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.

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Total Synthesis and Stereochemistry of Hybridalactone

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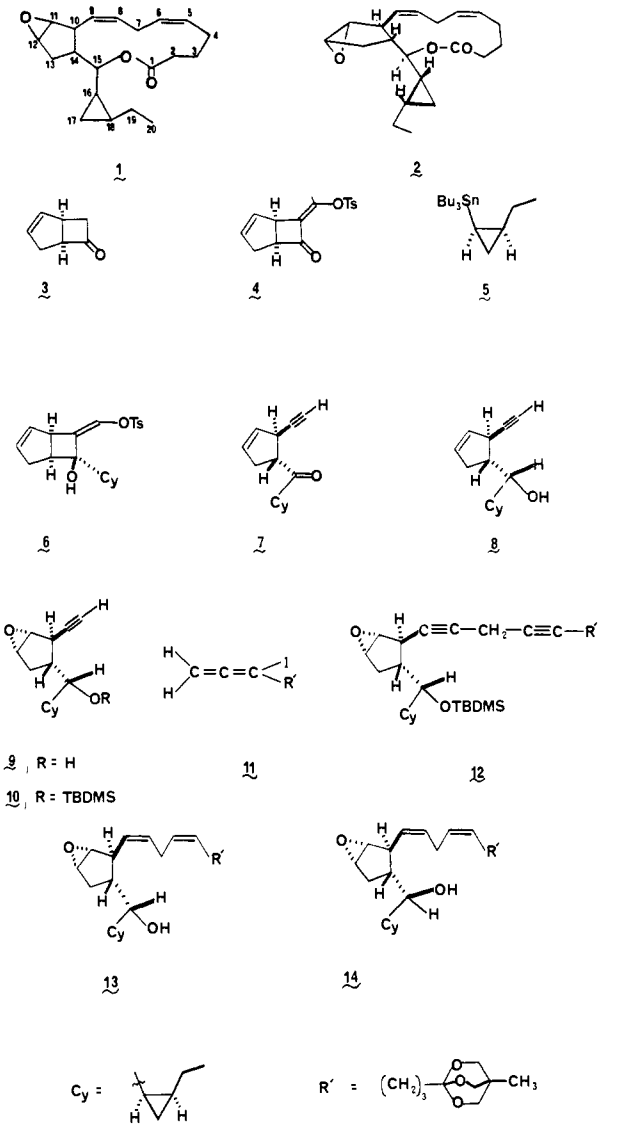
Hybridalactone, a macrocyclic lactone from the marine alga *Laurencia hybrida* was recently shown to have the gross structure **1** on the basis of proton magnetic resonance (^{1}H NMR) and mass



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

(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.

(1) Higgs, M. D.; Mulheirn, L. J. *Tetrahedron* **1981**, *37*, 4259.
(2) See: (a) Corey, E. J. *Experientia* **1982**, *38*, 1259; (b) *Ibid.* **1983**, *39*, 1084. (c) Corey, E. J.; Schmidt, G.; Shimoji, K. *Tetradron Lett.* **1983**, *24*, 3169.

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of hybridalactone, we have studied this substance in some detail. Reported here is a successful total synthesis, which was guided by a biogenetic surmise that correctly predicted the absolute stereochemistry[3] and also by conformational calculations that allowed assignment of relative configuration at C-14 and C-15.[3] The synthetic work led to the unambiguous proof of stereoformula **2** for hybridalactone. Subsequently, a sample of natural hybridalactone was obtained and structure **2** was demonstrated independently by X-ray crystallography.[3]

One starting point for the synthesis was the readily available dextro bicycloheptenone **3**, $[\alpha]^{23}_D$ + 61.4° (*c* 1, $CHCl_3$) (absolute configuration as shown), which was generously provided to us by Dr. C. J. Wallis of Glaxo Co.[4] Slow addition of **3** to a mixture of 2.1 equiv of powdered sodium hydride in dimethoxyethane (DME) and *tert*-butyl formate (2.1 equiv), with stirring at 20 °C and reaction at 20 °C for 3 h and subsequent tosylation at 0° for 45 min with tosyl chloride, afforded after extractive isolation and chromatography the *Z* β-tosyloxy enone **4** (49%), recovered **3** (19%), and the *E* isomer of **4** (3%).[5,6] The other key starting material for the synthesis, levorotatory 1(*R*)-(tributylstannyl)-2(*S*)-ethylcyclopropane (**5**), was synthesized from (−)-*cis*-1-(tributylstannyl)-2-(hydroxymethyl)cyclopropane by a process to be described separately.[7] Reaction of levo **5** with 2 equiv of *n*-butyllithium at 0 °C in tetrahydrofuran (THF) for 3 h afforded the corresponding *cis*-(2-ethylcyclopropyl)lithium, which was allowed to react with the *Z* β-tosyloxy enone **4** at −78 °C for 1 h to give after quenching at −78 °C, extractive isolation, and chromatography on silica gel (sg) a single cyclopropylcarbinol **6** in 76% yield. Acetylene-forming fragmentation of **6** was effected cleanly by reaction with tetra-*n*-butylammonium fluoride (commercial 1 M solution in 95:5 THF–H_2O) at 23 °C. After a reaction time of 10 h the initially formed cis ethynyl ketone was equilibrated to a 9:1 trans–cis mixture (TLC R_f values 0.57 and 0.34 on sg plates with 10:1 pentane–ether). After chromatography the desired trans ketone **7** (78%) was obtained along with the cis isomer (8%), which could be isomerized to **7** by K_2CO_3–CH_3OH treatment. Reduction of the trans ketone **7** with 1.1 equiv of lithium Selectride (Aldrich) in THF at −78 °C for 30 min afforded >92% of a 6:1 mixture consisting mainly of the (*R*)-carbinol **8** (shown) and the (*S*)-carbinol diastereomer (sg TLC R_f values 0.27 and 0.20, respectively, with 4:1 hexane–ether), which was used in further steps without separation.[8] Selective α-face epoxidation of **8** was accomplished[9] with 1.5 equiv of *tert*-butyl hydroperoxide and 4% by weight vanadyl acetylacetonate in methylene chloride at 23 °C for 6 h to give **9** in 87% yield. Reaction of **9** with 1.1 equiv of *tert*-butyldimethylsilyl triflate and 10 equiv of 2,6-lutidine in methylene chloride at −40 °C for 10 min[10] provided the corresponding silyl ether acetylene **10** in 97% yield. Lithiation of the terminal acetylene **10** (*n*-butyllithium–THF at −78 °C), conversion to the Gilman reagent (1.2 equiv of cuprous cyanide in 1:1 THF–hexamethylphosphoric triamide (HMPT), 30 min at 0 °C), and coupling with iodo allene OBO ortho ester **11**[11,12] at 0 °C for 5 h and 23 °C for 24 h afforded after extractive isolation and column chromatography on sg (pretreated with triethylamine) the diyne OBO ortho ester **12** in 86% yield. Hydrogenation (Lindlar Pd–$CaCO_3$, 1 atm H_2, 10:1 ethyl acetate–pyridine) at 23 °C and desilylation with tetra-*n*-butylammonium fluoride–THF produced the diene alcohol **13** and the C-15 diastereomer, ratio 6:1, in 92% overall yield. Inversion at C-15 to give **14** was effected by the following process: (1) oxidation of the 15-alcohols to 15-ketone by 2 equiv of pyridinium dichromate–5-Å molecular sieves–magnesium sulfate in methylene chloride at 23 °C, (2) reduction with 1.1 equiv of lithium Selectride in THF at −45 °C for 40 min, (3) chromatography to separate the desired 15(*S*)-carbinol **14** (75% yield) from a small amount of the 15-*R* diastereomer **13** (ca. 16%). The sg TLC R_f values with 2:1 ether–hexane were 0.35 for **14** and 0.28 for the 15-*R* diastereomer. The OBO ortho ester function was converted to carboxyl (96% yield) by the sequence: (1) exposure to aqueous sodium bisulfate–DME solution (pH ca. 3) for 1 min at 0 °C, (2) basification with lithium hydroxide and saponification at 23 °C for 30 min, (3) acidification and extractive isolation. Lactonization of the hydroxy acid was accomplished by the double activation method in 83% yield by the following process: (*1*) reaction with bis(4-*tert*-butyl-*N*-isopropylimidazol-2-yl) disulfide–triphenylphosphine (5 equiv of each)[13] in toluene at 0 °C for 30 min, (2) dilution with toluene and heating at reflux for 12 h under nitrogen, (3) sg chromatography. The product was indistinguishable from a sample of native hybridalactone (**2**) obtained by extraction of *L. hybrida*[14] as shown by identity of measured optical rotation, $[\alpha]^{23}_D$ −53 ± 2° (*c* 0.14, CH_3OH), ^1H NMR, infrared, and mass spectra and TLC mobility on sg in several solvent systems.

The 15-epimer of hybridalactone, synthesized from **13** by OBO ester hydrolysis and lactonization as descrbied above, was easily distinguished from hydribalactone; for example, in the ^1H NMR spectra $J_{14,15}$ is the 10 Hz for hybridalactone and 5 Hz for 15-*epi*-hybridalactone as expected from our conformational analysis studies,[3] and sg TLC R_f values were 0.30 for hybridalactone and 0.24 for the 15-epimer (20:1 hexane–ether).

The synthesis of hybridalactone recorded above was completed before an authentic sample of naturally derived hybridalactone became available and despite the fact that the pre-existing literature[1] did not distinguish between eight possible stereoisomers. The power of machine conformational analysis and biosynthetic arguments for such problems is nicely demonstrated.[15,16]

Supplementary Material Available: Spectral data for compounds **4–14** and synthetic **2** (2 pages). Ordering information is given on any current masthead page.

(3) Corey, E. J.; De, B.; Ponder, J. W.; Berg, J. M. *Tetrahedron Lett.* **1984**, *25*, 1015.

(4) The dextro ketone **3** is readily available from (±)-3 by resolution using the crystalline bisulfite addition product obtained from (−)-α-phenylethylamine, sulfur dioxide and 1 equiv of water; see: Collington, E. W.; Wallis, C. J.; Waterhouse, I. *Tetrahedron Lett.* **1983**, *24*, 3125.

(5) Satisfactory ^1H NMR, infrared, and mass spectral data were obtained for each synthetic intermediate. For optical rotations see ref 15. All temperatures in degrees Celsius.

(6) The stereochemical assignment to enol tosylate **4** and the *E* isomer was made from the appearance of ^1H NMR peaks due to CHOTs at δ 6.25 and 7.12 ($CDCl_3$ solvent), respectively. Although the yield for this step is probably not optimum, it was noted that the yields were definitely higher with *tert*-butyl formate than ethyl formate.

(7) Work of T. M. Eckrich in these laboratories to appear in *Tetrahedron Letters*. (±)-*cis*-1-(Tributylstannyl)-2-(hydroxymethyl)cyclopropane was resolved, and the enantiomers were correlated chemically with the known enantiomers of *cis*-2-methylcyclopropane carboxylic acid (Bergman, R. G. *J. Am. Chem. Soc.* **1969**, *91*, 7405).

(8) The stereochemistry of the major carbinol **8** follows from transformation (a) to 15-*epi*-hybridalactone (i.e., 15-*R* diastereomer) by chain extension and lactonization and (b) to hybridalactone itself by chain extension, carbinol inversion, and lactonization as described herein.

(9) Sharpless, K. B.; Michaelson, R. C. *J. Am. Chem. Soc.* **1973**, *95*, 6136.

(10) Corey, E. J.; Cho, H.; Rücker, C.; Hua, D. H. *Tetrahedron Lett.* **1981**, *22*, 3455.

(11) (a) Corey, E. J.; Kang, J. *J. Am. Chem. Soc.* **1981**, *103*, 4618; (b) *Tetrahedron Lett.* **1982**, *23*, 1651.

(12) It is convenient to have a short generic name for these useful oxabicyclo[2.2.2]octyl ortho esters, and we propose the term "OBO" ortho ester. The OBO ortho ester used in this work was prepared in 75% overall yield from the OBO ortho ester of 5-hexynoic acid (Corey, E. J.; Raju, N. *Tetrahedron Lett.* **1983**, *22*, 5571) by the sequence (1) lithiation with *n*-BuLi at −78 °C in THF containing 2 equiv of HMPT, (2) alkylation with (trimethylsilyl)-methyl triflate (Chiu, S. K.; Peterson, P. E. *Ibid.* **1980**, *21* 4047), and (3) reaction with 1 equiv of iodine, 1 equiv of silver trifluoroacetate, and 0.1 equiv of silver carbonate at −78 °C for 1 h in methylene chloride; the bromoallene corresponding to **11** can be made in 95% yield using 1.1 equiv of *N*-bromosuccinimide in methylene chloride at 23 °C in the last step.

(13) Corey, E. J.; Brunelle, D. J. *Tetrahedron Lett.* **1976**, 3409.

(14) We are indebted to Dr. Peter Leeming (Chas, Pfizer, U.K.) and Dr. A. Pettet (U. of Khartoum) for a supply of *Laurencia hybrida* and to Dr. M. D. Higgs, Shell Co., Amsterdam, for copies of the original spectra.

(15) Measured values of optical rotations of the various synthetic intermediates ($[\alpha]^{23}_D$ in $CHCl_3$) are as follows: **5**, −2.34° (*c* 4.6); **6** −203° (*c* 4.4); **7**, −320.4° (*c* 0.75); **8**, −177° (*c* 2.3); **10**, −5.8° (*c* 2); **13**, −7.1° (*c* 0.2); ketone from **13**, −60° (*c* 0.17); **14**, −1.4° (*c* 0.2). Rotations for last 3 measured in MeOH.

(16) This research was supported by grants from the National Institutes of Health and the National Science Foundation. We are grateful to T. M. Eckrich for providing reactant **5**.