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Articles

Enantiospecific Syntheses of α-(Fluoromethyl)tryptophan Analogues: Interactions with Tryptophan Hydroxylase and Aromatic L-Amino Acid Decarboxylase†

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α-Fluoromethyl amino acids are enzyme-activated irreversible inhibitors of amino acid decarboxylases. Aromatic L-amino acid decarboxylase (AADC) is the enzyme responsible for the final step in the biosynthesis of both dopamine and serotonin via decarboxylation of L-dopa and 5-hydroxy-L-tryptophan, respectively. Our goal is to utilize antagonists of the serotonin-producing enzymes (tryptophan hydroxylase and AADC) as the basis for a chemotherapeutic approach to the treatment of carcinoid tumors, a rare tumor type characterized by the overproduction of serotonin. We report here an enantiospecific synthesis of α(*S*)-(fluoromethyl)tryptophan [(*S*)-**11a**] and α(*S*)-(fluoromethyl)-5-hydroxytryptophan [(*S*)-**11b**], as well as the (*R*)-enantiomers, based upon recent methodology involving the face-selective alkylation of cyclic tryptophan tautomers. Our synthetic route provided both enantiomers of **11a** and **11b** with greater than 97% enantiomeric purity based upon evaluation of the NMR spectra of their Mosher's acid derivatives. (*S*)-**11a** was evaluated as a substrate for P815 tryptophan hydroxylase and determined to have an apparent K_m of 4.31 ± 1.07 mM, essentially half the value previously reported for the racemic mixture of **11a** with rat brain stem tryptophan hydroxylase. (*R*)-**11a** was not a substrate for P815 tryptophan hydroxylase. (*S*)-**11b** was evaluated as an enzyme-activated irreversible inhibitor of murine liver AADC and determined to have a K_I of 24.3 ± 3.01 μM and a k_2 of 2.26 ± 0.44 min^{-1}. (*R*)-**11b** was not an inhibitor of murine liver AADC.

Introduction

Amino acid decarboxylases are important enzymes in the biosynthetic pathways of endogenous biogenic amines such as γ-aminobutyric acid (GABA), histamine, dopamine, and serotonin (5-hydroxytryptamine). In 1978, Kollonitsch and co-workers[1] reported that α-(fluoromethyl) analogues of amino acids were potent and selective irreversible inhibitors of their target decarboxylases. Since that time there have been additional reports of α-(fluoromethyl) amino acids which were irreversible inhibitors of amino acid decarboxylases.[2] These inhibitors operate via formation of a Schiff base intermediate with the pyridoxal phosphate cofactor in the enzyme's active site, with subsequent β-elimination of fluoride ion upon enzyme-mediated decarboxylation. The elimination of fluoride ion produces a cofactor-bound electrophilic species which is postulated to undergo nucleophilic attack by a proximal residue in or near the active site, thus irreversibly inactivating the enzyme.[1]

Aromatic L-amino acid decarboxylase (AADC) is a ubiquitous enzyme responsible for the final step in the biosynthesis of the neurotransmitters dopamine and serotonin via decarboxylation of L-dopa and 5-hydroxy-L-tryptophan, respectively.[3] α(*S*)-(Fluoromethyl)dopa[1] and α(*R,S*)-(fluoromethyl)-5-hydroxytryptophan[2b] both inhibit AADC in an irreversible, time-dependent manner. Because AADC is common to both dopaminergic and

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serotonergic cells, it is not possible to selectively inhibit AADC in one versus the other of these two cell types with either α-(fluoromethyl)dopa or α-(fluoromethyl)-5-hydroxytryptophan. However, selectivity can be achieved by administration of precursor molecules which are not themselves AADC inhibitors, but are enzymatically converted to active AADC inhibitors in vivo by enzymes found only in either dopaminergic or serotonergic cells, and which act only on tyrosine-like or tryptophan-like substrates. Thus, Jung and co-workers[4] reported that α(*R*,*S*)-(fluoromethyl)tyrosine (a bioprecursor which does not inhibit AADC) was converted to α-(fluoromethyl)dopa (the active AADC inhibitor) via hydroxylation by tyrosine hydroxylase, an enzyme present only in dopaminergic cells and specific for tyrosine-like substrates. Analogously, Schirlin and co-workers[2b] showed that α(*R*,*S*)-(fluoromethyl)-tryptophan (a bioprecursor which does not inhibit AADC) was converted to α-(fluoromethyl)-5-hydroxytryptophan (the active AADC inhibitor) via hydroxylation by tryptophan hydroxylase, an enzyme present only in serotonergic cells and specific for tryptophan-like substrates.

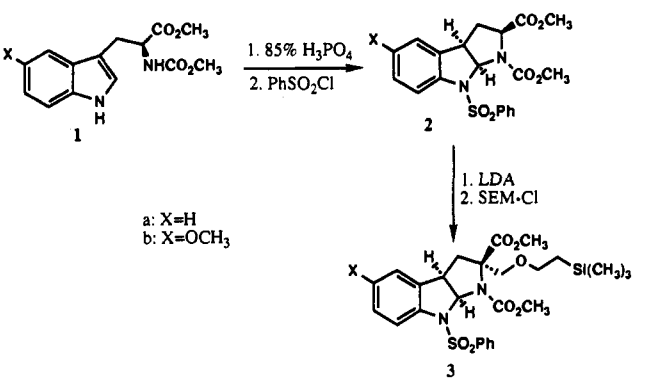
Our laboratory is interested in the development of agents useful for the treatment of carcinoid tumors. One of the hallmark features of these tumors is the overproduction of serotonin, and we are currently characterizing the enzymes in the serotonin-producing pathway (tryptophan hydroxylase and AADC) in human carcinoid material.[5] These tumors can utilize large amounts of dietary tryptophan (up to 60% in patients with advanced metastatic carcinoid as opposed to 1% in normal individuals) for conversion to serotonin.[6] We intend to exploit the demonstrated utility of α-(fluoromethyl)-5-hydroxytryptophan to irreversibly inhibit AADC as a means to selectively interact with AADC in carcinoid tumor tissue. We therefore required an efficient synthetic route to the desired α-(fluoromethyl)tryptophan congeners which could be readily modified to accommodate many desired substituents. Additionally, because several amino acid decarboxylases (including AADC) have been reported to exhibit specificity for α(*S*)-fluoromethyl amino acids,[1,2d,2e] we desired a methodology that would provide the target molecules with high levels of enantiomeric purity. The α-(fluoromethyl)tryptophan analogues reported by Schirlin[2b] were racemic mixtures and, to our knowledge, no determination of the enzymatic characteristics for the pure enantiomers has been reported.

Bourne and co-workers[7] recently reported that α-substituted tryptophan analogues can be prepared with high levels of enantiomeric purity via alkylation of the corresponding cyclic tautomers. On the basis of this methodology, we now report an efficient synthesis of α-(fluoromethyl)tryptophan analogues which results in products of greater than 97% optical purity.

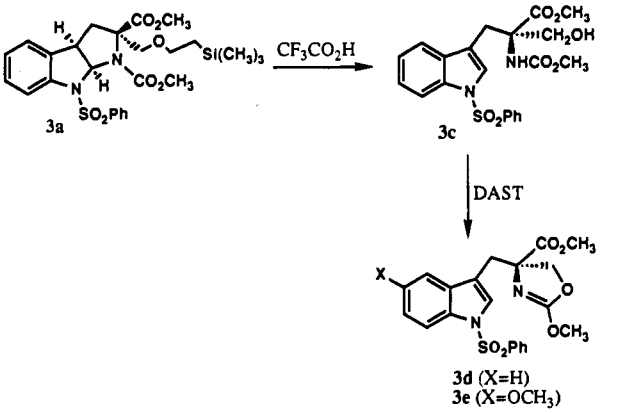
Chemistry

For purposes of comparison, both the (*S*)- and (*R*)-enantiomers of α-(fluoromethyl)tryptophan and α-(fluoromethyl)-5-hydroxytryptophan were desired. Both enantiomers of each entity were prepared via the same common synthetic route described below, beginning with the starting material possessing the desired stereochemistry. The structures in Schemes I–IV show the stereochemistry of the analogues used in preparation of the (*S*)-enantiomers. Likewise, the text in this section corresponds with the results obtained in the preparation of the (*S*)-enantiomers.

Scheme I



Scheme II

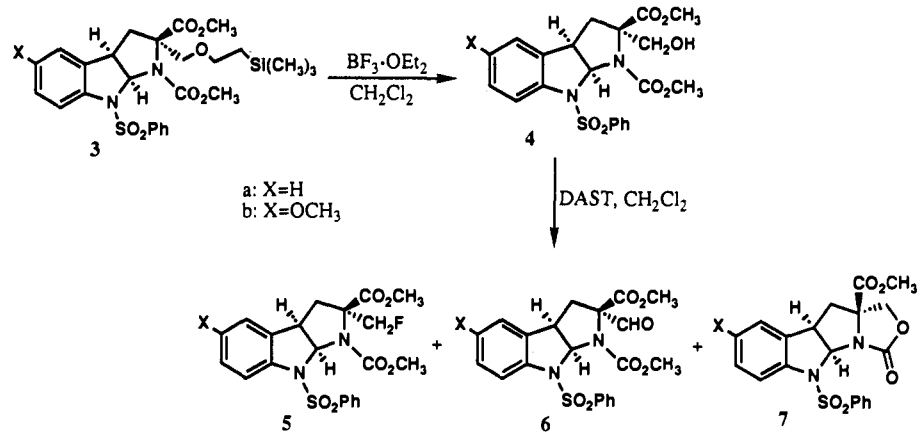


The fully protected cyclic tautomers **2a** and **2b** were prepared via cyclization of **1a** and **1b**[8] in 85% phosphoric acid followed by reaction with benzenesulfonyl chloride as described by Bourne[7] in 60–70% yield for the two steps (Scheme I). Formation of the lithium enolates of **2a** and **2b** with 1.5 equiv of lithium diisopropylamide followed by quenching with [2-(trimethylsilyl)ethoxy]methyl chloride (SEM-Cl) provided analogues **3a** and **3b** in 53–96% yield. Treatment of **3a** with trifluoroacetic acid for 1 h[9] provided the α-(hydroxymethyl) ring-open tryptophan analogue **3c** in 90% yield (Scheme II). Reaction of **3c** with (diethylamido)sulfur trifluoride (DAST) in methylene chloride provided only a trace of the desired α-(fluoromethyl) analogue **8a** and, as the predominant product, the oxazoline **3d**.

To circumvent this problem, a method was required to effectively deprotect the α-(hydroxymethyl) substituent while leaving the ring-closed tautomer intact. Efforts to deprotect **3a** with either tetrabutylammonium fluoride or potassium fluoride/18-crown-6 failed to provide any product, even after prolonged reaction times in refluxing THF or acetonitrile. However, the α-(hydroxymethyl) substituent was smoothly deprotected by treating **3a** and **3b** with 3 equiv of boron trifluoride etherate[10] in methylene chloride (Scheme III). The reaction was complete in less than 30 min at room temperature, providing **4a** and **4b** in 80–95% yield, with only trace amounts of the corresponding ring-open tautomers as determined by ^{1}H NMR. Analogue **4a** was recrystallized from acetone/hexane to provide the pure ring-closed tautomer; **4b** could not be induced to crystallize and was used as obtained after column chromatography, with trace amounts of ring-open tautomer.

Treatment of **4a** and **4b** with DAST provided the desired α-(fluoromethyl) analogues **5a** and **5b**, respectively, in 50–59% yield and, as the major side products (30–32%), the

Scheme III



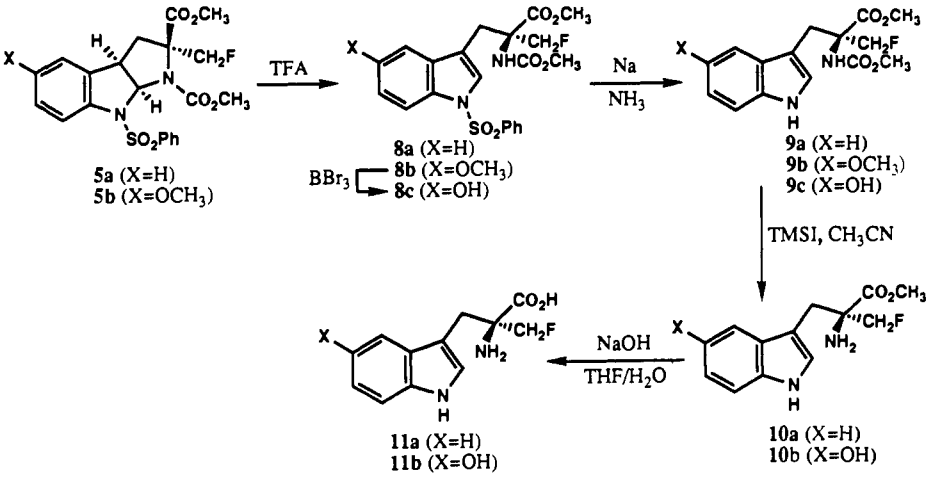
oxazolidinones **7a** and **7b** (Scheme III). A second side product in the fluorination of **4a** was the aldehyde **6a**, which constituted 2–4% of the reaction product; none of the analogous aldehyde **6b** was isolated in the fluorination of **4b**. However, the reaction mixture obtained from fluorination of **4b** included a small amount of the oxazoline **3e**, which arises from cyclization of the trace amount of the ring-open tautomer of alcohol **4b** discussed above. All of the above components were readily separated by column chromatography. The oxazolidinones **7a** and **7b** were apparently formed via fluoride ion-mediated deprotonation of the α-(hydroxymethyl) groups of **4a** and **4b**, followed by intramolecular attack of the resulting oxyanion upon the N_b-(methoxycarbonyl). In support of this mechanism, treatment of **4a** with 1.5 equiv of pyridine in methylene chloride rapidly produced **7a** as the sole product. It has been reported that oxazolidinone formation is a major side-reaction in the DAST fluorination of β-carbamoyl alcohols.[11] Additionally, aldehyde **6a** was prepared in 50% yield via the pyridinium chlorochromate (PCC) oxidation of alcohol **5a** in the presence of 3-Å molecular sieves.[12] Attempts to perform the PCC oxidation in the absence of molecular sieves failed to provide any product, even after extended reaction times. It is interesting to note that the excess DAST in the reaction mixture did not produce any of the corresponding α-(difluoromethyl) analogue via reaction with the aldehyde **6a**, as DAST has been widely used to prepare *gem*-difluorides from aldehydes and ketones.[13] Reaction of **6a** with 2 equiv of DAST in refluxing chloroform failed to produce the expected α-(difluoromethyl) analogue, even after 48 h. This may be due to steric congestion about the aldehyde moiety.

The ring-open tautomers **8a** and **8b** were obtained in 95–100% yield simply by stirring **5a** and **5b** in trifluoroacetic acid for 2 h at room temperature (Scheme IV). Reductive cleavage of the N_a-phenylsulfonamide protecting groups with sodium metal in refluxing liquid ammonia provided compounds **9a** and **9b** in 90% and 69% yields, respectively. Treatment of **9b** with boron tribromide in methylene chloride effectively cleaved the 5-methyl ether to provide compound **9c**, but only in 21% yield (14% yield from **8b** to **9c**). However, the yield for the two-step transformation of **8b** to **9c** was greatly increased by reversing the order of the reaction sequence. Cleavage of the 5-methyl ether of **8b** with boron trichloride provided analogue **8c** in 90% yield; subsequent reductive cleavage of the N_a-(phenylsulfonyl) with sodium metal provided the desired compound **9c** in 76% yield (68% yield from **8b** to **9c**), nearly a 5-fold increase in yield for the two steps relative to the opposite reaction sequence.

Removal of the N_b-(methoxycarbonyl) protecting group was first attempted with trimethylsilyl iodide (TMSI) in refluxing chloroform. Irie and co-workers[14] reported that N_b-(methoxycarbonyl)tryptophan methyl esters were efficiently deprotected with TMSI in refluxing chloroform, with 1.0 equiv of TMSI selectively cleaving the N_b-(methoxycarbonyl) group and greater amounts cleaving both the methoxycarbonyl and the methyl ester. However, reaction of **9a** with 1.0 equiv of TMSI in refluxing chloroform showed no product formation, even after 48 h at reflux. When the reaction was carried out in refluxing acetonitrile, the N_b-(methoxycarbonyl) group was cleaved in less than 30 min, affording analogues **10a** and **10b** from **9a** and **9c**, respectively, in 72–75% yield. Excess TMSI (up to 3 equiv) did not cleave the methyl esters; apparently the strongly electronegative fluorine atom greatly decreases the electron density at the two carbonyl oxygens, thus decreasing the nucleophilicity of the two carbonyl centers toward electrophilic reagents such as TMSI. Base-catalyzed hydrolysis of the methyl esters **10a** and **10b** with sodium hydroxide (1.2 and 2.4 equiv, respectively) in THF/water followed by neutralization with an equal amount of HCl provided the desired α(*S*)-(fluoromethyl)tryptophan (**11a**) and α(*S*)-(fluoromethyl)-5-hydroxytryptophan (**11b**). Analogue **11a** was obtained as the pure amino acid via anion exchange chromatography with a 75% mass recovery. However, **11b** rapidly decomposed upon exposure to aqueous base in the presence of air and was used as obtained, with 2.4 equiv of sodium chloride. HPLC and ^{1}H NMR analyses of the **11b**/NaCl mixture showed the presence of only one component, with no evidence of unreacted **10b**.

The same reaction sequence described above was utilized to prepare the (*R*)-enantiomers of **11a** and **11b**, starting with (*R*)-**1a** and (*R*)-**1b**.[15] The reaction yields of all the intermediates for the (*R*)-isomers were essentially the same as described above for the (*S*)-series, as were all of the physical and spectral properties, except for the signs of the optical rotations. Optical purity of the products was determined by preparation of the Mosher's acid amide[16] of analogue **10a**, as well as the corresponding (*R*)-enantiomer. Comparison of the ^{1}H NMR spectra (500 MHz, $CDCl_3$) of the derivatized (*S*)-**10a** and (*R*)-**10a** analogues showed very well defined differences in shifts for the Mosher's acid methoxyl group (δ 3.38 and 3.23, respectively) as well as the indole C-2 protons (δ 6.65 and 7.03, respectively). On the basis of the comparative

Scheme IV



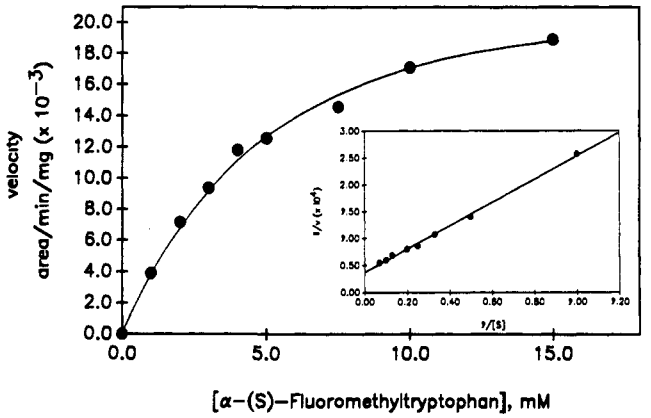


Figure 1. Reaction velocity dependence upon the concentration of α(*S*)-(fluoromethyl)tryptophan [(*S*)-**11a**] as a substrate for P815 tryptophan hydroxylase. **Inset**: Lineweaver–Burke plot for (*S*)-**11a** as a substrate for P815 tryptophan hydroxylase.

integration of these regions the enantiomeric purity of the products obtained via this synthetic route was determined to be greater than 97%, similar to that reported by Bourne and co-workers.[7]

Interactions with Tryptophan Hydroxylase and AADC.

Kinetic parameters were determined for α(*S*)-(fluoromethyl)tryptophan [(*S*)-**11a**] as a substrate for tryptophan hydroxylase using enzyme from cultured P815 murine mastocytoma cells, which are rich in tryptophan hydroxylase.[17] The production of α(*S*)-(fluoromethyl)-5-hydroxytryptophan [(*S*)-**11b**] from the hydroxylation of (*S*)-**11a** was monitored by reverse-phase HPLC using fluorometric detection. Figure 1 illustrates the dependence of reaction velocity upon the concentration of (*S*)-**11a**; the Lineweaver–Burke transformation of substrate and velocity data was linear (Figure 1, inset). The computer program described by Cleland[18] provided an apparent K_m value for (*S*)-**11a** with P815 tryptophan hydroxylase of 4.31 ± 1.07 mM (average of three trials ± standard deviation). The V_{max} value obtained from this study was 17.7 ± 4.11 μM/min per mg protein. The apparent K_m value we obtained correlated nicely with the value of 7.5 mM (for rat brain stem tryptophan hydroxylase) reported by Schirlin[2b] for the racemic mixture of **11a**, if one assumes that only the (*S*)-enantiomer is active. In support of this assumption, the (*R*)-enantiomer of **11a** was found to be inactive as a substrate for tryptophan hydroxylase at a concentration of 15 mM.

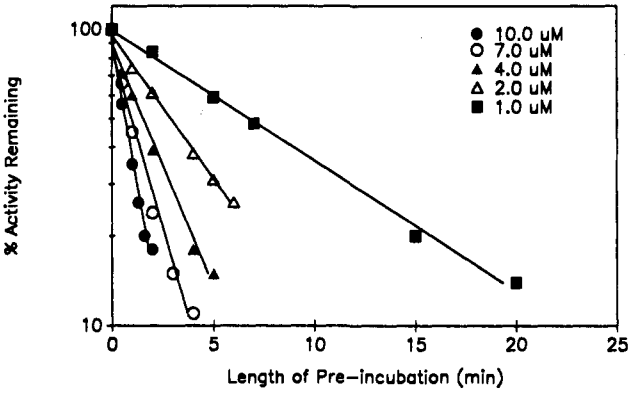


Figure 2. Time-dependent inactivation of murine liver AADC as a function of α(*S*)-(fluoromethyl)-5-hydroxytryptophan [(*S*)-**11b**] concentration.

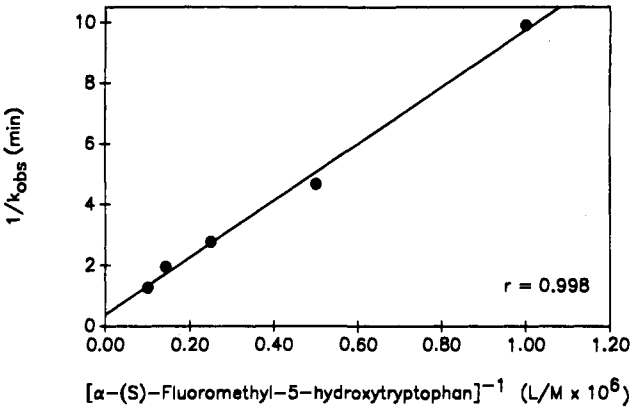


Figure 3. Kitz-Wilson plot for α(*S*)-(fluoromethyl)-5-hydroxytryptophan [(*S*)-**11b**] as an irreversible inhibitor of murine liver AADC.

α(*S*)-(Fluoromethyl)-5-hydroxytryptophan [(*S*)-**11b**] was evaluated as an irreversible inhibitor of AADC using murine (CD2F1 mice) liver as the enzyme source. Figure 2 illustrates the time-dependent inactivation of AADC activity as a function of inhibitor concentration. A Kitz-Wilson plot[19] of the data in Figure 2 was linear (Figure 3), and yielded an enzyme–inhibitor dissociation constant (K_I) value of 24.3 ± 3.02 μM (average of three trials ± standard deviation) and a first-order inactivation rate constant (k_2) of 2.26 ± 0.44 min^{-1} for (*S*)-**11b** as an irreversible inhibitor of murine liver AADC. No kinetic constants for α-(fluoromethyl)-5-hydroxytryptophan have previously been reported. α(*R*)-(Fluoromethyl)-5-hydroxytryptophan [(*R*)-**11b**] did not inhibit AADC at inhibitor concentrations of

10 μM, even after 20 min of preincubation. As illustrated in Figure 2, α(S)-(fluoromethyl)-5-hydroxytryptophan rapidly inactivated AADC activity at a concentration of 10 μM, once again emphasizing the stereospecificity of this enzyme.

As was noted in previous reports concerning the inactivation of AADC with α-(fluoromethyl) amino acids,[2b,d] the time-dependent decay of AADC activity deviated from pseudo-first-order kinetics as the AADC activity was substantially depleted (data not shown). This phenomenon was also reported for the irreversible inhibition of glutamate decarboxylase by α-(fluoromethyl)glutamic acid.[2c] It has been postulated that the biphasic inactivation of AADC by α-(fluoromethyl)dopa was due to the presence of differing isozymes of AADC,[2d] but there is no conclusive evidence to support this concept. An alternative explanation was recently suggested by Funaki and co-workers.[20] They proposed a mathematical model for enzyme-activated irreversible inhibitors which predicts deviation from pseudo-first-order kinetics dependent upon the partition ratio of the inhibitor. We found, however, that when data points were taken early in the inactivation process (e.g., before the enzyme is >85% inactivated), linear plots were obtained as shown in Figure 2.

Discussion

We have described a synthetic methodology for the preparation of α-(fluoromethyl)tryptophan analogues possessing high levels of enantiomeric purity (>97%). Using this methodology, we prepared both the (R)- and (S)-enantiomers of α-(fluoromethyl)tryptophan (**11a**) and determined that the (S)-enantiomer was a substrate for P815 tryptophan hydroxylase. The apparent K_m (4.31 ± 1.07 mM) was essentially half that reported for the racemic mixture of this analogue.[2b] The (R)-enantiomer of **11a** was not a substrate for tryptophan hydroxylase, consistent with the stereochemical requirements of the enzyme. We also prepared both the (R)- and (S)-enantiomers of α-(fluoromethyl)-5-hydroxytryptophan (**11b**). The (S)-enantiomer was a time-dependent irreversible inhibitor of murine liver AADC (K_I = 24.3 ± 3.02 μM; k_2 = 2.26 ± 0.44 min^{-1}), while the (R)-enantiomer was not an inhibitor of the enzyme at concentrations up to 10 μM, even with 20 min of preincubation.

Our current work concerning characterization of tryptophan hydroxylase and AADC from human carcinoid tumor tissue has revealed that these tumors possess significant levels of both enzymes.[5] We hope to utilize appropriately functionalized α-(fluoromethyl)tryptophan analogues as irreversible inhibitors of AADC to assist in our characterization, and to serve as the basis for a chemotherapeutic approach to the treatment of these tumors. Preliminary data have shown that α(R,S)-(fluoromethyl)dopa and α(R,S)-(difluoromethyl)dopa inhibit AADC from human carcinoid tumor tissue, lending further validity to our approach.[21] Synthetic methodologies are available to prepare tryptophan analogues of high enantiomeric purity bearing many varieties of substitution through direct functionalization of cyclic tryptophan analogues[22] or enantiospecific Fischer cyclization.[14] These methods, when combined with our synthetic route to α-(fluoromethyl)tryptophan analogues reported here, provide a reliable pathway to a variety of ring-substituted α-(fluoromethyl)tryptophan congeners possessing the desired stereochemistry.

Experimental Section

Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker AMX 500 (500 MHz) NMR spectrometer; chemical shifts are reported on the δ scale downfield from either TMS or TSP as internal standard. High-resolution mass spectra were obtained from the Department of Chemistry, University of Minnesota, Minneapolis, MN. Elemental analyses were obtained from Atlantic Microlab (Norcross, Georgia) and agree to within ±0.4% of theoretical values unless otherwise indicated. Optical rotations were measured with a JASCO DIP-370 digital polarimeter at ambient temperature using a path length of 50 mm. Thin-layer chromatography was performed with Merck Kieselgel 60F-254 precoated silica gel plates; R_f values are reported using EtOAc/hexane (2:1) as eluent unless otherwise indicated. Column chromatography was performed with Merck Kieselgel 60 silica gel (70–230 mesh) with indicated eluents. HPLC analysis was conducted with a Milton Roy Constametric II HPLC unit fitted with a 5-μm octadecyl column (0.45 × 25 cm; I.I.I Supplies Co., Meriden, CT) and a guard column of 7-μm Newguard RP-18 (0.32 × 1.5 cm; Brownlee Labs, Santa Clara, CA), using a Varian Fluorichrom fluorometric detector. Dry tetrahydrofuran was obtained by refluxing over sodium/benzophenone and was distilled immediately prior to use. L-Tryptophan and D-tryptophan were purchased from Aldrich Chemical Co. and used without further purification. L-5-Hydroxytryptophan was purchased from Sigma Chemical Co. and used without further purification. Protein was quantitated with the BCA protein assay (Pierce, Rockford, IL). α-(Fluoromethyl)dopa and α-(difluoromethyl)dopa used in our preliminary inhibition experiments with carcinoid material were generous gifts from Dr. Michael Palfreyman, Marion Merrell Dow Pharmaceuticals, Cincinnati, OH.

Dimethyl (2*S*,3a*R*,8a*S*)-8-(phenylsulfonyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-1,2-dicarboxylate [(+)-2a] was prepared in two steps from (S)-**1a** as described in refs 6 and 23, except without purification of the N_a-unprotected cyclic intermediate and recrystallized from acetone/hexane (1:1): mp 170 °C (lit.[23] mp 165–167 °C); R_f = 0.25; $[\alpha]_D$ = +82.0° (*c* 1.20, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.47 (m, 1 H), 2.59 (d, 1 H, *J* = 13 Hz), 3.14 (s, 3 H), 3.57 (bs, 3 H), 3.65 (bs, 1 H), 4.60 (bs, 1 H), 6.26 (bs, 1 H), 7.05 (m, 2 H), 7.24 (m, 1 H), 7.40 (t, 2 H, *J* = 7.5 Hz), 7.48 (bs, 1 H), 7.51 (t, 1 H, *J* = 7.5 Hz), 7.72 (d, 2 H, *J* = 7.5 Hz); EIMS *m/e* 416.1044 (M^+, requires 416.1039), 357, 275, 243, 216.

[(−)-2a]: mp 169.5–170 °C; $[\alpha]_D$ = −81.8° (*c* 1.15, $CHCl_3$).

Dimethyl (2*S*,3a*R*,8a*S*)-5-methoxy-8-(phenylsulfonyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-1,2-dicarboxylate [(+)-2b] was prepared in two steps from (S)-**1b**[15] as described above for the preparation of **2a** from **1a** and produced a white foam: R_f = 0.22; $[\alpha]_D$ = +170.9° (*c* 0.38, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.41 (m, 1 H), 2.52 (d, 1 H, *J* = 13 Hz), 3.19 (s, 3 H), 3.42 (bs, 1 H), 3.69 (s, 3 H), 3.75 (s, 3 H), 4.58 (bs, 1 H), 6.16 (bs, 1 H), 6.52 (d, 1 H, *J* = 2 Hz), 6.80 (dd, 1 H, *J* = 8, 2 Hz), 7.38 (t, 2 H, *J* = 7.5 Hz), 7.44 (d, 1 H, *J* = 7.5 Hz), 7.51 (t, 1 H, *J* = 7.5 Hz), 7.64 (d, 2 H, *J* = 7.5 Hz); EIMS *m/e* 446.1139 (M^+, requires 466.1148), 317, 305, 245. Anal. ($C_{21}H_{22}N_2O_7S \cdot H_2O$) C, H, N.

[(−)-2b]: $[\alpha]_D$ = −171.8° (*c* 1.10, $CHCl_3$).

Dimethyl (2*R*,3a*R*,8a*S*)-8-(Phenylsulfonyl)-2-[[2-(trimethylsilyl)ethoxy]methyl]-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-1,2-dicarboxylate [(+)-3a]. To a solution of (+)-**2a** (6.97 g, 16.8 mmol) in 100 mL of dry THF at −78 °C was added lithium diisopropylamide (2.0 M, 10.1 mL) via syringe under nitrogen. The resulting orange solution was stirred for 1 h and then [2-(trimethylsilyl)ethoxy]methyl chloride (5.59 g, 33.5 mmol), dissolved in 10 mL of THF, was added via syringe and the solution stirred at −78 °C for 4 h. The cold bath was removed and the solution allowed to warm to room temperature, at which time the reaction was quenched with 5 mL of methanol. The solvent was evaporated and the residue partitioned between water and EtOAc. The organic layer was collected and washed with 2 N HCl and brine, dried over sodium sulfate, and evaporated to provide a thick orange oil. Chromatography through silica gel (EtOAc/hexane, 1:1) provided the desired product as a light-

yellow oil (8.80 g, 96%): R_f = 0.69; $[\alpha]_D$ = +66.4° (c 0.91, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.01 (s, 9 H), 0.89 (t, 2 H, J = 8 Hz), 2.39 (d, 1 H, J = 13 Hz), 2.73 (dd, 1 H, J = 13, 8 Hz), 3.07 (s, 3 H), 3.30 (t, 1 H, J = 7 Hz), 3.54 (m, 3 H), 3.67 (s, 3 H), 4.14 (bs, 1 H), 6.20 (d, 1 H, J = 7 Hz), 6.97 (d, 1 H, J = 7.5 Hz), 7.06 (t, 1 H, J = 7.5 Hz), 7.25 (t, 1 H, J = 7.5 Hz), 7.35 (t, 2 H, J = 8 Hz), 7.48 (t, 1 H, J = 7.5 Hz), 7.54 (d, 1 H, J = 8 Hz), 7.61 (d, 2 H, J = 8 Hz); EIMS m/e 546.1860 (M^+, requires 546.1851), 503, 415, 383, 274. Anal. ($C_{26}H_{34}N_2O_7SSi$) C, H, N; C: calcd, 57.12; found, 58.01.

[(−)-3a]: 84% yield; $[\alpha]_D$ = −65.2° (c 1.36, $CHCl_3$).

Dimethyl (2*R*,3a*R*,8a*S*)-5-methoxy-8-(phenylsulfonyl)-2-[[2-(trimethylsilyl)ethoxy]methyl]-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-1,2-dicarboxylate [(+)-3b] was prepared from (+)-**2b** (6.0 g, 13.4 mmol) as described above for the preparation of **3a** from **2a** to provide the desired product (4.08 g, 53%) as a light yellow oil: R_f = 0.71; $[\alpha]_D$ = +130.5° (c 0.87, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.01 (s, 9 H), 0.89 (t, 2 H, J = 8 Hz), 2.32 (d, 1 H, J = 13 Hz), 2.66 (dd, 1 H, J = 13, 8 Hz), 3.12 (s, 3 H), 3.52 (m, 3 H), 3.74 (bs, 6 H), 4.14 (bs, 1 H), 6.11 (d, 1 H, J = 6 Hz), 6.46 (d, 1 H, J = 2.5 Hz), 6.68 (dd, 1 H, J = 8, 2.5 Hz), 7.34 (t, 2 H, J = 8 Hz), 7.48 (d, 1 H, J = 8 Hz), 7.49 (t, 1 H, J = 7.5 Hz), 7.55 (d, 2 H, J = 8 Hz); EIMS m/e 576.1943 (M^+, requires 576.1962), 533, 435, 407, 304. Anal. ($C_{27}H_{36}N_2O_8SSi \cdot {}^1/_2H_2O$) C, H, N.

[(−)-3b]: 97% yield; $[\alpha]_D$ = −128.9° (c 1.00, $CHCl_3$).

α(*R*)-(Hydroxymethyl)-N_b-(methoxycarbonyl)-N_a-(phenylsulfonyl)tryptophan Methyl Ester (3c). A sample of (+)-**3a** (820 mg, 1.50 mmol) was dissolved in 6 mL of trifluoroacetic acid and stirred at room temperature for 1 h. The solvent was evaporated and the residue dissolved in methylene chloride. The solution was washed with 5% sodium bicarbonate and brine, dried over sodium sulfate, and evaporated. Purification through a silica gel column (EtOAc/hexane, 2:1) provided a white foam (600 mg, 90%): R_f = 0.31; $[\alpha]_D$ = +61.1° (c 1.23, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.20 (d, 1 H, J = 14.6 Hz), 3.55 (d, 1 H, J = 14.6 Hz), 3.66 (s, 3 H), 3.71 (s, 3 H), 3.97 (d, 1 H, J = 11.5 Hz), 4.30 (d, 1 H, J = 11.5 Hz), 5.61 (s, 1 H), 7.26 (dt, 1 H, J = 7.5, 0.6 Hz), 7.32 (m, 2 H), 7.44 (m, 3 H), 7.54 (bt, 1 H, J = 7.5 Hz), 7.82 (d, 2 H, J = 7.5 Hz), 7.96 (d, 1 H, J = 8.3 Hz); EIMS m/e 446.1120 (M^+, requires 446.1145), 414, 355, 270. Anal. ($C_{21}H_{22}N_2O_7S$) C, H, N. Note: the reaction product sometimes contained trace amounts of the ring-closed tautomer **4a**, evident by NMR, which was indistinguishable from **3c** via silica gel chromatography. However, the two tautomers could be distinguished on alumina eluting with 3% methanol in methylene chloride (**3c**, R_f = 0.26; **4a**, R_f = 0.43).

(*R*)-2-Methoxy-4-(methoxycarbonyl)-4-[[*N*-(phenylsulfonyl)-3-indolyl]methyl]-2-oxazoline (3d). Obtained as the major product in the reaction of alcohol **3c** with 1.5 equiv of DAST at −78 °C in CH_2Cl_2 as a white crystalline solid: mp 96 °C; R_f = 0.38; $[\alpha]_D$ = −36.6° (c 1.29, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.20 (d, 1 H, J = 14.6 Hz), 3.26 (d, 1 H, J = 14.6 Hz), 3.74 (s, 3 H), 3.88 (s, 3 H), 4.21 (d, 1 H, J = 8.8 Hz), 4.68 (d, 1 H, J = 8.8 Hz), 7.23 (dt, 1 H, J = 8, 1 Hz), 7.31 (dt, 1 H, J = 8, 1 Hz), 7.44 (m, 3 H), 7.52 (t, 1 H, J = 7.5 Hz), 7.56 (dd, 1 H, J = 7.5, 0.5 Hz), 7.81 (dd, 2 H, J = 8, 1 Hz), 7.95 (dd, 1 H, J = 7.5, 0.5 Hz); EIMS m/e 428.1023 (M^+, requires 428.1040), 353, 270. Anal. ($C_{21}H_{20}N_2O_6S \cdot {}^1/_2H_2O$) C, H, N.

(*R*)-2-Methoxy-4-(methoxycarbonyl)-4-[[*N*-(phenylsulfonyl)-5-methoxy-3-indolyl]methyl]-2-oxazoline (3e) was obtained as an artifact in the reaction of alcohol **4b** (containing a trace amount of the ring-open tautomer, see text) as a white foam: R_f = 0.41; $[\alpha]_D$ = +20.8° (c 1.62, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.96 (s, 3 H), 3.13 (d, 1 H, J = 15.5 Hz), 3.36 (d, 1 H, J = 15.5 Hz), 3.82 (s, 6 H), 3.95 (d, 1 H, J = 9.2 Hz), 4.36 (d, 1 H, J = 9.2 Hz), 6.83 (d, 1 H, J = 2.5 Hz), 6.96 (dd, 1 H, J = 9.1, 2.5 Hz), 7.31 (s, 1 H), 7.45 (t, 2 H, J = 8 Hz), 7.53 (t, 1 H, J = 8 Hz), 7.79 (d, 2 H, J = 8 Hz), 7.89 (d, 1 H, J = 9.1 Hz). EIMS m/e 458.1153 (M^+, requires 458.1145), 399, 300, 159. Anal. ($C_{22}H_{22}N_2O_7S \cdot {}^1/_2H_2O$) C, H, N.

Dimethyl (2*R*,3a*R*,8a*S*)-2-(Hydroxymethyl)-8-(phenylsulfonyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-1,2-dicarboxylate [(+)-4a]. To a solution of (+)-**3a** (3.50 g, 6.41 mmol) in 150 mL of methylene chloride was added boron trifluoride etherate (2.73 g, 19.2 mmol) at 0 °C. The ice bath was removed, and after 1 h, the solution was poured into saturated sodium bicarbonate. The organic phase was collected and the aqueous phase extracted twice with methylene chloride. The combined organic extracts were washed with brine, dried over sodium sulfate, and evaporated to afford a light-orange oil. Purification through a silica gel column (EtOAc/hexane, 2:1) provided a white solid; recrystallization from acetone/hexane (1:1) produced shiny white plates (2.42 g, 85%): mp 149 °C; R_f = 0.33; $[\alpha]_D$ = +58.2° (c 1.12, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.09 (m, 1 H), 2.72 (d, 1 H, J = 12.5 Hz), 3.14 (s, 3 H), 3.46 (t, 1 H, J = 6.5 Hz), 3.60 (s, 3 H), 3.74 (dd, 1 H, J = 12.5, 2.8 Hz), 3.94 (t, 1 H, J = 12.5 Hz), 4.98 (bs, 1 H), 6.28 (d, 1 H, J = 6.5 Hz), 7.05 (d, 1 H, J = 7.5 Hz), 7.09 (t, 1 H, J = 7.5 Hz), 7.26 (dt, 1 H, J = 8, 1 Hz), 7.40 (t, 2 H, J = 7.5 Hz), 7.44 (d, 1 H, J = 8), 7.50 (t, 1 H, J = 7.5 Hz), 7.66 (dd, 2 H, J = 8, 1 Hz); EIMS m/e 446.1130 (M^+, requires 446.1148), 383, 369, 305, 270, 243. Anal. ($C_{21}H_{22}N_2O_7S$) C, H, N.

[(−)-4a]: 56% yield; mp 149 °C; $[\alpha]_D$ = −57.9° (c 1.05, $CHCl_3$).

Dimethyl (2*R*,3a*R*,8a*S*)-2-(hydroxymethyl)-5-methoxy-8-(phenylsulfonyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-1,2-dicarboxylate [(+)-4b] was prepared in an analogous manner from (+)-**3b** (3.85 g, 6.68 mmol) and boron trifluoride etherate (2.85 g, 20.0 mmol) as described above for the preparation of **4a** from **3a**, providing 2.18 g (69%) of the desired alcohol (+)-**4b** as a white foam: R_f = 0.35; $[\alpha]_D$ = +146.6° (c 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.04 (m, 1 H), 2.65 (d, 1 H, J = 13 Hz), 3.19 (s, 3 H), 3.23 (t, 1 H, J = 6.5 Hz), 3.72 (s, 3 H), 3.73 (m, 1 H), 3.75 (s, 3 H), 3.92 (t, 1 H, J = 13 Hz), 5.00 (bs, 1 H), 6.18 (d, 1 H, J = 6.5 Hz), 6.52 (dd, 1 H, J = 2.6, 0.8 Hz), 6.81 (dd, 1 H, J = 8.8, 2.6 Hz), 7.37 (t, 2 H, J = 7.5 Hz), 7.40 (t, 1 H, J = 8.8 Hz), 7.51 (dt, 1 H, J = 7.5, 1.2 Hz), 7.58 (dd, 2 H, J = 7.5, 1.2 Hz); EIMS m/e 476.1257 (M^+, requires 476.1251), 444, 335, 303. Anal. ($C_{22}H_{24}N_2O_8S \cdot H_2O$) C, H, N.

[(−)-4b]: 62% yield; $[\alpha]_D$ = −147.0° (c 1.04, $CHCl_3$).

Reaction of Alcohol 4a with DAST. To a solution of **4a** (1.90 g, 4.26 mmol) in 30 mL of methylene chloride was added DAST (1.03 g, 6.39 mmol) and the solution stirred at room temperature for 3 h. The solution was diluted with 70 mL of chloroform and then washed with 5% sodium bicarbonate, water, and brine. The organic phase was dried over sodium sulfate and evaporated to provide a thick, light-orange oil. TLC (silica, EtOAc/hexane, 2:1) showed two major components, the desired fluoride **5a** (R_f = 0.58) and the oxazolidinone **7a** (R_f = 0.28), as well as a trace amount of the aldehyde **6a** (R_f = 0.36). Upon dissolving of the residue in chloroform/methanol (1:3), the majority of the oxazolidinone **7a** separated as long white needles. Column chromatography of the mother liquor (silica gel, EtOAc/hexane, 1:1 → 2:1) provided the desired fluoride **5a** as a white foam (1.13 g, 59%), the aldehyde **6a** (62 mg, 3%), and additional oxazolidinone **7a** (total 565 mg, 32%).

Dimethyl (2*S*,3a*R*,8a*S*)-2-(fluoromethyl)-8-(phenylsulfonyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-1,2-dicarboxylate [(+)-5a]: R_f = 0.58; $[\alpha]_D$ = +116.7° (c 1.10, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.48 (d, 1 H, J = 13.5 Hz), 2.69 (dd, 1 H, J = 13.5, 8 Hz), 3.11 (s, 3 H), 3.39 (dd, 1 H, J = 8, 6.5 Hz), 3.73 (s, 3 H), 4.55 (dd, 1 H, J = 45.7, 9.5 Hz), 5.18 (dd, 1 H, J = 45.7, 9.5 Hz), 6.22 (d, 1 H, J = 6.5 Hz), 6.98 (d, 1 H, J = 7.5 Hz), 7.09 (t, 1 H, J = 7.5 Hz), 7.26 (t, 1 H, J = 8 Hz), 7.36 (t, 2 H, J = 7.5 Hz), 7.50 (t, 1 H, J = 7.5 Hz), 7.56 (d, 1 H, J = 8 Hz), 7.61 (d, 2 H, J = 8 Hz); EIMS m/e 448.1102 (M^+, requires 448.1104), 307, 275, 247, 211, 169, 130. Anal. ($C_{21}H_{21}FN_2O_6S$) C, H, N.

[(−)-5a]: 57% yield; $[\alpha]_D$ = −114.8° (c 0.95, $CHCl_3$).

Dimethyl (2*R*,3a*R*,8a*S*)-2-Formyl-8-(phenylsulfonyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-1,2-dicarboxylate [(+)-6a]. To a solution of **[(+)-4a]** (2.63 g, 6.81 mmol) in 100 mL of methylene chloride were added pyridinium chlorochromate (2.95 g, 13.6 mmol) and 2.63 g of powdered 3-Å molecular sieves, and the solution was stirred at room temperature for 6 h. The solution was filtered through Florisil (30 g) and evaporated to provide a light-green oil. Purification through a short silica gel column (EtOAc/hexane, 2:1) provided a white foam. Crystallization from EtOAc/hexane (1:1) afforded the desired product as white plates (1.32 g, 50%), identical in all respects with the sample obtained from the DAST fluorination of (+)-**4a** above: mp 158 °C; R_f = 0.36; $[\alpha]_D$ = +124.9° (c 1.24, $CHCl_3$); ^1H NMR

(500 MHz, $CDCl_3$) δ 2.49 (m, 1 H), 2.62 (d, 1 H, J = 13.5 Hz), 3.18 (s, 3 H), 3.58 (bs, 1 H), 3.69 (s, 3 H), 6.28 (d, 1 H, J = 6 Hz), 7.00 (d, 1 H, J = 7.5 Hz), 7.12 (t, 1 H, J = 7.5 Hz), 7.29 (t, 1 H, J = 8 Hz), 7.36 (t, 2 H, J = 8 Hz), 7.52 (t, 1 H, J = 7.5 Hz), 7.55 (d, 1 H, J = 8 Hz), 7.61 (d, 2 H, J = 8 Hz), 9.51 (s, 1 H); EIMS m/e 444.0996 (M^+, requires 444.0989), 383, 303, 274, 270, 242. Anal. ($C_{21}H_{20}N_2O_7S$) C, H, N.

Methyl (4a*R*,9b*R*,10a*S*)-5-(phenylsulfonyl)-3-oxo-4a,5,9b,10-tetrahydro-3*H*-oxazolo[3′,4′:1,5]pyrrolo[2,3-*b*]indole-10a(1*H*)-carboxylate [(+)-7a]: mp 209 °C, with softening at 204–205 °C; R_f = 0.28; $[\alpha]_D$ = +87.2° (*c* 1.41, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.24 (dd, 1 H, J = 13, 8 Hz), 2.94 (s, 3 H), 2.95 (t, 1 H, J = 13 Hz), 4.15 (t, 1 H, J = 8 Hz), 4.23 (d, 1 H, J = 9.4 Hz), 4.34 (d, 1 H, J = 9.4 Hz), 6.42 (d, 1 H, J = 7.5 Hz), 7.00 (dt, 1 H, J = 7.5, 0.7 Hz), 7.11 (d, 1 H, J = 7.5 Hz), 7.22 (t, 1 H, J = 7.5 Hz), 7.44 (d, 1 H, J = 8 Hz), 7.51 (dt, 2 H, J = 8, 1.5 Hz), 7.59 (tt, 1 H, J = 7.5, 0.7 Hz), 8.07 (td, 2 H, J = 8, 1.5 Hz); EIMS m/e 414.0883 (M^+, requires 414.0886), 351, 273, 169, 130. Anal. ($C_{20}H_{18}N_2O_6S$) C, H, N.

[(−)-7a]: 30% yield; mp 205.5 °C; $[\alpha]_D$ = −87.8° (*c* 1.25, $CHCl_3$).

Reaction of 4b with DAST was conducted in the same manner as for the reaction of **4a** with DAST described above. Starting with 3.50 g (7.35 mmol) of (+)-**4b** and 1.78 g (11.0 mmol) of DAST, 1.75 g (50%) of the fluoride (+)-**5b** as a white foam, 950 mg (30%) of the oxazolidinone (+)-**7b** as white needles from chloroform/methanol, and 130 mg of the oxazoline (+)-**3e** as a white foam were obtained.

Dimethyl (2*S*,3a*R*,8a*S*)-2-(fluoromethyl)-5-methoxy-8-(phenylsulfonyl)-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-1,2-dicarboxylate [(+)-5b]: R_f = 0.58; $[\alpha]_D$ = +186.9° (*c* 1.02, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.42 (d, 1 H, J = 13.5 Hz), 2.63 (dd, 1 H, J = 13.5, 8 Hz), 3.15 (s, 3 H), 3.19 (m, 1 H), 3.75 (s, 3 H), 3.79 (s, 3 H), 4.53 (dd, 1 H, J = 45.8, 9.5 Hz), 5.18 (bd, 1 H, J = 45.8 Hz), 6.14 (d, 1 H, J = 6.2 Hz), 6.48 (dd, 1 H, J = 1.5, 0.8 Hz), 6.81 (dd, 1 H, J = 7.5, 1.5 Hz), 7.36 (t, 2 H, J = 7.5 Hz), 7.50 (t, 2 H, J = 7.5 Hz), 7.51 (d, 1 H, J = 7.5 Hz), 7.56 (d, 1 H, J = 7.5 Hz); EIMS m/e 478.1212 (M^+, requires 478.1206), 337, 227. Anal. ($C_{22}H_{23}FN_2O_7S \cdot H_2O$) C, H, N; H: calcd, 5.08; found, 4.65.

[(−)-5b]: 32% yield; $[\alpha]_D$ = −185.5° (*c* 0.94, $CHCl_3$).

Methyl (4a*R*,9b*R*,10a*S*)-8-methoxy-5-(phenylsulfonyl)-3-oxo-4a,5,9b,10-tetrahydro-3*H*-oxazolo[3′,4′:1,5]pyrrolo[2,3-*b*]indole-10a(1*H*)-carboxylate [(+)-7b]: mp 195 °C; R_f = 0.26; $[\alpha]_D$ = +154.6° (*c* 1.22, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.20 (dd, 1 H, J = 13, 8 Hz), 2.92 (d, 1 H, J = 13 Hz), 3.08 (s, 3 H), 3.75 (s, 3 H), 4.02 (t, 1 H, J = 8 Hz), 4.26 (d, 1 H, J = 9 Hz), 4.33 (d, 1 H, J = 9 Hz), 6.33 (d, 1 H, J = 7.5 Hz), 6.63 (d, 1 H, J = 2.5 Hz), 6.76 (dd, 1 H, J = 9, 2.5 Hz), 7.39 (d, 1 H, J = 9 Hz), 7.48 (t, 2 H, J = 8 Hz), 7.57 (tt, 1 H, J = 8, 1.3 Hz), 7.98 (dd, 2 H, J = 8, 1.3 Hz); EIMS m/e 444.1008 (M^+, requires 444.0988), 303, 199, 160. Anal. ($C_{21}H_{20}N_2O_7S$) C, H, N.

[(−)-7b]: 31% yield; mp 195 °C; $[\alpha]_D$ = −155.2° (*c* 1.01, $CHCl_3$).

α(*S*)-(Fluoromethyl)-N_b-(methoxycarbonyl)-N_a-(phenylsulfonyl)tryptophan Methyl Ester [(*S*)-8a]. A 1.10-g portion of (+)-**5a** was dissolved in 7 mL of trifluoroacetic acid and stirred at room temperature for 2 h. The solution was poured into 80 mL of chloroform and the resulting solution washed with water, 5% sodium bicarbonate, and brine. The organic phase was dried over sodium sulfate and evaporated to provide the desired product as a white foam (1.04 g, 95%): R_f = 0.63; $[\alpha]_D$ = +69.9° (*c* 1.10, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.13 (d, 1 H, J = 14 Hz), 3.56 (d, 1 H, J = 14 Hz), 3.64 (s, 3 H), 3.68 (s, 3 H), 4.77 (dd, 1 H, J = 45, 9 Hz), 5.08 (dd, 1 H, J = 45, 9 Hz), 5.59 (s, 1 H), 7.23 (t, 1 H, J = 7.5 Hz), 7.30 (t, 1 H, J = 7.5 Hz), 7.31 (d, 1 H, J = 7.5 Hz), 7.43 (m, 3 H), 7.54 (t, 1 H, J = 7.5 Hz), 7.82 (d, 2 H, J = 7.5 Hz), 7.96 (d, 1 H, J = 7.5 Hz); EIMS m/e 448.1098 (M^+, requires 448.1104), 270. Anal. ($C_{21}H_{21}FN_2O_6S$) C, H, N.

[(*R*)-8a]: 97% yield; $[\alpha]_D$ = −68.4° (*c* 1.11, $CHCl_3$).

α(*S*)-(Fluoromethyl)-5-methoxy-N_b-(methoxycarbonyl)-N_a-(phenylsulfonyl)tryptophan methyl ester [(*S*)-8b] was prepared from (+)-**5b** (1.62 g) in 10 mL of trifluoroacetic acid as described above for the preparation of **8a** from **5a**: R_f = 0.61; $[\alpha]_D$ = +62.5° (*c* 1.06, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.08 (d, 1 H, J = 13 Hz), 3.54 (d, 1 H, J = 13 Hz), 3.65 (s, 3 H), 3.71 (s, 3 H), 3.80 (s, 3 H), 4.75 (dd, 1 H, J = 45, 9 Hz), 5.05 (dd, 1 H, J = 45, 9 Hz), 5.58 (s, 1 H), 6.87 (s, 1 H), 6.91 (d, 1 H, J = 7.5 Hz), 7.43 (t, 2 H, J = 7.5 Hz), 7.53 (t, 1 H, J = 7.5 Hz), 7.78 (d, 2 H, J = 7.5 Hz), 7.84 (d, 1 H, J = 7.5 Hz); EIMS m/e 478.1222 (M^+, requires 478.1206), 300, 159. Anal. ($C_{22}H_{23}FN_2O_7S$) C, H, N.

[(*R*)-8b]: 94% yield; $[\alpha]_D$ = −62.7° (*c* 1.05, $CHCl_3$).

α(*S*)-(Fluoromethyl)-N_b-(methoxycarbonyl)tryptophan-Methyl Ester [(*S*)-9a]. Into a dry flask fitted with a Dewar condenser and containing 1.00 g (2.23 mmol) of (*S*)-**8a** was condensed approximately 25 mL of anhydrous ammonia at −78 °C. The cold bath was removed and the solution allowed to come to reflux. Over a 5-min period, 260 mg of sodium metal was added in small slices. The solution was stirred for 30 min and then the Dewar condenser removed. The reaction was quenched with 5 mL of methanol and 1 g of ammonium chloride and the ammonia allowed to evaporate. The solution was partitioned between EtOAc and water and the organic layer collected, washed with water and brine, dried over sodium sulfate, and evaporated to afford a colorless oil. The product was purified via column chromatography (silica gel, EtOAc/hexane, 2:1) to provide the desired product (613 mg, 90%) as a colorless oil: R_f = 0.55; $[\alpha]_D$ = +89.8° (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.25 (d, 1 H, J = 14 Hz), 3.63 (d, 1 H, J = 14 Hz), 3.68 (s, 3 H), 3.70 (s, 3 H), 4.83 (dd, 1 H, J = 46, 9 Hz), 5.10 (dd, 1 H, J = 46, 9 Hz), 5.58 (s, 1 H), 6.98 (d, 1 H, J = 2.5 Hz), 7.11 (t, 1 H, J = 7.5 Hz), 7.18 (t, 1 H, J = 7.5 Hz), 7.35 (d, 1 H, J = 8.2 Hz), 7.52 (d, 1 H, J = 8.2 Hz), 8.12 (s, 1 H); EIMS m/e 308.1169 (M^+, requires 308.1172), 277, 233, 144, 130. Anal. ($C_{15}H_{17}FN_2O_4$) C, H, N.

[(*R*)-9a]: 82% yield; $[\alpha]_D$ = −89.0° (*c* 1.24, $CHCl_3$).

α(*S*)-(Fluoromethyl)-5-methoxy-N_b-(methoxycarbonyl)-tryptophan methyl ester [(*S*)-9b] was prepared from (*S*)-**8b** (1.55 g, 3.24 mmol) as described above for the preparation of **9a** from **8a** to provide the desired product in 69% yield (760 mg) as a white foam: R_f = 0.49; $[\alpha]_D$ = +76.9° (*c* 1.08, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.21 (d, 1 H, J = 14.2 Hz), 3.53 (d, 1 H, J = 14.2 Hz), 3.68 (s, 6 H), 3.85 (s, 3 H), 4.82 (dd, 1 H, J = 46.5, 9.2 Hz), 5.09 (dd, 1 H, J = 46.5, 9.2 Hz), 5.58 (s, 1 H), 6.84 (dd, 1 H, J = 8.8, 2.4 Hz), 6.94 (d, 1 H, J = 2.4 Hz), 6.99 (d, 1 H, J = 2.4 Hz), 7.23 (d, 1 H, J = 8.8 Hz), 8.03 (bs, 1 H); EIMS m/e 338.1264 (M^+, requires 338.1275), 160, 145. Anal. ($C_{16}H_{19}FN_2O_5$) C, H, N.

α(*S*)-(Fluoromethyl)-5-hydroxy-N_b-(methoxycarbonyl)-N_a-(phenylsulfonyl)tryptophan Methyl Ester [(*S*)-8c]. To a solution of (*S*)-**8b** (865 mg, 1.81 mmol) in 30 mL of methylene chloride at −78 °C was added boron tribromide (1.0 M in methylene chloride, 9.05 mL, 9.05 mmol) via syringe under nitrogen. The solution was stirred for 2 h at −78 °C and then for 30 min at room temperature. The reaction mixture was recooled to −78 °C and quenched with 3 mL of methanol. After warming to room temperature, the solution was poured into chloroform (100 mL) and then washed with water, 5% sodium bicarbonate, and brine. After drying over sodium sulfate the solution was evaporated to provide a colorless oil. The product was purified through a short silica gel column (EtOAc/hexane, 2:1) to afford the desired product as a white foam (671 mg, 80%): R_f = 0.45; $[\alpha]_D$ = +60.8° (*c* 1.02, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.05 (d, 1 H, J = 14.3 Hz), 3.47 (d, 1 H, J = 14.3 Hz), 3.66 (s, 3 H), 3.71 (s, 3 H), 3.74 (dd, 1 H, J = 46.2, 9.1 Hz), 5.03 (s, 1 H), 5.04 (dd, 1 H, J = 46.2, 9.1 Hz), 5.58 (bs, 1 H), 6.81 (d, 1 H, J = 2.5 Hz), 6.83 (s, 1 H), 7.26 (d, 1 H, J = 8 Hz), 7.43 (t, 2 H, J = 8 Hz), 7.52 (t, 1 H, J = 7.5 Hz), 7.79 (m, 3 H); EIMS m/e 464.1041 (M^+, requires 464.1050), 286. Anal. ($C_{21}H_{21}FN_2O_7S \cdot 1.5H_2O$) C, H, N,; H: calcd, 4.92; found, 4.35.

[(*R*)-8c]: 65% yield; $[\alpha]_D$ = −59.8° (*c* 1.28, $CHCl_3$).

α(*S*)-(Fluoromethyl)-5-hydroxy-N_b-(methoxycarbonyl)-tryptophan methyl ester [(*S*)-9c] was prepared from (*S*)-**8c** (635 mg, 1.37 mmol) and sodium (95 mg, 4.11 mmol), as described above for the preparation of **9a** from **8a**, to provide 336 mg (76%) of the desired product as a light-tan foam: R_f = 0.46; $[\alpha]_D$ = +30.0° (*c* 0.62, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 3.18 (d, 1 H, J = 14.4 Hz), 3.26 (d, 1 H, J = 14.4 Hz), 3.59 (s, 3 H), 3.66 (s, 3 H), 4.64 (dd, 1 H, J = 37.6, 9.3 Hz), 4.77 (dd, 1 H, J = 37.6, 9.3 Hz), 6.65 (dd, 1 H, J = 8.3, 1.8 Hz), 6.88 (d, 1 H, J = 1.8 Hz), 6.94 (s, 1 H), 7.15 (d, 1 H, J = 8.3 Hz); EIMS m/e 324.1124 (M^+, requires 324.1119), 148. Anal. ($C_{15}H_{17}FN_2O_5 \cdot {}^1/_4H_2O$) C, H, N.

[(*R*)-9c]: 66% yield; $[\alpha]_D$ = −30.6° (*c* 1.00, MeOH).

α(S)-(Fluoromethyl)tryptophan Methyl Ester [(S)-10a]. To a solution of (S)-**9a** (906 mg, 2.94 mmol) in 10 mL of acetonitrile was added trimethylsilyl iodide (706 mg, 3.53 mmol) and the solution was refluxed for 30 min. The solvent was evaporated and the residue dissolved in chloroform. The solution was washed with 5% sodium bicarbonate, dried over sodium sulfate, and evaporated to provide a light-yellow oil. Addition of EtOAc/hexane (1:2) quickly produced shiny white crystals which were collected and recrystallized from EtOAc/hexane (1:2) to provide 445 mg of shiny white needles. The crystals were collected and the combined mother liquors chromatographed through a short silica gel column (EtOAc) to provide an additional 105 mg of desired product (total yield 550 mg, 75%): mp 141 °C (lit.[2b] mp 144 °C, racemate); R_F = 0.12; $[\alpha]_D$ = +13.3° (c 1.23, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.01 (d, 1 H, J = 14.2 Hz), 3.23 (d, 1 H, J = 14.2 Hz), 3.68 (s, 3 H), 4.45 (dd, 1 H, J = 47.3, 8.7 Hz), 4.79 (dd, 1 H, J = 47.3, 8.7 Hz), 7.06 (d, 1 H, J = 2.3 Hz), 7.13 (dt, 1 H, J = 8, 1 Hz), 7.20 (dt, 1 H, J = 8, 1 Hz), 7.36 (d, 1 H, J = 8 Hz), 7.60 (d, 1 H, J = 8 Hz), 8.16 (bs, 1 H); EIMS m/e 250.1115 (M^+, requires 250.1118), 230, 191, 130.

[(R)-10a]: 74% yield; mp 141–142 °C; $[\alpha]_D$ = −13.6° (c 1.20, $CHCl_3$).

α(S)-(Fluoromethyl)-5-hydroxytryptophan methyl ester [(S)-10b] was prepared from (S)-**9c** (336 mg, 1.04 mmol) and trimethylsilyl iodide (625 mg, 3.12 mmol) as described above for the preparation of **10a** from **9a** to provide 200 mg (72%) of desired product as a white foam: R_f = 0.28 (EtOAc); $[\alpha]_D$ = +30.1° (c 1.05, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 2.88 (dd, 1 H, J = 14.4, 0.6 Hz), 3.13 (dd, 1 H, J = 14.4, 0.6 Hz), 3.65 (s, 3 H), 3.43 (dd, 1 H, J = 47.5, 9.0 Hz), 4.76 (dd, 1 H, J = 47.5, 9.0 Hz), 6.67 (ddd, 1 H, J = 8.8, 2.3, 0.4 Hz), 6.87 (dd, 1 H, J = 2.3, 0.6 Hz), 7.00 (d, 1 H, J = 0.4 Hz), 7.16 (dd, 1 H, J = 8.8, 0.6 Hz); EIMS m/e 267.1093 (M^+, requires 267.1097), 246, 207, 146.

[(R)-10b]: 25% yield; $[\alpha]_D$ = −28.9° (c 0.85, MeOH).

α(S)-(Fluoromethyl)tryptophan [(S)-11a]. A 100-mg portion (0.40 mmol) of (S)-**10a** was dissolved in 4 mL of THF and 2 mL of water and then 5.60 mL of aqueous sodium hydroxide (0.09995 M, 1.4 equiv) added. The solution was allowed to stand for 24 h in the dark at room temperature and was then neutralized with aqueous HCl (0.1019 M, 5.50 mL, 1.4 equiv). The solution was evaporated in vacuo to provide a white solid, a mixture of (S)-**11a** and 1.4 equiv of NaCl. Purification through Amberlite IR-120 (H^+ form) afforded (S)-**11a** as a white solid (71 mg, 75%): mp 184–188 °C dec; $[\alpha]_D$ = +27.7° (c 1.17, 1 N HCl); ^1H NMR (500 MHz, D_2O/DCl) δ 3.39 (d, 1 H, J = 15 Hz), 3.61 (d, 1 H, J = 15 Hz), 4.84 (dd, 1 H, J = 48, 10 Hz), 5.15 (dd, 1 H, J = 48, 10 Hz), 7.19 (t, 1 H, J = 8 Hz), 7.28 (t, 1 H, J = 8 Hz), 7.38 (s, 1 H), 7.55 (d, 1 H, J = 8 Hz), 7.66 (d, 1 H, J = 8 Hz); FAB-MS m/e 237.1040 (M + H^+, requires 237.1039). Anal. ($C_{12}H_{13}FN_2O_2 \cdot {}^3/_4H_2O$) C, H, N.

[(R)-11a]: 70% yield; mp 182–185 °C dec; $[\alpha]_D$ = −26.9° (c 1.28, 1 N HCl).

α(S)-(Fluoromethyl)-5-hydroxytryptophan [(S)-11b] was prepared from 100 mg (0.376 mmol) of (S)-**10b** as described above for the preparation of **11a** from **10a**, except using 2.4 equiv of sodium hydroxide and HCl instead of 1.4 equiv. After 24 h, the solution was evaporated in vacuo to provide a light-tan solid, a mixture of (S)-**11b** and 2.4 equiv of NaCl, which was used without further purification for enzymatic analysis: ^1H NMR (500 MHz, D_2O) δ 3.16 (d, 1 H, J = 14 Hz), 3.35 (d, 1 H, J = 14 Hz), 4.69 (dd, 1 H, J = 47, 10 Hz), 4.96 (dd, 1 H, J = 47, 10 Hz), 6.85 (d, 1 H, J = 8 Hz), 7.09 (s, 1 H), 7.26 (s, 1 H), 7.39 (d, 1 H, J = 10 Hz); FAB-MS m/e 253.0983 (M + H^+, requires 253.0988).

Tryptophan Hydroxylase: Crude Enzyme Preparation. P815 murine mastocytoma cells (American Type Culture Collection, Rockville, MD) were cultured in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Crude tryptophan hydroxylase solutions were prepared at 4 °C by washing 200 million cells twice with Locke's solution containing 5% glucose, adding a volume of 0.3 M Tris–acetate, pH 7.6, equal to cellular weight, and sonicating at 100 W for 1.5 min total. The preparation was then divided into aliquots and stored at −70 °C. The crude enzyme preparation so obtained displayed an apparent K_m of 135 ± 1.53 μM (average of three trials ± standard deviation) with L-tryptophan as substrate.

AADC: Crude Enzyme Preparation. Livers from four or five CD2F1 mice (National Cancer Institute, Bethesda, MD) were removed following etherization, rinsed in a small amount of ice-cold 0.1 M potassium phosphate, pH 7.5 buffer, pooled, and minced in 2 volumes of the same buffer. The preparation was homogenized with 10 strokes in a prechilled, power-assisted, Teflon-pestle tissue grinder and centrifuged at 10000g for 30 min at 4 °C. The supernatant was made 0.1 mM in dithiothreitol before division into aliquots and storage at −70 °C. The crude enzyme preparation so obtained displayed an apparent K_m of 45 ± 4.73 μM (average of three trials ± standard deviation) with 5-hydroxy-L-tryptophan as substrate.

HPLC Analyses of Enzymatic Reactions. Simultaneous separation of **11a**, **11b**, tryptophan, 5-hydroxytryptophan, and serotonin was afforded by a modification of the isocratic HPLC technique described by Yamaguchi.[24] The mobile phase consisted of 20% methanol, 10 mM potassium phosphate, pH 5.0, at a flow rate of 1 mL/min for the AADC assays; the methanol content was decreased to 10% for the tryptophan hydroxylase assays. HPLC analyses were monitored by fluorescence detection (excitation 280 nm, emission 300–400 nm).

Tryptophan Hydroxylase Assay with 11a. Assay mixtures were those described by Fujisawa[25] and contained, in addition to 0.34 mg of protein of crude P815 tryptophan hydroxylase, final concentrations of 60 mM Tris–acetate, pH 7.6; 2 mg/mL catalase; 25 mM dithiothreitol; 0.1 mM ferrous ammonium sulfate; 0.2 mM 2-amino-4-hydroxy-6-methyltetrahydropteridine hydrochloride; and the desired concentration of substrate (**11a**), to a final volume of 100 μL. Reactions at 37 °C were started by addition of substrate following a 10 min preincubation and were terminated after 45 min by addition of 10 μL of 60% perchloric acid. Following incubation on ice for 5 min, protein was removed by centrifugation at 10000g for 5 min, and the supernatants were analyzed by the HPLC technique described above. The retention time for the product of the enzymatic reaction with **11a** was identical with that observed for synthetic **11b**.

AADC Inhibition Assay with 11b. Assay mixtures were basically those described by Sourkes[26] and contained, in addition to 3.58 mg/mL protein of crude murine liver AADC, final concentrations of 60 mM Tris–acetate, pH 7.6; 0.1 mM pyridoxal-5-phosphate; and 1 mM pargyline hydrochloride to inhibit monoamine oxidase A and B. Reactions at 37 °C were started by addition of inhibitor (**11b**) and, following a defined preincubation period, aliquots were removed and added to a solution of 5-hydroxytryptophan (1.0 mM final concentration of 5-hydroxytryptophan). After incubating for 10 min, the reaction was quenched by the addition of 60% perchloric acid. After incubation on ice for 5 min, protein was removed by centrifugation at 10000×g for 5 min, and the superantants were analyzed by the HPLC technique described above. Concentrations of 5-hydroxytryptamine (5-HT) product were determined from a 5-HT standard curve and the percentage of AADC activity remaining was determined by comparison with controls run in the absence of inhibitor.

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