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Examination of the New α-(2′*Z*-Fluoro)vinyl Trigger with Lysine Decarboxylase: The Absolute Stereochemistry Dictates the Reaction Course

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Mechanism-based enzyme inactivators (MBEIs)[1,2] are useful bioorganic tools to study enzyme mechanism and to modulate metabolic flux. Moreover, of the over 300 enzyme-targeted drugs in the FDA orange book, most may be considered broadly "mechanism-based".[3] As such, some combinatorial libraries are now built around MBEI trigger motifs.[4] Recently, "suicide triggers" have also found application in screens for catalytic antibodies[5] and in activity-based proteomics.[6]

We describe here the first successful halovinyl trigger for amino acid decarboxylase (AADC) inactivation, of which we are aware. Among MBEIs for AADCs, DFMO (α-difluoromethylornithine), used clinically for parasitic diseases and as a potential chemopreventive agent,[7] probably is the benchmark. The mechanism of action of the α-difluoromethyl trigger in the ornithine decarboxylase (ODC) active site has now been elucidated by a combination of MS[8] and X-ray methods,[9] and the non-enantioselective nature of its inactivation has been established.[10]

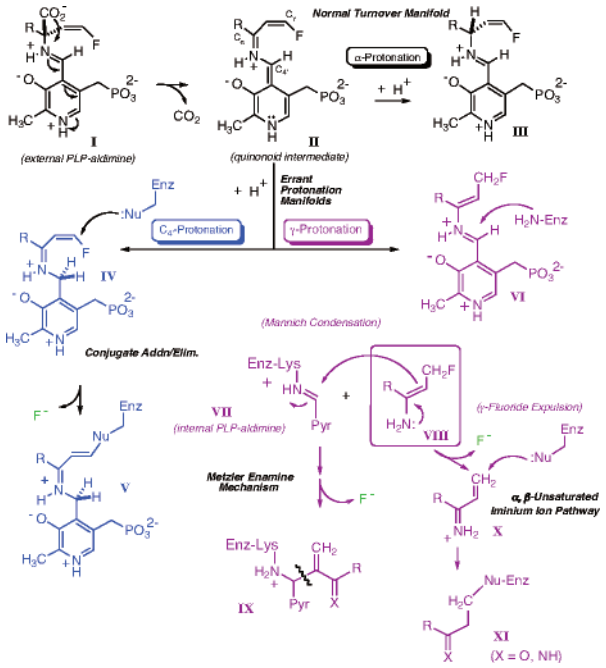
We set out to develop an α-(2′-fluoro)vinyl (2′FV) trigger for AADC inactivation with projected mechanism(s) as outlined in Scheme 1. The design envisions the normal transaldimination (to **I**) and α-decarboxylation (gives **II**) steps, followed by an errant protonation at either $C_{4'}$ of the cofactor (to **IV**) or at Cγ of the "suicide substrate". The former pathway finds precedence in the now crystallographically established[11] Michael addition mechanism[12] of the antiepileptic drug, Vigabatrin, though for this target, GABA transaminase, $C_{4'}$, is the normal locus of protonation. The latter Cγ pathway can branch into a nucleophilic Metzler enamine pathway,[13] and/or into a second electrophilic pathway, initiated by γ-fluoride expulsion out of intermediate **VIII**,[14] rather than Mannich condensation.

Note again that all three putative AADC inactivation pathways require that the enzyme be directed into aberrant protonation of quinonoid intermediate **II**. The literature provides insight as to how this detour pathway might be promoted. It is known that installation of an α-methyl group, to generate a quaternary AA, results in increased errant ($C_{4'}$) protonation in the cognate AADC (from typically 0.01−0.1% up to 4% frequency for ODC).[15] So, the placement of the (2′FV) trigger at a quaternary α-center is critical to our design and is to serve the dual purpose of (i) dictating specificity for AADCs over enzymes that labilize the α-proton (e.g., transaminases, racemases, and β- and γ-replacement enzymes), and (ii) promoting the requisite errant protonation.

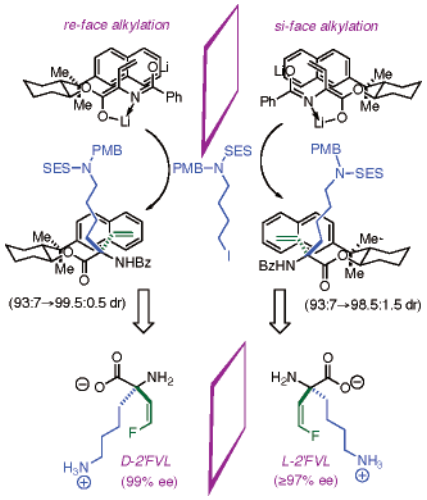
We chose lysine decarboxylase (LDC) as a model enzyme in which to test this new AADC trigger, as we had access to multi-milligram quantities of the *Hafnia alvei* LDC.[16] Furthermore, this class of bacterial LDC was reported to be resistant to covalent modification with the α-difluoromethyl trigger.[17]

A stereoselective synthesis of each antipode of the targeted inactivator was achieved as outlined in Scheme 2. The quaternary center is introduced by alkylation of a chiral vinylglycine-derived dianionic dienolate.[18] As illustrated, our working model for such

Scheme 1. Projected Mechanisms for the 2′FV-AADC Trigger



Scheme 2. Stereoselective Introduction of the 2′FV-AADC Trigger



systems involves (i) the use of α-nitrogen-based amidate chelation to control enolate geometry and (ii) the application of auxiliaries of the "arylmenthyl" variety to control facial selectivity.

The use of dienolates outfitted with the Comins auxiliary (available in both antipodal forms)[19] here provides a potentially generalizable method to access both enantiomers of sought after quaternary AAs. Following side-chain installation, conversion of the α-vinyl group to a 2′*Z*-fluorovinyl group proceeds smoothly, following our recently disclosed protocol.[20]

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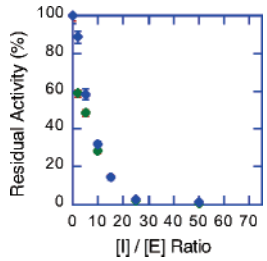

Figure 1. Titration of LDC with L-2′FVL (two trials, color-coded)

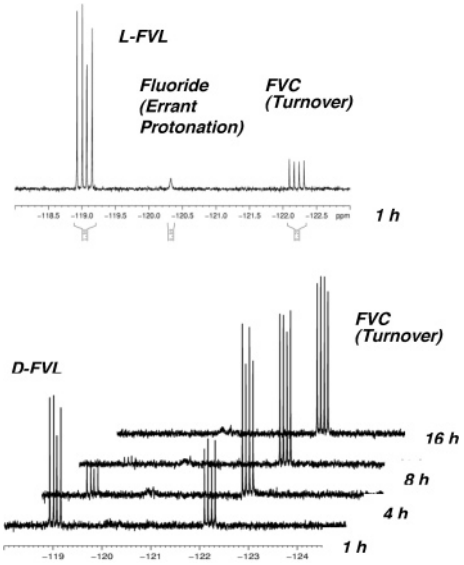

Figure 2. ^{19}F NMR spectral acquisitions monitoring the reaction course taken by the L- (top) and D-enantiomer (bottom) of α-(2′*Z*-fluoro)vinyllysine ([I]/[E] = 75:1), upon incubation with *Hafnia alvei* LDC (15 μM) at pH 6.0, for the indicated times. Parallel kinetic assays show 92% inactivation for the L-antipode in 10 min, and complete knockout in <1 h, accounting for the invariant NMR spectrum for L-FVL (essentially unchanged @ t = 16 h, see Supporting Information).

L-α-(2′Z-Fluoro)vinyllysine displays time dependent inactivation of LDC, whereas its mirror image displays little to no inactivation. Kitz−Wilson of the L-2′FVL data, yields $k_{inact} = 0.26 \pm 0.07$ min^{-1} and $K_I = 86 \pm 22$ μM. This is comparable to the k_{inact} values seen for both antipodes of DFMO and (*S*)-Vigabatrin. The K_I value is higher than that seen for L-DFMO (~1.3 μM),[10] but significantly lower than that seen for (*S*)-Vigabatrin (~3 mM).[21] Inactivation was functionally irreversible (dialysis). Curvature in ln(E_t/E_0) versus t plots was observed, suggestive of significant partitioning into natural or unnatural turnover pathways. In fact, a titration of the enzyme with varying *I*/*E* ratios provides an estimate of the partition ratio (total turnovers per inactivation event) of ~20 ± 3 (Figure 1).

Fluorine serves both a mechanistic and a labeling role in this trigger. ^{19}F NMR monitoring,[22] of the individual FVL-antipodes (Figure 2) shows the D-enantiomer to be exclusively a *substrate*, whereas the L-antipode is a *suicide substrate*. Inactivation of 15 μM LDC produces 168 ± 14 μM α-(2′fluoro)vinylcadaverine (FVC) turnover product (matches authentic sample) and 70 ± 13 μM fluoride.[23] The fluoride value was confirmed with an ion-specific electrode (78 ± 5 μM).

Since all errant protonations are projected to release fluoride, one can estimate that 1 in 3.4 decarboxylations leads to errant protonation (29%!), with 1 in 5 errant protonation events leading to LDC inactivation. This gives an overall partition ratio of 16 ± 2. The high errant protonation rate (~8 times the maximum value produced by α-methylation) seen in this model AADC active site is promising, as altered protonation is required for trigger actuation. γ-Protonation provides the simplest mechanism for release of four excess equivalents of fluoride, but this does not exclude any of the three pathways put forth for the inactivation step itself. This must await the results of further studies. Given the success in driving errant protonation with L-FVL and its favorable k_{inact}, it will be of interest to examine this trigger in other AADC active sites. The remarkable enantio-discrimination observed stands in stark contrast to the case of DFMO and underscores the value of interrogating individual antipodes in MBEI studies.

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Supporting Information Available: Details of the asymmetric synthesis, enzyme inactivation, and ^{19}F NMR studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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