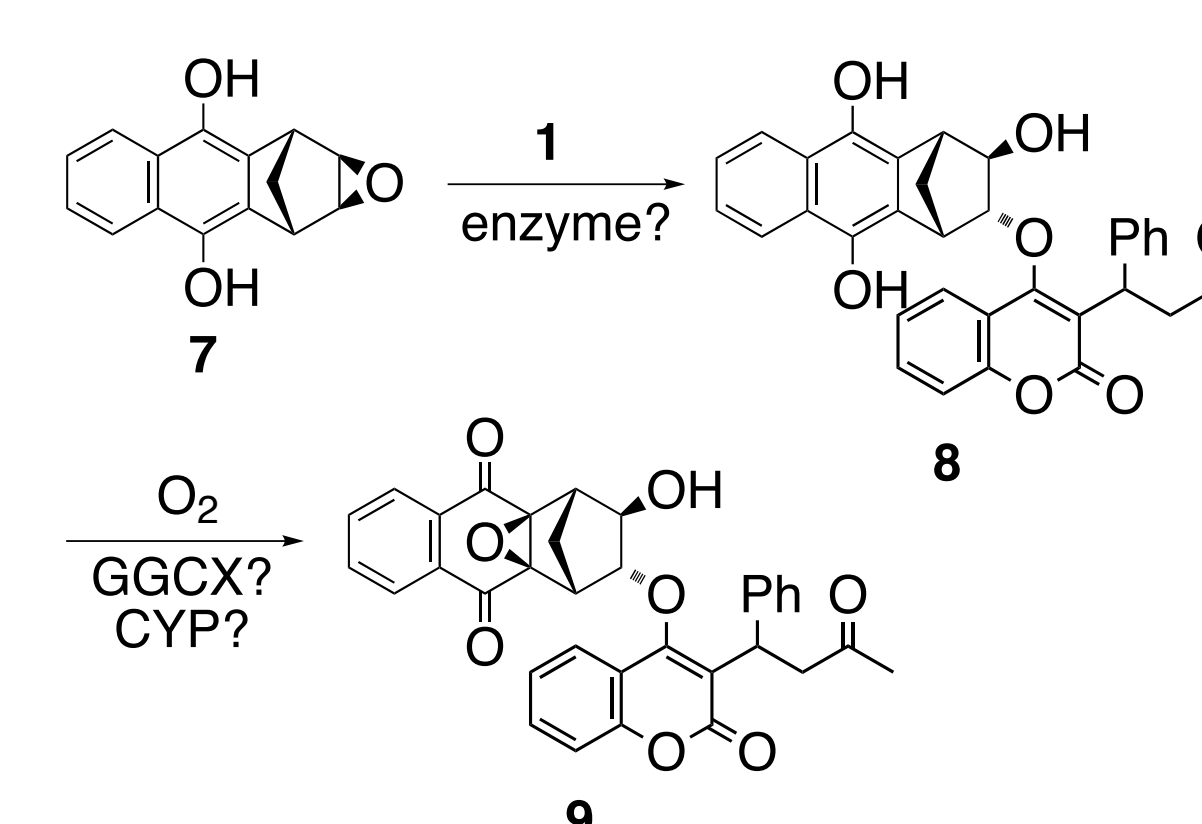
Characterization of Epoxide



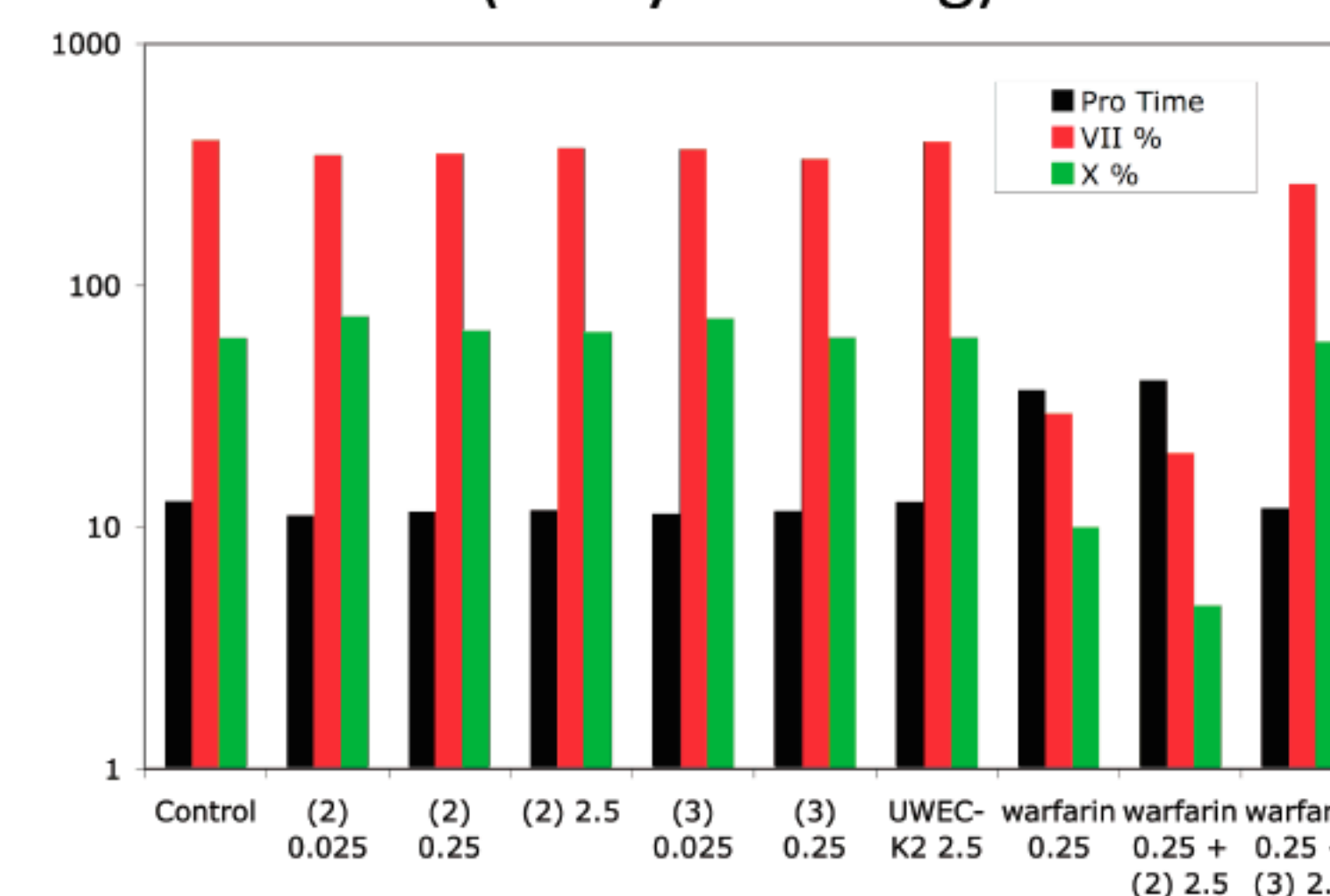
Feeding Results

- The epoxide exhibits anti-warfarin properties, restoring coagulation in warfarin-treated rats, during the first 4 days of the program.
- After 10 days, the anti-coagulation characteristic of warfarin was restored and enhanced four-fold.
- Based on these results, we hypothesize that the epoxide first removes warfarin from circulation by forming an inactive conjugate.
- The conjugate is slowly oxidized *in vivo* to give an epoxide which is analogous to KO.

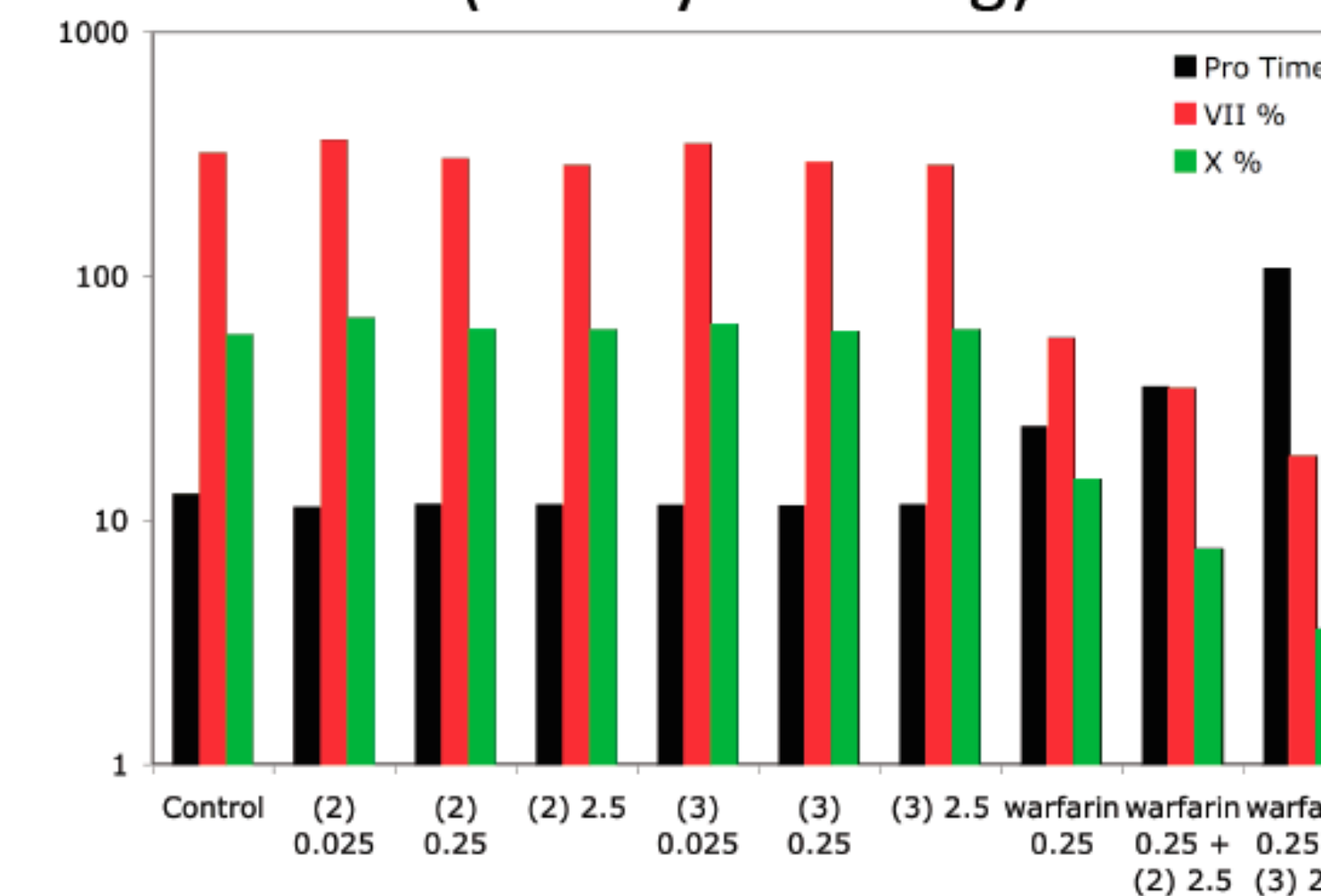
Proposed Action of Warfarin Enhancers



Results of Rat Feeding Studies (4 days feeding)



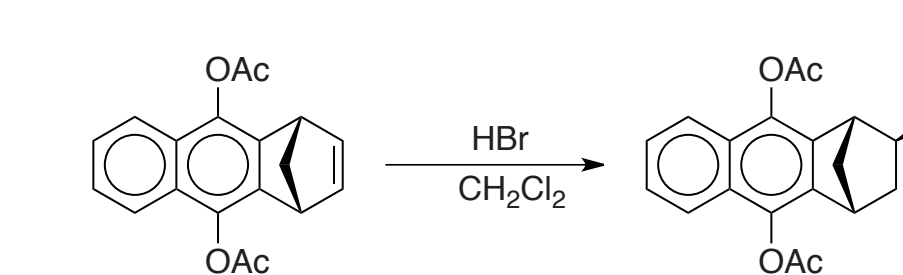
Results of Rat Feeding Studies (10 days feeding)



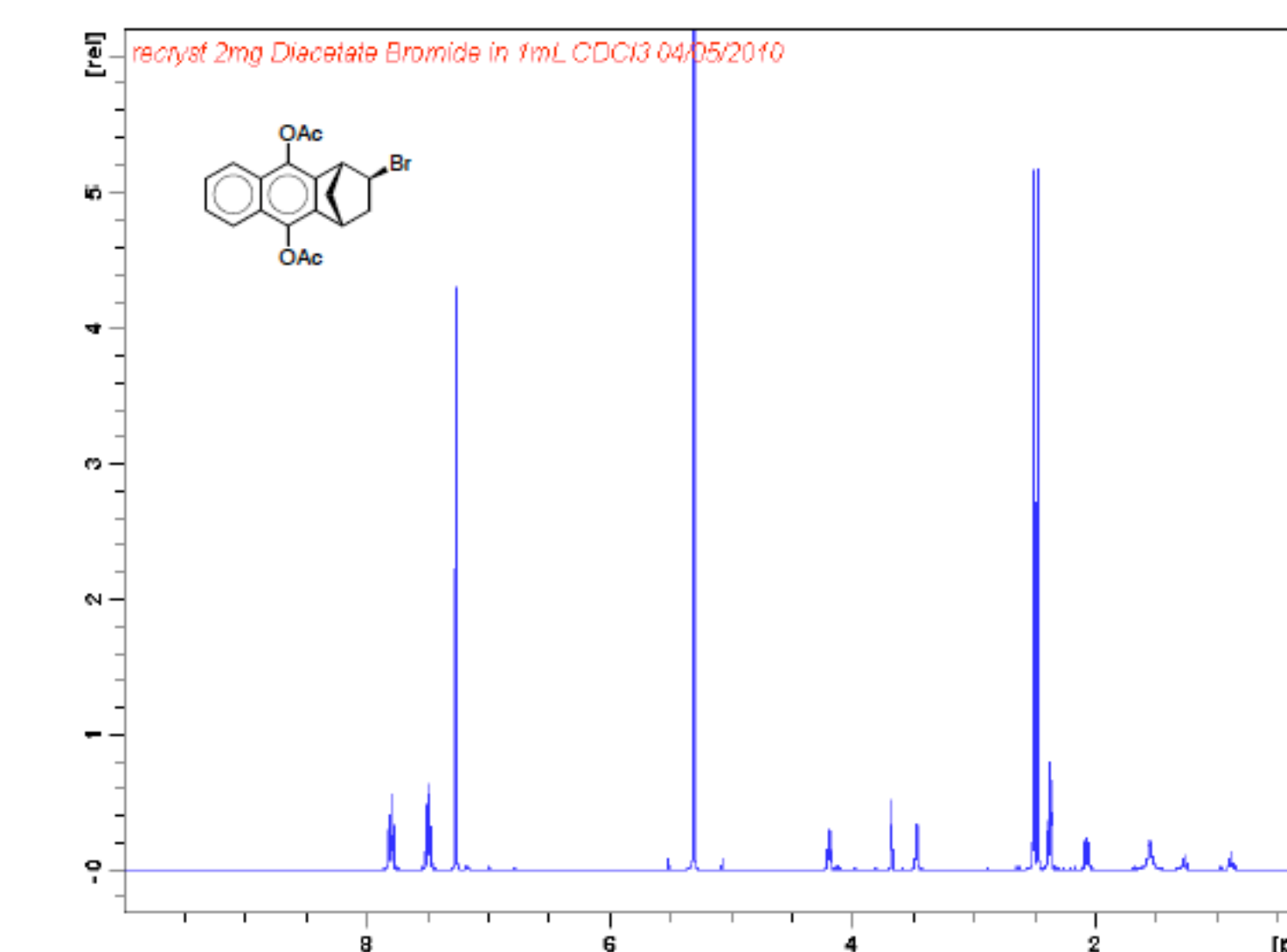
Reference: Stromich, J.J.; Weber, A.K.; Mirzaei, Y.R.; Caldwell, M.D.; Lewis, D.E. *Bioorg. Med. Chem. Lett.* 2010, 20, 1928-1932.

Synthesis of Bromide Precursor

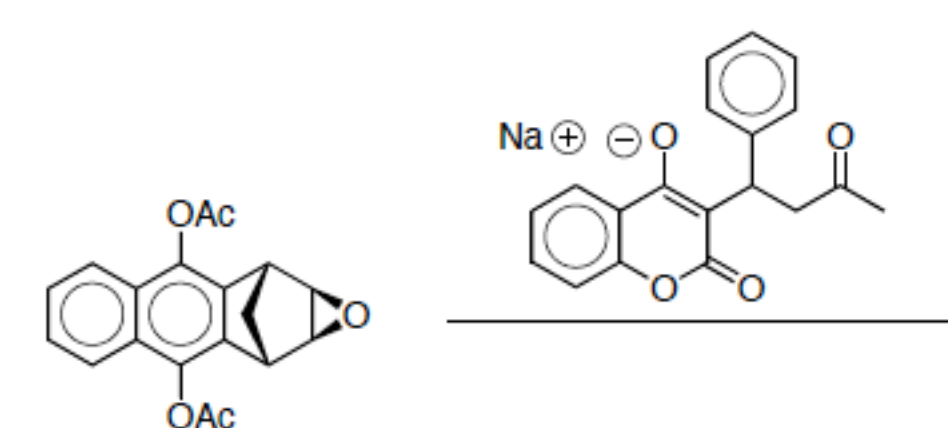
- The diacetate was dissolved in minimal dichloromethane.
- Hydrogen bromide gas was bubbled through the diacetate solution.
- The product was recrystallized from ethyl acetate-hexane to yield a pale grey solid.



Characterization of Bromide

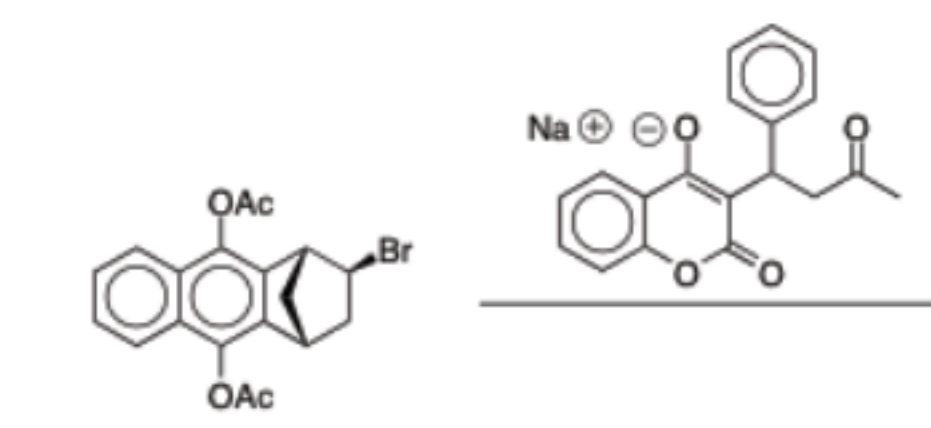


Conjugation of Epoxide with Warfarin



- Reactions have been run in acetone, acetonitrile, CH₂Cl₂-H₂O (Bu₄NBr Phase Transfer), and DMSO
- Conjugation unsuccessful thus far
- Current work involves utilization of triethylamine-HCl buffer in DMSO

Conjugation of Bromide with Warfarin



- Reactions have been run in DMSO, acetonitrile, and CH₂Cl₂-H₂O (Bu₄NBr Phase Transfer)
- Conjugation unsuccessful thus far
- Current work involves generation of the silver salt of warfarin