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Synthesis and Deployment of an Elusive Fluorovinyl Cation Equivalent: Access to Quaternary α-(1'-Fluoro)vinyl Amino Acids as Potential PLP Enzyme Inactivators

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fluorovinyl cation equivalent, α-fluorovinyl amino acids, mechanism-based enzyme inhibitors, lysine decarboxylase, quaternary amino acids, pyridoxal phosphate, PLP-enzymes, β,γ-unsaturated amino acids, fluorinated synthons, time dependent enzyme inactivation, α-branched amino acids

Supporting Information

ABSTRACT: Developing specific chemical functionalities to deploy in biological environments for targeted enzyme inactivation lies at the heart of mechanism-based inhibitor (MBI) development, but also is central to other protein-tagging methods in modern chemical biology including activity-based protein profiling (ABPP) and proteolysis-targeting chimeras (PROTACS). We describe here a previously unknown class of potential PLP enzyme inactivators; namely, a family of quaternary, α-(1'fluoro)vinyl amino acids, bearing the side chains of the cognate amino acids. These are obtained by the capture of suitably protected amino acid enolates with β,β-difluorovinyl phenyl sulfone, a new 1'-fluorovinyl cation equivalent, and an electrophile that previously eluded synthesis, capture and characterization. A significant variety of biologically relevant AA-side chains are tolerated including those for alanine, valine, leucine, methionine, lysine, phenylalanine, tyrosine and tryptophan. Following addition/elimination, the resulting *transoid* α-(1'-fluoro)-β-(phenylsulfonyl)vinyl AA esters undergo smooth sulfone-stannane interchange to stereoselectively give the corresponding *transoid* α-(1'fluoro)-β-(tributylstannyl)vinyl AA esters. Protodestannylation and global deprotection then yields these sterically encumbered and densely functionalized, quaternary amino acids. The α-(1'fluoro)vinyl trigger, a potential allene-generating functionality originally proposed by Abeles, is now available in a quaternary AA context for the first time. In an initial test of this new inhibitor class, α-(1'-fluoro)vinyllysine is seen to act as a time dependent, irreversible inactivator of lysine decarboxylase from *Hafnia alvei*. The enantiomers of the inhibitor could be resolved and each is seen to give time dependent inactivation with this enzyme. Kitz-Wilson analysis reveals similar inactivation parameters for the two antipodes, L-α-(1'-fluoro)vinyllysine (K_i = 630 ± 20 μM; $t_{1/2}$ = 2.8 min) and D-α-(1'-fluoro)vinyllysine (K_i = 470 ± 30 μM; $t_{1/2}$ = 3.6 min). The stage is now set for exploration of the efficacy of this trigger in other PLP-enzyme active sites.

INTRODUCTION

Mechanism-based inhibitors (MBIs) offer advantages of specificity and functional irreversibility.[1] Indeed, there continues to be great interest in "covalent drugs"[2] or compounds that behave as functionally irreversible enzyme inactivators,[3] as this confers advantages in terms of dosing (lower time of systemic exposure), and pharmacokinetics (covalent capture), as well as potentially lower susceptibility to resistance.[4] Nearly one-third of all enzymes being targeted have an FDA-approved covalent drug;[5] while about 65% of enzyme-targeted drugs have a mechanistic design.[6] The development of tailored functional groups for a specific mechanism or active site constellation is critical for single enzyme covalent inhibitor development, including PROTACS (proteolysis targeting chimeras)[7] and CLIPTACS (click-formed-PROTACS)[8] approaches, as well as methods that target entire mechanistic classes, such as ABPP (activity-based protein profiling).[9] Appropriate 'warheads' are also key to developing qABP imaging reagents (quenched fluorescent activity-based probes)[10] among the most innovative small molecule agents being deployed in the fields of cancer diagnostics.[11]

There is particular interest in developing inhibitors that bear fluorinated functionality,[12] for mechanistic reasons (electronegativity of fluorine, leaving group ability of fluoride) and for analytical purposes, as ^{19}F is a bio-orthogonal NMR nucleus[13] and used as a spectroscopic window into protein-ligand interactions.[14] Moreover, ^{18}F-substitution may offer opportunities for PET-imaging,[15] and for enhancing metabolic stability.[16] Unnatural, unsaturated/α-branched amino acids are also of interest as building blocks for modified non-ribosomal peptide synthesis[17] and programmed unnatural amino acid mutagenesis at the ribosome.[18] Quaternary AAs also find application in driving the formation of useful secondary structure when strategically incorporated into α-[19] and β-peptides[20] as well as hybrid α/β-peptides,[21] or in promoting interesting helical motifs such as the 3_{10}-helix[22] and the 2.05-helix.[23] Elements of side chain unsaturation can serve as useful conformational constraints[24] and contribute to overall peptide stability via stapling technology.[25]

MBIs bearing tailored fluorinated functional groups designed to inactivate their target enzymes based upon mechanism have led to clinically useful therapeutics. Examples include α-difluoromethylornithine (DFMO; trypanosomiasis/African sleeping sickness)[26] and 5-fluorouracil (cancer chemotherapy).[27] That said, outfitting AAs with the α-(1'-fluoro)vinyl functionality in place of the α-proton has not been possible heretofore. This would be required if one wished to deploy this trigger in the ac-

tive sites of PLP-dependent amino acid decarboxylase (AADC) or retroaldolase enzymes, for example. Described in this communication is the first method that allows access to quaternary α-(1'-fluoro)vinyl amino acids (AAs), with wide side chain tolerance, as potential inactivators of a range of PLP-dependent enzymes.

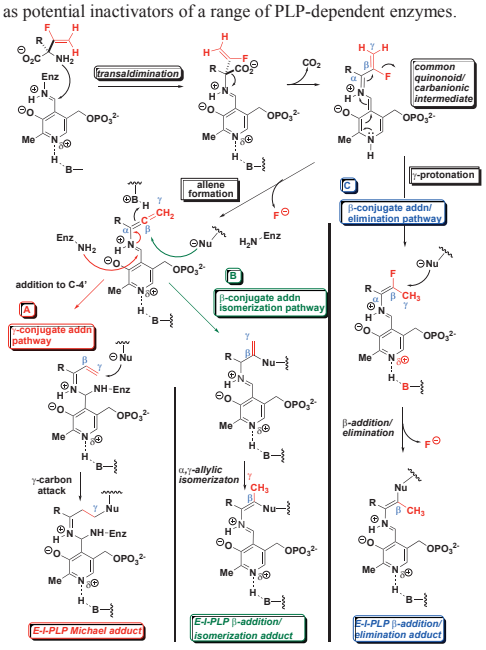


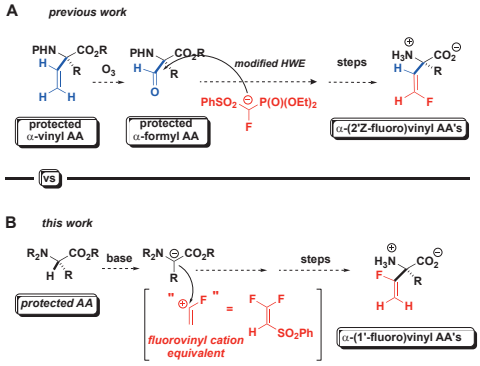
Figure 1. Potential PLP-enzyme inactivation mechanisms for quaternary, α-(1'-fluoro)vinyl amino acids, following the design of Abeles for α-(1'-fluoro)vinylglycine, illustrated for an AADC mechanism, highlighting both γ-conjugate addition (via an alleneimine intermediate) and β-conjugate addition (both via an alleneimine and via γ-protonation) pathways.

The β,γ-unsaturated trigger design is bio-inspired and derives from the parent compound, α-vinylglycine, a fungal natural product, and an inactivator of both PLP-dependent transaminases[28] and of ACC synthase,[29] a key enzyme in the biosynthesis of the plant ripening hormone, ethylene. The design of the (α-1'fluoro)vinyl trigger is due to Abeles, who envisioned that such a motif could lead to the formation of a reactive allene-type intermediate in a PLP enzyme active site (Figure 1).[30] In fact, that pioneering work showed great promise for this trigger, with (α-1'fluoro)vinylglycine serving as an efficient mechanism-based inhibitor for tryptophan synthase. Despite this promise, previous reports for (1'-fluoro)vinyl amino acids have been limited to the glycine scaffold,[30-31] as chemistry has not been available to install the (1'-fluoro)vinyl group in a quaternary setting. In this context, it is noteworthy that in recent years, there has been growing interest in the use of fluorovinyl functionality to inhibit other enzymes, for example, by installing mono- and (*bis*-fluoro)vinyl moieties at the γ-position of various GABA analogues[32] to generate compounds with clinical promise for the inhibition of GABA transaminase (epilepsy and drug addiction)[33] and ornithine aminotransferase (hepatocellular carcinoma).[34]

There has been a longstanding effort in this research group and elsewhere to develop synthetic methods toward L-vinylglycine,[35] functionalized β,γ-unsaturated AAs,[36,37] as well as quaternary α-vinyl AAs,[38] as mechanism-based inhibitors for PLP enzymes. Building on these achievements, we have been focusing on constructing quaternary, β,γ-unsaturated AAs that retain the native α-carboxyl and α-amino groups and the cognate side chain, but additionally feature a *position-specifically fluorinated vinylic trigger*, also appended to the α-carbon. In the first such endeavor, we were able to establish a general approach to α-(2'Z-fluoro)vinyl AAs from the parent α-vinyl AAs via a β-carbon excision/fluoromethylenation sequence that avails itself of the McCarthy reagent (modified HWE reagent, Scheme 1A). An initial test of this motif for PLP enzyme inactivation showed great promise and highlighted the value of incorporating fluorine into the inactivation trigger.[39] This chemistry begins from quaternary, α-vinyl AAs, and so, leverages the chemistry we have developed to access these unnatural AAs. That said, the chemistry is not particularly convergent and is limited to accessing 2'-fluorinated vinylic triggers. Clearly, a fundamentally new approach would be needed to introduce fluorine into the 1'-position of the vinylic α-branch. Only then would it be possible to evaluate the Abelesian inactivator design illustrated in Figure 1 in amino acid contexts beyond glycine.

Herein we present a solution to this synthetic challenge. A convergent approach is taken, disconnecting at the α-carbon; resulting in a formal α-(1'-fluoro)vinylation of AA-enolates (Scheme 1B). Key features of the chemistry include (i) the synthesis and characterization of a previously elusive (1'-fluoro)vinyl cation equivalent, (ii) its capture via AA-derived enolates across a range of biologically relevant side chains and a spectrum of protecting groups, and (iii) global deprotection to the free, quaternary α-(1'-fluoro)vinyl AAs. Finally, in the first test of the new, quaternary α-(1'-fluoro)vinyl trigger in a PLP-enzyme active site; lysine decarboxylase (LDC) from *Hafia alvei* is seen to undergo time-dependent, irreversible inactivation with (±)-α-(1'-fluoro)vinyllysine.

Scheme 1. Toward Quaternary, Fluorovinyl Amino Acids



A – Quaternary, α-(2'Z-fluoro)vinyl AAs via *excision/(2'-fluoro)methylenation* of α-vinyl AAs
B – Quaternary, α-(1'-fluoro)vinyl AAs via *convergent, α-(1'-fluoro)vinylation* of AA-enolates

RESULTS AND DISCUSSION

Development of a Reactive Fluorovinyl Cation Equivalent. Given the great interest in fluorinated alkene functionality in chemical biology, for example, as latent triggers for enzyme inac-

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tivation,[30,32c,32d,39a] as masked aldehyde[32a] or carboxylate[32b] equivalents, or as peptide bond isosteres,[40] there has been a significant effort in methodology development in this area. Approaches to monofluorovinyl group installation most commonly are formal nucleophilic fluoromethylenations, whereby a EWG-CY(-)-F reagent is condensed with a carbonyl center. Included in this category are modified Horner-Wadsworth-Emmons (HWE) condensations [Y = $P(O)(OR)_2$],[39,41] fluoro-Peterson olefinations [Y = SiR_3],[42] and a range of quite effective Julia-Kocienski couplings [Y = SO_2Ar].[43,44] Recently, in a particularly elegant approach, Hoveyda, Schrock and coworkers have described the first viable cross-metathesis route for formal fluoromethylation.[45] That said, by their very nature, all of these approaches are limited to the installation of 2'-fluorovinyl groups, and, as noted are rather linear as the terminal fluorovinyl group is installed one carbon at a

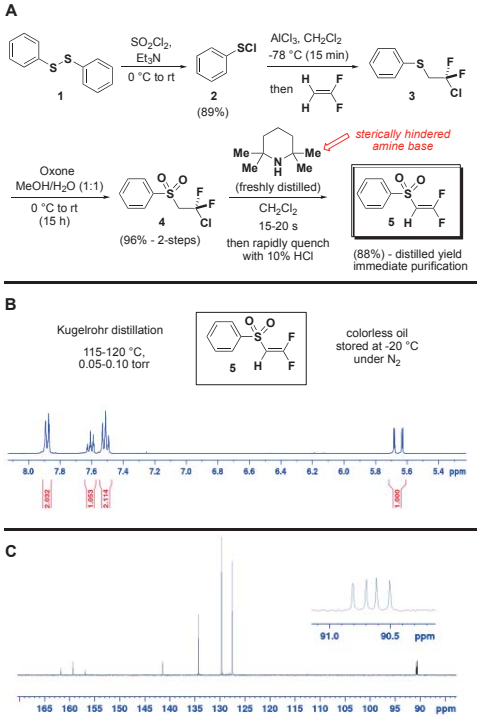


Figure 2. **A**- Synthesis of β,β-difluorovinyl phenyl sulfone **5** which following Kuglerohr distillation is isolated as a transparent oil. **B**-^{1}H NMR spectrum of **5** after purification; reaction run for 20 s. **C**-^{13}C NMR spectrum of **5** showing both geminal and vicinal C-F splitting.

time.

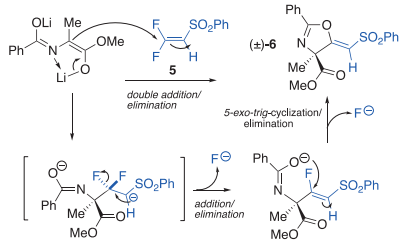
In terms of the (1'-fluoro)vinyl group, the best methods so far reported utilize transition metal-mediated cross-couplings with terminal (α-stannyl)fluoromethylene[46] or dihalomethylene species,[47] including a C-H activation-based approach described recently.[41a] However, these cross-couplings are restricted to C(sp^2)-C(sp^2)-couplings and work best for the installation of a (1'-fluoro)styrenyl unit [a related photoredox catalysis entry has also been described[48]], rather than the (1'-fluoro)vinyl "trigger" targeted here for chemical biology applications.

According□□□□□□□□□□ly, we set out to develop a viable □(1'-fluoro)vinyl cation equivalent that could be condensed directly with an amino acid-derived enolate to allow the construction of quaternary, α-(1'-fluoro)vinyl for the first time. β,β-Difluorovinyl phenyl sulfone was pursued as an attractive candidate (Figure 2). To be sure, there is keen interest in electrophilic, fluorinated α,β-unsaturated sulfones, particularly as dienophiles for [4π+2 π] cycloadditions[49] and as reactive electrophiles in conjugate addition reactions.[50,51] Indeed, a careful examination of the literature uncovers previous attempts to synthesize β,β-difluorovinyl phenyl sulfone as a potential Diels-Alder dienophile. However, these accounts also reveal that despite considerable effort, this species has remained elusive. Early on, Feiring[52] had attempted to synthesize this compound, but observed that unwanted hydrofluorination of the fluorovinyl compound yielded β,β,β-trifluoroethyl phenyl sulfone. In later studies, Percy was able to synthesize chlorodifluoroethyl phenyl sulfone from chlorodifluoroethanol[53] using a modified protocol previously reported by Kotsuki.[54] In this case, β,β-difluorovinyl phenyl sulfone was believed to form transiently, but decomposed under all conditions examined, with again the only isolable product being β,β,β-trifluoroethyl sulfone.

We are pleased to report here that one can access and fully characterize the targeted electrophile in four steps from readily available diphenyl disulfide (**1**), as delineated in Figure 2. Specifically, treatment of **1** with sulfuryl chloride provides phenyl sulfenyl chloride (**2**), which upon exposure to vinylidene fluoride in the presence of aluminium trichloride cleanly affords β-chloro-β,β-difluorosulfide (**3**). This species can be smoothly oxidized to β-chloro-β,β-difluoroethyl phenyl sulfone (**4**) under the agency of Oxone™. At this point in the synthesis, viable conditions to afford the β,β-difluorovinyl phenyl sulfone (**5**) were carefully explored.

It was found that the conditions used in the key dehydrochlorination of intermediate **4**, as well as those involved in the purification and isolation of **5** were critical to success here. Use of a highly-hindered amine base; namely exceptionally brief exposure (15-20 s) of **4** to freshly distilled 2,2,6,6-tetramethylpiperidine, followed by an acid quench, and rapid extraction, led to reproducibly good yields of **5.** Analytically pure material could be obtained by Kugelrohr distillation @ ≤ 0.1 torr vacuum. As can be seen from Figure 2B and 2C, one obtains spectroscopically homogeneous reagent in this way, even on a gram scale (see Experimental Section).

Scheme 2. Double Add'n/Elimination with AA-Derived Dianions



AA-Enolate Capture with the New Fluorovinyl Cation Equivalent. With the elusive electrophile in hand, we next turned our attention to the capture of **5** with amino acid-derived enolate nucleophiles. Dianions were examined initially. Previous experience in our laboratory in unnatural, quaternary amino acid synthe-

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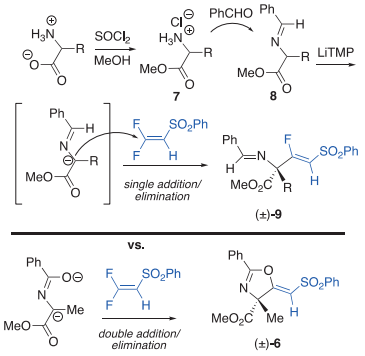
sis had demonstrated the utility of N-benzoyl AA-ester derived dianions, particularly as they display excellent nucleophilicity toward building hindered, quaternary α-centers through alkylation. This includes the construction of racemic, quaternary, α-vinylic AAs, through alkylation of AA-amidate enolate dianions, bearing a range of side chains, with ethylene oxide, as a readily available, though only modestly reactive, vinyl cation equivalent.[55] This also includes quaternizations in the converse order in which the vinyl group is installed first, and then quaternization is achieved by alkylation of an N-benzoyl vinylglycine-derived amidate/ester enolate with a range of side chain electrophiles in S_N2-fashion.[38c] Therefore, we initially attempted to quaternize the Cα-center with the dianionic enolate amidate generated from N-benzoyl-protected methyl alaninate with two equivalents of LDA. When this strategy was employed here, it appears that the desired *intermolecular* AA-enolate addition/elimination reaction apparently did occur to efficiently capture difluoromethylene sulfone electrophile **5**. However, under the conditions of the reaction, a second, *intramolecular* AA-amidate addition/elimination apparently follows suit, quite efficiently, presumably via a Baldwin-favored[56] *5-exo-trig*-type transition state leading to adduct 6, isolated in 66% yield after just 30 min at -78 °C (Scheme 2).

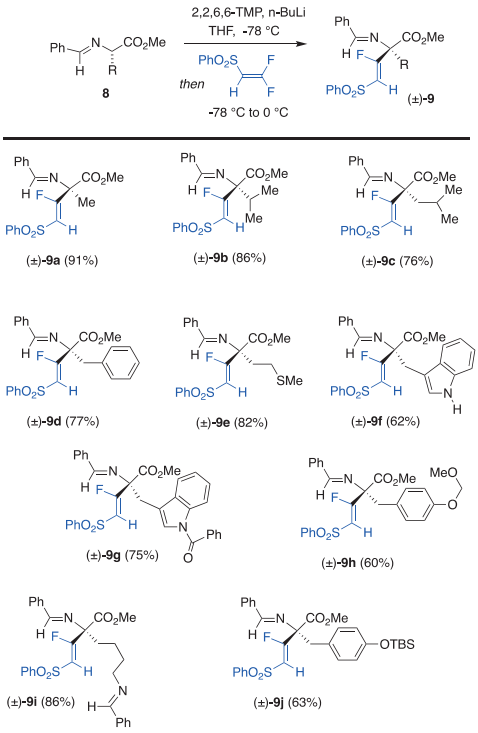
In light of this observation, and given that **5** is likely a considerably better electrophile than ethylene oxide, it was decided to examine the behavior of less nucleophilic monoanionic enolates derived from N-benzylidene-AA esters. The requisite N-benzylidene methyl esters were prepared from the corresponding free amino acids by methyl esterification ($SOCl_2$/MeOH), followed by incubation of the crude ester HCl-salt with benzaldehyde and NEt_3 to afford **8a-j**. In case of the tryptophan, the benzoyl protection for the indole nitrogen was examined (**8g**), while for the tyrosine phenol both MOM acetal (**8h**) and TBS ether protection were employed (**8j**).

Scheme 3. Single vs. Double Addition/Elimination



We were now set to examine the key C-C bond formation step. Enolate formation for compounds **8a-j** was achieved with lithiated 2,2,6,6-tetramethylpiperdine (LiTMP) at -78 °C. Subsequent addition of (1'-fluoro)vinyl cation equivalent **5** at -78 °C, followed by allowing to warm to room temperature, resulted in smooth introduction of the (1'-fluoro)vinylsulfonyl functionality

Scheme 4. α-(1'-Fluoro)vinylation of AA-Derived Enolates with 5



in 1-2 h time generally. Pleasingly, we were able to achieve good yields of the targeted, quaternary amino acids bearing a α-(1'-fluoro)vinyl sulfone derivatives **9a-j**, avoiding the double addition/elimination product previously observed (see Schemes 3 and 4; 10 examples: 60-91% yield). The condensation is stereoselective as exclusively the *transoid* (nominally *Z*) addition/elimination products are obtained, as inferred from the 29-34 Hz vicinal $J_{H\text{-}F}$ coupling constants observed **9a-j**.[50b,57] This condensation proceeds efficiently across a diverse array of AA ester enolates including those bearing thioether (Met), silyl ether (Tyr), acetal (Tyr), imine (Lys), amide (Trp) functionality. Moreover, even though a quaternary AA-center is being formed, sterically encumbered AA ester enolates, such as those carrying either α- (Val) or β-alkyl branching (Leu), give some of the highest yields. And finally, the free indole ring in Trp was also tolerated (**f** series) which proved quite useful here.

Fluorovinyl Sulfone-Fluorovinyl Stannane Interchange.

Treatment of the α-(1'fluoro)vinyl phenyl sulfones with $HSnBu_3$ and AIBN in refluxing benzene led to clean sulfone-stannane interchange, providing products **10a-i** in very good to excellent isolated yields (Scheme 5). Only the *transoid* (formally *Z*) quaternary□□□e□□□□□, □α-(□1'-fluoro-2'tributylstannyl) vinyl-AAs were observed as judged by the consistent vicinal $J_{H\text{-}F}$ values observed (71-73 Hz)[58] across all side chains. This i□□□□□□s notable because radical sulfide-

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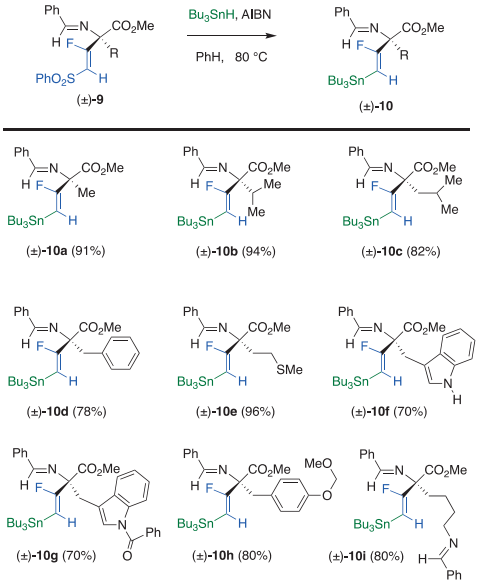
Scheme 5. α-Fluoro Sulfone-Stannane Interchange: Entry into Quaternary, Fluoro-Trialkylstannylvinyl AAs

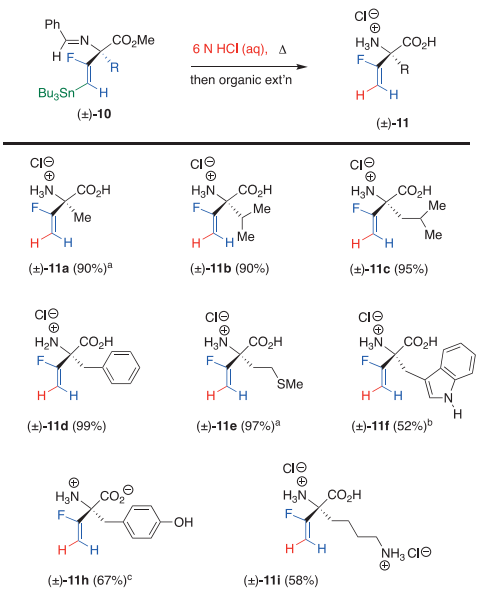


stannane interchange reactions are known to sometimes lead to scrambling of alkene geometry.[58b,59] That said, for α-fluorovinylsulfones, both we[39b] and McCarthy[60] have observed that radical vinyl sulfone/stannane proceeds with clean retention of configuration, with examples of both geometric isomers in McCarthy's case, providing strong experimental evidence for a stereospecific transformation, at least in these cases. In this regard, it is important to note that classic studies showed that simple vinyl stannanes can be isomerized upon heating in the presence of trialkyltin radical.[61] And more recently, Wnuk and coworkers similarly observed that exposure of α-fluorovinylsulfones to ArS-radical with heating also leads to equilibration of double bond geometry in generating the targeted α-fluorovinyl sulfides.[62] The authors propose radical alkene addition/elimination mechanisms for these substitution reactions, with free rotation in the intermediate radicals serving as the equilibration mechanism. Considering these observations, future experiments will be needed to establish whether the transformation is truly stereospecific. It can be said that, for the interesting β-fluorovinylsulfones under study here, the observed stannyldesulfonylation is at the very least highly stereoselective, producing exclusively the *transoid* β-fluorovinylstannanes, embedded as the α-branch in this new class of sterically encumbered, functionally dense, quaternary, amino acids.

Protodestannylation/Global Deprotection.

Pleasingly, as can be seen from Scheme 6, we were able efficiently to "unmask" the α-(1'fluoro)vinyl appendage as well as globally deprotect to the free amino acids, in one step. Namely, under acidic conditions (6 N HCl, Δ), protodestannylation was accompanied by imine cleavage and ester hydrolysis. For the β-fluorovinyl stannanes **10a-i**, assuming that the protostannylation transformation proceeds via initial alkene protonation, hypercon-

Scheme 6. Global Deprotection to Quaternary, α-(1'-Fluoro)vinyl AAs



a – In these cases, though the fluorovinyl AA-HCl salt was clean by NMR, we elected to generate the free, quaternary, α-(1'-fluoro)vinyl AA via ion exchange chromatography [Dowex-50; 96% from **11a-HCl**; (Ala); 94% from **11e-HCl** (Met)]. **b** – In this case, Dowex 50 cation exchange chromatography also led to an improvement in purity (~15%, by NMR) of the fluorovinyl AA (76% from **11f-HCl**). **c** – In this case, MOM cleavage was performed with TFA in CH_2Cl_2, with accompanying protodestannylation and aldimine cleavage, followed by via base-catalyzed ester saponification. The crude product was acidified and purified via Dowex 50 cation exchange chromatography (66% yield of **11h**).

jugative β-stannyl cation stabilization presumably overides the expected destabilizing contribution of the α-fluoro substituent. The subsequent loss of a Bu_3SnAr^+ equivalent would then likely be promoted via chloride ion. In the tryptophan case, owing to complication with N-benzoyl deprotection (**g** series), it was found to be advantageous to move the free indole (**f** series) system forward, ultimately providing homogeneous α-(1'fluoro)vinyltryptophan (**11f**). In the case of the MOM-protected tyrosine derivative, side chain acetal deprotection was found to proceed efficiently in TFA/CH_2Cl_2 than in 6N HCl (aq). In this case, the quaternary AA-ester was ultimately cleaved under basic conditions (see entry **11h**, Scheme 6 and the SI for details). The target α-(1'fluoro)vinyl AAs were obtained as either the clean HCl salts following extraction of the organic byproducts, or if desired/needed for purification, as the charge-balanced zwitterionic quaternary, α-(1'fluoro)vinyl AAs, following cation exchange chromatography (Dowex 50; NH_4OH elution).

Enzyme Inhibition Studies. With the first members of this new class of potential PLP enzyme MBI in hand, we set out to test for inactivation behavior in a model active site. Lysine decarboxylase (LDC) was chosen as, in our hands, this enzyme had proven to be a useful model system to in which to examine the behavior of mechanism-based inhibitor candidates.[12b,39a] LDC was purified from the native bacterial source, *Hafnia alvei*, as this organism

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naturally produces high titers of the enzyme. Following sequential hydrophobic chromatography (Phenyl-Sepharose) and size exclusion chromatography (Sephacryl S300), homogeneous LDC was obtained, as evidenced by both specific activity and gel electrophoresis (SDS-PAGE).

Enzyme inhibition was initially assayed using a ^{14}C-radiolabel assay, whereby ^{14}C-CO_2 generated from the decarboxylation of U-^{14}C-labeled L-lysine was captured by as an organic carbonate salt, via benzethonium hydroxide-soaked filter paper positioned above the assay solution as illustrated in Figure 3A. Inhibitor candidate (±)-**11i** was incubated with *H. alvei* LDC and 2.5 mM L-lysine, across an inhibitor concentration range of 0.2-10 mM, for 30 min vs. a control in which no inhibitor was present. Following a TFA quenching and scintillation counting, percent inhibiton was calculated as (cpm-inhibitor run/cpm control incubation) x 100. The data clearly evidenced time dependent inhibition *H. alvei* LDC (Note: The three-dimensional structure for *H. alvei* LDC has not yet been solved. The LDC homology model depicted in Figure 3 was generated using SWISS-MODEL from a total of 1279 templates. The top 5 models and their sequence identities are 5fl2, 5fkx, 3q16 (all inducible LDC from *E. coli*; 88%), 5fkz (constitutive LDC-*E. coli*; 70%) and 2ycp (tyrosine-phenol lyase-*Citrobacter freundi*; 11%).

To better characterize the nature of the inactivation, we set out to perform a Kitz-Wilson kinetic characterization.[63] As these initial assays were conducted with racemic inhibitor, we first set out to resolve the individual antipodes of α-(1'-fluoro)vinyllysine. Pleasingly, it was discovered that this quaternary, α-fluorovinyl AA could be selectively benzoylated at the ε-amino group under Schotten-Baumen conditions. Moreover, D- and L-N$^{\varepsilon}$-benzoyl-α-(1'-fluoro)vinyllysine proved to be separable by chiral HPLC (Chirobiotic T column – teicoplanin-based chiral stationary phase – see SI for details). The first eluting enantiomer was assigned the D-stereochemistry, and the latter as L-, based upon the specific rotations of the corresponding, free α-(1'-fluoro)vinyllysine antipodes by comparison with the rotations of a significant range of quaternary, α-vinylic AAs (see SI for a complete tabulation).

Following separation and N$^{\varepsilon}$-debenzoylation, the individual enantiomers of α-(1'-fluoro)vinyllysine were assayed for time dependent inhibition of *H. alvei* LDC utilizing a complementary time point assay specific for the cadaverine product as illustrated in Figure 3C and described in the Experimental Section and in the SI. The assay utilized is a modification of the original Lenhoff assay[64] and involves base quenching at specific time points and covalent capture of the diamine product with TNBS (2,4,6-trinitrobenzene sulfonate). The resultant Kitz-Wilson primary and secondary plots for each antipodal inhibitor candidate are presented in Figure 3C. Interestingly, each exhibits time dependent inactivation of LDC; the L-antipode gives K_i = 620 ± 20 μM; k_{inact} = 0.25 ± 0.03 min^{-1} ($t_{1/2}$ ~2.8 min) whereas the D-antipode shows K_i = 470 ± 30 μM; kinact = 0.19 ± 0.04 min^{-1} ($t_{1/2}$ ~3.6 min).

That both antipodes of this new MBI-class effectively inactivate the LDC target enzyme here is reminiscent of the behavior of the antipodes of DFMO with ornithine decarboxylase.[26c] As DFMO is utilized to treat African sleeping sickness,[26d] and in clinical trials as a chemotherapeutic,[65] this property should not be regarded as limiting the potential utility of this inhibitor in chemical biology or biomedicine. That said, it is somewhat surprising

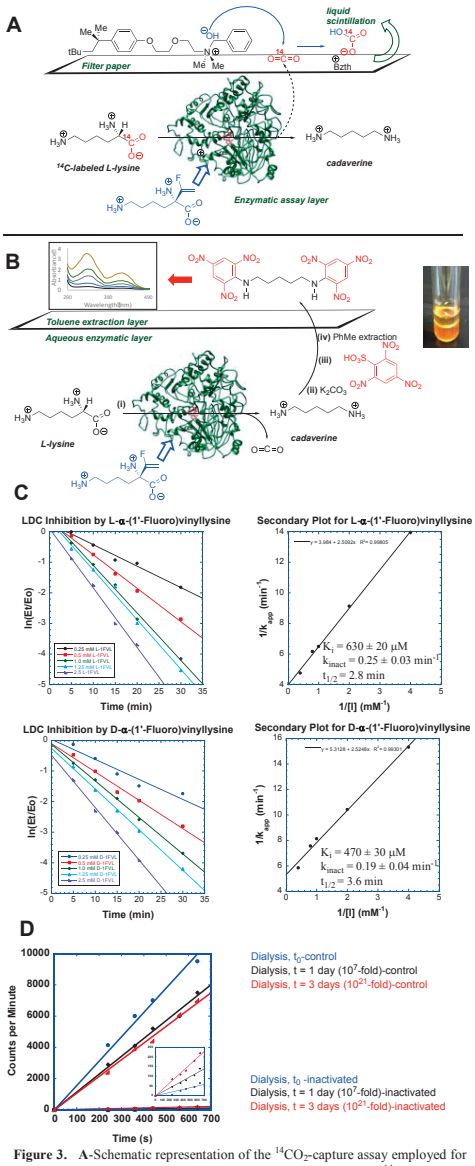

Figure 3. **A**-Schematic representation of the $^{14}CO_2$-capture assay employed for LDC activity. LDC from *Hafnia alvei* was incubated with U-^{14}C-labeled L-lysine (2.5 mM conc; 7.14 nCi in 200 μL total volume) in an Eppendorf tube. Evolved $^{14}CO_2$ was captured with base (benzethonium hydroxide) soaked filter paper... **B**-Schematic of the complementary UV-based Lenhoff assay that measures formation of cadaverine product with time (details in the SI). **C**-Primary and secondary Kitz-Wilson plots for the time dependent inactivation of *H. alvei* LDC by L- and D-α-(1'-fluoro)vinyllysine. **D**-Results of dialysis experiments. LDC (1.5 U) was first inactivated for 1 h with 10 mM (±)-α-(1'-fluoro)vinyllysine (98.9% inactivation). Each cycle was run as a 1:250-fold dilution against 100 μM PLP, 100 mM KPO_4, pH 6.0. A total of 9 cycles of dialysis (~10^{21}-fold dilution) was run. The insert (lower right corner) shows an expansion of the cpm vs. time data for the inactivated enzyme samples as a function of dialysis cycle.

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that both enantiomers inactivate LDC here given the quite different behavior with the α-(l'-fluoro)vinyl trigger,[39a] for example. Clearly, future investigations are warranted here to understand the mechanistic underpinnings of these different enantiospecifities of inhibition.

Consistent with the postulated mechanisms for this new class of AADC inactivator (Figure 1), inhibition was seen to be essentially irreversible. This was established by extensive dialysis experiments. Specifically, LDC was initially incubated with 10 mM (±)-**11i** for 1h leading to 98.9% inhibition relative to control. Each dialysis cycle was run as a 1:250 fold dilution, as described in detail in Figure 3. Each dialysis cycle was run for 3h and three dialysis cycles were performed per day. This amounts to a 10^7-fold dilution after Day 1 and a 10^{21}-fold dilution after Day 3. The data are plotted in Figure 3C and indicate that after Day 1, 98.2% inhibition was observed (0.7% reactivation), and after Day 3, 96.9% inhibtion was observed (2% overall reactivation).

Two additional important control experiments were conducted; namely, external nucleophile and substrate protection experiments. In the former experiment, LDC inactivation was run as described above with 4 mM **11i** alone and in the presence of 2 mM 3-mercaptopropanesulfonate. After 1.5 h of incubation with inhibitor, no external nucleophile protection was observed (i.e. ~99% inactivation in both assays). These results suggest that reactive species formed from **11i** in the LDC active site (includes the Cγ- and Cβ-centered electrophilic species illustrated in Figure 1) cannot be intercepted by the external nucleophile in competition with active site nucleophiles. Perhaps more importantly, this experiment suggests that the proposed electrophilic species are generated in the active site, and are not freely diffusible prior to inactivating the enzyme. In the latter substrate protection experexperiment, significant substrate protection was observed with 2.5 mM L-lysine reducing the effect of a 1 h preincubation with 2 mM (±)-α-(1'-fluoro)vinyllysine from 94% inactivation to 75% inactivation (see Experimental Section and SI), consistent with the title compound acting as an active site-directed mechanism-based inactivator.

CONCLUSIONS

This work describes a long sought-after method to synthesize, purify and store the elusive electrophile β,β-difluorovinyl phenyl sulfone (**5**). As noted, this will make this potent electrophile available to the synthetic community for study in Diels-Alder or related cycloaddition reactions, as well as for examination in conjugate/addition chemistry, in general. Perhaps, most importantly, in our view, this electrophile serves as a useful (1'-fluoro)vinyl cation equivalent, condensing with enolates of N-benzylidene AA esters to provide entry to the interesting class of quaternary, α-(1'-fluoro)vinyl AAs, bearing native side chains, for the first time. This puts the chemical biology community in a position to examine the Abelesian α-(1'fluoro)-vinyl trigger design (Figure 1) in AADC active sites broadly.

The key point is that the chemistry communicated herein provides access to amino acids that bear the requisite α-amino and α-carboxyl groups, but also the key cognate side chain, for delivery to the targeted enzyme active site, in additional to the masked (α-(1'-fluoro)vinyl warhead. In the first test of this concept, LDC from *Hafnia alvia* is seen to be inactivated by both L and D-α-(1'fluoro)-vinyllysine. The inactivation is functionally irreversible (extensive dialysis) and active site-directed (substrate protection observed). Moreover, external nucleophile protection is not observed consistent with the putative inactivation mechanism(s) (Figure 1).

It is important to note that although the new α-(1'-fluoro)vinyl trigger is clearly sterically encumbering, the K_i values observed here 470-630 μM, for the two antipodal LDC inactivators are similar to the K_m value (640 μM) of L-lysine with the enzyme. Moreover, it is well to note that perhaps the most clinically important vinylic amino acid, γ-vinyl-GABA (Vigabatrin), a useful drug for epilepsy[66] and a compound gaining interested for drug addiction as well,[67] exhibits similar kinetic parameters for inactivation of its PLP-enzyme target, GABA transaminase (K_i = 3 mM; $t_{1/2}$ = 1.9 min).[68]

It should also be noted that the class of sterically encumbered (±)-α-(1'fluoro)-vinyl AAs described here may be regarded as a potentially novel solution to both the AADC and the retro-aldolase inactivation problem (while presumably remaining inert to PLP enzymes that labilize the Cα-H bond, including transaminases, racemases and β- and γ-replacement/elimination enzymes). The former enzymes labilize the Cα-CO_2^- bond and exist in both PLP- and pyruvamide-classes whereas the latter enzymes labilize the Cα-Cβ(OH) bond and utilize exclusively PLP cofactors.

In addition to the LDC model system examined herein, the PLP-dependent AADCs include glutamate DC[69] (biosynthesis of the inhibitory neurotransmitter, GABA), aromatic AADC (biosynthesis of both dopamine and serotonin), ornithine DC (rate-limiting step in the polyamine pathway, of interest both for both the development of anti-parasitic[70] and anti-neoplastic[65,71] agents), arginine DC[72] (alternative pathway into polyamine biosynthesis, via agmatine) and histidine DC[73] (histamine biosynthesis). The pyruvamide-dependent AADCs include S-adenosylmethionine (SAM) DC[74] from all three kingdoms of life (also central to the polyamine biosynthetic pathway), aspartate α-DC[75] (key step in coenzyme A biosynthesis, potential antibiotic target), phosphatidyl serine DC[76] (phospholipid biosynthesis) as well as other variants of both histidine DC[77] and arginine DC.[78] In this context, the methodology/protection strategy reported here shows real promise for building potential new inhibitors for ODC (lysine chemistry here) and for aromatic AADC (tyrosine and tryptophan side chains established here vs. the DOPA and 5-hydroxytryptophan side chains that presumably would target this active site).

Moreover, the class of PLP-dependent retroaldolases continues to grow, and should be targetable with this same MBI approach. The classic member of this family is L-serine hydroxymethyl transferase (SHMT),[79] involved in the one-carbon cycle associated with pyrimidine biosynthesis, a three-enzyme pathway in which the other two enzymes are targeted by the clinical chemotherapeutics 5-fluorouracil (thymidylate synthase) and methotrexate (dihydrofolate reductase - DHFR). More recently, PLP dependent aldolases for L-threonine,[80] D-threonine,[81] phenylserine[82] and α-methylserine,[83] with a number of these demonstrating considerable side chain tolerance.[84] Thus, the chemistry presented here is expected to be enabling tool across a broad spectrum of chemical biological studies.

Finally, it should be noted that the intermediate fluorovinyl-sulfonyl AAs are also worthy of future examination. Namely, recent investigations of peptides or amino acids bearing fluorinated α,β-unsaturated aryl sulfone functionality seek to build "designer" covalent enzyme inhibitors in this manner.[2a] It is expected that the chemistry reported herein, and the direct access to fluorovinyl-sulfonylated amino acids that it enables will be of great utility to the chemical biology and medicinal chemistry communities in these endeavors as well.

EXPERIMENTAL SECTION

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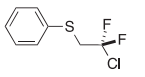
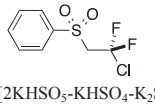
2-Chloro-2,2-difluoroethyl Phenyl Sulfide (3)



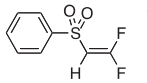
To a cooled solution (-78 °C) of phenylsulfenyl chloride (**2**) (10.0 g, 69.2 mmol) in methylene chloride (70 mL) was added $AlCl_3$ (4.62 g, 34.6 mmol, 0.5 eq) and the solution was allowed to stir at -78 °C for 15 min. Dry 1,1-difluoroethylene was bubbled into the reaction vessel for 10-15 min and the resulting mixture was stirred until a noticeable color change occurred (~ 30 min). Typically, solution color evolved from an initial orange hue, to yellow, to finally a brown color. The reaction was quenched by addition of 10% HCl (aq). Following the addition of CH_2Cl_2 and extraction with several portions thereof, the combined organics were dried ($MgSO_4$), filtered, and concentrated to give **3**, which was carried forward directly to the next step without further purification.

2-Chloro-2,2-difluoroethyl Phenyl Sulfone (4)



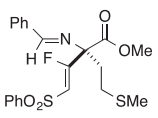
To a cooled solution (0 °C) containing crude residue **3** in MeOH/H_2O (1:1, 500 mL total volume) was added Oxone®, (potassium monopersulfate tri-salt [$2KHSO_5$-$KHSO_4$-K_2SO_4 (nominal MW = 614.8), 127 g, 207 mmol, 3.0 eq] and the suspension was stirred for an additional 15 h at room temperature. Subsequently, the reaction mixture was diluted with $CHCl_3$ (300 mL), and the organics were extracted with H_2O (3 x 150 mL), dried over $MgSO_4$, filtered, and concentrated. Purification by SiO_2 column chromatography [4:1 hexane:EtOAc] afforded homogeneous **4** (16.0 g, 96 % over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 (t, *J* = 12.0 Hz, 2H), 7.62-7.58 (m, 2H), 7.74-7.78 (m, 1H), 7.99-8.01 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 64.7 (t, *J* = 27.0 Hz), 122.9 (t, *J* = 292.0 Hz) 128.8, 129.7, 135.0, 138.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ -50.86 (t, *J* = 11.28 Hz, 2F); HRMS (FAB, 3-NBA) *m/z* calcd for $C_8H_8ClF_2O_2S$ $[m+H]^+$ 240.9896, obsd 240.9906.

2,2-Difluorovinyl Phenyl Sulfone (5)



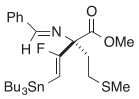
To a solution of **4** (1.0 g, 4.2 mmol) in methylene chloride (10.4 mL) was added freshly distilled 2,2,6,6-tetramethylpiperidine (2,2,6,6-TMP , 0.70 mL, 4.1 mmol, 1.0 eq) in one steady portion over 15-20 s. Immediately following this addition, the reaction was quenched by addition of 10% HCl (aqueous, 10 mL), followed by further stirring for an additional min. Following extraction (Et_2O - 3 x 10 mL), the combined organics were washed with brine, dried (Na_2SO_4) and concentrated. The resulting oil was purified via kugelrohr distillation (bp 115-120 °C, 0.05-0.10 torr) to afford **5** as a clear, colorless oil (0.85 g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.65 (dd, *J* = 2.4, 22.4 Hz, 1H), 7.55-7.60 (m, 2H), 7.65-7.69 (m, 1H), 7.93-7.95 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 90.7 (dd, *J* = 23.8, 13.8 Hz), 127.5, 129.7, 134.3, 141.5 (d, *J* = 2.5 Hz), 159.3 (t, *J* = 305 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ -68.2 (dd, *J* = 11.4, 2.3 Hz, 1F), -63.17 (dd, *J* = 11.3, 18.8 Hz, 1F); HRMS (FAB, 3-NBA) *m/z* calcd for $C_8H_6F_2O_2S$ $[m+Li]^+$ 211.0211, obsd 211.0212.

(±)-Methyl N-Benzylidine-α-(1'Z-fluoro-2'-phenylsulfonyl) vinylmethioninate (9e)



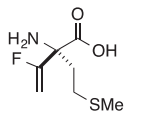
To a cooled (-78 °C) 1.6 M solution of 2,2,6,6-TMP (0.56 mL, 3.3 mmol, 1.1 eq) in THF (3.0 mL) was added 1.6 M *n*BuLi (2.70 mL, 3.3 mmol, 1.1 eq) dropwise via syringe and the mixture was allowed to warm to 0 °C and stirred (30 min) then recooled to -78 °C. Then, a 1.6 M solution of N-benzylidine methionine methyl ester **8e** (750 mg, 3.0 mmol, 1.0 eq), in THF (1.25 mL) was added via cannula. The mixture was warmed to 0 °C for 30 min before being cooled once again to -78 °C. A solution of freshly distilled 2,2-diflurovinyl phenyl sulfone, **5** (780 mg, 3.3 mmol, 1.3 eq), in THF (1.10 mL; 2.4 M conc) was added, dropwise, via cannula at -78 °C. The resulting reaction mixture was stirred at -78 °C for 10-20 min and then warmed to 0 °C and monitored by TLC [80:20 Hex:EtOAc containing Et_3N (3% v/v)] until complete (~ 30 min). The reaction was quenched by addition of $NaHCO_3$ (aq, sat'd) and the resultant crude reaction mixture was extracted with Et_2O (3x). The combined organics were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification by SiO_2 column chromatography [80:20 hexane:EtOAc containing Et_3N (3% v/v)] afforded **9e** (939 mg, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.96 (s, 3H), 2.16-2.22 (m, 1H), 2.26-2.43 (m, 3H), 3.69 (s, 3H), 6.77 (d, *J* = 32.0 Hz, 1H), 7.44 (t, *J* = 7.5 Hz, 2H), 7.50 (app t, *J* = 7.5, 7.0 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 2H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.73 (d, *J* = 7.6 Hz, 2H), 8.01 (d, *J* = 8.0 Hz, 2H), 8.18 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.7, 27.8, 36.3, 53.5, 73.0 (d, *J* = 28.8 Hz) 114.9 (d, *J* = 6.3 Hz), 127.7, 129.06, 129.08, 129.3, 132.6, 133.9, 135.0, 142.0, 163.0, 166.8 (d, *J* = 279.0 Hz), 168.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ -91.7 (d, *J* = 33.8 Hz, 1F); HRMS (ESI) *m/z* calcd for $C_{21}H_{22}F NO_4S_2$ $[m+Na]^+$ 458.0872, obsd 458.0878.

(±)-Methyl-N-Benzylidine-α-(1'-fluoro-2'Z-tributylstannyl) vinylmethioninate (10e)



To a 100 mM solution of **9e** (570 mg, 1.3 mmol, 1.0 eq) in toluene (14 mL) was added Bu_3SnH (0.35 mL, 1.3 mmol, 1.1 eq) and AIBN (42 mg, 0.26 mmol, 0.2 eq). The resulting solution was bubbled with argon for 10 min and then refluxed until TLC [80:20 Hex:EtOAc containing Et_3N (3%v/v)] indicated consumption of starting material. The solution was concentrated under reduced pressure and further purified by SiO_2 column chromatography [100% hexane containing Et_3N (3%v/v) to 92:8 hexane:EtOAc containing Et_3N (3% v/v)] to afford **10e** (740 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 7.6 Hz, 9H), 0.96-1.0 (m, 6H), 1.31 (sext, *J* = 7.2 Hz, 6H), 1.48-1.55 (m, 6H), 2.09 (s, 3H), 2.27-2,40 (m, 2H), 2.53-2.70 (m, 2H), 3.77 (s, 3H), 5.42 (d, *J* = 72.0 Hz, 1H), 7.40-7.46 (m, 3H), 7.79 (dd, *J* = 8.0, 2.0 Hz, 2H), 8.25 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.5, 13.9, 15.7, 27.4, 29.0, 29.3, 37.5, 52.9, 74.8, (d, *J* = 37.0 Hz), 102.0 (d, *J* = 44.0 Hz), 128.8, 128.9, 131.6, 136.1, 161.7, 164.6 (d, *J* = 247.0 Hz), 171.0; ^{19}F NMR (283 MHz, $CDCl_3$) δ -82.6 (d, *J* = 73.3 Hz, 1F); HRMS (ESI) *m/z* calcd for $C_{27}H_{44}FNO_2SSn$ $[m+Na]^+$ 608.1996, obsd 608.1968.

(±)-α-(1'-Fluoro)vinylmethionine (11e)



To 6 N HCl (10 mL) was added **10e** (360 mg, 0.68 mmol) and the solution was stirred at 80 °C overnight. Upon completion, the aqueous phase was extracted with CH_2Cl_2 (5 x 10 mL) to remove impurities. The aqueous layer was concentrated under vacuum while gently heating (~ 50° C) to give **11e** (147 mg, 97%) as the HCl-salt which was determined to be clean by ^{1}H NMR. The HCl-salt

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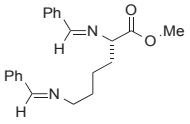
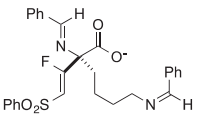
can be neutralized by loading onto a DowexWx50 cation exchange column and eluting with 3N NH_4OH, concentrating the principal fraction, in vacuo, to yield **11e** (116 mg, 94% of product from HCl-salt). ^{1}H NMR (400 MHz, D_2O) δ 2.16 (s, 3H), 2.27-2.35 (m, 1H), 2.38-2.46 (m, 1H), 2.51-2.58 (m, 1H), 2.63-2.70 (m, 1H), 5.04 (dd, *J* = 72.8, 4.8 Hz, 1H), 5.13 (dd, *J* = 40.8, 4.8 Hz, 1H); ^{13}C NMR (100 MHz, D_2O) δ 14.1, 27.2, 32.3 (d, *J* = 4.0 Hz), 64.6 (d, *J* = 27.0 Hz), 95.4 (d, *J* = 18.0 Hz), 160.5 (d, *J* = 255.0 Hz, 1C), 170.5; ^{19}F NMR (376 MHz, D_2O) δ -108.2 (dd, *J* = 52.6, 18.8 Hz, 1F); HRMS (CI, isobutane) *m/z* calcd for $C_7H_{13}FNO_2S$ $[m+H]^+$ 194.0646, obsd, 194.0657.

Methyl (N,N'-Dibenzylidene)lysinate (8i)

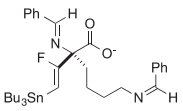


To a 0.5 M solution of L-lysine methyl ester dihydrochloride, commercially available on Sigma-Aldrich (1.0 g, 4.2 mmol, 1.0 eq) in CH_2Cl_2 (20 mL) was added $MgSO_4$ (1.5 g, 12 mmol, 3.0 eq) and Et_3N (1.2 mL, 8.4 mmol, 2.0 eq). The mixture was stirred for 15 minutes at room temperature. Next, benzaldehyde (0.85 mL, 8.4 mmol, 2.0 eq) was added and the reaction was further stirred overnight whereby TLC [90:10 CH_2Cl_2:MeOH containing NH_4OH (2% v/v); ninhydrin stain] indicated complete disappearance of starting material. The mixture was filtered and partitioned between $NaHCO_3$ (aq)) and CH_2Cl_2 (3 x 50 mL). The organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Subsequent SiO_2-column chromatography [90:10 hexane:EtOAc containing Et_3N (10% v/v)] afforded **8i** (1.2 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (m, 2H), 1.74 (m, 2H), 2.02 (m, 2H), 3.57 (t, 2H), 3.70 (s, 3H), 3.99 (t, 1H), 7.39 (t, 6H), 7.75 (d, 2H), 8.23 (d, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.64, 30.51, 33.18, 52.18, 61.32, 73.40, 128.09, 128.62, 128.65, 129.06, 129.78, 130.55, 131.18, 135.72, 136.33, 161.06, 163.46, 172.68; HRMS (ESI) *m/z* calcd for $C_{21}H_{24}N_2O_2$ $[m+Na]^+$ 359.4242, obsd 359.4239.

(±)-Methyl N,N'-Dibenzylidine-4'-(*tert*-butyldimethylsilyloxy)-α-(1'-fluoro-2'Z-phenylsulfonyl)vinyllysinate (9i)



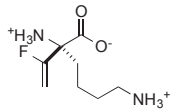
To a cooled (-78 °C) 1.6 M solution of 2,2,6,6-tetramethylpiperdine (0.56 mL, 3.3 mmol, 1.1 eq) in THF (3.0 mL) was added 1.2 M nBuLi (2.70 mL, 3.3 mmol, 1.1 eq) dropwise via syringe and the mixture was allowed to warm to 0 °C and stirred (30 min) then recooled to -78 °C. Then, a 1.2 M solution of **8i** (1.0 g, 3.0 mmol, 1.0 eq) in THF (3.0 mL) was added via cannula. The mixture was warmed to 0 °C for 30 min before being cooled once again to -78 °C. By cannulation, a 2.4 M THF (1.10 mL) solution of freshly distilled 2,2-difluorovinyl phenyl sulfone 5 (790 mg, 3.9 mmol, 1.3 eq) in THF (0.5 mL) was added. The resulting reaction mixture was stirred at -78 °C for 10-20 min, warmed to 0 °C and monitored by TLC [80:20 Hex:EtOAc containing Et_3N (3% v/v)] until completion (~ 30 min). Quenching with $NaHCO_3$ (aq) was followed by Et_2O extraction, and the combined organics were washed with brine, dried (Na_2SO_4) and concentrated. SiO_2 flash chromatography (70:30:10 hexane:EtOAc:Et_3N) then gave **9i** (1.34 g, 86%) following ^{1}H NMR (700 MHz, $CDCl_3$) δ 1.17 (m, 1H), 1.35 (m, 1H), 1.60 (q, 2H), 2.11 (t, 2H), 3.48 (m, 2H), 3.66 (s, 3H), 6.80 (d, *J* = 32.0 Hz, 1H), 7.40 (m, 6H), 7.46 (m, 1H), 7.51 (m, 2H), 7.52 (m, 1H), 7.63 (d, 2H), 7.69 (s, 2H), 8.00 (d, 2H), 8.18 (d, 2H); ^{13}C NMR (176 MHz, $CDCl_3$) δ 20.8, 30.9, 36.0, 53.4, 61.1, 73.3 (d, *J* = 28.7 Hz) 114.5 (d, *J* = 6.6 Hz), 127.7, 128.2, 128.8, 128.9, 129.0, 129.3, 130.7, 132.3, 133.7, 135.3, 136.4, 142.2, 161.3, 162.6, 167.4 (d, *J* = 286.5 Hz), 169.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ -90.7 (d, *J* = 33.8 Hz, 1F); HRMS (ESI) *m/z* calcd for $C_{29}H_{29}FN_2O_4S$ $[M+Na]^+$ 543.6086, obsd 543.6079



(±)-Methyl N,N'-Dibenzylidine-α-(1'-fluoro-2'Z-tributylstannyl)vinyllysinate (10i)

To a 100 mM solution of **9i** (510 mg, 1.3 mmol, 1.0 eq) in benzene (9.2 mL, 100 mM), was added Bu_3SnH (0.35 □L, 1.3 mmol, 1.1 eq) and AIBN (42 mg, 0.26 mmol, 0.2 eq) gave **10i** (696 mg, 80%) following SiO_2 column chromatography [100% hexane containing Et_3N (10% v/v) to 92:8 hexane:EtOAc containing Et_3N (10% v/v)]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (m, 12H), 0.96 (m, 6H), 1.27 (m, 10H), 1.35 (m, 12H), 1.51(m, 2H), 1.70 (m, 2H), 3.59 (t, 2H), 3.74 (s, 3H), 5.44 (d, 1H, *J* = 72.4 Hz), 7.41 (m, 6H), 7.65 (t, 2H), 7.76 (t, 2H), 8.25 (d, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ10.42, 13.84, 21.58, 27.30, 29.17, 31.18, 37.26, 52.63, 61.45, 75.21 (d, *J* = 36.2 Hz) 101.21 (d, *J* = 43.8 Hz), 128.12, 128.66, 128.7, 129.1, 129.9, 130.5, 131.3, 136.2, 136.4, 161.0, 161.2, 165.4 (d, *J* = 247.2 Hz), 171.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ -82.7 (d, *J* = 71.4 Hz, 1F); HRMS (FAB) *m/z* calcd for $C_{35}H_{51}FN_2O_2Sn$ $[M+Na]^+$ 692.5026, obsd 692.5030.

(±)-α-(1'-Fluoro)vinyllysine (HCl) (11i)



To 6N HCl (2 mL) was added **9i** (0.51 g, 0.75 mmol) and solution stirred at 80° C overnight. Upon completion the aqueous phase was extracted with CH_2Cl_2 to remove impurities. The aqueous phase was washed with two more portions of CH_2Cl_2 and concentrated under vacuum while gently heating (~ 50° C) to afford **11i** (114 mg, 58%) as the HCl-salt. ^{1}H NMR (700 MHz, D_2O) δ 1.37 (m, 1H), 1.55 (m, 1H), 1.75 (m, 2H), 2.04 (m, 1H), 2.14 (m, 1H), 3.026 (t, 2H), 4.99 (dd, *J* = 51.1, 4.9 Hz, 1H), 5.13 (dd, *J* = 18.9, 4.9 Hz, 1H); ^{13}C NMR (176 MHz, D_2O) δ 20.2, 26.5, 32.0, 38.9, 64.6 (d, *J* = 27.7 Hz), 95.5 (d, *J* = 17.7 Hz), 160.6 (d, *J* = 256.0 Hz), 170.8; ^{19}F NMR (376 MHz, D_2O) δ -108.2 (dd, *J* = 48.8, 18.8 Hz, 1F); HRMS (CI, methane) calcd for $C_8H_{16}FN_2O_2$ $[M+H]^+$ 191.1190, obsd, 191.1191.

Purification of Lysine Decarboxylase from *Hafia alvei*. Enzyme was purified from the native source by growing *Hafnia alvei* (12 L culture) on a medium of bactopeptone, yeast extract, D-glucose (all @ 10 g/L) containing $MgSO_4$-heptahydrate (2 g/L) and L-lysine (6 g/L) at 37 °C and 250 rpm, harvesting in late log phase(~ 72 h). Centrifugation was performed at 5000 rpm producing 40 g of wet cells. Cells were frozen at -80 °C, and then thawed on ice just prior to breakage. Cells were resuspended in 1 mL/g of 10 mM phosphate buffer at pH 7; phenylmethanesulfonyl fluoride (PMSF) was then added to give a final concentration of 2 mM. Cells were then sonicated on ice for five cycles @ 45 s each. Following centrifugation (10,000 rpm, 10 min), the supernatant was decanted, followed by protein precipitation with 60% ammonium sulfate and centrifugation (5000 rpm, 20 min). The ammonium sulfate precipitate was resuspended in of 100 mM phosphate buffer, pH 7 (@1 mL/g of solid), followed by purification by chromatography on phenyl sepharose resin. Following an initial wash with one column volume of 100 mM KPO_4 buffer, protein was eluted with a linear salt gradient (100 mM →10 mM KPO_4 buff-

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er). Fractions were monitored at 280 nm and 420 nm to detect protein and LDC internal aldimine, respectively. SDS PAGE revealed purified LDC along with truncated protein. Thus, final purification was conducted with an additional size exclusion step (Sephacryl S-300 column). size exclusion column. Fractions containing LDC were concentrated via sequential ultrafiltration (Amicon cell, Ar pressure) and centrifugation-facilitated filtration (Centricon cell) to a total volume of 400 μL. Homogeneous LDC (98 U/mg) was obtained, utilizing the standard assay described below and a Lowry assay for protein. The specific activity and monomeric MW (82 kDa) were used to estimate LDC concentration.

Inhibition Studies/Radioactive Assay. LDC activity was determined utilizing a radioactivity-based assay whereby one measures ^{14}C-labeled CO_2 released from L-(U-^{14}C)-lysine (Amersham or Perkin-Elmer). The standard assay was run in 100 mM KPO_4 buffer, pH 6.0, 100 μM PLP, 86 mU LDC, and 2.5 mM ^{14}C-L-lysine (92.5 nCi). The was achieved as follows: First, the labeled L-Lys substrate solution (40 mM; 7.14 μCi/mL) was prepared from 300 μL (46.7 mM L-lysine) plus 2.5 μCi L-(U-^{14}C)-lysine (50 μL of a 50 μCi/mL stock solution). Then, for each time point, a separate 1.5 mL Eppendorf tube was prepared containing assay buffer (100 mM KPO_4 buffer, pH 6.0, 172.5 μL) to which were added sequentially (i) PLP stock solution (5 mM in buffer, 4 μL), (ii) labeled L-lysine (12.5 μL of 40 mM; 7.14 μCi/mL) and (iii) LDC (11 μL of stock solution of 7.8 U/mL). The top of the Eppendorf tube was overlaid with a sheet of filter paper soaked in benzethonium hydroxide solution (200 μL, 1M solution in MeOH). Time points were taken by quenching with an equal volume (200 μL) of TCA to quench, followed by scintillation counting. LDC inhibition assays were run using (±)-α-(1'-fluoro)vinyllysine concentrations of 0.5, 1, 2, 4, and 10 mM. Activity assays were run vs. control (uninhibited enzyme solution) as described (*vide supra*).

Inhibition Studies/Modified Lenhoff UV Assay. The assay performed is a modified version of the originally reported assay by Lenhoff and coworkers.[64] For every activity measurement, a minimum of four time points was obtained. 10 μL of LDC (0.006 U) was added to each time point sample along with 280 μL 5 mM potassium phosphate buffer pH 6.0. The reaction was initiated by the addition of 2.5 mM L-lysine (10 μL of a 100 mM L-lysine stock solution). The reaction was quenched at various time points by the addition of 300 μL 1.0 M K_2CO_3. Solutions were incubated at 37 °C for 7 min followed by the addition of 300 μL of a 22 mM solution of 2,4,6-trinitrobenzenesulfonic acid (TNBS) followed by a second 7 min incubation at 37 °C. The reactions were then cooled to room temp and 600 μL of toluene was added and the contents vortexed for 1 min. After complete separation of phases, the 200 μL of the toluene layer was pipetted into a quartz cuvette (1 cm path length) and absorbance was measured at 340 nm via UV spectrophotometer..

LDC K_i and k_{inact} Activity Experiments. Time dependent inactivation kinetic assays were conducted utilizing the resolved individual enantiomers, L and D-α-(1'-fluoro)vinyllysine at concentrations of 0.25, 0.5, 1, 1.25, and 2.5 mM. Activity assays were run vs. control (uninhibited enzyme solution) as described (*vide supra*). Each sample was run after the following incubation times; 5, 10, 15, 20, and 30 minutes (see SI for the initial data, as well as primary and secondary Kitz-Wilson analysis).

Substrate Protection Control. To test whether inhibition is active site-directed, substrate protection experiments were performed. Thus, inactivation levels were compared for 1 h preincubation of 2 mM (±)-α-(1'-fluoro)vinyllysine vs. 1 h preincubation of 2 mM (±)-α-1'-fluoro)vinyllysine in the presence of 2.5 mM L-lysine. Following pre-incubation, activity was measured in the standard radiochemical assay at 2.5 mM L-lysine concentration. The results show a 94% knockdown of activity (vs. control = no inhibitor) in the non-protected case vs. a 75% knock-down in the substrate-protected case, evidencing significant protection.

External Nucleophile Control. Enzyme inactivation experiments were run with incubation in the presence of 4 mM inhibitor in the presence or absence of 2 mM 3-mercaptopropanesulfonate vs. control for 1 h. No appreciable change in inactivation levels was seen (see SI for primary data).

Dialysis. Dialysis was performed on a sample of LDC (1.5 U) inactivated for 1 h with (±)-10 mM (±)-α-(1'-fluoro)vinyllysine (>99.5% inactivation). Each cycle was run as a 1:250-fold dilution (2 mL dialysis bag suspended in 500 mL buffer) against 100 μM PLP, 100 mM KPO_4, pH 6.0. Each cycle was performed for ≥ 3 h @ 3 cycles per day. Parallel dialysis runs were conducted for the control LDC sample with the only difference being the omission of inactivator. Aliquots were taken at t = 0 (initial activity readings), 1 (3 cycles of dialysis, ~10^7-fold dilution) and 3 days (9 cycles of dialysis, ~10^{21}-fold dilution) from both the control and the inactivated LDC samples and activity was measured using the standard ^{14}C-radioactive assay (*vide supra*).

ASSOCIATED CONTENT

Supporting Information. Detailed experimental procedures; characterization of new compounds, including copies of NMR spectra; kinetic data and details of inhibitor characterization (160 pages). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes The authors declare no competing financial interests.

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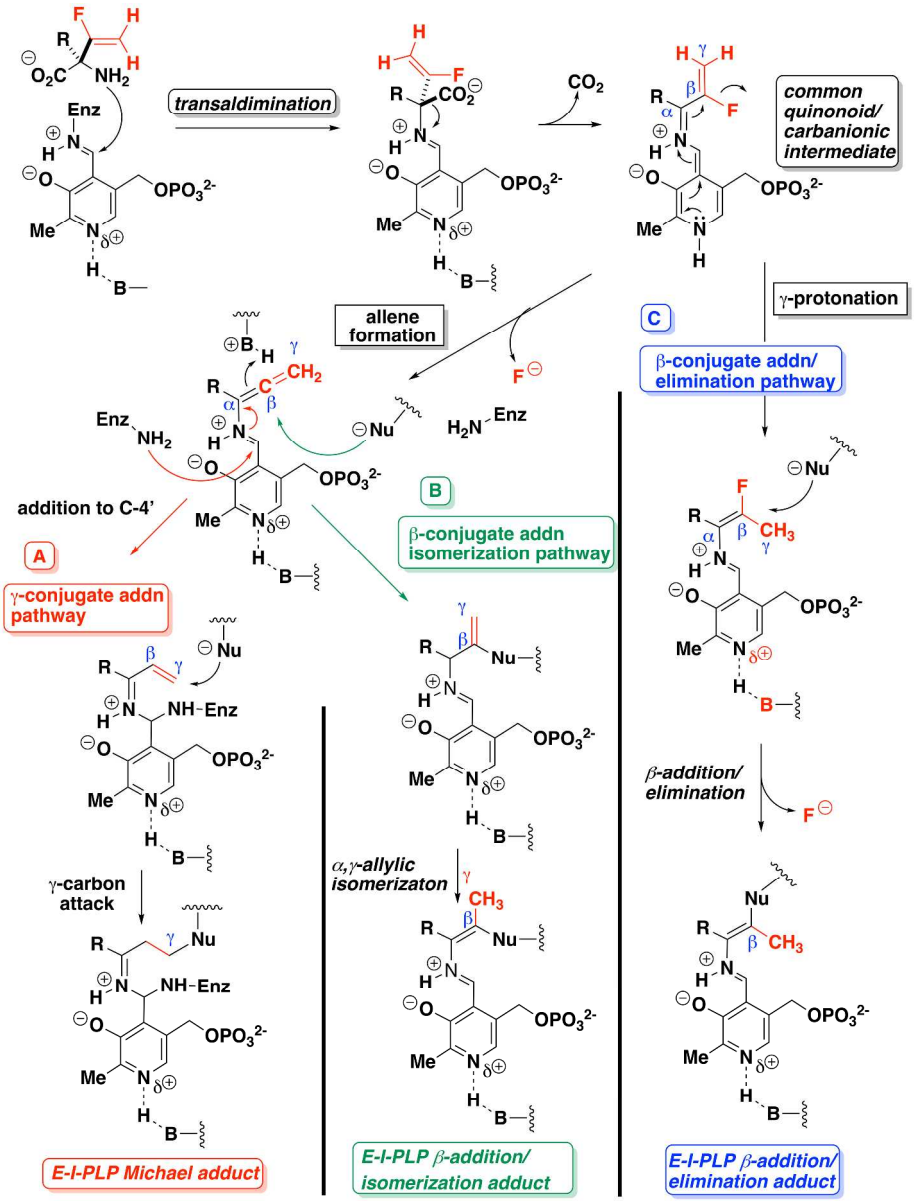


Figure 1

224x292mm (600 x 600 DPI)

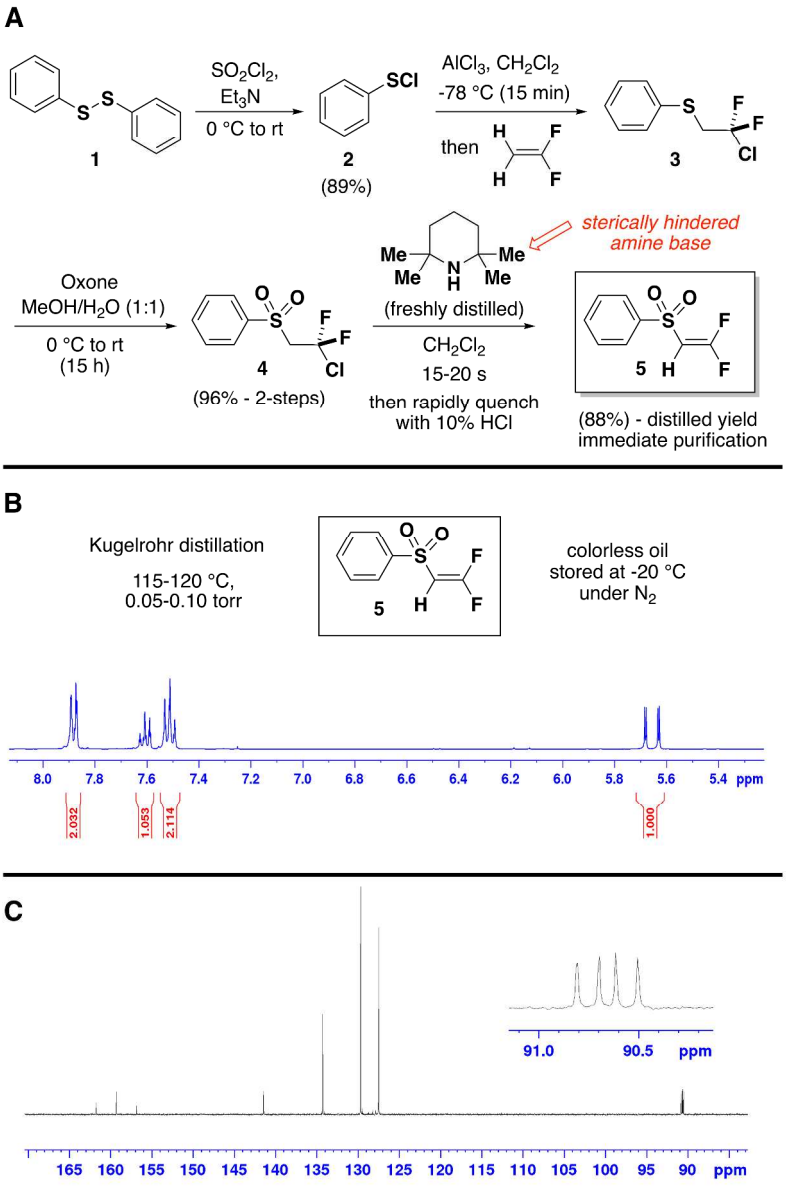
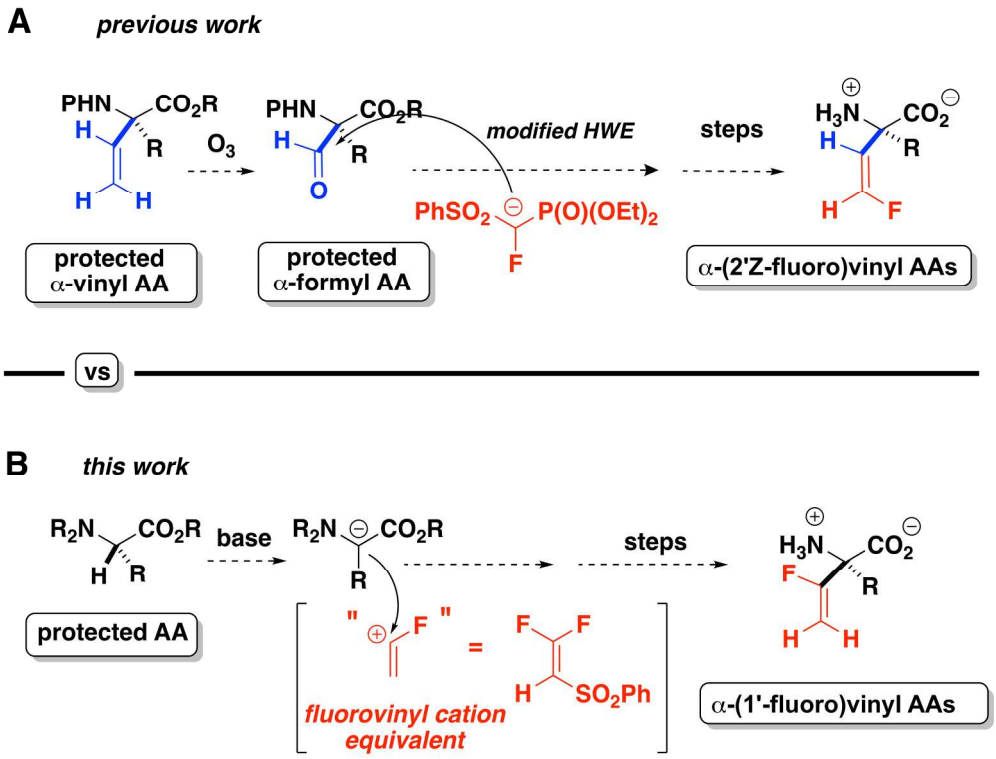


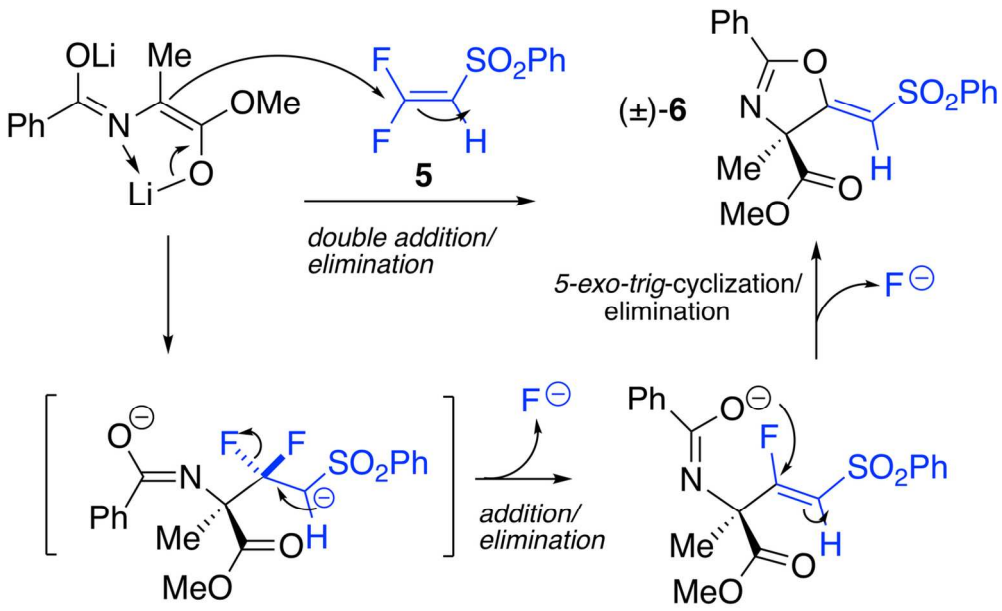
Figure 2

217x330mm (600 x 600 DPI)



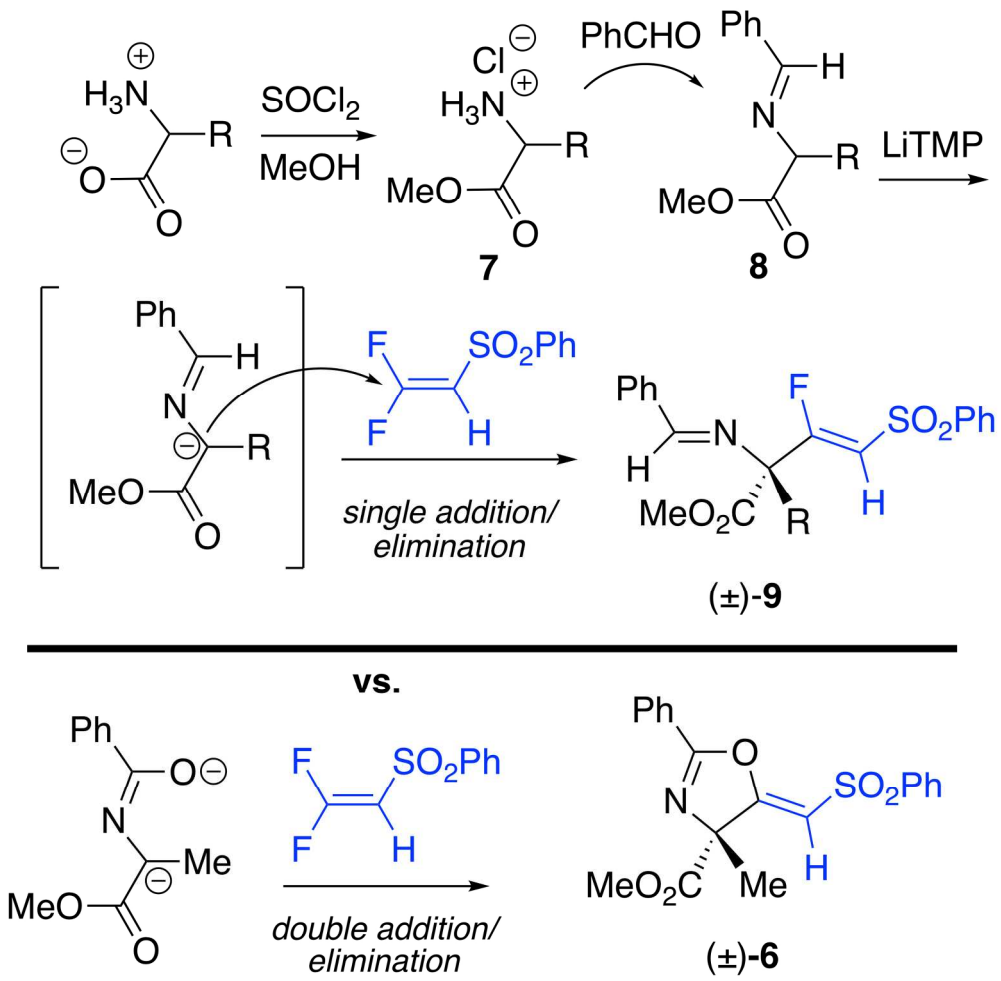
Scheme 1

109x82mm (600 x 600 DPI)



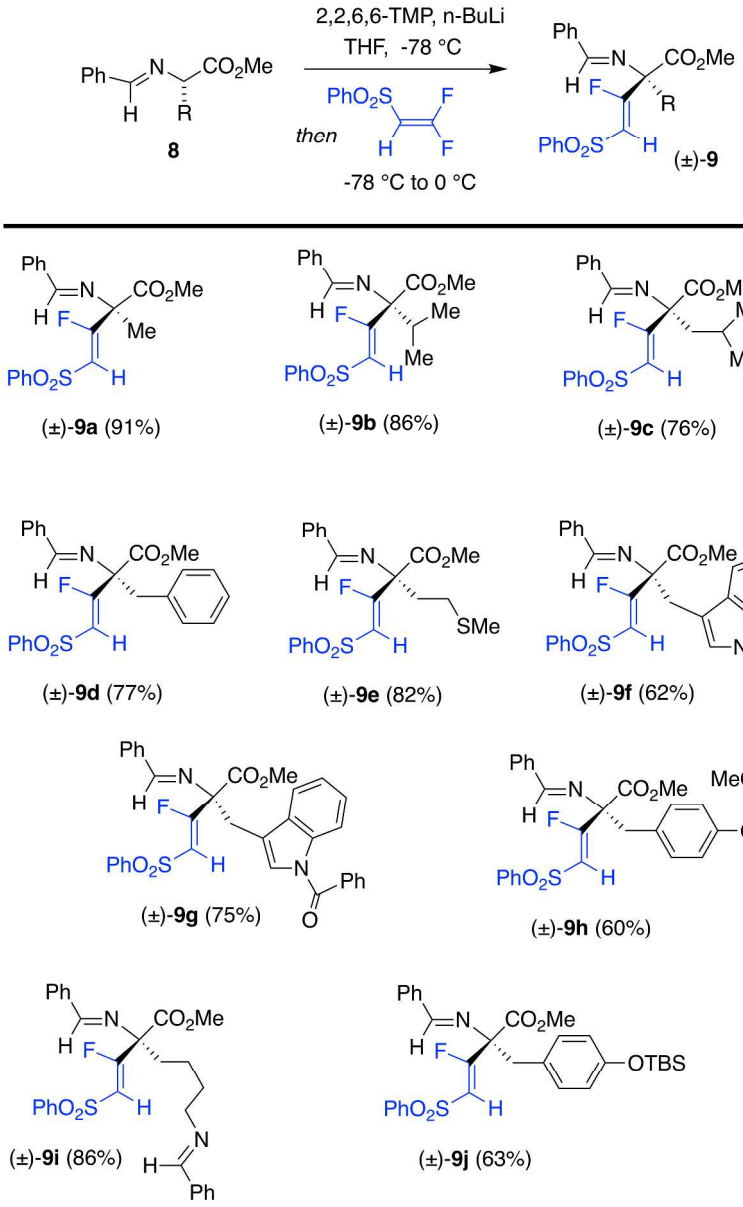
Scheme 2

64x39mm (600 x 600 DPI)



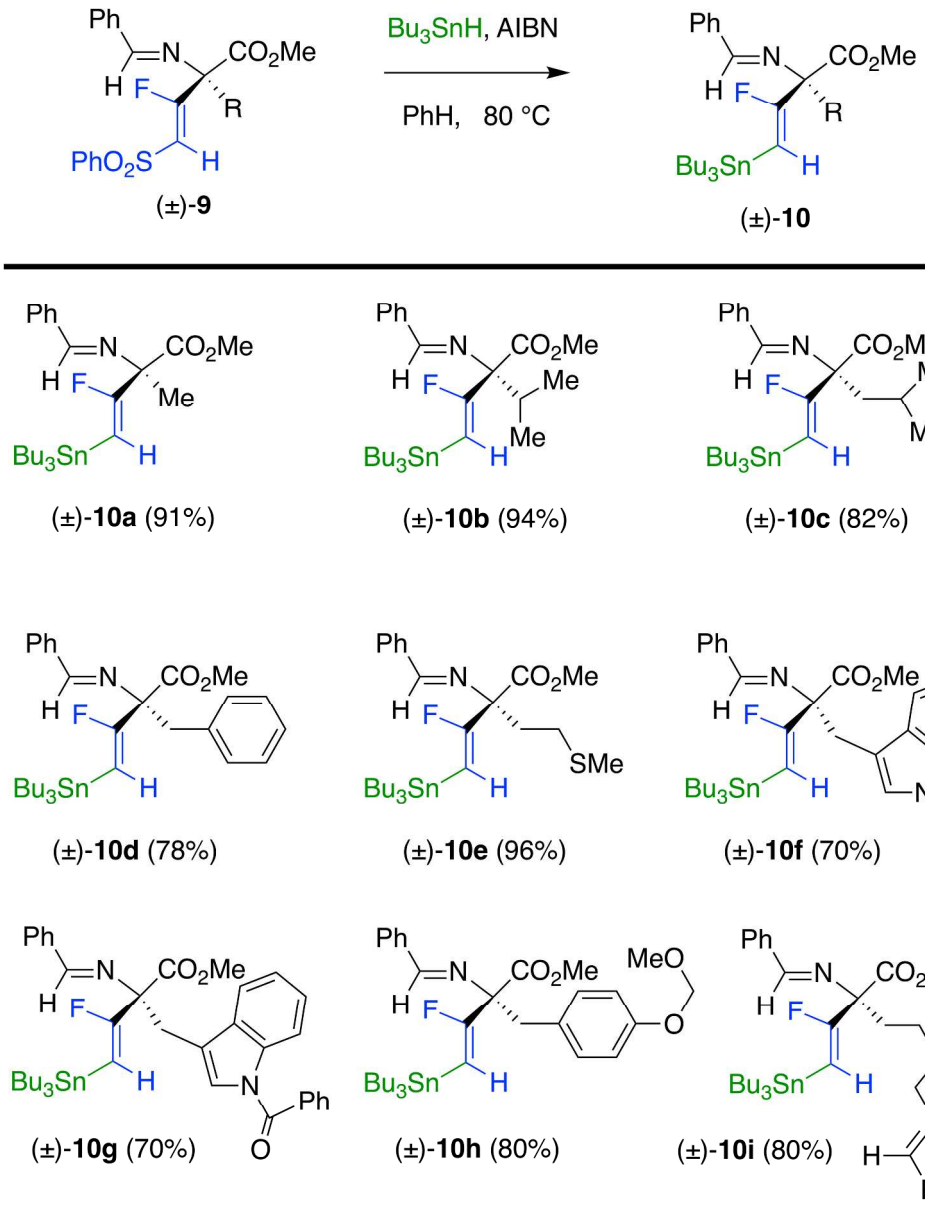
Scheme 3

96x94mm (600 x 600 DPI)



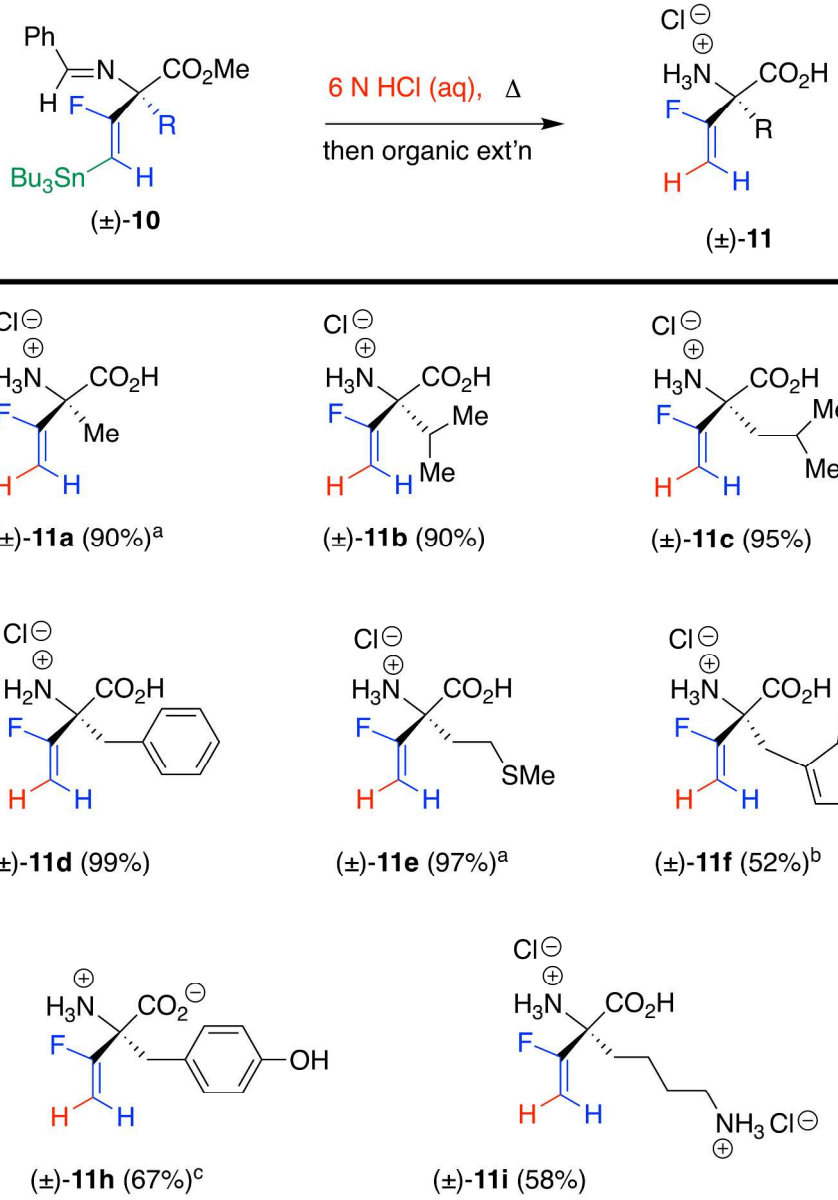
Scheme 4

197x300mm (600 x 600 DPI)



Scheme 5

158x192mm (600 x 600 DPI)



Scheme 6

159x195mm (600 x 600 DPI)

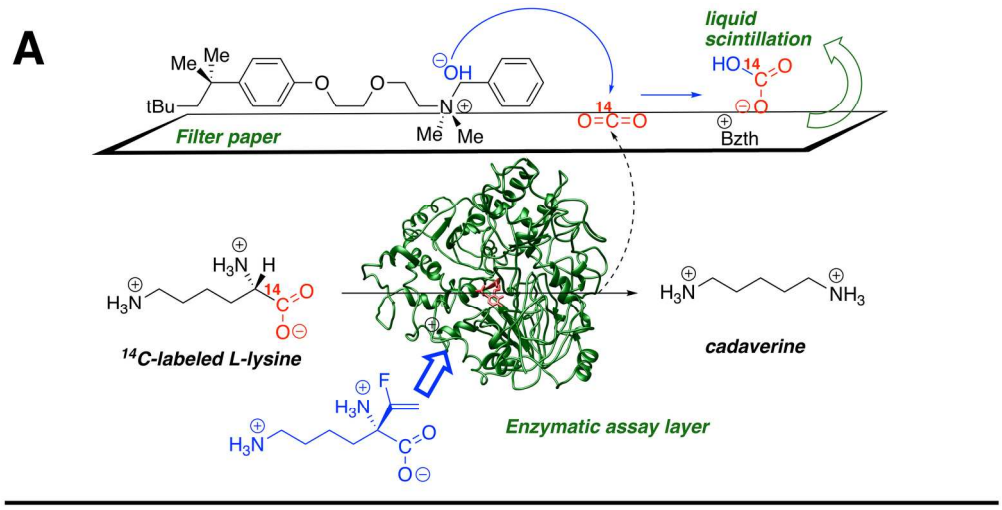


Figure 3A

92x46mm (600 x 600 DPI)

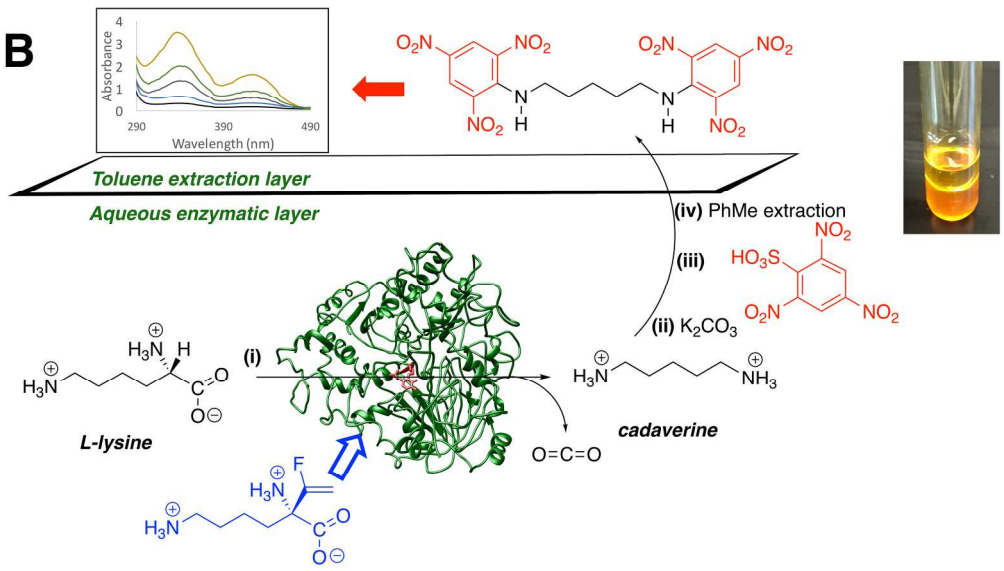


Figure 3B

104x58mm (600 x 600 DPI)

C

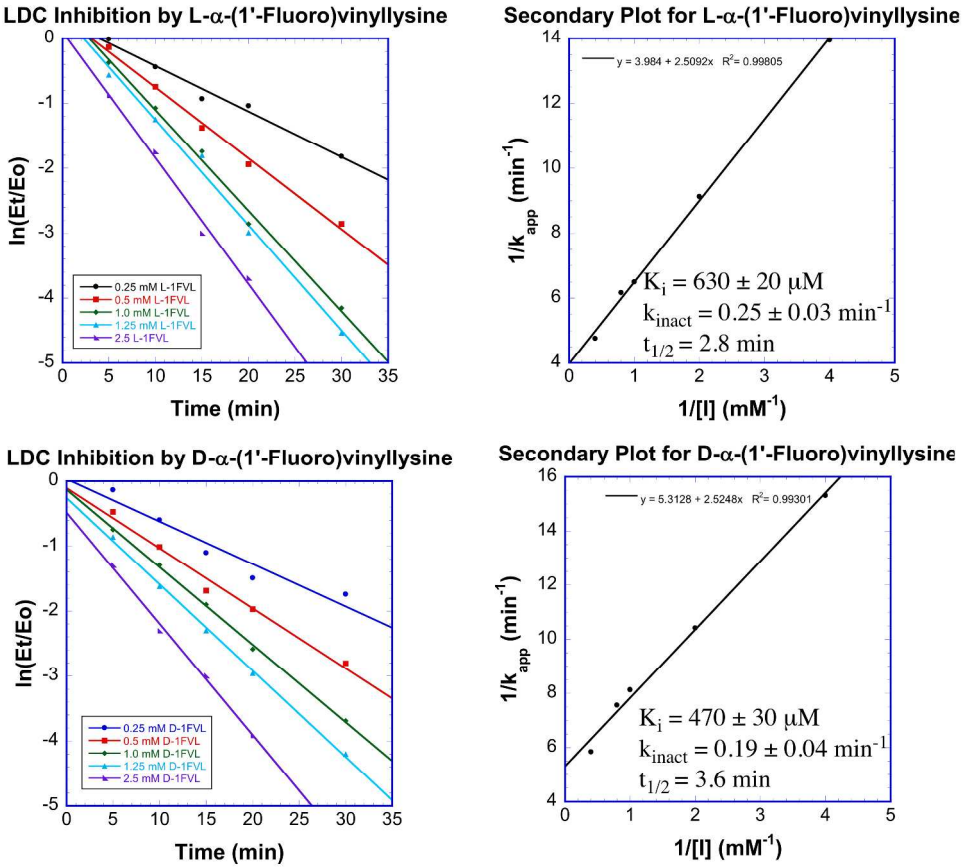


Figure 3C

176x166mm (600 x 600 DPI)

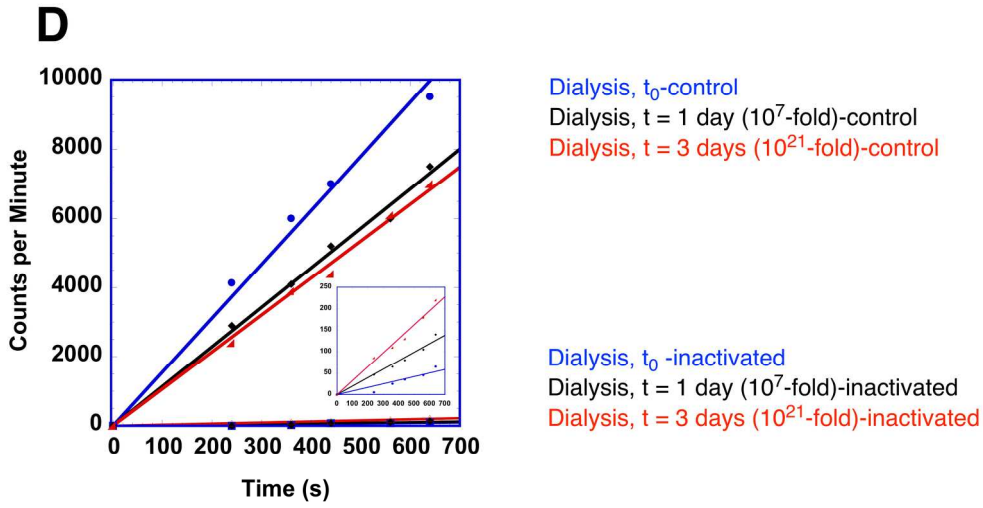
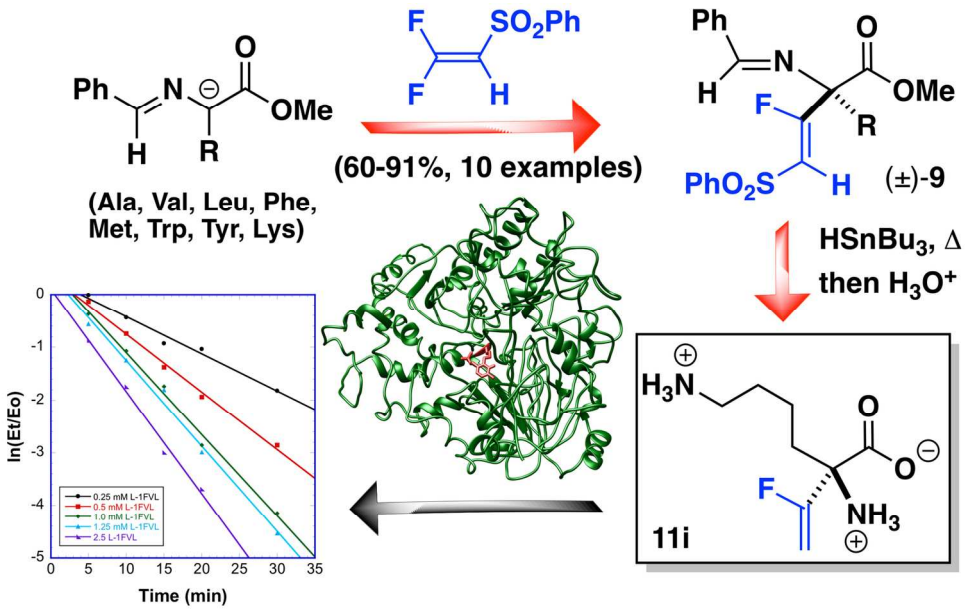


Figure 3D

96x52mm (600 x 600 DPI)



TOC graphic

74x45mm (600 x 600 DPI)