# Investigation of Phosphate Pro-Drug Led Inhibition of ENO2 in ENO1-Deleted Cells

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## Introduction

- Inhibiting the glycolytic gene ENO2 is a known target for the destruction of ENO1-deleted tumor cells such as Glioblastoma.
- The current best drug for targeting ENO2 is IBuVCY27 derived from HEX.
- Three drugs similar in structure to IBuVCY27 may prove more effective at inhibiting ENO2.

## Methods

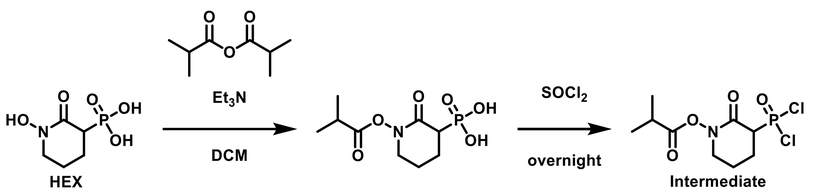


**Fig 1.** Synthesis of the intermediate from HEX.

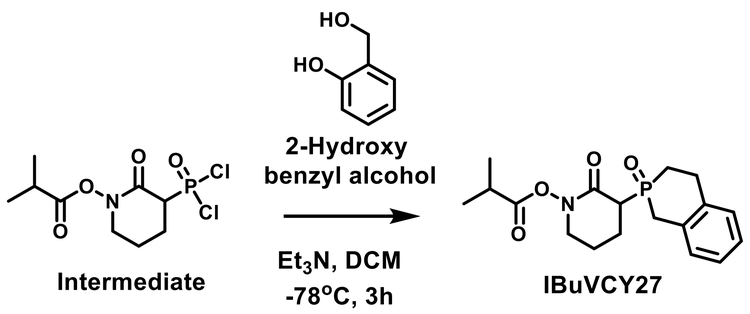


**Fig 2.** Synthesis of IBuVCY27 using 2-Hydroxy benzyl alcohol.

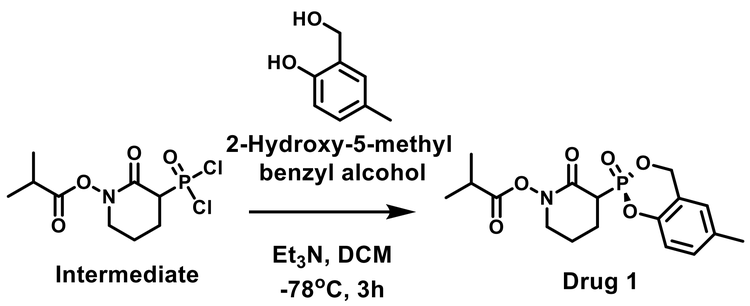


**Fig 3.** Synthesis of Drug 1 using 2-Hydroxy-5-methyl benzyl alcohol.

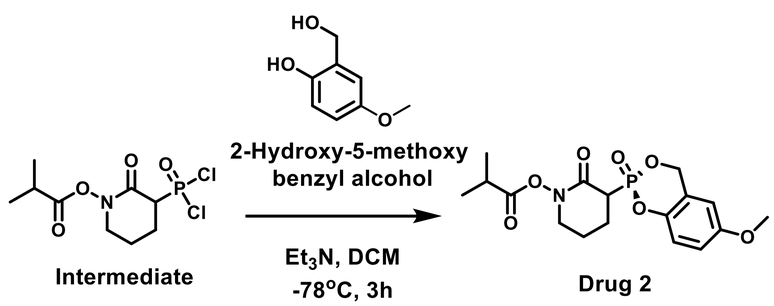


**Fig 4.** Synthesis of Drug 2 using 2-Hydroxy-5-methoxy benzyl alcohol.

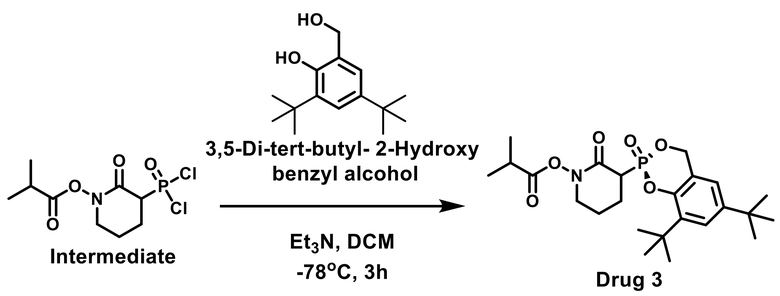


**Fig 5.** Synthesis of Drug 3 using 3,5-Di-tert-butyl-2-hydroxy benzyl alcohol.

## Results

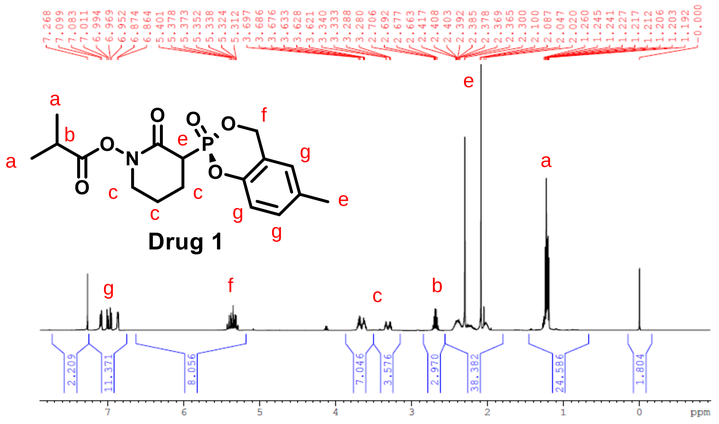


**Fig 6.** Proton NMR of Drug 1. The peaks on the graph represent the hydrogen atoms from the structure. It is used to confirm the compound. Each drug was confirmed this way.

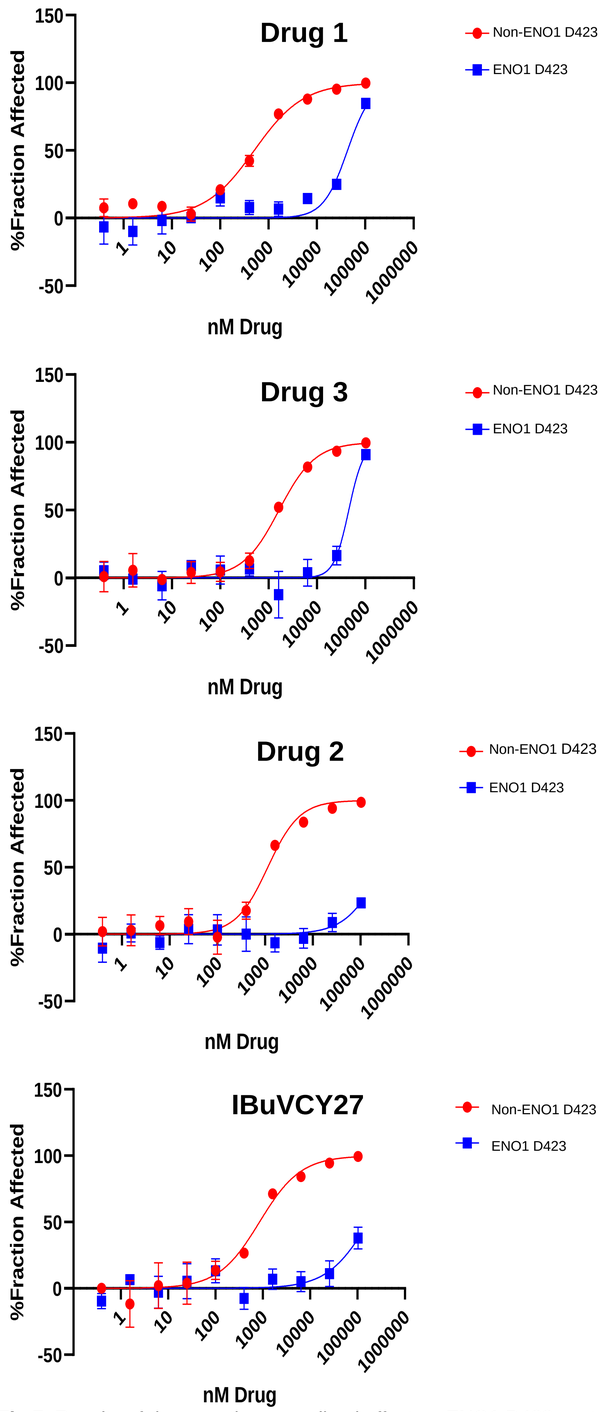


**Fig 7.** Results of drugs vs the normalized effect on ENO1 D423 control cells. Each point represents the different concentration of drug added to the cells. As the concentration increases the percentage of cells affected increases.

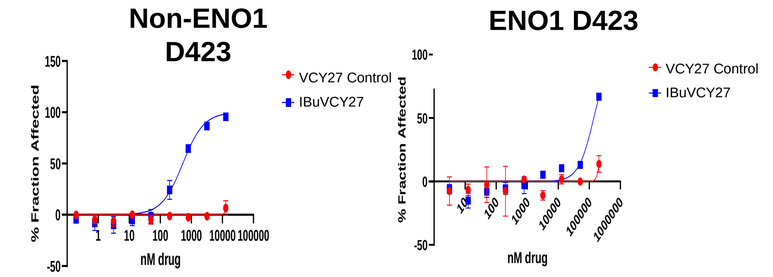


**Fig 8.** Previous run of IBuVCY27.

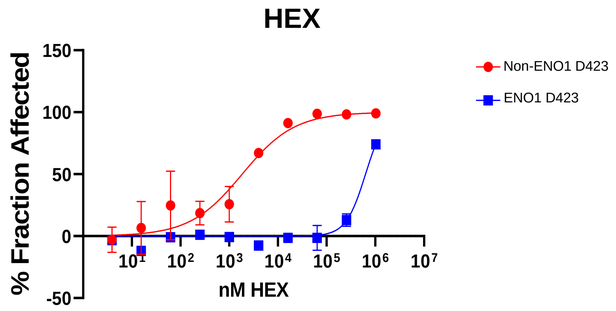


**Fig 9.** Results of an unaltered HEX run. For comparisons.

## Comparison of the Drugs

| Drug Type | EC50 Non-ENO1 D423 | EC50 ENO1 D423 | ENO1/D42 3 Ratio |
|---|---|---|---|
| Drug 1 | 506 | 42,973 | 84.91 |
| Drug 2 | 1,135 | 276,377 | 243.50 |
| Drug 3 | 1,640 | 45,336 | 27.64 |
| IBuVCY27 | 850 | 172,583 | 203.04 |
| Previous IBuVCY27 | 530 | 139,318 | 262.47 |
| HEX | 1,842 | 627,605 | 340.72 |

**Fig 10.** The EC50 Non-ENO1 D423, says which compounds requires the lowest concentration to affect the most cells.

## Conclusion

- Drugs 1, 2, and 3, were successfully made and confirmed
- Drug 1, Drug 2, and IBuVCY27 appear the best compounds.
- The next step would be testing the stability of these drugs and advance to testing *in vivo* with mice in order to find the best drug.

## References

- Lin, YH., Satani, N., Hammoudi, N. *et al.* An enolase inhibitor for the targeted treatment of *ENO1*-deleted cancers. *Nat Metab* **2,** 1413–1426 (2020). https://doi.org/10.1038/s42255-020-00313-3

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