Allenic Suicide Substrates. New Inhibitors of Vitamin B_6 Linked Decarboxylases†

A. L. Castelhano, D. H. Pliura, G. J. Taylor, K. C. Hsieh, and A. Krantz*

Syntex Inc., Mississauga, Ontario, Canada L5M 2B3

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α-Amino acids that are fully functionalized at the α-carbon often possess useful biological activity.[1] In a number of instances unsaturation in the form of an α-vinyl or an α-ethynyl substituent radically alters the properties of the parent amino acids, converting them from substrates to potent inactivators of their target enzymes.[2] Many of these compounds are of recent vintage and have been conceived as suicide inhibitors on the grounds that a highly reactive Michael acceptor capable of trapping an active site nucleophile, will be unmasked during the course of enzyme catalysis.[3-8]

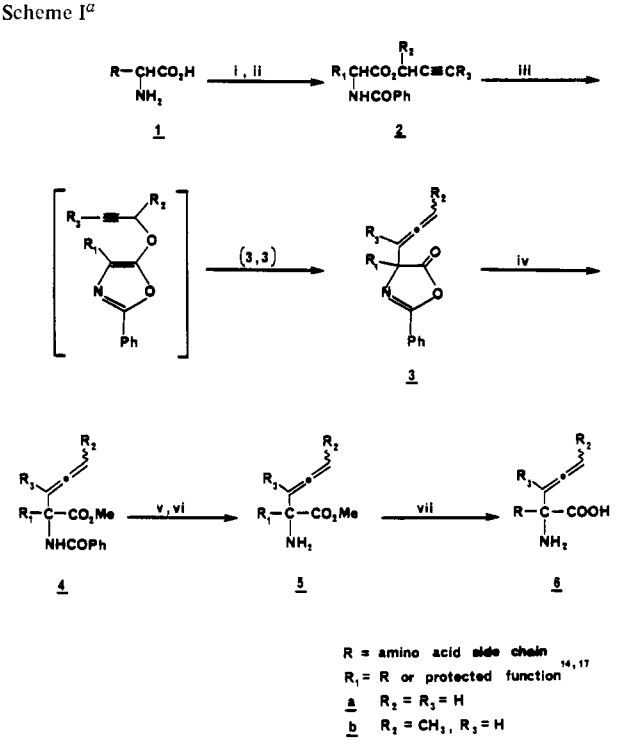
α-Allenic α-amino acids are attractive candidates for the specific inhibition of vitamin B_6 linked decarboxylases. Besides possessing the latent reactivity of β,γ-unsaturated α-amino acids, the chirality bestowed by an asymmetric allenic element can be exploited as a further refinement in the design of inhibitors of narrow specificity.

Despite their potential as "mechanism-based" inhibitors, not a single example of α-allenic α-amino acids has been reported. In principle, the Claisen rearrangement is an elegant way of translocating a three-carbon unit with concomitant interconversion of propargylic and allenic moieties.[9] A recent article by Bartlett[10] has discussed the limitations of effecting the ester–enolate Claisen rearrangement en route to β,γ-unsaturated α-amino acids. An alternative approach mediated by oxazolones leads to *N*-benzoyl-α-amino acids that carry an unsaturated side chain but suffers from the difficulty of hydrolyzing the resulting benzamide without affecting the side chain.[10,11]

We have now established the generality of the conversion of α-benzamido propargylic esters **2**, to 4-allenic oxazolones **3**, and defined the hydrolytic conditions that must be met in order to salvage α-allenic α-amino acids. The general route to α-allenic α-amino acids is outlined in Scheme I.

Treatment of the appropriate α-amino acid with benzoyl chloride under Schotten–Baumann conditions[12] followed by esterification with propargylic alcohols[13] gives the corresponding amido propargylic esters **2** in good yield.[14] 4-Allenic oxazolones **3** are formed by treating amido propargylic esters with triphenylphosphine, triethylamine, and carbon tetrachloride in acetonitrile[11a] at room temperature to generate putative oxazoles which spontaneously rearrange. Methanolysis of the oxazolones **3** gives benzamido esters. With the exception of the histidine analogue, isolated yields for the conversion of **3** to **4** are 50–100%. Although the hydrolysis of the benzamido group in *N*-benzoyl α-allenic histidine takes place in 20% HCl at 80 °C within 2 days, a ketone side product (2-amino-2-(4-imidazolylmethyl)-4-oxopentanoic acid (**7**)) is also formed in about 30% yield. Acid hydrolysis of the other amido esters requires more stringent conditions and generally results in hydration of the allenic moiety. Deprotection of most of the α-allenic α-benzamido esters that we have investigated is accomplished in yields of 50–80% by treating the precursor benzamido esters with freshly prepared Meerwein's reagent,[15] evaporating CH_2Cl_2, and hydrolyzing the residual imidate with 10% aqueous acetic acid.[16] Saponification (1.0 N NaOH in MeOH) of the amino ester **5** gives, in excellent yield, after ion-exchange chromatography (Bio-Rad Ag 50W-X8 column eluting with 20% aqueous pyridine), the α-allenic α-amino acids **6**.[17]

Scheme I[a]



[a] (i) PhCOCl, OH^-; (ii) $HOCHR_2C{\equiv}CR_3$, DCC, DMAP; (iii) Ph_3P, CCl_4, NEt_3, CH_3CN; (iv) MeOH, NEt_3; (v) $Et_3O^+BF_4^-$, CH_2Cl_2; (vi) 10% HOAc; (vii) 1.0 N NaOH/MeOH. The N^{im}-benzoylated **3a**, R_1 = (*N*-benzoylimidazolyl)methyl, is hydrolyzed first to the acid corresponding to **4a** and then treated with 20% HCl at 80 °C for 2 days. En route to allenic ornithine, the δ-lactam, rather than the amino ester **5a**, is isolated prior to acid hydrolysis.

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Our preliminary results indicate that α-allenic α-amino acids can be potent, time-dependent inactivators of vitamin B_6 linked amino acid decarboxylases. For example, α-allenic DOPA (**6a**, R = 3,4-dihydroxybenzyl; R_2 = R_3 = H) rapidly inactivates porcine kidney aromatic amino acid decarboxylase (AADC, EC 4.1.1.26) with t_{50} = 6 min at 100 μM inhibitor, ([I]/[E] = 64) at 37 °C and pH 6.8. By comparison, α-vinyl- and α-ethynyl-DOPA at 100 μM are reported to have t_{50} = 20 min under similar conditions. In the presence of the substrate 5-hydroxy-L-tryptophan (500 μM), inactivation of AADC by α-allenic DOPA is retarded such that t_{50} = 12 min at 100 μM inhibitor. The protection afforded by natural substrates demonstrates the active-site-directed nature of the inactivation. Biphasic, complete (>90%), and essentially irreversible[18] inactivation is characteristic of the inhibition of mammalian AADC by α-allenic aromatic amino acids. α-Vinyl- and α-ethynyl-DOPA were reported to inactivate by pseudo-first-order kinetics[1c] but inactivation is incomplete (<70%), and up to 85% of the original activity can be recovered after exhaustive dialysis.[1c,2]

An important aspect of this work is that the diastereomeric pairs of chiral allenic aromatic amino acids **6b** (R = 3-hydroxybenzyl) differ in their abilities to inactivate mammalian and bacterial AADC.[19] There is little variation (t_{50} = 20, 22, and 35 min at [I] = 2 mM) in the abilities of allenic *m*-tyrosine inhibitors **6a** or the separate diastereomeric pairs of **6b** (isomers I and II,[20] respectively) to inactivate bacterial tyrosine decarboxylase (EC 4.1.1.25). However, one diastereomeric pair (isomer I) is at least an order of magnitude more effective than the other (isomer II) against mammalian AADC (t_{50} = 4.5 and 85 min, respectively, at [I] = 100 μM).[21]

This work demonstrates that the chirality of the allene can have a significant effect on the potency and specificity of the suicide inhibitor. Studies of the differential inactivation of vitamin B_6 linked enzymes by chiral allenic amino acids are continuing.

(17) Compounds **6** (R_2 = R_3 = H), including α-allenic Phe, Tyr, Glu, His, Lys, or DOPA were obtained as racemates and were fully characterized by IR, ^{1}H and ^{13}C NMR, mass spectra, and micro analysis. For example, α-allenic *m*-tyrosine: mp 242–245 ° dec; IR (KBr) ν_{max} 1960 cm^{-1} (C=C=C); ^{1}H NMR δ (D_2O) 3.2 (AB, J_{AB} = 13.29 Hz, 2 H, CH_2Ph), 5.15 (m, 2 H, CH_2=C), 5.17 (m, 1 H, HC=C), 6.8–7.6 (m, 4 H, Ph). Anal. Calcd for $C_{12}H_{13}NO_3$: C, 65.7; H, 6.00; N, 6.40. Found: C, 64.93; H, 6.22; N, 6.26. α-Allenic histidine·2HCl: mp 205 °C dec; IR (KBr) ν_{max} 1977 cm^{-1} (C=C=C); ^{1}H NMR δ (D_2O) 3.5 (AB, J_{AB} = 14.57 Hz, 2H, CH_2), 5.25 (m, 2 H, H_2C=C), 5.7 (m, 1 H, HC=C) , 7.45 (s, 1 H, Im CH), 8.75 ppm (s, 1 H, ImCH); ^{13}C NMR δ (D_2O) 209.0 (C=C=C); MH^+ 194. α-Allenic histidine·H_2O: anal. Calcd for $C_9H_{13}NO_3$: C, 51.18; H, 6.20; N, 19.89. Found: C, 51.04; H, 6.37; N, 20.13. α-Allenic ornithine·HCl: mp 210 °C dec; IR (KBr) ν_{max} 1962 cm^{-1} (C=C=C); ^{1}H NMR δ (D_2O) 1.94 (m, 4 H, CH_2), 3.1 (t, 2 H, CH_2N), 5.2 (m, 2 H, H_2C=C), 5.6 (m, 1 H, HC=C); ^{13}C NMR δ (D_2O) 208.9 (C=C=C); MH^+ 171. α-Allenic glutamic acid: mp 171 °C dec; IR (KBr) ν_{max} 1962 cm^{-1} (C=C=C); ^{1}H NMR δ (D_2O) 2.3–2.8 (m, 4 H, CH_2), 5.25 (m, 2 H, H_2C=C), 5.6 (m, 1 H, HC=C); ^{13}C NMR δ (D_2O) 209.1 (C=C=C); MH^+ 186. α-Allenic DOPA: mp 230–240 °C dec; IR (KBr) ν_{max} 1955 cm^{-1} (C=C=C); ^{1}H NMR δ (D_2O) 3.15 (AB, 2 H, *J* = 14.5 Hz, CH_2Ph), 5.15 (app d, 2 H, H_2C=C); 5.65 (app t, 1 H, HC=C); 6.8 (m, 3 H, Ph); M^+ 235, MH^+ 236.

(18) No more than 10% of AADC activity was recovered after Sephadex G-25 gel filtration or exhaustive dialysis at pH 7.2 in the presence of exogenous PLP for mammalian AADC inactivated by the α-allenic analogues of DOPA, *m*-tyrosine, or phenylalanine.

(19) DOPA decarboxylase (mammalian AADC) from porcine kidney was purified by minor modification to procedures outlined in: (a) Borri-Voltattorni, C.; Minelli, A.; Vecchini, P.; Fiori, A.; Turano, C. *Eur. J. Biochem.* **1979**, *93*, 181. (b) Rudd, E. A.; Thanassi, J. W. *Biochemistry* **1981**, *20*, 7469. L-Tyrosine decarboxylase *ex Streptococcus faecalis* was purchased from Sigma Chemical Co.

(20) Diastereomeric pairs of chiral allenic *m*-tyrosine analogues **6b** (R = 3-hydroxybenzyl, R_2 = CH_3, R_3 = H) were isolated by semipreparative HPLC-RP-18 eluting with 15% (v/v) CH_3CN in 30 mM ammonium acetate at pH 6.0. Isomer I designates the first diastereomeric pair to elute under these conditions followed by isomer II.

(21) Incubations were carried out with inhibitors at 37 °C and pH 6.8 against mammalian DOPA decarboxylase[19] or at pH 5.5 with bacterial L-tyrosine decarboxylase. Residual activities were determined by HPLC/electrochemical monitoring of dopamine or *p*-tyramine production by mammalian or bacterial enzymes, respectively.

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Total Synthesis and Stereochemistry of Hybridalactone

E. J. Corey* and Biswanath De

Department of Chemistry, Harvard University
Cambridge, Massachusetts 02138

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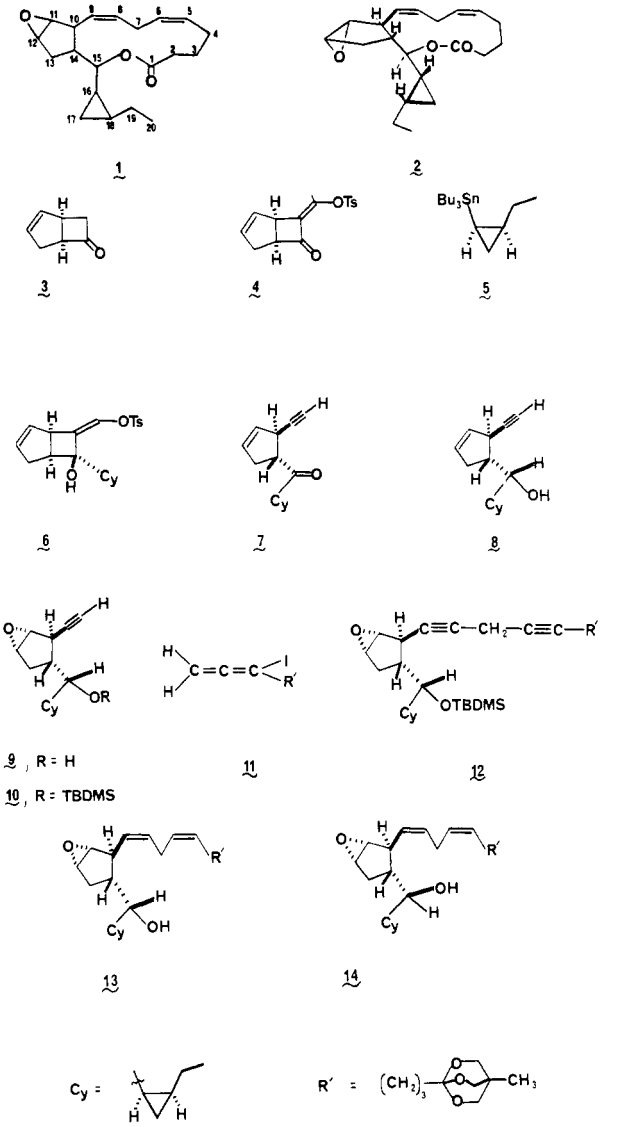
Hybridalactone, a macrocyclic lactone from the marine alga *Laurencia hybrida* was recently shown to have the gross structure **1** on the basis of proton magnetic resonance (^{1}H NMR) and mass



spectral (MS) studies.[1] Although a partial assignment of stereochemistry was also made (Δ^5- and Δ^8-double bonds both *Z*, H-10/H-11 trans, H-10/H-14 trans, H-11/H-12 cis, H-16/H-18 cis), neither the absolute configuration nor the relative configurations at carbons 14–16 were ascertained. Because of our interest in novel eicosanoids[2] and the intriguing question of the biosynthesis

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