16, 111113-35-0; 17, 103336-30-7; 18, 111113-36-1; 19, 111113-37-2; 20, 111187-51-0; BOC-Phe-OH, 13734-34-4; BOC-His(Tos)-OH·DCHA, 65057-34-3; BOC-Pro-OH, 15761-39-4; BOC-Trp(CHO)-OH, 47355-10-2; BOC-Sta-OEt, 67010-43-9; BOC-Sta-OH, 58521-49-6; BOC-Leuψ[CH(OTBDMS)CH_2]Val-OH, 103335-80-4; BOC-Leu-H, 58521-45-2; H-Val-OBzl, 21760-98-5; BOC-Leuψ[CH_2NH]Val-OBzl, 82252-38-8; BOC-Leuψ[CH_2NH]Val-OH, 82252-39-9; renin, 9015-94-5.

Synthesis and Biological Properties of α-Mono- and α-Difluoromethyl Derivatives of Tryptophan and 5-Hydroxytryptophan

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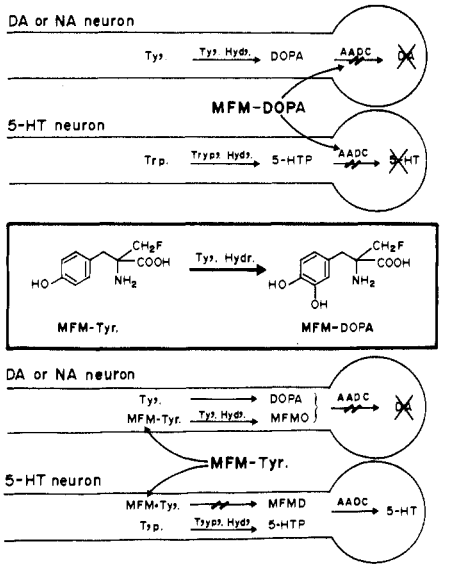
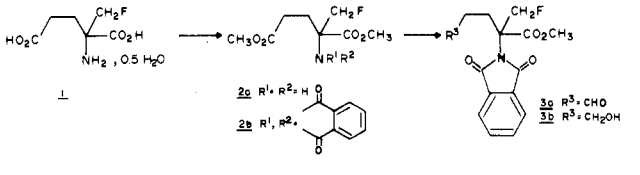
The syntheses of α-mono- and α-difluoromethyl derivatives of tryptophan and 5-hydroxytryptophan are described. In an attempt to selectively regulate serotonin synthesis, α-(mono- and difluoromethyl)tryptophan were tested in vivo as precursors (or prodrugs) of their 5-hydroxy analogues. Although α-(mono- and difluoromethyl)-5-hydroxytryptophans are potent irreversible inhibitors of aromatic amino acid decarboxylase (equipotent to α-difluoromethyl-Dopa), only α-(monofluoromethyl)tryptophan affects the level of serotonin in vivo (small decrease), α-(difluoromethyl)tryptophan being a very poor substrate of the activating (or helper) enzyme, tryptophan hydroxylase.

Over the past years, we and others have reported the synthesis of a number of halomethyl analogues of various amino acids and described their inhibitory properties of the corresponding α-amino acid decarboxylases both in vitro and in vivo.[1-4] Thus, both α-(difluoromethyl)- and α-(monofluoromethyl)-Dopa are time-dependent irreversible inhibitors of aromatic amino acid decarboxylase (AADC)[5,6] although their respective potencies and therefore potential applications are different. Difluoromethyl-Dopa inhibits AADC essentially in peripheral organs but never to the extent where the decarboxylation of endogenous Dopa becomes rate-limiting in catecholamine synthesis.[7] This compound could therefore in conjunction with Dopa replace Carbidopa in the treatment of parkinsonism. α-(Monofluoromethyl)-Dopa on the contrary inhibits AADC in peripheral organs as well as in brain and causes a time- and dose-dependent inhibition of biogenic amine synthesis.[8] In rat brain, for instance, α-(monofluoromethyl)-Dopa depletes in parallel dopamine and norepinephrine as well as serotonin, because the AADC's in the three types of neurons are inhibited to a degree where decarboxylation becomes rate-limiting. We recently demonstrated that α-(fluoromethyl)-Dopa can be generated from the *p*-tyrosine analogue by the action of tyrosine hydroxylase both in vitro and in vivo.[9,10] α-(Fluoromethyl)-*p*-tyrosine functions as a bioprecursor of the potent AADC inhibitor. This compound depletes selectively norepinephrine and, at higher doses, dopamine but has no effect on serotonin even after prolonged administration. The molecular basis for the increased selectivity of α-(monofluoromethyl)-*p*-tyrosine is illustrated in Scheme I.[11a] On the basis of the analogy of that result, we report here the synthesis and biological evaluation of α-(halomethyl)tryptophan and 5-hydroxytryptophan as an attempt to selectively regulate serotonin synthesis. The availability of a means to deplete specifically this important biogenic amine in combination with the use of selective antagonists of the various 5-HT receptors subtypes[11b] should advance our understanding of the role of serotonin.

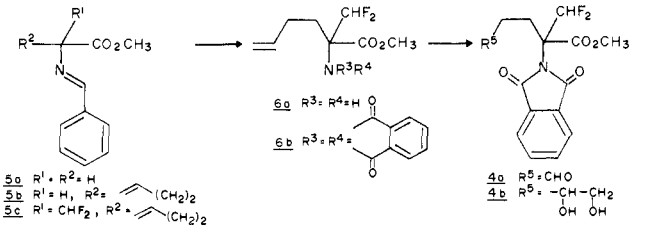
Scheme I. Postulated Mechanism for the Inhibition of AADC in Catecholaminergic and Serotoninergic Neurons by MFMD and MFMT



Scheme II



Scheme III



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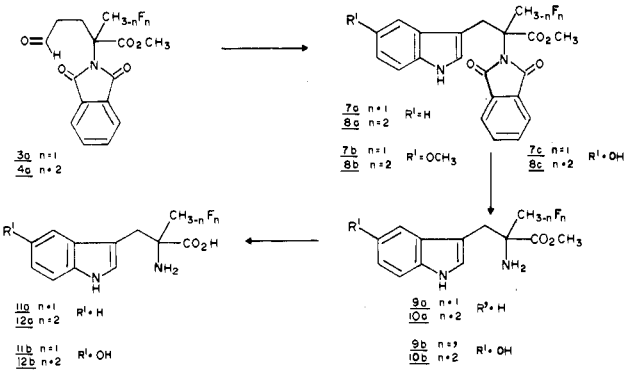
Scheme IV



Chemistry

α-(Mono- and difluoromethyl)tryptophans (**11a** and **12a**) and their 5-hydroxy derivatives (**11b** and **12b**) have been prepared by total synthesis, via a Fischer indole ring formation as shown in Scheme IV.

The synthetic approaches to the key aldehyde intermediates **3a** and **4a** are outlined in Scheme II and III, respectively.

Aldehyde 3a. (Fluoromethyl)glutamic acid semihydrate **1**[12] was converted in two steps ($SOCl_2$, CH_3OH·$NaHCO_3$; $PhtCl_2$) to the diester **2b**. Reduction of **2b** with 2.1 equiv of DIBAL-H occurred selectively at the terminal ester functionality and yielded 93% of methyl 2-(fluoromethyl)-5-hydroxy-2-phthalimidopentanoate (**3b**), the oxidation of which (DMSO, $(COCl)_2$) gave the expected aldehyde **3a** in 45% overall yield from **1**.

Aldehyde 4. Alkylation of the sodium salt of the butenyl-substituted Schiff base methyl ester of glycine **5b** with chlorodifluoromethane provided, as expected from our previous work,[13a] upon mild acidic hydrolysis the olefinic α-difluoromethyl amino ester **6a**. Phthaloylation of the amino functionality, followed by a two-step oxidation sequence of the double bond (OsO_4, NMMO; $NaIO_4$), afforded the expected aldehyde **4a** in 59% yield from **6a**.

Reaction of aldehydes **3a** or **4a** wtih phenylhydrazine (or (4-methoxyphenyl)hydrazine), under mild acidic conditions, resulted in the formation of the corresponding unstable hydrazones, which, upon being heated in a mixture of sulfuric acid and methanol underwent Fischer indole ring closure to yield the tryptophan derivatives **7a** (or **7b**) and **8a** (or **8b**), respectively.

Dealkylation of the hydroxyl protecting group of compounds **7b** and **8b** was performed, prior to the amine deprotection, with boron tribromide.

Removal of the phthalimido protecting groups via hydrazinolysis of **7a** or **7c** allowed the isolation of pure amino esters **9a** or **9b**, respectively.

Compounds **10a** or **10b** were isolated after hydrazinolysis of **8a** or **8c**, respectively.

Finally, saponification (0.1 N NaOH) of the methyl esters **9a** or **10a** afforded the α-(mono- and difluoromethyl)tryptophans **11a** and **12a**, respectively, while their 5-OH derivatives **11b** and **12b** were obtained under similar reaction conditions from **9b** and **10b**.

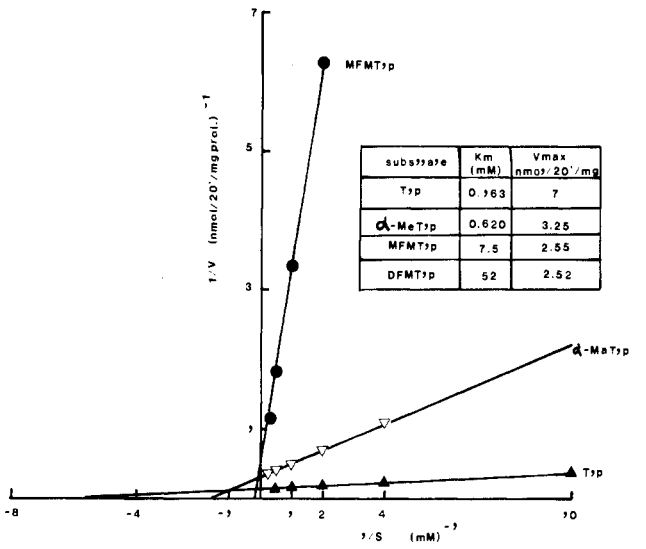


Figure 1. Kinetic parameters for the hydroxylation of tryptophan analogues by partially purified tryptophan hydroxylase. Rat brain stem soluble tryptophan hydroxylase (35000*g* supernatant) was incubated with various concentrations of tryptophan analogues for 20 min at 37 °C. The reaction was stopped with perchloric acid, and the supernatants were analyzed by reversed-phase HPLC with fluorometric detection and with corresponding 5-hydroxy analogues as references.

In Vitro Biochemistry

L-Tryptophan, α-methyltryptophan, and α-(monofluoromethyl)- or α-(difluoromethyl)tryptophan **11a** or **12a** were incubated with a partially purified preparation of tryptophan hydroxylase (see the Experimental Section for details). Formation of hydroxy derivatives was followed by reverse-phase HPLC with electrochemical and fluorimetric detection[14] using the authentic 5-hydroxy derivatives **11b** and **12b** as standards. Hydroxylation takes place

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(12) (a) Kollonitsch, J.; Barash, L.; Doldouras, G. A. *J. Am. Chem. Soc.* **1970**, *92*, 7494. (b) Could alternatively be prepared from amino nitrile A (U.S. Patent 4 405 530, issued September 20, 1983) (A) $CH{=}CHCH_2C(CH_2F)(CN)NH_2$. Gerhart, F., manuscript in preparation.

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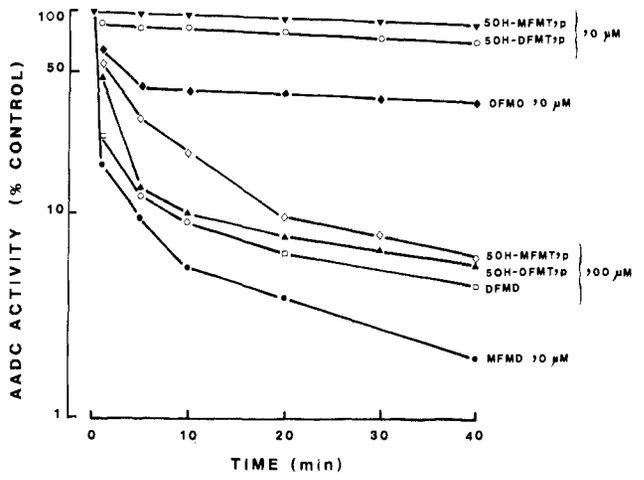

Figure 2. Time-dependent inhibition of AADC from hog kidney by the fluoromethyl derivatives of 5-hydroxytryptophan and Dopa. Partially purified hog kidney AADC was incubated with the fluorinated Dopa, tryptophan, and 5-hydroxytryptophan analogues at various concentrations. At the indicated time intervals, aliquots were withdrawn and assayed for residual enzyme activity with [1-^{14}C]Dopa as substrate. (AADX activity control: 2 μmol per h per 25 μL).

with all three α-substituted tryptophan derivatives. It was already known that α-methyltryptophan was a substrate of tryptophan hydroxylase;[15] we included it here for the sake of comparison.

Figure 1 compares in Lineweaver–Burk coordinates the kinetic parameters of tryptophan and its α-methyl and α-monofluoromethyl analogues. The affinity of the difluoromethyl analogue was too low for representation on the same scales; however, the calculated K_M and V_{max} appear in the inserted table.

The rate of hydroxylation at saturating concentration of substrates as measured by V_{max} remains relatively unaffected by the nature of the α-substituent; for instance, V_{max} of α-(difluoromethyl)tryptophan **12a** is about one-third that of L-tryptophan. However, the changes in K_M are of several orders of magnitude. Steric hindrance can certainly be invoked to explain the fourfold difference in affinity between tryptophan and α-methyltryptophan. Substitution of the hydrogens in the methyl group has an even more dramatic effect: each fluorine atom decreases the affinity by almost 1 order of potency. Steric influence of fluorine substitution is usually considered as negligible; therefore, it can be assumed that this drop in affinity is related to electronic factors, in particular to the pK_a of the amino group. In other series of α-fluoromethyl analogues of amines or α-amino acids, it was found that there was a decrease of 1 pK_a unit per atom of fluorine.[16] Substrate activity of a series of compounds for a given enzyme is best measured by k_{cat}/K_M or when the concentration of enzyme is unknown by V_{max}/K_M. It appears from the table of Figure 1 that introduction of an α-methyl substituent causes an eightfold decrease of the ratio V_{max}/K_M, with L-tryptophan as reference; the α-fluoromethyl substituent and the α-difluoromethyl substituent cause a 130-fold or a 1000-fold decrease, respectively, of V_{max}/K_M.

Neither α-(monofluoromethyl)- nor α-(difluoromethyl)tryptophan (**11a** or **12a**, respectively) inhibit partially purified AADC from hog kidney. The 5-hydroxy derivatives **11b** and **12b**, however, produce a time- and concentration-dependent decrease of enzyme activity. In Figure 2, the effectiveness of the 5-hydroxytryptophan analogues is compared to the corresponding Dopa analogues. It must first be noted that the rate of enzyme activity decay does not follow pseudo-first-order kinetics for any of the compounds. This had already been reported for α-(difluoromethyl)-Dopa;[7] the reasons for such a behavior are not understood. It appears, however, from Figure 2 that the two 5-hydroxytryptophan analogues are equipotent to α-(difluoromethyl)-Dopa. These three compounds are at least 3 times weaker inhibitors of AADC than α-(monofluoromethyl)-Dopa.

In Vivo Biochemistry

Twenty-four hours after oral administration to rats, both α-(monofluoromethyl)- and α-(difluoromethyl)-5-hydroxytryptophan (100 mg/kg) produce a 50% reduction of brain AADC activity. No significant effect was seen, however, on biogenic amine metabolism.

During the in vivo studies on α-(monofluoromethyl)-*p*-tyrosine, it was realized that the methyl ester of this compound gave less variability of biological effects than the free amino acid; therefore the methyl esters of α-(monofluoromethyl)- and α-(difluoromethyl)tryptophan **9a** and **10a** were used for in vivo evaluation. The two methyl esters (100 mg/kg per dose) were given orally, three consecutive times at 12-h interval, and the animals were killed 6 h after the last dose. The methyl ester of α-(difluoromethyl)tryptophan **10a** produced no measurable change in AADC activity and serotonin or 5-hydroxyindoleacetic acid concentration. After this treatment schedule, α-(monofluoromethyl)tryptophan methyl ester **9a** caused a significant accumulation of 5-hydroxytryptophan and small but significant decreases of serotonin and 5-hydroxyindole acetic acid, while norepinephrine levels were not affected.

Discussion and Conclusion

Enzyme-activated irreversible inhibitors share with affinity labeling agents the advantages of potency and duration of action in vivo as their effects do not depend on the continuous presence of the inhibitory agent. In addition, the former class of enzyme inhibitors has increased selectivity and therefore hopefully reduced toxicity as the reactive group is unveiled by the catalytic mechanism of the target enzyme. However, whenever organ or tissue selectivity is desirable, additional features are necessary. Some selectivity may be gained by designing prodrugs for altered transport properties.[17] It seemed to us more promising to take advantage of the enzyme machinery, specific to the metabolic cascade to inhibit, for the purpose of generating the actual inhibitor locally, from a properly designed bioprecursor.

Two examples of such an approach have appeared recently in the literature. The first one has already been briefly described in the introduction (Scheme I)[9,10] and served as a model for the present study. In short, blockade of biogenic amine synthesis in brain by means of AADC inhibition can be restricted to catecholaminergic neurons by replacing α-(monofluoromethyl)-Dopa by its tyrosine analogue. In this case the enzyme activating the bioprecursor is tyrosine hydroxylase. The second example is the elegant inhibition of monoamine oxidase A restricted to the central nervous system.[18,19] In this work, a moderately

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selective MAO-A inhibitor, β-(fluoromethylene)-*m*-tyramine, is generated from an amino acid bioprecursor, β-(fluoromethylene)-*m*-tyrosine, by the action of neuronal AADC. To avoid liberation of the amine in peripheral tissues, AADC needed to be blocked by a peripherally acting inhibitor such as Carbidopa.[19] The necessary conditions for such an approach to be successful have been discussed:[11] (a) the bioprecursor should not inhibit the target enzyme directly, (b) it should not be an irreversible inhibitor of the enzyme selected for the activation process (helper enzyme), (c) it should, on the contrary, be a reasonably good substrate of this enzyme, and (d) after activation, the transformed product should be a potent inhibitor of the enzyme selected for inhibition (target enzyme).

In the present study, we planned to use tryptophan hydroxylase as activating or helper enzyme to generate an irreversible inhibitor of AADC, the target enzyme. We verified first that the α-(monofluoromethyl)- and α-(difluoromethyl)tryptophan **11a** and **12a** have no direct inhibitory action on AADC. The two compounds are substrates of tryptophan hydroxylase and do not inhibit it in a time-dependent manner as the appearance of the hydroxylated species is linear with time. In addition, the products of hydroxylation identified by HPLC as being the 5-hydroxy analogues are time-dependent inhibitors of AADC. So that in theory all conditions were met.

Indeed, α-(monofluoromethyl)tryptophan **11a** affects serotonin metabolism in vivo in the expected manner: accumulation of 5-hydroxytryptophan, decrease of AADC activity, and decrease of serotonin and 5-HIAA concentrations. This compound was the best substrate of tryptophan hydroxylase. α-(Difluoromethyl)tryptophan **12a**, being a poorer substrate of the activating enzyme, probably did not yield enough of the 5-hydroxy compound to block effectively AADC. The same structure–activity relationship had been encountered with the α-fluoromethyl analogues of tyrosine (as reported in ref 10): α-(difluoromethyl)tyrosine is a poorer substrate than α-(monofluoromethyl)tyrosine of tyrosine hydroxylase and we could not demonstrate any effect on catecholamine metabolism in vivo with the former compound.

The small decrease of serotonin concentration caused by α-(monofluoromethyl)tryptophan **11a** in this study did not produce any gross behavioral change in the animals. Longer treatment times would be needed to enhance the depletion and to study the pharmacological effects of a selective blockade of serotonin biosynthesis.

Experimental Section

Melting points were determined on a Büchi or a Mettler FP5 melting point apparatus and are uncorrected, as are boiling points. Microanalyses were conducted on a Perkin-Elmer 240 CHN analyser. Infrared spectra were taken on a Perkin-Elmer IR-577 spectrophotometer. Ultraviolet spectra were recorded on a Beckman DU-7 spectrophotometer. ^{1}H nuclear magnetic resonance spectra were recorded on a Varian Associates T-60 (60 MHz) or EM-390 (90 MHz) spectrometer or on a Brücker AM-360 (360 MHz) spectrometer and are reported in parts per million from internal tetramethylsilane or 3-(trimethylsilyl)propionic acid-d_4 sodium salt on the δ scale. ^{19}F nuclear magnetic resonance spectra were recorded on a Brücker AM-360 (338.8 MHz) spectrometer and are reported in parts per million on the φ scale. Trifluoroacetic acid or C_6F_6 was used as the standard.

Data are presented as follows: solvent, chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet; m = multiplet), coupling constants, and interpretation. Mass spectrometry was performed on a Finnigan TSQ GC/MS/MS system.

Solvents and reagents were dried prior to use when deemed necessary. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride and diisopropylamine from solid KOH; dichloromethane was distilled from P_2O_5; methanol was dried by using the method described by Lund and Bjerrum.[23] Dimethyl sulfoxide was distilled from calcium hydride. Reactions were routinely followed by ^{1}H NMR analysis of aliquots or by thin-layer chromatography (TLC) analysis. Analytical TLC were performed with Merck precoated silica gel 60F-254 plates that were 0.25 mm thick. Seven eluant systems were used: system A, ethyl acetate; system B, ethyl acetate–cyclohexane (1:1); system C, ethyl acetate–petroleum ether (25:75); system D, diethyl ether; system E, methanol; system F, chloroform–methanol (8:2); system G, ethanol–NH_4OH (17%) (8:2).

HPLC analysis was performed on an Ultrasphere IP-C_{18} column (250 × 4.6 mm i.d., 5-μm particle size) with isocratic elution with a 76/24 (v/v) mixture of 0.1 M NaH_2PO_4 and methanol containing 5×10^{-3} M hexanesulfonic acid, at a pH of 2.85 (adjusted using H_3PO_4), and a flow rate of 1 mL/min and at 25 °C. Detection was performed by fluorescence (λ_{exc} = 300 and λ_{em} = 340) followed by electrochemical detection (ECD) (oxidation potential = 0.85 V versus an Ag/AgCl reference electrode). Retention times (t_R) are given in minutes and are presented as follows: retention time measured by fluorescence detection (t_R(A)), retention time measured by electrochemical detection (t_R(B)).

Unless otherwise specified, reaction workups consisted of drying of the solvent over anhydrous $MgSO_4$ and removal of the solvent by evaporation at reduced pressure. Bulb-to-bulb distillation was accomplished in a Büchi GKR-50 Kugelrohrapparat at the oven temperature and pressure indicated. Reactions described as run under nitrogen employed a mercury bubbler arranged so that the system could alternatively be evacuated and filled with inert gas and left under a positive pressure. Lithium diisopropylamide (LDA) was prepared in the following manner: a solution of diisopropylamine (1 M) in THF (1 equiv) was cooled to −70 °C followed by addition of a hexane solution of *n*-BuLi (2 M, 1 equiv) via syringe. The cooling bath was removed and the temperature of the reaction mixture was allowed to rise to −20 °C where it was maintained for a few minutes. The resulting solution of diisopropylamide was then cooled to the temperature desired for subsequent operations.

α-(Fluoromethyl)glutamic Acid Dimethyl Ester (2a). A suspension of (fluoromethyl)glutamic acid semihydrate (**1**; 3.60 g, 19.1 mmol) in dry methanol (50 mL) was cooled with ice and treated slowly with thionyl chloride (10 mL). During this treatment, the amino acid dissolved. After being heated under reflux overnight, the mixture was evaporated to dryness. The residue was dissolved in water, treated with an excess of 10% aqueous sodium bicarbonate, and extracted three times with dichloromethane (3 × 50 mL). After the mixture was washed with water, drying ($MgSO_4$) and evaporation gave the title compound as a yellow oil (2.4 g, 61%): ^{1}H NMR ($CDCl_3$) δ 1.60–2.6 (4 H, m, CH_2CH_2), 1.77 (2 H, br s, NH_2), 3.63 (3 H, s), 3.73 (3 H, s) (2 OCH_3), 4.43 (2 H, AB part of ABX, J_{AB} = 9 Hz, J_{AX} = J_{BX} = J_{H-F} = 47 Hz, CH_2F).

Dimethyl 2-(Fluoromethyl)-2-phthalimidoadipate (2b). A solution of **2a** (2.40 g, 11.6 mmol) and triethylamine (3.5 g, 35 mmol) in dry dichloromethane (30 mL) was cooled with ice, and phthaloyl dichloride (2.3 g, 11.3 mmol), dissolved in dichloromethane (10 mL), was added with stirring. The ice bath was removed, and stirring was continued overnight at room temperature. The solution was washed three times with 1 N HCl and two times with water, then dried ($MgSO_4$), and evaporated to give a yellow oil. This oil (3.7 g), which consisted mainly of the isophthaloyl derivative, was dissolved in dry dichloromethane (30 mL), triethylamine (3.5 g) was added, and the mixture was heated at reflux for 3 days. Workup as described above gave the title compound as a yellow oil (3.2 g, 79%): ^{1}H NMR ($CDCl_3$) δ 2.3–3.0 (4 H, m, CH_2CH_2), 3.57 (3 H, s), 3.80 (3 H, s) (2 OCH_3), 5.07 (2 H, AB part of ABX, J_{AB} = 9 Hz, J_{AX} = J_{BX} = J_{H-F} = 48 Hz, CH_2F), 7.80 (4 H, s, H aromatic).

Methyl 2-(Fluoromethyl)-5-hydroxy-2-phthalimidopentanoate (3b). Under an atmosphere of nitrogen, a solution

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of **2b** (2.50 g, 7.1 mmol) added in dry THF (70 mL) was cooled to −78 °C, and 1 M DIBAL-H in THF (15 mL) was added with stirring. The mixture was allowed to warm up to room temperature, and stirring was continued for an additional $1^1/_2$ h. After acidification with 1 N HCl, the THF was removed under vacuum. To the residue was added water and sodium chloride, and the mixture was extracted with dichloromethane (3 × 50 mL). After the mixture was washed with water, drying ($MgSO_4$), treatment with charcoal, and evaporation gave **3b** as a colorless oil (2.13 g, 93%): ^{1}H NMR ($CDCl_3$) δ 1.4–2.8 (4 H, m, CH_2CH_2), 3.67 (2 H, t, J = 7 Hz, CH_2OH), 3.80 (3 H, s, OCH_3), 5.03 (2 H, AB part of ABX, J_{AB} = 9 Hz, $J_{AX} = J_{BX} = J_{H-F}$ = 47 Hz, CH_2F), 7.77 (4 H, s, H aromatic).

Methyl 2-(Fluoromethyl)-5-oxo-2-phthalimidopentanoate (3a). Under an atmosphere of nitrogen, a solution of oxalyl chloride (3.88 g, 30 mmol) in dry dichloromethane (60 mL) was cooled to −60 °C, and a solution of dry DMSO (4.68 g, 60 mmol) in dichloromethane (20 mL) was added dropwise. Stirring was continued for an additional 5 min at −60 °C, and then **3b** (9.2 g, 27 mmol), dissolved in CH_2Cl_2 (40 mL), was added slowly at the same temperature. After the solution was stirred for 25 min at −60 °C, triethylamine (16.4 g, 162 mmol) in CH_2Cl_2 (10 mL) was added from a dropping funnel, stirring was continued for 10 min at −60 °C, and the mixture was allowed to warm up to room temperature. The solution was washed with water, 1% HCl, and with water again until neutral. Drying and evaporation gave the crude title compound as a yellow oil (9.7 g), which still contained some dichloromethane and DMSO. This material was used for the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 2.1–3.1 (4 H, m, CH_2CH_2), 3.77 (3 H, s, OCH_3), 5.03 (2 H, AB part of ABX, J_{AB} = 9 Hz, $J_{AX} = J_{BX} = J_{H-F}$ = 47 Hz, CH_2F), 7.77 (4 H, s, H aromatic), 9.77 (1 H, s, CHO).

Methyl 2-(Benzylideneamino)-5-hexenoate (5b). To a suspension of sodium hydride (9.44 g of a 45% dispersion in oil, washed three times with pentane, 0.177 mol) in anhydrous tetrahydrofuran (300 mL) was added, at room temperature under nitrogen, a solution of **5a**[13a] (31.40 g, 0.177 mol) in anhydrous tetrahydrofuran (50 mL). The mixture was heated for 30 min to 55–60 °C. The temperature then was allowed to decrease to room temperature and 1-bromo-4-butene (23.90 g, 0.177 mol) was added. Stirring at room temperature was continued overnight. Water was added and the solvent evaporated in vacuo to leave an oil, which was dissolved in diethyl ether. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification by fractional distillation of the crude product yielded 11.10 g of **5b** (27%) as a yellow oil: bp 132–134 °C (0.11 mm); ^{1}H NMR ($CDCl_3$) δ 1.70–2.20 (4 H, m, CH_2CH_2), 3.70 (3 H, s, OCH_3), 3.80–4.15 (1 H, m, CHN), 4.75–5.20 (2 H, m, CH=CH_2), 5.40–6.10 (1 H, m, CH=CH_2), 7.20–7.50 (3 H, m) and 7.55–7.90 (2 H, m) (H aromatic), 8.25 (1 H, s, CH=N).

Methyl 2-(Benzylideneamino)-2-(difluoromethyl)-5-hexenoate (5c). To a suspension of sodium hydride (2.66 g of a 45% dispersion in oil, washed three times with pentane, 0.050 mol) in anhydrous tetrahydrofuran (80 mL) was added, at room temperature under nitrogen, a solution of **5b** (11.10 g, 0.048 mol) in anhydrous tetrahydrofuran (20 mL). After the mixture was heated at 50 °C for 2.5 h, a stream of chlorodifluoromethane was rapidly bubbled through the carbanion solution maintained at 50 °C. Water was added and the solvent was evaporated in vacuo, yielding an oil, which was dissolved in diethyl ether. The organic layer was washed with water and brine and dried over $MgSO_4$. Concentration in vacuo and distillation yielded 9.35 g of **5c** (69%) as a colorless oil: bp 155 °C (0.075 mm, Kugelrohr); ^{1}H NMR ($CDCl_3$) δ 2.00–2.30 (4 H, m, CH_2CH_2), 3.75 (3 H, s, OCH_3), 4.80–5.20 (2 H, m, CH=CH_2), 5.45–6.10 (1 H, m, CH=CH_2), 6.15 (1 H, t, J_{H-F} = 55 Hz, CHF_2), 7.20–7.45 (3 H, m) and 7.50–7.85 (2 H, m) (H aromatic), 8.32 (1 H, s, CH=N).

Methyl 2-Amino-2-(difluoromethyl)-5-hexenoate (6a). A mixture of 1 N HCl (100 mL) and **5c** (19.49 g, 0.069 mol) was stirred at room temperature for 3 h. The aqueous solution was washed with diethyl ether, concentrated in vacuo, and saturated with sodium bicarbonate. The basic aqueous mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$. Concentration in vacuo gave an oil purified by chromatography on silica gel (medium pressure, eluant ethyl acetate/cyclohexane, 1:4) to afford 9.30 g of pure **6a** (70%): ^{1}H NMR ($CDCl_3$) δ 1.54–2.45 (6 H, m, CH_2CH_2 and NH_2), 3.74 (3 H, s, OCH_3), 4.85–5.20 (2 H, m, CH=CH_2), 5.45–6.15 (1 H, m, CH=CH_2), 5.85 (1 H, t, J_{H-F} = 55 Hz, CHF_2); TLC (system B) 0.42 (I_2).

Methyl 2-(Difluoromethyl)-2-phthalimido-5-hexenoate (6b). To a mixture of **6a** (7.10 g, 0.0368 mol) and triethylamine (11.10 g, 0.110 mol) in anhydrous dichloromethane (75 mL) was added at 0 °C under nitrogen a solution of *o*-phthaloyl dichloride (7.47 g, 0.0368 mol) in anhydrous dichloromethane (25 mL). The temperature was allowed to rise slowly to room temperature. Stirring was continued while the mixture was heated at reflux for 16 h. The solution was concentrated at atmospheric pressure to a volume of 30 mL. Triethylamine (4.35 g) was added and the mixture was again heated at reflux for 12 h. Dichloromethane (100 mL) was added at room temperature and the mixture was washed with 1 N HCl (3 × 50 mL) and water and dried over $MgSO_4$. Concentration of the organic layer in vacuo left the crude phthalimido derivative which was purified by chromatography on silica gel (medium pressure, eluant ethyl acetate/cyclohexane, 15:85) to afford 9 g of pure **6b** (75%) as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.95–2.80 (4 H, m, CH_2CH_2), 3.80 (3 H, s, OCH_3), 4.75–5.18 (2 H, m, CH=CH_2), 5.40–6.10 (1 H, m, CH=CH_2), 6.58 (1 H, t, J_{H-F} = 54 Hz, CHF_2), 7.75 (4 H, m, H aromatic); TLC (system B) 0.52 (UV, I_2). Anal. ($C_{16}H_{15}F_2NO_4$) C, H, N.

Methyl 2-(Difluoromethyl)-5,6-dihydroxy-2-phthalimidohexanoate (4b). A mixture of **6b** (8.67 g, 0.0268 mol), *N*-methylmorpholine *N*-oxide (0.8 H_2O) (3.87 g, 0.0295 mol), and osmium tetraoxide (6.4 mL of 5% solution in *t*-BuOH) in acetone (280 mL) and water (4.5 mL) was heated at reflux under nitrogen for 6 h. After the mixture was cooled to room temperature, sodium disulfite (0.30 g) in water (4 mL) and Celite (0.60 g) were added. The mixture was stirred for 0.5 h and then filtered. The filtrate was concentrated in vacuo to about a $^1/_{10}$ of its initial volume, and water (20 mL) was added. The pH was adjusted to 1 with 1 N HCl and the solution was extracted with ethyl acetate (3 × 80 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The crude oily residue was purified by chromatography on silica gel (medium pressure, eluant ethyl acetate/cyclohexane, 4:1, and finally pure ethyl acetate) to yield 8 g of **4b** (83%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.30–1.80 (2 H, m) and 2.35–2.85 (2 H, m) (CH_2CH_2), 3.15–3.85 (5 H, m, $HOCH_2CHOH$), 3.77 (3 H, s, OCH_3), 6.50 (1 H, t, J_{H-F} = 54 Hz, CHF_2), 7.72 (4 H, m, H aromatic); TLC (system A) 0.29 (UV, I_2); IR ($CHCl_3$) 3450 (br), 1730 cm^{-1}; MS (DCI/CH_4), m/z (relative intensities) 358 (MH^+, 50), 340 (20), 106 (100).

Methyl 2-(Difluoromethyl)-5-oxo-2-phthalimidopentanoate (4a). A mixture of sodium metaperiodate (4.61 g, 0.0215 mol) and **4b** (7.00 g, 0.0196 mol) in tetrahydrofuran/water (2:1, 150 mL) was stirred at room temperature for 2 h. Water (50 mL) was added and the solution was extracted with diethyl ether (3 × 100 mL). The combined organic layers were dried over $MgSO_4$ and evaporated in vacuo to yield 5.90 g of **4a** (93%), which was used in the next step without purification: ^{1}H NMR ($CDCl_3$) δ 2.50–3.05 (4 H, m, CH_2CH_2), 3.80 (3 H, s, OCH_3), 6.53 (1 H, t, J_{H-F} = 54 Hz, CHF_2), 7.71 (4 H, m, H aromatic), 9.95 (1 H, s, CHO); TLC (system B) 0.36 (UV, I_2); IR 1750, 1725 cm^{-1}; MS (DCI/CI/NH_3), m/z (relative intensities) 326 (MH^+, 2), 343 (MNH_4^+, 100).

Methyl 2-(Difluoromethyl)-3-(3-indolyl)-2-phthalimidopropionate (8a). A solution of phenylhydrazine (1.92 g, 0.0178 mol) in tetrahydrofuran (5 mL) was added to aldehyde **4a** (5.77 g, 0.0178 mol) in tetrahydrofuran/water (1:1, 90 mL). The pH was adjusted to 5 (with 1 M AcOH) and the mixture was stirred for 1 h at room temperature. The solution rapidly became yellow. Water was then added and the mixture was extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over $MgSO_4$. Filtration and removal of the solvent in vacuo left 6.67 g of phenylhydrazone as a yellow oil, which was further used without purification. TLC (system A): 0.57 (UV).

The crude phenylhydrazone (6.67 g, 0.016 mol) was heated at reflux in a 1:1 mixture of methanol and 1 M H_2SO_4 (64 mL) for 4 h. Water was added and the solution was extracted with diethyl ether (4 × 50 mL). The combined organic phases were dried over $MgSO_4$. Filtration and removal of the solvent in vacuo left an oil, which was purified by column chromatography (MPLC, silica gel, ethyl acetate/cyclohexane, 1:1) to yield 1.88 g of pure **8a** (29%)

as a yellow foam: ^{1}H NMR ($CDCl_3$) δ 3.77 (3 H, s, OCH_3), 4.00 (2 H, AB, J_{AB} = 15 Hz, ν_{AB} = 36 Hz, CH_2), 6.73 (1 H, t, J_{HF} = 54 Hz, CHF_2), 6.83–7.67 (m) and 7.68 (m) (9 H, H aromatic), 8.23 (1 H, br s, NH); TLC (system D) 0.47 (UV). Anal. ($C_{21}H_{16}F_2N_2O_4$) H; C: calcd, 63.32; found, 62.67. N: calcd, 7.03; found, 6.51.

2-(Difluoromethyl)tryptophan Methyl Ester (10a). A solution of **8a** (1.88 g, 4.7 mmol) in 1 M ethanolic hydrazine hydrate (4.7 mL) was heated at reflux for 17 h under nitrogen. The solvent was removed in vacuo and the solid residue was dissolved in methanol (5 mL) and 1 N HCl (5 mL). The mixture was heated at reflux for 2.5 h. The solvent was evaporated in vacuo and the residue was triturated with water. The solid suspension was filtered off. The pH of the filtrate was adjusted to 9–10 with 1 M sodium carbonate and the solution was extracted with ethyl acetate. Usual workup afforded the expected amine **8b**, which was purified by chromatography (MPLC, silica gel, ethyl acetate). Recrystallization from a mixture of ethyl acetate/pentane yielded 0.80 g of pure **10a** (63%): mp 98 °C; ^{1}H NMR ($CDCl_3$) δ 1.83 (2 H, br s, NH_2), 3.20 (2 H, AB, J_{AB} = 14 Hz, ν_{AB} = 17.2 Hz, CH_2), 3.58 (3 H, s, OCH_3), 5.97 (1 H, t, J_{HF} = 55 Hz, CHF_2), 6.80–7.80 (5 H, m, aromatic), 8.64 (1 H, br s, NH); ^{19}F NMR ($CDCl_3/C_6F_6$) 29.03 (1 F, dd, $J(F_AF_B)$ = 279 Hz, $J(HF_A)$ = 56 Hz), 34.38 (1 F, dd, $J(F_AF_B)$ = 279 Hz, $J(HF_B)$ = 55.5 Hz); UV_{max} (H_2O) 215 nm (ϵ 34 403), 270 (sh), 277 (5853), 286 (4761); IR (KBr) 3225 (br), 1755 cm^{-1}; TLC (system F) 0.61 (UV, ninhydrin); MS (DCI/NH_3), m/z (relative intensities) 269 (MH^+, 100), 286 (MNH_4^+, 37), 147 (1), 130 (3). Anal. ($C_{13}H_{14}F_2N_2O_2$) C, H, N.

2-(Difluoromethyl)tryptophan (12a). A solution of **10a** (0.116 g, 0.43 mmol) in a mixture of tetrahydrofuran (4 mL) and 0.1 N sodium hydroxide (8.6 mL, 2 equiv) was stirred at room temperature for 16 h. A solution of 0.1 N HCl (8.6 mL) was then added and the solvent was evaporated in vacuo, leaving a white solid. The free amino acid was obtained by passage over an ion-exchange resin (Amberlite IR 120 H^+, eluted with water and a gradient of NH_4OH). The ninhydrin-positive fractions were collected and evaporated to dryness, yielding 0.080 g of pure **12a** (73%) as a white solid recrystallized from water/acetone: mp 209 °C; ^{1}H NMR (D_2O) δ 3.43 (2 H, AB, J_{AB} = 15 Hz, ν_{AB} = 17.4 Hz, CH_2), 6.41 (1 H, t, J_{HF} = 54 Hz, CHF_2), 7.15–7.80 (5 H, m, aromatic); ^{19}F NMR (D_2O/CF_3CO_2H) –51.45 (1 F, dd, $J(F_AF_B)$ = 279.6 Hz, $J(HF_A)$ = 52 Hz), –56.30 (1 F, dd, $J(F_AF_B)$ = 279.6 Hz, $J(HF_B)$ = 54 Hz); UV_{max} (H_2O) 218 nm (ϵ 30 667), 271 (sh), 278 (5257), 287 (4295); TLC (system E) 0.64 (UV, ninhydrin); HPLC, one peak, 10.96 (t_R(A)), 11.03 (t_R(B)); MS (DCI/NH_3), m/z (relative intensities) 272 (MNH_4^+, 100), 255 (MH^+, 68), 191 (11), 130 (4). Anal. ($C_{12}H_{12}F_2N_2O_2 \cdot 0.5H_2O$) C, H, N.

Methyl 2-(Fluoromethyl)-3-(3-indolyl)-2-phthalimidopropionate (7a). Compound **7a** was prepared from **3a** (6.63 g, 21.6 mmol) and phenylhydrazine (2.30 g, 21.6 mmol) in 46% yield in a manner similar to that described for the synthesis of **8a** from **4a**. **7a** (3.80 g) was isolated as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 3.73 (3 H, s, OCH_3), 3.90 (2 H, AB, J_{AB} = 14 Hz, CH_2), 5.10 (2 H, AB part of ABX, J_{AB} = 9 Hz, J_{AX} = J_{BX} = J_{HF} = 47 Hz, CH_2F), 6.70–7.60 (5 H, m, H indole), 7.67 (4 H, s, H phthalimide), 8.10 (1 H, br s, NH); TLC (system C) 0.20 (UV); MS (EI/70 eV), 380 (M^+), 233, 130.

2-(Fluoromethyl)tryptophan Methyl Ester (9a). Compound **9a** was prepared from **7a** (3.50 g, 9.2 mmol) in 42% yield in a manner similar to that described for the synthesis of **10a** from **8a**. **9a** (colorless solid, 0.77 g) was isolated: mp 144 °C; ^{1}H NMR ($CDCl_3$) δ 2.70 (2 H, br s, NH_2), 3.17 (2 H, AB, J_{AB} = 14 Hz, CH_2), 3.70 (3 H, s, OCH_3), 4.67 (2 H, AB part of ABX, J_{AB} = 9 Hz, J_{AX} = J_{BX} = J_{HF} = 47 Hz, CH_2F), 6.90–7.90 (5 H, m, aromatic), 8.37 (1 H, br s, NH); ^{19}F NMR ($CDCl_3/C_6F_6$) –63.99 (t, J_{HF} = 47 Hz); UV_{max} (CH_3CN) 221 nm (ϵ 36 989), 274 (sh), 279 (5756), 289 (4832); IR (KBr) 3180 (br), 1745 cm^{-1}; TLC (system F) 0.62 (UV, ninhydrin); MS (EI/70 eV), 250 (M^+), 229, 191, 130. Anal. ($C_{13}H_{15}FN_2O_2$) C, H, N.

2-(Fluoromethyl)tryptophan (11a). Compound 11a was prepared from **9a** with 0.1 N sodium hydroxide (1.4 equiv) in a manner similar to that described for the synthesis of **12a** from **10a**. A solution of 0.1 N HCl (1.4 equiv) was aded after completion of the reaction, and the solvent was evaporated in vacuo, leaving a white solid, a mixture of 11a and sodium chloride (1.4 equiv), which was used as such for further experiments: ^{1}H NMR (D_2O/DCl) δ 3.50 (2 H, AB, J_{AB} = 15 Hz, CH_2), 5.03 (2 H, AB part of ABX, J_{AB} = 10 Hz, J_{AX} = J_{BX} = J_{HF} = 45 Hz, CH_2F), 7.00–7.80 (5 H, m, aromatic); ^{19}F NMR (D_2O, DCl/CF_3CO_2H) –151.61 (t, J_{HF} = 45 Hz); UV_{max} (H_2O) 218 nm (ϵ 28973), 273 (sh), 279 (5208), 287 (4281); TLC (system G) 0.68 (UV, ninhydrin); HPLC, one peak, 13.75 (t_R(A)), 13.83 (t_R(B)); MS (DCI/NH_3), m/z (relative intensities) 237 (MH^+, 100), 254 (MNH_4^+, 65), 219 (5), 197 (8), 173 (20).

Methyl 2-(Difluoromethyl)-3-(5-methoxy-3-indolyl)-2-phthalimidopropionate (8b). Compound **8b** was prepared from **4a** (4.90 g, 0.015 mol) and (*p*-methoxyphenyl)hydrazine hydrochloride (2.62 g, 0.015 mol) in 60% yield in a manner similar to that described for the synthesis of **8a** from **4a**. **8b** (3.90 g) was isolated and recrystallized from ethyl acetate/pentane: mp 140 °C; ^{1}H NMR ($CDCl_3$) δ 3.64 (3 H, s, CO_2CH_3), 3.73 (3 H, s, OCH_3), 3.97 (2 H, AB, J_{AB} = 14 Hz, ν_{AB} = 37 Hz, CH_2), 6.70 (1 H, t, J_{HF} = 54 Hz, CHF_2), 6.57–7.23 (4 H, m, aromatic), 7.63 (4 H, m, H phthalimide), 8.15 (1 H, br s, NH); TLC (system D) 0.48 (UV, I_2). Anal. ($C_{22}H_{18}F_2N_2O_5$) C, H, N.

Methyl 2-(Difluoromethyl)-3-(5-hydroxy-3-indolyl)-2-phthalimidopropionate (8c). Boron tribromide (1 M) in dichloromethane (25.5 mL) was added at –78 °C, under nitrogen, to a solution of **8b** (2.20 g, 5.1 mmol) in anhydrous dichloromethane (30 mL). The temperature was allowed to rise to room temperature over a period of 3 h and the mixture was stirred 1 h at that temperature. The mixture was poured over crushed ice and water and was extracted with ethyl acetate (4 × 50 mL). The combined organic phases were washed with diluted aqueous sodium bicarbonate and brine and dried over $MgSO_4$. Chromatography (MPLC, silica gel, ethyl acetate/cyclohexane, 3:7) and recrystallization from ethyl acetate/pentane yielded 0.43 g of pure **8c** (20%): ^{1}H NMR ($CDCl_3$) δ 3.72 (3 H, s, CO_2CH_3), 3.88 (2 H, AB, J_{AB} = 14 Hz, ν_{AB} = 37.4 Hz, CH_2), 5.53 (1 H, br s, OH), 6.47–7.20 (m, aromatic) and 6.67 (t, J_{HF} = 54 Hz, CHF_2) (5 H), 7.63 (4 H, m, H phthalimide), 8.25 (1 H, br s, NH); TLC (system B) 0.15 (UV, I_2). Anal. ($C_{21}H_{16}F_2N_2O_5$) C, H, N.

2-(Difluoromethyl)-5-hydroxytryptophan Methyl Ester (10b). Compound **10b** was prepared from **8c** (1.42 g, 3.4 mmol) in 74% yield in a manner similar to that described for the synthesis of **10a** from **8a**. Compound **10b** was isolated as a white foam (0.72 g): mp <60 °C; ^{1}H NMR ($CDCl_3$ + 20% CD_3OD) δ 3.10 (2 H, AB, J_{AB} = 13 Hz, ν_{AB} = 15.4 Hz, CH_2), 5.98 (1 H, t, J_{HF} = 55 Hz, CHF_2), 6.50–7.50 (5 H, m, aromatic); ^{19}F NMR ($CDCl_3/C_6F_6$) 29.07 (1 F, dd, $J(F_AF_B)$ = 279 Hz, $J(HF_A)$ = 56 Hz), 34.40 (1 F, dd, $J(F_AF_B)$ = 279 Hz, $J(HF_B)$ = 55 Hz); UV_{max} (H_2O) 202 nm (ϵ 20 170), 216 (sh), 276 (4246), 295 (3567); IR (KBr) 3400 (br), 1740 cm^{-1}; TLC (system E) 0.62 (UV, ninhydrin); MS (DCI/NH_3), m/z (relative intensities) 285 (MH^+, 100), 302 (MNH_4^+, 34), 146 (4). Anal. ($C_{13}H_{14}F_2N_2O_3$) C, H; N: calcd, 9.85; found, 9.32.

2-(Difluoromethyl)-5-hydroxytryptophan (12b). A solution of **10b** (0.029 g, 0.1 mmol) in a mixture of tetrahydrofuran (2 mL) and 0.1 N NaOH (2.4 mL) was stirred at room temperature, under nitrogen, and in the dark for 20 h. A solution of 0.1 N HCl (2.4 mL) was then added. The solvent was removed in vacuo, leaving a solid, a mixture of **12b** and sodium chloride (2.4 equiv), which was tested as such: ^{1}H NMR (D_2O) δ 3.20 (2 H, AB, J_{AB} = 15 Hz, ν_{AB} = 9.5 Hz, CH_2), 6.23 (1 H, t, J_{HF} = 53 Hz, CHF_2), 6.50–7.45 (4 H, m, aromatic); ^{19}F NMR (D_2O, DCl/CF_3CO_2H) –56.45 (1 F, dd, $J(F_AF_B)$ = 281.4 Hz, $J(HF_A)$ = 54 Hz), –51.35 (1 F, dd, $J(F_AF_B)$ = 281.4 Hz, $J(HF_B)$ = 53 Hz); UV_{max} (H_2O) 205 nm (ϵ 16 653), 274 (4419), 295 (3618); TLC (system G) 0.69 (UV, ninhydrin); HPLC, one peak, 4.76 (t_R(A)), 4.83 (t_R(B)); MS (DCI/NH_3), m/z (relative intensities) 288 (MNH_4^+, 100), 271 (MH^+, 27), 185 (19), 148 (11), 134 (26).

Methyl 2-(Fluoromethyl)-3-(5-methoxy-3-indolyl)-2-phthalimidopropionate (7b). Compound **7b** was prepared from **3a** (21.7 g, 0.070 mol) and (*p*-methoxyphenyl)hydrazine hydrochloride (11.7 g, 0.067 mol) in 38% yield in a manner similar to that described for the synthesis of **8a** from **4a**. **7b** (11 g) was isolated after purification by chromatography (silica gel, ethyl acetate/petroleum ether, 6:10): ^{1}H NMR ($CDCl_3$) δ 3.67 (3 H, s, CO_2CH_3), 3.77 (3 H, s, OCH_3), 3.85 (2 H, AB, J_{AB} = 15 Hz, CH_2), 5.10 (2 H, AB part of ABX, J_{AB} = 9 Hz, J_{AX} = J_{BX} = J_{HF} = 47 Hz, CH_2F), 6.60–7.30 (4 H, m, H indole), 7.66 (4 H, s, H phthalimide), 8.25 (1 H, br s, NH); TLC (system B) 0.50 (UV,

I_2); MS (DCI/CH_4), m/z (relative intensities) 411 (MH^+, 100), 379 (6), 160 (21).

Methyl 2-(Fluoromethyl)-3-(5-hydroxy-3-indolyl)-2-phthalimidopropionate (7c). Compound **7c** was prepared from **7b** (7.38 g, 0.018 mol) in a manner similar to that described for the synthesis of **8c** from **8b**. Two grams of **7c** was isolated after purification by chromatography (silica gel, ethyl acetate/cyclohexane, 4:6): ^1H NMR ($CDCl_3$) δ 3.70 (3 H, s, CO_2CH_3), 3.80 (2 H, AB, J_{AB} = 15 Hz, CH_2), 5.06 (2 H, AB part of ABX, J_{AB} = 9 Hz, $J_{AX} = J_{BX} = J_{HF}$ = 47 Hz, CH_2F), 6.40–7.30 (4 H, m, H indole), 7.63 (4 H, s, H phthalimide), 8.00 (1 H, br s, NH); TLC (system A) 0.43 (UV, I_2).

2-(Fluoromethyl)-5-hydroxytryptophan Methyl Ester (9b). Compound **9b** was prepared from **7c** (2.3 g, 5.8 mmol) in 71% yield in a manner similar to that described for the synthesis of **10a** from **8a**. **9b** (1.1 g) was isolated after purification by chromatography (silica gel, ethyl acetate) as a white solid: ^1H NMR ($CDCl_3$ + CD_3OD), 3.00 (2 H, AB, J_{AB} = 14 Hz, CH_2), 4.58 (2 H, AB part of ABX, J_{AB} = 9 Hz, $J_{AX} = J_{BX} = J_{HF}$ = 47 Hz, CH_2F), 6.58–7.37 (4 H, m, aromatic); ^{19}F NMR ($CDCl_3/C_6F_6$) −63.92 (t, J(HF) 47 Hz); UV_{max} (CH_3CN) 204 nm (ϵ 23 235), 220 (sh), 277 (5598), 294 (4262), 310 (sh); TLC (system E) 0.65 (UV, ninhydrin); MS (DCI/CH_4), m/z (relative intensities) 267 (MH^+, 100), 250 (27), 146 (37). Anal. ($C_{13}H_{15}FN_2O_3$) H; C: calcd, 58.64; found, 58.13. N: calcd, 10.52; found, 9.55.

2-(Fluoromethyl)-5-hydroxytryptophan (11b). Compound **11b** was prepared from **9b** in a manner similar to that described for the synthesis of **12b** from **10b**, with 0.1 N NaOH (2.4 equiv). After completion of the reaction, a solution of 0.1 N HCl (2.4 equiv) was added. The solvent was removed in vacuo, leaving a solid, a mixture of **11b** and sodium chloride (2.4 equiv), which was tested as such: ^1H NMR (D_2O) δ 3.30 (2 H, AB, J_{AB} = 14 Hz, CH_2), 4.82 (2 H, AB part of ABX, J_{AB} = 10 Hz, $J_{AX} = J_{BX} = J_{HF}$ = 46 Hz, CH_2F), 6.80–7.45 (4 H, m, aromatic); ^{19}F NMR (D_2O/CF_3CO_2H) −151.3 (t, J_{HF} = 46 Hz); UV_{max} (H_2O) 203 nm (ϵ 19 045), 275 (4341), 293 (3613); TLC (system G) 0.62 (UV, ninhydrin); HPLC, one major peak (95%), 5.04 ($t_R(A)$), 5.11 (t_R(B)); minor contamination by **9b** (5%), 13.96 ($t_R(A)$), 14.04 t_R(B)); MS (CDI/NH_3), m/z (relative intensities) 134 (100), 148 (44).

Biochemistry. Preparation of Partially Purified Tryptophan Hydroxylase and Assay. Tryptophan hydroxylase was extracted from the brain stem of rats by the procedure of Hamon et al.[20] This supernatant either was used as enzyme source or was further purified by affinity chromatography on a dimethyltetrahydropteridine/agarose column.[21] The assays on the different tryptophan analogues were run under the conditions described by Hamon et al.[22] The formation of the 5-hydroxytryptophan derivatives was followed by reversed-phase HPLC with fluorometric detection.[14]

Aromatic Amino Acid Decarboxylase Preparation, Assay, and Inhibition. As in previous studies, AADC for in vitro work was partially purified from hog kidney. Methods of assay and inhibition and ex vivo measurements have all been previously described.[6-9]

In Vivo Studies. Male Sprague–Dawley rats (200–250 g) were used. Drugs were given orally as aqueous solutions containing 1% ascorbic acid. At appropriate times, the animals were decapitated, and the brain was split sagitally: half was used for AADC activity determination after homogenization in 9 volumes of 50 mM potassium buffer, pH 7.2, containing 10^{-5} M of PLP and 10^{-2} M of mercaptoethanol,[5,8] and the other half was homogenized in 0.2 N $HClO_4$ containing α-methyl-Dopa as an internal standard and was used for the determination of catecholamine, indoleamine, amino acids, and acidic metabolites by reversed-phase HPLC with electrochemical detection.[14] Treated animals were compared to a group of control animals, that received the vehicle, i.e, an aqueous solution of 1% ascorbic acid.

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