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(*E*) and (*Z*)-β-Fluoromethylene-*m*-Tyrosines: Resolution and Determination of Configuration

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Abstract: The geometrical isomers of β-fluoromethylene-*m*-tyrosine (*E* and *Z*) were prepared and the *R* and *S* enantiomers of the biologically active (*E*)-isomer were separated with an enantiomeric excess >95% by the kinetic resolution of the racemic mixture using the enzyme α-chymotrypsin. The enantiomers of the (*Z*)-isomer were obtained by an acid catalyzed isomerization reaction. The absolute configurations of the isolated enantiomers were determined based on their CD spectral characteristics and chiral HPLC behavior. The *R* and *S* enantiomers of (*E*)-β-fluoromethylene-*m*-tyrosine were also analyzed by single crystal X-ray crystallography.

INTRODUCTION

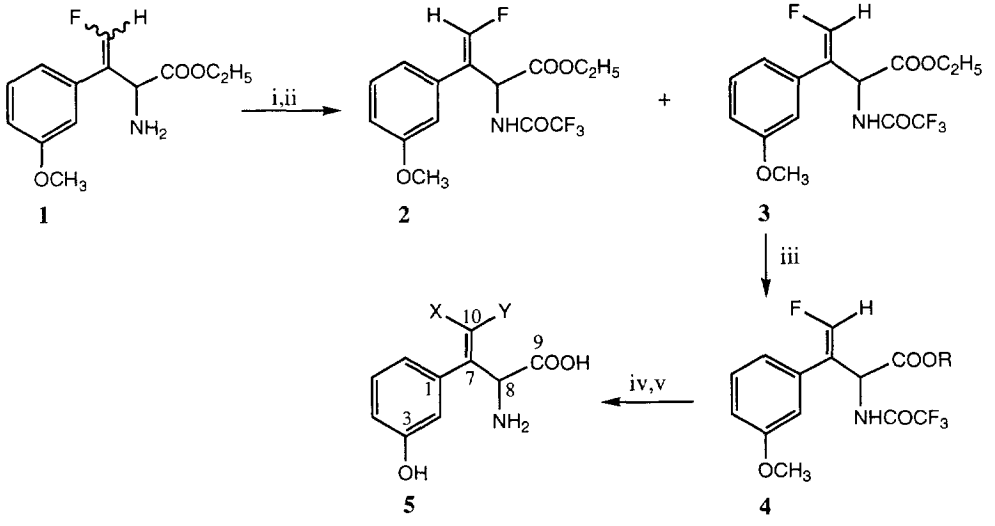
(*E*)-β-Fluoromethylene-*m*-tyrosine [(*E*)-FMMT] has been synthesized as a member of the enzyme-activated irreversible inhibitors.[1] In the brain, (*E*)-FMMT is decarboxylated by the enzyme aromatic *L*-amino acid decarboxylase (AAAD; EC 4.1.1.26) present in monoamine nerve terminals to yield (*E*)-β-fluoromethylene-*m*-tyramine which selectively inhibits the enzyme monoamine oxidase (MAO; EC 1.4.3.4).[2,3] MAO is a key enzyme in dopamine neurotransmission, which is linked to neurological disorders such as Parkinson's and Huntington's diseases as well as depression[4] and the effects of drugs of abuse (e.g., cocaine, methamphetamine).[5] Non-invasive mapping of MAO present in monoamine neurons in human brain can be advantageously followed using an appropriately radiolabeled (*E*)-FMMT and positron emission tomography (PET).[6] Radiosynthetic efforts in this regard have previously utilized ^{18}F-fluorination of a *D,L*-mixture of (*E*)-FMMT[7] or a protected derivative of (*E*)-FMMT.[8] In both cases racemic products of aromatic ring fluorinated derivatives of (*E*)-FMMT were isolated and utilized in animal experiments. While these racemic mixtures have been used for the preliminary PET studies, enzyme specificity appears restricted

to the L-enantiomer.[1b] The presence of positron emitter labeled D-amino acids generally complicates the quantitation of the kinetic data.[6]

To probe *in vivo* MAO present in monoamine neurons, closely associated with AAAD,[9] we have investigated the preparation of the enantiomers of (*E*) and (*Z*)-FMMT. Reported herein are the resolution of the enantiomers of (*E*)-FMMT and their corresponding (*Z*)-isomer counterparts. The absolute configurations of all these enantiomers were established based on circular dichroism spectral data and correlated with analytical chiral HPLC characteristics.

RESULTS AND DISCUSSION

Synthesis. The synthesis of racemic (*E*)-β-fluoromethylene-*m*-tyrosine (**5a** + **5c**) was achieved by two general routes.[1a,c] The first route involving a Wittig-type reaction yielded predominantly the (*E*)-isomer while the second one incorporating an oxazoline intermediate gave a mixture of (*E*)- and (*Z*)-isomers. In spite of the overall low yields of the final product,[10] the first method is preferred because of the better control of stereochemistry. In our attempts to prepare the pure isomers **2** and **3** as substrates for the enzyme-catalyzed hydrolysis and separation of enantiomers, we followed the Wittig route for the preparation of (*E*) and (*Z*) mixture of the protected derivative **1**.[1c] Protection of the amino group in **1** yielded the trifluoroacetyl derivatives **2** and **3** [(*Z*)/(*E*) = 15/85] which were separated easily by flash chromatography (Scheme 1).



a: X=F; Y=H (*E,S*) c: X=F; Y=H (*E,R*) a: R=H; (*S*) b: R=C_2H_5; (*R*)
b: X=H; Y=F (*Z,S*) d: X=H; Y=F (*Z,R*)

i = $(CF_3CO)_2O$; ii = Chromatography; iii = α-Chymotrypsin;
iv = HBr; v = Amberlite IRA 400 (acetate).

Scheme 1

Separation of Enantiomers. The pure geometrical isomers **2** and **3** were subjected to enzyme catalyzed hydrolysis for the separation of the enantiomers. α-Chymotrypsin is an enzyme that primarily and specifically catalyzes the hydrolysis of amide bonds of proteins and peptides containing aromatic *L*-amino acid residues of tryptophan, tyrosine and phenylalanine.[11] This enzyme which also catalyzes the hydrolysis of esters of simple aromatic *L*-amino acids[12] was chosen for the enzymatic resolution of racemic FMMT derivatives. The protected derivatives **2** and **3** were thus subjected to α-chymotrypsin enzyme catalyzed hydrolysis. Poor solubility of both isomers in aqueous media required the use of organic co-solvents. The presence of organic solvents in analogous reactions is known to cause a reduction in the rate of hydrolysis[13] and the enzyme selectivity.[14] However, the enzyme α-chymotrypsin retains its activity even at high ratios of organic solvents.[15] Thus, the racemic (*E*)-isomer **3** in DMF (8% v/v organic to aqueous phase) was hydrolyzed in an ammonium acetate solution of α-chymotrypsin at pH 8.0[16] to yield the derivatives **4a** and **4b**. Interestingly, the nature of the organic solvent did have an effect on the course of the enzyme hydrolysis of **3**. For example, when methanol was substituted for DMF, **4a** was obtained only in 13% yield while the corresponding methyl ester was the major product. The reaction of the acyl-enzyme intermediate,[17] formed between **4a** and α-chymotrypsin, with methanol is facile and leads to the formation of the methyl ester. This reaction is reminiscent of the selective esterification of N-acetyl-*L*-tyrosine and N-acetyl-*L*-tryptophan mediated by α-chymotrypsin in water-ethanol medium.[18]

Hydrolysis of the racemic (*Z*)-isomer **2** was unsuccessful under the conditions utilized for the reaction of **3**. When a mixture of **2** and **3** was hydrolyzed, only the *E*-isomer served as the substrate for the enzyme. However, no data was obtained on the interaction of the (*Z*)-isomer **2** with α-chymotrypsin. It could be speculated that the geometrical difference between the amino acid derivative **2** (*Z*) and **3** (*E*) alters the orientation and the distance between a distal end of the aromatic ring and the α-carbonyl group of the ester moiety, and thus affecting the ability of the molecule to fit productively into the active site of α-chymotrypsin.[19] Steric hindrances that arise from β-branching of the side chain in aromatic *L*-amino acids have been shown generally to decrease the reactivity of such substrates towards α-chymotrypsin catalyzed hydrolysis.[20] However, stereomodels do not display any convincing differences between **2** and **3** that could account for the enzyme selectivity. Interestingly, the Michaelis-Menten constant (Km) of the racemic (*Z*)-FMMT for the enzyme aromatic *L*-amino acid decarboxylase is an order of magnitude higher than that of the *E*-isomer[1c] and the structurally related (*Z*)-2-(3,4-dihydroxyphenyl)-3-fluoroallylamine is a six times less potent inhibitor of semicarbazide-sensitive amine oxidase than its *E* counterpart.[21]

It has been recently reported that carbonic anhydrase (carbonate hydrolyase, EC 4.2.2.1) selectively hydrolyses the *D*-enantiomers of N-acetylamino acid methyl esters.[22] However, this enzyme failed to hydrolyze the derivative **3** suspended in phosphate buffer at pH 7.5 or dissolved in acetonitrile.

Deprotection of the derivatives of (*E*)-FMMT (**4a** and **4b**) was achieved by acid hydrolysis with 48% HBr. The geometry of the double bond was retained in the products (**5a** and **5b**) when the hydrolysis was performed in the dark. It was also observed that the hydrolysis was complete in 30 min under this condition. A similar result was obtained with phenol added as a scavenger of the elemental bromine that could be formed during HBr hydrolysis[23] and potentially influencing the (*E*) to (*Z*) isomerization.[2] Hydrobromides of enantiomeric amino acids thus obtained were treated with Amberlite IRA-400 (acetate form) to yield the free

amino acids (Scheme 1). Since an enzymatic resolution of the enantiomers of (*Z*)-FMMT (**5b** and **5d**) could not be achieved, a preparative HPLC method was resorted to. When the hydrolysis of **4a** (or **4b**) was conducted under ambient light conditions for 4h, the product contained a mixture of **5a** and **5b** (or **5c** and **5d**) in roughly equal amounts; the mixture was separated on a C-18 reversed phase HPLC column. The isolated amino acids **5a-d** all exhibited excellent enantiomeric purities (see experimental section).

Determination of Configurations. The configurations of the resolved enantiomers of (*E*) and (*Z*)-FMMT were assigned by comparison of their circular dichroism (CD) spectra with those of the related aromatic α-amino acids of known configurations. It has been shown that compounds of a given family with the same configurations exhibit CD spectra of similar pattern and magnitude.[24] For example, positive Cotton effect curves around 200-220 nm were recorded for a variety of natural *L*-amino acids[25] as well as unnatural *L*-amino acids with olefinic chromophores in the side chain[26] and all of them were shown to have *S* configuration. Further, aromatic amino acids with *S* configurations generally exhibit a relatively weak negative Cotton effect band around 260 nm.[24] Based on these generalizations, the configurations of α-methyl aromatic α-amino acids,[27] and more recently 2,3-methano aromatic amino acids,[28] have been assigned.

The room temperature CD spectra of the enantiomers **5a-d** in water are shown in Figure 1.

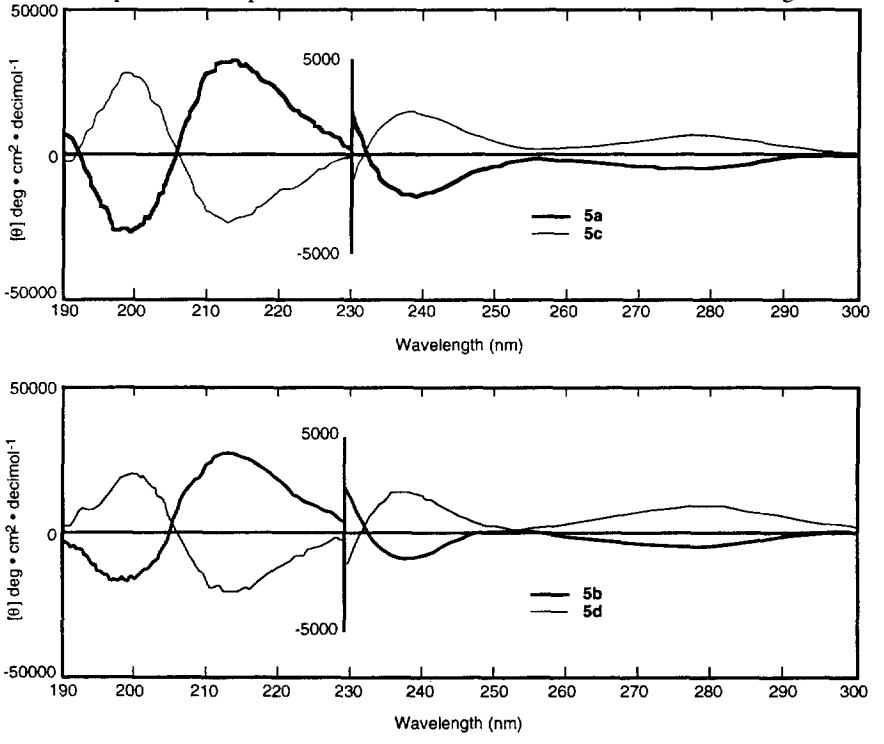
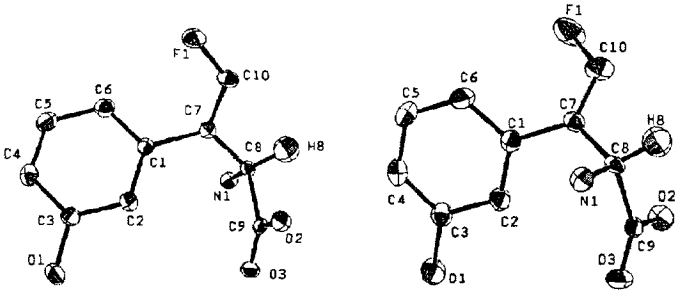


Figure 1. Circular dichroism spectra of the enantiomers of (*E*)- and (*Z*)-FMMT.

Each enantiomer exhibited four different Cotton effect bands. The Cotton effect appearing around 240 nm is attributed to the $n \rightarrow \pi^*$ transition of the carboxyl group while the other three electronic absorption bands are associated with the phenyl group.[29] The strong absorption bands observed near 199 nm and 214 nm are designated in Platt's terminology[30] as the $^1B_{a,b}$ and ^1L_a transitions, respectively.[29] The weak band around 280 nm is associated with the ^1L_b transition. The electronic absorption due to the fluorovinyl group is probably masked by the phenyl absorption bands. The strong positive Cotton effect ^1L_a bands and the weak negative ^1L_b exhibited by the (*E*) and (*Z*)-isomers **5a** and **5b** are akin to *L*-*m*-tyrosine[31] and α-methyl aromatic α-amino acids[27] with *S* configuration. The CD spectra of the (*E*) and (*Z*)-isomers **5c** and **5d** were similar in magnitude, but opposite in sign to those of **5a** and **5b**, indicating these to be *R* and *S* enantiomers, respectively. Similarly observed Cotton effect bands have been used to assign the configurations of 2,3-methano-*m*-tyrosines and 2,3-methanophenylalanines.[28]

The configurational assignments based on CD spectral data were corroborated by the chiral HPLC elution characteristics of the enantiomers. The chiral ligand exchange HPLC system (see experimental section) used in this regard utilized a column with *L*-proline as the selector ligand bonded to C-18 stationary phase and a mobile phase containing Cu (II) ions.[32] In this chiral HPLC system, the *D* (*R*)-enantiomers of phenylalanine, tyrosine and dopa eluted before the corresponding *L* (*S*)-enantiomers. A similar elution pattern was recently observed for fluorinated dopa and *m*-tyrosine analogs.[33] Accordingly, the *S* enantiomers of (*E*) and (*Z*)-isomers **5a** and **5b**, (the configurations assigned based on their CD spectral characteristics) were retained longer in the chiral HPLC column than their corresponding *R* enantiomers **5c** and **5d**. This chiral HPLC system also enabled the determination of the enantiomeric excess of the amino acids **5a-d**.

X-ray Crystal Structure Analyses. The enantiomers **5a** and **5c**, were also investigated by X-ray structural analysis with samples crystallized from water. The ORTEP plots of these derivatives are shown in Figure 2.



5a **5c**

Figure 2. X-ray crystal structures of **5a** and **5c**

The overall geometry of **5a** and **5c** resembled those of ethyl 2-amino-3-(3-methoxyphenyl)-4-fluoro-3-butenoate,[1c] dopa,[34] α-methyldopa,[35] tyrosine,[36] *o*- and *m*-tyrosines[37] and α-methyl-*m*-tyrosine.[38] The presence of the fluorovinyl moiety did, however, have a profound effect on several bond angles. For instance, the bond angles C(1)-C(7)-C(8) in **5a** and **5c** (120°) were wider than the corresponding angles in α-methyl-*m*-tyrosine (116.6°)[38] and *m*-tyrosine (114.3°).[37b] Further, the fluorovinyl group generally caused a widening of the angle C(7)-C(8)-C(9) by ~5° in comparison to the corresponding angle in *m*-tyrosine[37b] or dopa.[34] It is quite interesting to note that the fluoromethylene group had an impact on the O(1)-C(3) bond. For example, the bond angle O(1)-C(3)-C(4) was increased by 4.2° while C(2)-C(3)-O(1) was decreased by 4.6° in **5a** in comparison to the related angles in *m*-tyrosine. An analogous shift in the bond angles has been observed between α-methyl-*m*-tyrosine[38] and *m*-tyrosine.[37b] However, such an effect has not been detected between dopa[34] and α-methyldopa.[35] Unfortunately, all attempts to establish the absolute configurations of the enantiomeric pairs **5a** and **5b** based on the anomalous scattering of the oxygen atom[39] were met with limited success.

SUMMARY

We have demonstrated the use of the enzyme α-chymotrypsin for the resolution of the *R* and *S* enantiomers of (*E*)-FMMT. The facile isomerization of the exocyclic double bond, also permitted the isolation of the enantiomers of (*Z*)-FMMT from the corresponding (*E*)-isomers.

EXPERIMENTAL SECTION

General. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. NMR spectra were recorded with a Bruker AM-360 WB spectrometer (operating at 360.14 MHz for ^{1}H; 90.57 MHz for ^{13}C; 338.87 MHz for ^{19}F). Chemical shifts were referenced to internal (TMS for ^{1}H and ^{13}C) or external (DSS for ^{1}H, 1,4-dioxane at 67.4 ppm for ^{13}C and $CFCl_3$ for ^{19}F) standards. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Ultraviolet spectra were recorded with a Beckman DU-50 spectrophotometer. Circular dichroism spectra in water at 20-22°C were obtained with a JASCO J-600 spectropolarimeter in the spectral region of 190-300 nm. The sample cell length was 1cm for measurements in the 190-300 nm region and 0.1cm for the 230-300 nm region. The slit was programmed for a spectral band width of 1.0 nm. Sample concentrations for the molecular ellipticity parameter [θ] are expressed in mM. High resolution electron impact (EI HRMS) mass spectral data were collected on a VG Analytical AutoSpec mass spectrometer and fast atom bombardment high resolution mass spectral data (FAB HRMS) were collected on a ZAB 7070 mass spectrometer. The elemental analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, TN. Enantiomeric and isomeric purities of amino acids were assessed by analytical HPLC [Chiral Pro Cu=Si 100 Polyol column (Serva), 5 μm, 4.6 x 250 mm; eluent 1 mM $CuSO_4$, 50 mM KH_2PO_4 , pH 4.0, flow rate 1 mL/min; uv detection at 279 nm and Econosphere C18 3μ column (Alltech), 4.6 x 150 mm; 0.02 M sodium acetate, pH 3.5, flow rate 1 mL/min; uv detection at 279 nm, respectively]. Semipreparative HPLC separations and purifications of (*E*) and (*Z*)-isomers of

enantiomeric amino acids were performed with an Econosil C18 semipreparative column (Alltech), 10 μm, 500 x 10 mm; mobile phase A: 3% methanol in 0.1% acetic acid, flow rate 5 mL/min; uv detection at 279 nm. HPLC system included Beckman 110B solvent delivery module and Kratos Spectroflow 773 uv absorbance detector. The enzymes α-chymotrypsin and carbonic anhydrase were purchased from Sigma.

(*Z*) and (*E*)-Ethyl 2-trifluoroacetylamino-3-(3-methoxyphenyl)-4-fluoro-3-butenoate (**2** and **3**). Ethyl 2-amino-3-(3-methoxyphenyl)-4-fluoro-3-butenoate (**1**) (4.70 g, 18.8 mmol) obtained as a yellow oil[1c] was cooled in an ice bath and trifluoroacetic anhydride (37.2 g, 177 mmol) at 0°C was added. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The trifluoroacetic anhydride was removed under reduced pressure and the oily product was coevaporated three times with ethanol (3 x 100 mL). The residue was dissolved in dichloromethane (200 mL) and consecutively washed with water, saturated $NaHCO_3$ and water. The organic extract was dried ($MgSO_4$), filtered and evaporated to leave a dark brown oil which solidified upon standing in a desiccator. Flash chromatography of this product on silica gel (10% ether in petroleum ether) provided the two isomers **2** and **3**. The *Z*-isomer (**2**) (622 mg, 10%) was obtained as a pale yellow oil (analytical sample distilled at 110 -116°C/0.06 mm Hg), while the *E*-isomer (**3**) crystallized as colorless needles (3.96 g, 61%). Recrystallization of **3** from petroleum ether afforded the analytical sample, mp 59-60°C.

***Z*- isomer (2):** ^{1}H NMR ($CDCl_3$/TMS) δ 1.30 (t, 3H, J = 7.2 Hz, CH_3), 3.81 (s, 3H, OCH_3), 4.30 (m, 2H, CH_2), 5.65 (d, 1H, J = 7.3 Hz, CH), 6.82 (m, 2H, H-2, H-4), 6.84 (d, $^2J_{H,F}$ = 82.0 Hz, 1H, CHF), 6.90 (dd, 1H, J = 2.5 Hz and 8.0 Hz, H-6), 7.22 (broad d, 1H, NH), 7.27 (t, 1H, J = 8.0 Hz, H-5); ^{19}F NMR ($CDCl_3$/$CFCl_3$) δ -76.3 (s, CF_3), -122.6 (d, $^2J_{H,F}$ = 82.0 Hz, CHF); UV (96% EtOH) λ_{sh} 239 nm (ε = 4940), λ_{max} 280 nm (ε = 1910). Anal. Calcd for $C_{15}H_{15}NO_4F_4$: C, 51.58; H, 4.33; N, 4.01; F, 21.76. Found: C, 51.83; H, 4.39; N, 4.50; F, 21.42.

***E* - isomer (3):** ^{1}H NMR ($CDCl_3$/TMS) δ 1.27 (t, 3H, J = 7.3 Hz, CH_3), 3.79 (s, 3H, OCH_3), 4.26 (q, 2H, J = 7.3 Hz, CH_2), 5.18 (d, 1H, J = 7.1 Hz, CH), 6.74 (m, 2H, H-2, H-4), 6.90 (dd, 1H, J = 2.0 Hz and 7.5 Hz, H-6), 7.02 (d, 1H, $^2J_{H,F}$ = 81.0 Hz, CHF), 7.09 (broad d, 1H, NH), 7.29 (t, 1H, J = 7.5 Hz, H-5); ^{19}F NMR ($CDCl_3$/$CFCl_3$) δ -76.3 (s, CF_3), -121.9 (d, $^2J_{H,F}$ = 81.0 Hz, CHF). UV (96% EtOH) λ_{sh} 242 nm (ε = 4520), λ_{max} 280 nm (ε = 1710). Anal. Calcd for $C_{15}H_{15}NO_4F_4$: C, 51.58; H, 4.33; N, 4.01. Found: C, 51.60; H, 4.37; N, 3.94.

***S*-(*E*)-2-Trifluoroacetylamino-3-(3-methoxyphenyl)-4-fluoro-3-butenoic acid (4a).** A solution of **3** (3.49 g, 10 mmol) in dimethylformamide (15 mL) was added dropwise over a period of 15 min to a solution of α-chymotrypsin (250 mg) in 0.5 M ammonium acetate (200 mL) at pH 8.0. Upon the addition of **3** a precipitate formed and a small drop in pH was observed. After 2 h, 1N NH_4OH was added to readjust the pH to 8.0. The reaction mixture was stirred for 24 h at room temperature. The precipitated solid was dissolved in dichloromethane (200 mL) and the organic phase containing **4b** and some unreacted **3** was separated. The above enzyme hydrolysis procedure was repeated with the recovered **3/4b** mixture. The aqueous phase was

acidified to pH 3.0 with 1N HCl and extracted with ethyl acetate (3 x 150 mL). The ethyl acetate extracts were combined, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a solid residue. Flash chromatography of this solid product on silica gel (eluent: 20% methanol in dichloromethane) gave 1.15 g (71%) of pure **4a**, mp 170 - 172°C. $[\alpha]_D^{20}$ + 99.0 (c 1.0, H_2O); UV (96% EtOH) λ_{sh} 240 nm (ε = 2850), λ_{max} 279 nm (ε = 1280); ^1H NMR (D_2O/DSS) δ 3.82 (s, 3H, OCH_3), 5.02 (s, 1H, CH), 6.92 - 6.97 (m, 2H, H-2, H-4), 7.00 (dd, 1H, J = 2.1 Hz and 8.1 Hz, H-6), 7.08 (d, 1H, $^2J_{H,F}$ = 82.0 Hz, CHF), 7.38 (t, 1H, J = 8.1 Hz, H-5); ^{19}F NMR (D_2O/$CFCl_3$) δ -73.68 (s, CF_3), -123.80 (d, $^2J_{H,F}$ = 82.0 Hz, CFH); HRMS Calcd for $C_{13}H_{11}NO_4F_4$: 321.0624. Found: 321.0621.

***R*-(*E*)-Ethyl-2-trifluoroacetylamino-3-(3-methoxyphenyl)-4-fluoro-3-butenoate (4b)**. The *R* enantiomer **4b** was obtained as a crystalline solid upon evaporation of the dichloromethane extract (see above). The analytical sample was obtained by recrystallization from petroleum ether, mp 58-60°C; $[\alpha]_D^{20}$-15.0 (c 1.25, EtOH 98%); UV (96% EtOH) λ_{sh} 242 nm (ε = 4490), λ_{max} 281 nm (ε = 1730); The ^1H and ^{19}F NMR spectra of this product were identical to those of the racemic derivative **3**. Anal. Calcd for $C_{15}H_{15}NO_4F_4$: C, 51.58; H, 4.33; N, 4.01. Found: C, 51.42; H, 4.57; N, 3.83.

***S*-(*E*)-β-Fluoromethylene-*m*-tyrosine (5a)**. A solution of **4a** (160 mg, 0.5 mmol) in 48% HBr (2mL) (freshly distilled from $SnCl_2$) was refluxed for 30 min in dark. The HBr was evaporated under reduced pressure, the residue dissolved in water (5 mL) and passed through an Amberlite resin (acetate form) (5 mL) column. [The resin was regenerated by passing 3% aqueous acetic acid through Amberlite IRA-400 (OH)]. The column was eluted with water (10 x 2.5 mL). Ninhydrine positive fractions were pooled and lyophilized to obtain 100 mg (95%) of **5a**, mp 218-220°C (d) (water); $[\alpha]_D^{20}$ + 36.0 (c 1.0, H_2O); UV (H_2O) λ_{max} 216 nm (ε = 7173), λ_{sh} 239 nm (ε = 3700), λ_{max} 279 nm (ε = 1920); (ee 96%, chiral HPLC); CD (c 0.284, H_2O) $[\theta]_{277}$ -740, $[\theta]_{240}$ -2180, $[\theta]_{232}$ 0,$[\theta]_{214}$ +32130,$[\theta]_{205}$ 0,$[\theta]_{198}$ -25550,$[\theta]_{192}$ 0; ^1H NMR (D_2O/DSS) δ 4.49 (s, 1H, CH), 6.83 - 6.88 (m, 2H, H-2, H-4), 6.93 (dd, 1H, J = 2.2 Hz and 8.3 Hz, H-6), 7.12 (d, 1H, $^2J_{H,F}$ = 81.0 Hz, CHF), 7.34 (t, 1H, J = 8.3 Hz, H-5); ^{13}C NMR (D_2O/1,4-dioxane) δ 56.4 (d, $^3J_{C,F}$ = 3.7 Hz, C-8), 116.7 (C-2), 117.0 (C-4), 119.0 (d, $^2J_{C,F}$ = 5.4 Hz, C-7), 121.9 (C-6), 131.3 (C-5), 132.1 (C-1) 151.1 (d, $^1J_{C,F}$ = 267.7 Hz, C-10), 156.7 (C-3), 72.4 (C-9); ^{19}F NMR (D_2O/$CFCl_3$) δ -118.90 (d, $^2J_{H,F}$ = 81.0 Hz, CFH). Anal. Calcd for $C_{10}H_{10}NO_3F$: C, 56.87; H, 4.77; N, 6.63. Found: C, 56.65; H, 4.71; N, 6.55.

***S*-(*Z*)-β-Fluoromethylene-*m*-tyrosine (5b)**. A solution of **4a** (100 mg, 0.3 mmol) in 48% HBr (2mL) was refluxed for 4 h, and worked up as described above. ^1H NMR spectrum of the product (59 mg, 90%) showed a mixture of *E/Z* isomers (60/40). These isomers were separated by the semipreparative HPLC using mobile phase A (described above) to give 17 mg of pure **5b**, mp 168-170°C (d); $[\alpha]_{20}^{D}$ + 67.3 (c 0.40, H_2O); UV (H_2O) λ_{max} 214 nm (ε = 7609), λ_{sh} 239 nm (ε = 3640), λ_{max} 280 nm (ε = 1630); (ee 90%, chiral HPLC); CD (c 0.265, H_2O) $[\theta]_{278}$ -860, $[\theta]_{238}$ -1430,$[\theta]_{233}$ 0,$[\theta]_{213}$ +27360,$[\theta]_{205}$ 0,$[\theta]_{198}$ -16440; ^1H NMR (D_2O/DSS) δ 4.99 (s, 1H, CH), 6.84 (d, 1H, J= 2.0 Hz, H-2), 6.89 - 6.95 (m, 2H, H-4, H-6), 7.08 (d, 1H, $^2J_{H,F}$ = 83.0 Hz, CHF), 7.32 (t, 1H, J = 8.0 Hz, H-5); ^{13}C NMR (D_2O/1,4-dioxane) δ 52.1 (d, $^3J_{C,F}$ = 3.7 Hz, C-8), 115.8

(C-2), 116.8 (C-4), 119.6 (d, $^2J_{C,F}$ = 6.5 Hz, C-7), 121.1 (C-6), 131.3 (C-5), 133.8 (d, $^3J_{C,F}$ = 5.8 Hz, C-1), 151.4 (d, $^1J_{C,F}$ = 270 Hz, C-10), 156.7 (C-3), 172.4 (C-9); ^{19}F NMR ($D_2O/CFCl_3$) δ -119.3 (d, $^2J_{H,F}$ = 83.0 Hz, CFH). HRMS: Calcd for $C_{10}H_{10}NO_3F$: 211.0644. Found: 211.0633.

***R*-(*E*)-β--Fluoromethylene-*m*-tyrosine (5c).** A suspension of **4b** (400 mg, 1.15 mmol) in 48% HBr (4 mL) was refluxed in the dark for 30 min and worked up as described earlier for **5a**. Yield 217 mg (90%), mp 222 - 224°C (d); $[\alpha]_D^{20}$ - 36.0 (c 1.0, H_2O); UV (H_2O) λ_{max} 214 nm (ε = 7077), λ_{sh} 240 nm (ε = 3130), λ_{max} 279 nm (ε = 1740); (ee 96%, chiral HPLC); CD (c 0.284, H_2O) $[\theta]_{277}$ +980, $[\theta]_{239}$ +2260, $[\theta]_{232}$ 0, $[\theta]_{213}$ - 22920, $[\theta]_{206}$ 0, $[\theta]_{199}$ +28050,$[\theta]_{191}$ 0; The ^{1}H, ^{13}C and ^{19}F NMR spectra of this compound were identical to those of **5a**. Anal. Calcd for $C_{10}H_{10}NO_3F.H_2O$: C, 52.40; H, 5.28; N, 6.11. Found: C, 52.58; H, 5.03; N, 6.21.

***R*-(*Z*)-β-Fluoromethylene-*m*-tyrosine (5d).** A suspension of **4b** (200 mg, 0.57 mmol) in 48% HBr (2mL) was refluxed for 4 hours under ambient light conditions and worked up as described above to yield 102 mg (84%) of *E/Z* mixture (40/60). This isomeric mixture was separated by the semipreparative HPLC using mobile phase A (described earlier) to give 45 mg of pure **5d**, mp 180 - 182°C (d); $[\alpha]_D^{20}$ - 69.1 (c 0.73, H_2O); UV (H_2O) λ_{max} 215 (ε = 7547), λ_{sh} 239 nm (ε = 3640), λ_{max} 280 nm (ε = 1630); (ee 96%, chiral HPLC); CD (c 0.249, H_2O) $[\theta]_{278}$ +1320, $[\theta]_{238}$ +2250, $[\theta]_{232}$ 0, $[\theta]_{213}$ -20000, $[\theta]_{206}$ 0, $[\theta]_{199}$ +21750; The NMR spectral characteristics of this product were identical to those of **5b**. Anal. Calcd for $C_{10}H_{10}NO_3F.\ 0.75H_2O$: C, 53.45; H, 5.16; N, 6.23. Found: C, 53.28; H, 5.01; N, 6.01.

Single Crystal X-ray Diffraction Analysis. Colorless, needle shaped crystals of **5a** and **5c** were obtained by slow crystallization from water at 4°C. Crystal data, data collection parameters and refinement results are provided in Table 1. The unit cell parameters of each crystal were determined by a least squares fit to the ± 2θ of 25 reflections distributed through all octants of reciprocal space and measured using graphite monochromated CuKα or MoKα radiation. Three standard reflections were measured after every 97 reflections to monitor the intensity and orientation and found no significant variations. The intensity data were corrected for Lorentz and polarization effects. No absorption correction was applied. The structures were solved by applying the direct methods using the SHELXS86[40] computer program. The refinement was performed by a full-matrix least squares routine. The heavy atoms were refined anisotropically while the hydrogen atoms (excluding water molecules) were considered riding and refined with fixed isotropic U. The atomic coordinates for the X-ray structures of **5a** and **5c** have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, United Kingdom.

Table 1. Crystal Data, Data Collection and Refinement Parameters

	5a	**5c**
formula	$C_{10}H_{10}O_3NF$	$C_{10}H_{10}NO_3F$
fw	211.19	211.19
cryst syst	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$
cryst dimens, mm	0.30 X 0.25 X 0.22	0.10 X 0.10 X 0.25
a, Å	10.816 (2)	10.891 (3)
b, Å	14.863 (2)	14.927 (5)
c, Å	5.779 (1)	5.777 (1)
Z	4	4
V, $Å^3$	929.0 (3)	939.2 (5)
r (calcd), g cm^{-3}	1.51	1.49
radiation	Mo $K\alpha$	Cu $K\alpha$
abs coeff (μ), cm-1	1.17	10.20
F(000), e	440	440
temp, K	156	296
diffractometer	Picker (Crystal Logic)	AFC5R (Rigaku Rotating anode)
index ranges	h (0 to 15) k (0 to 20) l (0 to 8)	h (0 to 12) k (0 to 17) l (0 to 6)
scan mode, speed (deg/min)	θ-2θ, 6.0	θ-2θ, 16.0
2θ range, deg	1 - 60	1 - 120
total data collected	1604	896
unique data	1604	896
observed data used	994 ($F>6\sigma(F)$)	661 ($F>6\sigma(F)$)
no. of parms refined	136	136
final shift/error, max and avg	0.003, 0.001	0.004, 0.001
max resid density, e/$Å^3$	0.54	0.49
$R = \Sigma \lvert \lvert F_O \rvert - \lvert F_C \rvert \rvert / \Sigma \lvert F_O \rvert$	0.055	0.041
$R_W = (\Sigma w (\lvert F_O \rvert - \lvert F_C \rvert)^2 / \Sigma w(\lvert F_O \rvert)^2)^{1/2}$	0.063	0.052
$GOF = (\Sigma w (\lvert F_O \rvert - \lvert F_C \rvert)^2 / (No-Nv))^{1/2}$	1.713	1.275

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