Fluorination of (*E*)-β-(Fluoromethylene)-*m*-Tyrosine: Regioselective Synthesis of 4-Fluoro-(*E*)-β-(Fluoromethylene)-*m*-Tyrosine

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Received August 30, 1994[⊗]

Fluorination of (*R*)- and (*S*)-(*E*)-β-(fluoromethylene)-*m*-tyrosine (**1**) by acetyl hypofluorite yielded a mixture of the corresponding 2-fluoro- (**2a**), 6-fluoro- (**2b**), 4-fluoro- (**2c**), and 2,6-difluoro- (**2d**) derivatives along with a pair of diastereomeric products of addition across the vinylic double bond. A regioselective synthesis of 4-fluoro-(*E*)-β-(fluoromethylene)-*m*-tyrosine has also been developed based on a fluorodestannylation reaction with elemental fluorine followed by acid hydrolysis. This reaction sequence yielded minor byproducts, namely, 4-fluoro-(*Z*)-β-(fluoromethylene)-*m*-tyrosine (**11**), 4,6-difluoro- and 2,4-difluoro-(*E*)-β-(fluoromethylene)-*m*-tyrosines (**12a** and **12b**). All these products were completely separated by semipreparative HPLC and fully characterized by NMR and mass spectroscopy. Single crystal X-ray crystallographic analyses of 2-fluoro-, 4-fluoro-, and 6-fluoro-(*E*)-β-(fluoromethylene)-*m*-tyrosines unequivocally established the structures of these amino acids.

Introduction

The enzyme activated mechanism based inhibition of specific enzymes is a relatively recent concept that has significance in biochemical research.[1] This approach has led to the synthesis of novel inhibitors having both site selectivity as well as enzyme specificity.[2] For instance, (*E*)-β-(fluoromethylene)-*m*-tyrosine [(*E*)-FMMT] (**1**) has recently been developed as a prodrug[3] for the site selective inhibition of the mitochondrial enzyme monoamine oxidase (MAO), an enzyme that has implications in several brain degenerative diseases.[4] The amino acid (*E*)-FMMT in itself is not an inhibitor of MAO. In-vivo, the fluoromethylene-*m*-tyrosine **1** undergoes aromatic-L-amino acid decarboxylase (AAAD)-mediated decarboxylation in the monoaminergic neurons of the brain to produce (*E*)-β-(fluoromethylene)-*m*-tyramine.[5] This tyramine derivative discriminately inhibits MAO associated with monoamine nerve terminals.[6]

Probing of brain AAAD-MAO contained in dopaminergic terminals would be instrumental in the "biochemical dissection" of brain neurotransmission, especially in disease states (e.g., Parkinson's disease).[4] Positron emission tomography (PET), a noninvasive biochemical imaging technique,[7] would enable investigation of the MAO system in primates using a positron emitter labeled analog of the amino acid **1**. An interesting avenue in this regard would be substitution with the positron emitting ^{18}F ($t_{1/2}$ = 110 min) atom on the aromatic ring. Such an approach of fluorination of aromatic amino acids has yielded novel and useful biochemical probes.[8] Thus, to investigate the biochemical properties, and as a prelude to radiosyntheses, we now report fluorination of the recently resolved *R* and *S* enantiomers of (*E*)-β-(fluoromethylene)-*m*-tyrosine[9] and a regioselective synthesis of 4-fluoro-(*E*)-FMMT (**2c**) based on a fluorodestannylation reaction.

Results and Discussion

Direct Fluorination. Aromatic amino acids dissolved in acidic solvents have been reacted with electrophilic fluorinating agents such as fluorine or acetyl hypofluorite as a convenient technique for the preparation of the fluorinated analogs.[10] This procedure invariably yields a mixture of regioisomers which can be separated using semipreparative HPLC methods. The racemic (*E*)-FMMT in acetic acid/trifluoroacetic acid mixture was recently fluorinated with acetyl hypofluorite to yield a mixture of the corresponding 2-fluoro-, 6-fluoro-, and 2,6-difluoro analogs.[11] Since only the *S*-enantiomer of the natural amino acids is generally biologically active we have resolved the racemic amino acid **1**[9] using the enzyme chymotrypsin[12] and investigated the direct fluorination of the enantiomers.

Interestingly, in our hands, the reaction of acetyl hypofluorite with **1** (*R* and *S* enantiomers or racemic)

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1994.

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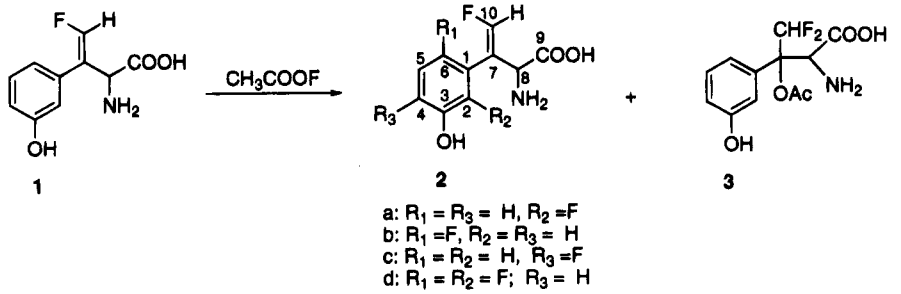
Scheme 1



gave a mixture of products that not only contained the ring fluorinated analogs **2a** (13%), **2b** (26%), and **2d** (6%) as reported earlier[11] but also a diastereomeric pair of addition products **3** (7%) as well as the 4-fluoro derivative **2c** (<1%) (Scheme 1). Using semipreparative HPLC, the mixture was completely separated and the structures of the ring fluorinated amino acids were determined based on ^1H and ^{19}F NMR, mass spectroscopy, and X-ray crystallography. The configurations of the fluoro analogs **2a**, **2b**, and **2d** obtained from the enantiomers of (*E*)-FMMT were analyzed by circular dichroism (CD) spectra. For example, *S* enantiomers of L-*m*-tyrosine[13] and several L-α-methyl aromatic α-amino acids[14] exhibit a strong positive Cotton effect ^1L_a band at 214 nm and a weak negative ^1L_b band at 280 nm. The fluorinated amino acids **2a**, **2b**, and **2d** obtained from the *S* enantiomer of **1** showed analogous Cotton effect curves suggesting a *S* configuration for these products. A similar comparison of CD spectra for the assignment of configurations of amino acids is well known.[15] Additional evidence for the configuration of these products was obtained from a copper (II)/L-proline ligand exchange chiral HPLC system[16] in which the *R* enantiomers of fluorinated aromatic amino acids eluted before the *S* isomers.[17] The isolated fluoro amino acids **2a**, **2b**, and **2d** obtained from *R* and *S* enantiomers of **1** also behaved analogously in this chiral HPLC system. Further, this chiral HPLC enabled the determination of the enantiomeric excess of the ring fluorinated amino acids.

The diastereomeric pair **3** was found to contain two isomers in a ratio of 9:1 by ^{19}F and ^1H NMR. The major diastereomer was isolated and analyzed by NMR and mass spectroscopy. Its configuration, however, was not determined.

Fluorodestannylation. It is generally known that the biochemical characteristics of fluorinated aromatic amino acids depend largely upon the position of the fluorine on the ring.[8] Based on the positive biochemical results obtained with 4-fluoro-*m*-tyrosine,[18] a derivative related to (*E*)-FMMT, it could be surmised that 4-fluoro-(*E*)-β-(fluoromethylene)-*m*-tyrosine (**2c**) could be an useful analog. However, very little information is available on this derivative primarily due to the difficulties encountered in its synthesis.[19] Further, the direct fluorination of the amino acid **1** gave **2c** only in very poor yields (vide supra). In general, the regioselectivity for the fluorination of aromatic compounds can be substantially improved using fluorodemetalation reactions. Aryl–mercury or Group IVb metal (Si, Ge, Sn) bonds are susceptible to fluorodemetalation reactions.[20,21] However, among such organometallics, the aryltin compounds have proven as the most versatile in their reaction toward an array of common fluorinating agents such as fluorine, acetyl hypofluorite, and oxygen difluoride.[22] Thus, the trimethylstannyl group substituted at C-4 on the aromatic ring of (*E*)-FMMT (**1**) has been chosen as a likely precursor for 4-fluoro-(*E*)-FMMT (**2c**). Further, such a precursor would readily allow the rapid synthesis of the ^{18}F-labeled counterpart of **2c**.[22]

The synthetic route employed for the preparation of the tin derivative **8** is shown in Scheme 2. Protection of phenolic functions with the t-Boc group has previously been shown to be advantageous during the fluorination reaction of the tin derivatives with F_2.[17b] However, the t-Boc derivative **5**, obtained from **4**, resisted all attempts at iodination. On the other hand, iodination of the free phenolic derivative **4** with trifluoroacetyl hypoiodite yielded the 4-iodo- (**6a**), 2-iodo- (**6b**), and 2,4-diiodo (**6c**) derivatives in a ratio 7:1:2, respectively. Interestingly, the presence of iodine at ortho positions, as in **6b** and **6c**, caused a steric hindrance to the free rotation around the C(1)–C(7) bond (Scheme 1). Thus, two sets of ^{19}F NMR signals in a ratio of 67:33 for the fluorovinyl and the trifluoroacetyl groups in **6b** and **6c** were observed. The ^1H NMR spectral data for the iodination products were compatible with the assigned structures. The phenolic function in **6a** was protected as methoxy or acetoxy groups (**7a** and **7b**) before the Pd(0)-catalyzed

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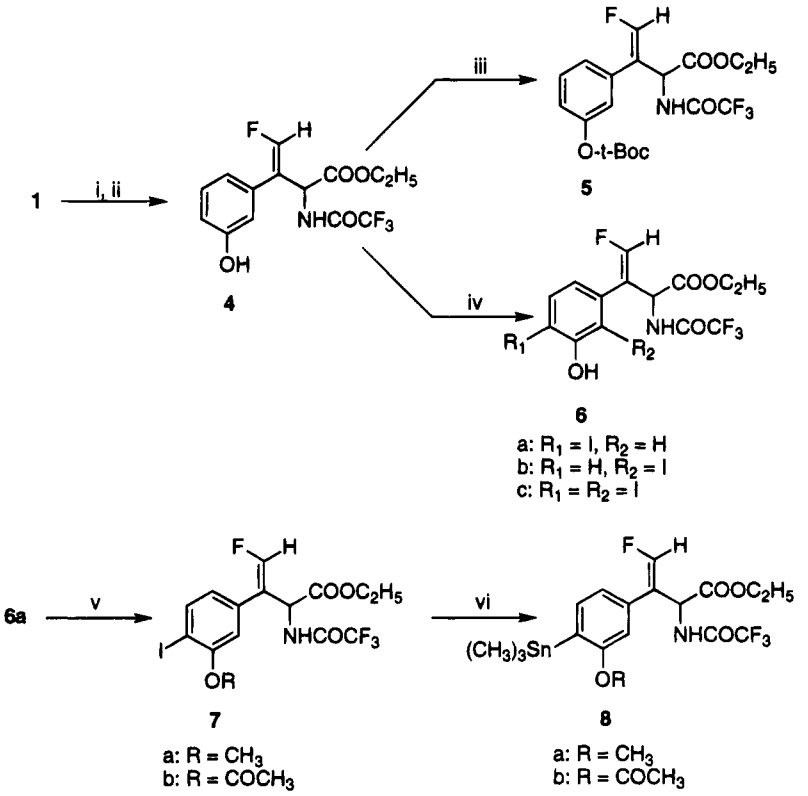
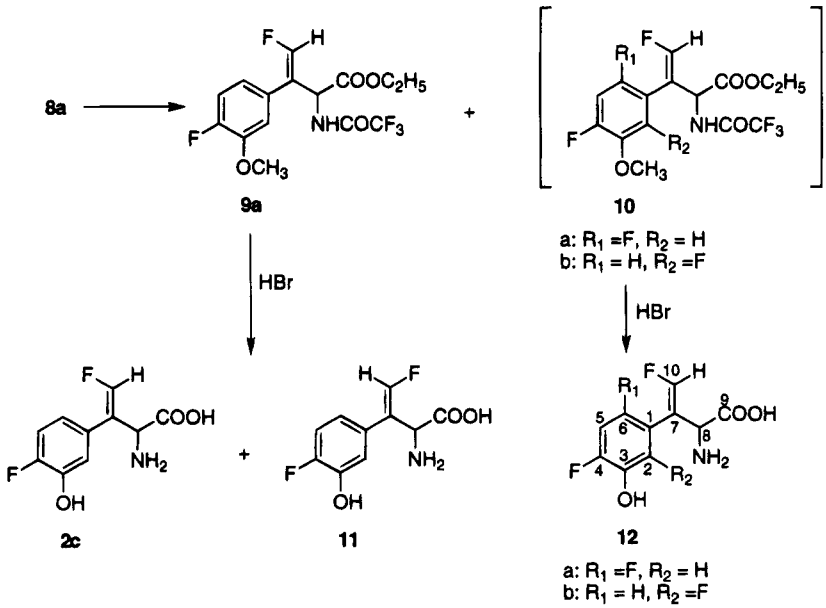
Scheme 2[a]



[a] (i) C_2H_5OH/HCl; (ii) $(CF_3CO)_2O$; (iii) $O[CO_2C(CH_3)_3]_2$; (iv) $AgOCOCF_3/I_2$; (v) CH_2N_2 or $(CH_3CO)_2O$; (vi) $(CH_3)_6Sn_2/(Ph_3P)_4Pd$.

Scheme 3



oxidative coupling reaction with hexamethylditin.[23] The trimethylstannyl precursors **8a** and **8b** were isolated as colorless oils and were characterized by ^1H, ^{13}C, and ^{119}Sn NMR. In the ^1H NMR spectra of **8a** and **8b**, upfield shifts (0.4 ppm) for the protons on C-5 (compared with the corresponding chemical shifts in the iodo compounds **7a** and **7b**), adjacent to the trimethyltin moiety, were observed with characteristic side bands due to proton–tin coupling. Such side bands were also found for protons on C-6 and C-2. Sharp singlets at δ 0.26 ppm and 0.31 ppm (^1H NMR) and δ −9.32 ppm and −9.23 ppm (^{13}C NMR) along with appropriate tin satellites and δ −32.04 and −27.47 ppm (^{119}Sn NMR) were distinguished for the trimethylstannyl substitution on the C-4 of the aromatic ring in **8a** and **8b**, respectively.

Fluorodestannylation reaction of the tin derivative **8a** in freon-11 was carried out with elemental fluorine (1% in argon) at room temperature (Scheme 3). A relatively slow rate of bubbling of diluted fluorine (~5 μmol/min) was found to be necessary for optimal fluorination. With a molar ratio of 1:1.1 of **8a**:F_2 and a reaction period of 45 min, the 4-fluoro derivative **9a** was isolated in 43% yield

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Table 1. ^{13}C NMR Chemical Shifts for 2c

carbon	δ (ppm) expt	δ (ppm) calcd[a]
C-1	128.7	128.4
C-2	121.0	119.8
C-3	146.3 ($^2J_{C,F}$ = 14.0 Hz)	145.6
C-4	154.2 ($^1J_{C,F}$ = 242.8 Hz)	153.9
C-5	119.2 ($^2J_{C,F}$ = 19.0 Hz)	120.3
C-6	124.1 ($^3J_{C,F}$ = 6.4 Hz)	124.9
C-7	120.6 ($^2J_{C,F}$ = 9.4 Hz)	
C-8	58.0 ($^3J_{C,F}$ = 8.0 Hz)	
C-9	174.1	
C-10	152.8 ($^1J_{C,F}$ = 267.9 Hz)	

[a] Values calculated[25] using the chemical shift data for **1**.[9]

along with the minor products **10a** and **10b**. Nearly half of the unreacted starting material was recovered (see Experimental Section). However, fluorination of the tin derivative **8b** (**8b**:F_2 = 1:2.5) gave only 21% of the corresponding fluoro derivative **9b** along with 35% of unreacted starting material. The position of the fluorine on the aromatic ring in **9a** was determined based on ^1H and ^{19}F NMR data. The ^1H NMR of the aromatic region exhibited a pair of doublet of doublets at δ 7.07 and 6.81 which were assigned to H-5 and H-2, respectively. The signal due to H-6 appeared as a doublet of doublet of doublets due to coupling with H-5 and H-2 as well as fluorine on C-4. A similar ^1H NMR pattern might also emerge if the fluorine was on C-6. However, the ^{19}F NMR chemical shift for the fluorine on the aromatic ring in **9a** (−134.6 ppm) is characteristic for the presence of an *o*-methoxy group.[24] These NMR data are consistent with the fluorine on C-4 in the aromatic ring. Further evidence in this regard was obtained from the structural data of **2c**, the hydrolysis product of **9a**.

Hydrolysis of **9a** with HBr in the dark gave 4-fluoro-(*E*)-FMMT (**2c**). The close agreement of the experimental ^{13}C NMR chemical shifts of **2c** (Table 1) with those of the calculated values[25] for the aromatic region strongly suggests C-4 as the position for the fluorine on the ring. Further, the ^1H NMR signals of the aromatic region were almost identical to that of the related 4-fluoro-*m*-tyrosine.[26] Convincing evidence for the structure of **2c** was, however, obtained from a single crystal X-ray crystallographic analysis given latter in the discussion.

The minor products **10a** and **10b** were characterized after hydrolysis with HBr to give **12a** and **12b**. The high resolution mass spectra of both these amino acids indicated difluorination of the aromatic ring. The positions of the fluorine on the ring in **12a** and **12b** were discerned from the ^1H NMR double irradiation experiments. For example, in the aromatic spectral part of **12a** two sets of doublet of doublets, each integrating for one proton, showed no coupling between them suggesting that they were para to each other. Further, the more downfield shifted signal (7.09 ppm) that displayed strong ortho coupling with two fluorines was assigned to H-5 and the second set of doublet of doublets arising due to *m*-fluorine couplings was ascribed to H-2, respectively. In the case of **12b**, the ^1H NMR spectrum showed a doublet of doublet of doublets (ddd) at 6.73 ppm and a doublet of triplets (dt) at 7.01 ppm, each integrating for one proton.

Table 2. ^{19}F Chemical Shifts for Fluorinated Derivatives of 1

compd	vinyl-F	δ (ppm)[a] ArF expt	δ (ppm)[a] ArF calcd[b]
(*E*)-FMMT[c] (**1**)	−118.90		
2-fluoro-(*E*)-FMMT (**2a**)	−113.6	−135.4	
6-fluoro-(*E*)-FMMT (**2b**)	−113.7	−122.9	
4-fluoro-(*E*)-FMMT (**2c**)	−119.0	−134.3	
2,6-difluoro-(*E*)-FMMT (**2d**)	−110.0	−119.5 (F-6)	−118.6
		−130.5 (F-2)	−131.4
4,6-difluoro-(*E*)-FMMT (**12a**)	−113.3	−118.8 (F-6)	−118.6
		−128.8 (F-4)	−130.3
2,4-difluoro-(*E*)-FMMT (**12b**)	−113.3	−129.7 (F-4)	−130.3
		−130.7 (F-2)	−131.4

[a] In D_2O with $CFCl_3$ as an external standard. [b] Values calculated using the chemical shift additivity method[27] with the ^{19}F chemical shift of fluorobenzene in D_2O at −111.7 ppm. Substituent chemical shift values were obtained from ^{19}F NMR spectra of *m*-difluorobenzene, fluorophenols, and monofluoro-(*E*)-FMMT derivatives in D_2O: *m*-fluoro, +4.0; *p*-OH, −11.3; *o*-OH, −24.1; fluoromethylene amino acid moiety at *ortho*, +0.4; *para*, +1.5. [c] Value from ref 9.

Based on the coupling constant ($J_{H,H}$ = 8.8 Hz) in the ddd signal as well as decoupling of the downfield proton, an ortho proton−proton arrangement was discerned. Such an arrangement of protons is possible only with **12b** and its 2,6-difluoro isomer. However, the ^1H NMR spectrum of 2,6-difluoro-(*E*)-FMMT (**2d**) does not correspond to that of **12b**. Thus, on the basis of the splitting pattern, the upfield signal (6.73 ppm) was assigned to H-6 and H-5 was ascribed to the downfield resonance in **12b**. The ^1H NMR signals due to the vinylic and amino acid moieties in **12a** and **12b** were similar to that of **1**.

The ^{19}F NMR spectral data for **12a** and **12b** along with the related derivatives, for comparison, are given in Table 2. The ^{19}F chemical shifts for the difluoro derivatives have been calculated based on the additivity method reported recently.[27] The close agreement between the experimental and calculated values for the difluoro amino acids confirms the above ^1H NMR spectral analyses. It is quite interesting to note the effect of the ring fluorination on the ^{19}F chemical shift of the exocyclic fluorine (Table 2). The almost identical chemical shifts of the vinylic fluorine in (*E*)-FMMT (**1**) and the 4-fluoro derivative **2c** indicate lack of electronic interactions between the aromatic and the aliphatic fluorines in **2c**. On the other hand, substitution of a fluorine on C-2 or C-6 causes a significant downfield shift (~5 ppm) of the fluoromethylene signal. The presence of fluorine on both C-2 and C-6, as in **2d**, led to a more pronounced downfield shift of the vinylic fluorine.

The hydrolysis of **9a** with HBr conducted under ambient light conditions caused an isomerization of the vinylic double bond and yielded 4-fluoro-(*Z*)-β-(fluoromethylene)-*m*-tyrosine (**11**). A similar isomerization reaction has been recently utilized in the preparation of (*Z*)-β-(fluoromethylene)-*m*-tyrosine.[9] It is interesting to note, however, that the difluoro derivatives **12a** and **12b** failed to undergo an analogous isomerization.

X-ray Crystallographic Analyses. The single crystal X-ray structure determination of the fluorinated amino acids **2a**, **2b** (*R* and *S*) and **2c** were carried out to (a) establish unequivocally the structures of these analogs and (b) to investigate the effect of ring fluorination on the aromatic ring and the side chain that contains the

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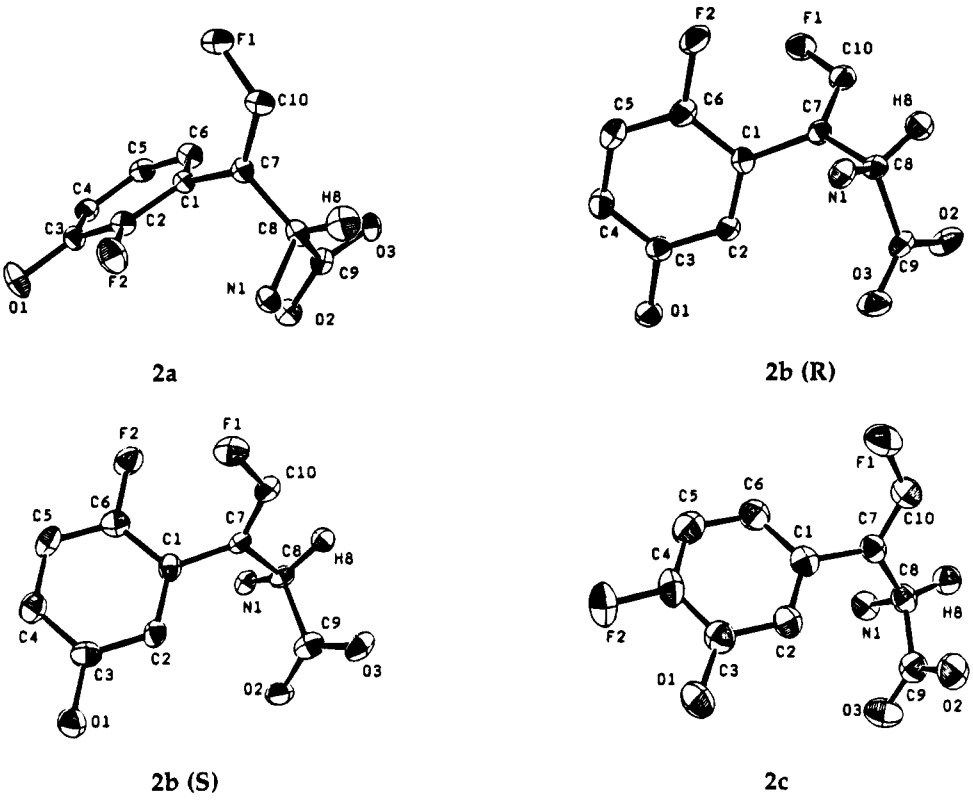
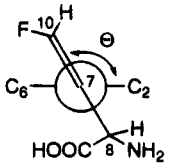


Figure 1. X-ray structures of **2a**, **2b**, and **2c**. Ellipsoids are drawn at 50% probability, except for the H-8 atom.



1: 130.3°
2a: 109.3°
2b: 119.4°
2c: 127.3°

Figure 2. The dihedral angle between the plane of the phenyl ring and of the fluorovinyl group.

fluorovinyl and amino acid moieties (Figure 1). Further, the X-ray crystal structure analysis of underivatized free amino acids containing fluorine are rather rare. Even though the crystal structures of the fluoro derivatives **2a–c** generally resembled that of the parent amino acid **1**,[9] conspicuous differences in certain bond angles were noted due to the different positions of fluorine on the aromatic ring. Among the three analogs **2a–c**, the 4-fluoro isomer **2c** differed by the least amount from the parent amino acid **1**. For example, the bond angles C(6)–C(1)–C(2) in **2a–c** were decreased by 3.4°, 2.8°, and 1.0°, respectively in comparison to that found in **1**.[9] A similar trend was also observed in the angles around the carbon atom in the aromatic ring that was connected to the fluorine atom. Thus, the 2-fluoro derivative **2a** increased the angle C(1)–C(2)–C(3) by 4.6° and the 6-fluoro analog **2b** widened C(5)–C(6)–C(1) by 3.2° while the 4-fluoro isomer **2c** increased the angle C(3)–C(4)–C(5) by only 2.0° in comparison with the corresponding angles in **1**. Similarly, the dihedral angle between the plane of the phenyl ring and of the double bond [C(2)–C(1)–C(7)–C(10)] (Figure 2) varied analogously.

In summary, the direct fluorination of (*E*)-β-(fluoromethylene)-*m*-tyrosine could be used for the preparation of the corresponding 2- and 6-fluoro analogs **2a** and **2b**. The 4-fluoro isomer **2c**, on the other hand, could be conveniently synthesized based on a regioselective fluorodestannylation reaction. The ready accessibility of $[^{18}F]$-F_2 from small cyclotrons would enable the synthesis of the ^{18}F-labeled counterpart of **2c** for positron emission tomographic applications.

Experimental Section

General. ^1H, ^{13}C, ^{19}F, and ^{119}Sn NMR spectra were recorded at 360.14, 90.57, 338.87, and 130.30 MHz, respectively. ^1H and ^{13}C spectra obtained in $CDCl_3$ were referenced to internal TMS whereas the data collected in D_2O were referenced to external DSS (for ^1H) and external 1,4-dioxane at 67.4 ppm (for ^{13}C). External $CFCl_3$ in $CDCl_3$ was used as the standard for all ^{19}F NMR spectra. ^{119}Sn NMR resonances were referenced to external $(CH_3)_4Sn$ in $CDCl_3$. Circular dichroism spectra in water at 20–22°C were obtained in the spectral region of 190–300 nm. The sample cell length was 1 cm for measurements in the 190–300 nm region and 0.1 cm 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. The melting points reported are uncorrected. The elemental analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, TN.

All organic extracts were dried with $MgSO_4$ and the solvents were always evaporated under reduced pressure. Flash chromatography was performed with silica gel (40–63 μm).

Caution: *Fluorine and acetyl hypofluorite are highly toxic and reactive gases. However, they can be handled safely by following the procedures developed specifically for such gases.*[28]

Direct Fluorination Procedure. Into a solution of **1** (*S* enantiomer[9]) (25 mg, 0.12 mmol) in 1:1 glacial acetic acid and trifluoroacetic acid (5 mL) at 0°C was bubbled acetyl hypofluorite (0.13 mmol) (prepared by passing 1% F_2 in argon through a $NaOAc{\cdot}3H_2O$ cartridge[29]) over a period of 25 min. The solvents were then evaporated at room temperature. The residue was dissolved in water (1 mL) and the mixture was

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(29) Bida, G. T.; Satyamurthy, N.; Barrio, J. R. *J. Nucl. Med.* **1984**, *25*, 1327–1334.

separated by semipreparative HPLC using an Econosil C18 column (10 μm, 500 × 10 mm) and a mobile phase of 0.02 M NaOAc (pH = 3.5) with a flow rate of 5.0 mL/min. The effluent of the column was monitored with a uv detector (279 nm). The elution sequence, with increasing retention times, was **1** < **2a** < **2b** < **2d** < **3**. The base line separation of the reaction mixture allowed for an easy collection of pure products. Collected fractions were lyophilized and rechromatographed on the same HPLC column with a mobile phase of 3% CH_3OH in 0.1% HOAc with a flow rate of 5.0 mL/min to remove the salts. After lyophilization, the following solid products were obtained: **1** (4 mg, 16%), **2a** (3 mg, 13%), **2b** (6 mg, 26%), **2d** (1.5 mg, 6%) and **3** (2 mg, 7%). Product **2c** [^{19}F NMR signals at −119.0 (d, $^2J_{H,F}$ = 81.0 Hz) and −134.3 (s)] appeared in trace amounts in the fraction containing **2a**. The fluorination of the *R* enantiomer of **1** as well as the racemic (*E*)-FMMT mixture gave a similar product composition from which pure products were isolated by preparative HPLC as described above. The enantiomeric excess of these products were determined by chiral HPLC as previously reported.[16,17]

(*S*)-2-Fluoro-(*E*)-β-(fluoromethylene)-*m*-tyrosine (2a): white solid, crystallized from water, mp 210−212 °C (d); $[\alpha]^{20}_D$ +40.8 (*c* 0.77, H_2O); UV (H_2O) λ_{max} 273 nm (ϵ = 1220); (ee 90%, chiral HPLC); CD (*c* 0.2287, H_2O) $[\theta]_{210}$ +35500, $[\theta]_{199}$ 0, $[\theta]_{195}$ −3150, $[\theta]_{193}$ 0; ^{1}H NMR (D_2O) δ 4.56 (s, 1H), 6.78−6.81 (m, 1H), 6.08−7.14 (m, 2H), 7.21 (d, 1H, $^2J_{H,F}$ = 80.9 Hz); ^{19}F NMR (D_2O) δ −113.6 (d, $^2J_{H,F}$ = 80.9 Hz), −135.4 (s). Anal. Calcd for $C_{10}H_9NO_3F_2 \cdot 1.5H_2O$: C, 46.88; H, 4.72, N, 5.47. Found: C, 46.47; H, 4.69; N, 5.44. FAB HRMS M^+ + H calcd for $C_{10}H_{10}NO_3F_2$ 230.0629, found 230.0630.

(*S*)-6-Fluoro-(*E*)-β-(fluoromethylene)-*m*-tyrosine (2b): white solid, recrystallized from water, mp 205−206 °C (d); $[\alpha]^{20}_D$ + 43.0 (*c* 0.31, H_2O); UV (H_2O) λ_{max} 283 nm (ϵ = 1810), (ee 94%, chiral HPLC); CD (*c* 0.1545, H_2O) $[\theta]_{284}$ +1060, $[\theta]_{213}$ +15600, $[\theta]_{202}$ 0, $[\theta]_{199}$ −3650; ^{1}H NMR (D_2O) δ 4.55 (s, 1H), 6.75 (dd, 1H, J = 3.1 Hz and 5.5 Hz), 6.93 (ddd, 1H, J = 8.7 Hz, 3.7 Hz and 3.7 Hz), 7.1 (dd, 1H, J = 9.2 Hz and 9.2 Hz), 7.20 (d, 1H, $^2J_{H,F}$ = 80.4 Hz); ^{19}F NMR (D_2O) δ −113.7 (d, $^2J_{H,F}$ = 80.4 Hz), −122.9 (s). Anal. Calcd for $C_{10}H_9NO_3F_2 \cdot 1.25H_2O$: C, 47.72; H, 4.61; N, 5.56. Found: C, 47.82; H, 4.40; N, 5.31. FAB HRMS M^+ + H calcd for $C_{10}H_{10}NO_3F_2$ 230.0629, found 230.0637.

(*S*)-2,6-Difluoro-(*E*)-β-(fluoromethylene)-*m*-tyrosine (2d): white solid, mp 197−199 °C (d); $[\alpha]^{20}_D$ + 32.0 (*c* 0.5, H_2O); UV (H_2O) λ_{max} 278 nm (ϵ = 1960); (ee 40%, chiral HPLC); CD (*c* 0.1942, H_2O) $[\theta]_{280}$ +2950, $[\theta]_{207}$ +39500, $[\theta]_{194}$ 0; ^{1}H NMR (D_2O) δ 4.56 (s, 1H), 6.95 (dd, 1H, *J* = 9.0 Hz and 9.0 Hz), 7.05 (ddd, 1H, *J* = 9.7 Hz, 9.7 Hz and 6.1 Hz), 7.29 (d, $^2J_{H,F}$ = 80.0 Hz); ^{19}F NMR (D_2O) δ −110.0 (d, $^2J_{H,F}$ = 80.0 Hz), −119.5 (s), −130.5 (s). FAB HRMS M^+ + H calcd for $C_{10}H_9NO_3F_3$ 248.0534, found 248.0516.

2-Amino-3-acetoxy-3-(3-hydroxyphenyl)-4,4-difluorobutyric Acid (3). This product after first HPLC pass (see above) was found to contain a diastereomeric mixture of **3** in a ratio 9:1 (^{1}H and ^{19}F NMR). Subsequent HPLC purification to remove the salts gave the major isomer as a white solid, mp 201 − 203 °C; UV (H_2O) λ_{max} 272 nm (ϵ = 1780), λ_{sh} 279 nm (ϵ = 1570), λ_{sh} 215 nm (ϵ = 7530); ^{1}H NMR (D_2O), δ 2.02 (s, 3H), 4.87 (s, 1H), 6.37 (t, 1H, $^2J_{H,F}$ = 54.8 Hz), 6.90 (d, 1H, *J* = 8.0 Hz), 7.04 (d, 1H, *J* = 2.2 Hz), 7.08 (d, 1H, *J* = 8.0 Hz), 7.32 (t, 1H, *J* = 8.0 Hz); ^{19}F NMR (D_2O) δ −129.74 (AB part of an ABX system, $J_{F1,F2}$ = 277.0 Hz, $J_{H,F1}$ = $J_{H,F2}$ = 55.0 Hz). CI HRMS (ammonia) M^+ + H calcd for $C_{12}H_{14}NO_5F_2$ 290.0840, found 290.0844.

Ethyl 2-[(Trifluoroacetyl)amino]-3-(3-hydroxyphenyl)-4-fluoro-3-butenoate (4). A suspension of **1**[5] (0.70 g, 3.3 mmol) in anhydrous EtOH (50 mL) at 0°C was saturated with HCl gas for 30 min and then heated under reflux for 5 h. The solvent was removed and the viscous oil dissolved in water (100 mL). After neutralization with 1 N NaOH the product was extracted with ethyl acetate (200 mL). The organic phase was dried and evaporated to give a pale yellow oil (0.67 g, 84%) which was used in the next step without further purification. Trifluoroacetic anhydride (10.4 g, 50 mmol) was added to the above ester at −78 °C, and the mixture was allowed to warm up to room temperature and was stirred for 5 h. The anhydride was evaporated and the residue coevaporated with ethanol (2 × 30 mL). The residual syrup was dissolved in ether (100 mL) and washed with 1 N HCl (3 × 100 mL), saturated $NaHCO_3$ solution (3 × 100 mL), and brine (3 × 100 mL). The organic phase was dried and evaporated to dryness, and the residue was flash chromatographed (CH_2Cl_2/ether, 30:1) to give a pale yellow oil (0.72 g, 77%): ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H, *J* = 7.0 Hz), 4.25 (q, 2H, *J* = 7.0 Hz), 5.17 (d, 1H, *J* = 7.2 Hz), 6.69 (d, 1H, *J* = 2.0 Hz), 6.74 (d, 1H, *J* = 7.6 Hz), 6.83 (dd, 1H, *J* = 7.6 and 2.0 Hz), 7.01 (d, 1H, $^2J_{H,F}$ = 80.0 Hz), 7.15 (broad d, 1H), 7.24 (t, 1H, *J* = 7.6 Hz); ^{19}F NMR ($CDCl_3$) δ −76.30 (s), −121.85 (d, $^2J_{H,F}$ = 80.0 Hz). Anal. Calcd for $C_{14}H_{13}O_4NF_4$. 0.25 H_2O: C, 49.49; H, 4.02; N, 4.12. Found: C, 49.33; H, 4.27; N, 4.11.

Ethyl 2-[(Trifluoroacetyl)amino]-3-[[3-(*tert*-butoxycarbonyl)oxy]phenyl]-4-fluoro-3-butenoate (5). A solution of di-*tert*-butyl dicarbonate (55.0 mg, 0.25 mmol) in anhydrous DMF (2 mL) was added dropwise to a solution of **4** (33.0 mg, 0.1 mmol) in anhydrous DMF (2 mL) and triethylamine (0.5 mL) under N_2 and stirred for 16 h at room temperature. The reaction mixture was then evaporated and the residue was flash chromatographed (CH_2Cl_2) to afford the product as a colorless oil (23.0 mg, 55%): ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, *J* = 7.2 Hz), 1.55 (s, 9H), 4.24 (q, 2H, *J* = 7.2 Hz), 5.19 (d, 1H, *J* = 7.1 Hz), 7.03 (d, 1H, $^2J_{H,F}$ = 80.4 Hz), 7.05 (m, 2H), 7.17 (dd, 1H, *J* = 8.5 and 1.5 Hz), 7.22 (broad d, 1H), 7.38 (t, 1H, *J* = 8.5 Hz); ^{19}F NMR ($CDCl_3$) δ −76.30 (s), −121.23 (d, $^2J_{H,F}$ = 80.4 Hz). Anal. Calcd for $C_{19}H_{21}O_6NF_4$: C, 52.42; H, 4.86; N, 3.22. Found: C, 52.99; H, 5.12; N, 3.08.

Ethyl 2-(Trifluoroacetyl)-3-(3-hydroxy-4-iodophenyl)-4-fluoro-3-butenoate (6a). A solution of I_2 (0.50 g, 1.99 mmol) in anhydrous CH_2Cl_2 (35 mL) was added dropwise to the solution of **4** (0.67 g, 1.99 mmol) and silver trifluoroacetate (0.48 g, 2.2 mmol) in CH_2Cl_2 (25 mL) over a period of 25 min. The mixture was stirred, protected from light, at room temperature for 20 h. A precipitate that formed was filtered off, the solvent evaporated and the residue chromatographed (CH_2Cl_2). The product **6a**, the major component of the reaction mixture, was isolated as a colorless viscous oil (0.48 g, 53%) and was used in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, *J* = 7.3 Hz), 4.26 (q, 2H, *J* = 7.3 Hz), 5.17 (d, 1H, *J* = 7.1 Hz), 5.62 (broad s, 1H), 6.52 (dd, 1H, *J* = 7.6 and 2.2 Hz), 6.83 (d, 1H, *J* = 2.2 Hz), 7.01 (d, 1H, $^2J_{H,F}$ = 80.3 Hz), 7.21 (broad d, 1H), 7.66 (t, 1H, *J* = 7.7 Hz); ^{19}F NMR ($CDCl_3$) δ −76.24 (s), −120.86 (d, $^2J_{H,F}$ = 80.3 Hz).

Ethyl 2-[(Trifluoroacetyl)amino]-3-(3-hydroxy-2-iodophenyl)-4-fluoro-3-butenoate (6b). White crystalline **6b** (27 mg, 7.4%), mp 143−144°C (petroleum ether), was obtained as a minor product from the above chromatographic separation. UV (96% EtOH) λ_{max} 288 nm (ϵ = 3010), λ_{sh} 235 nm (ϵ = 6480), λ_{max} 208 nm (ϵ = 21 800); ^{1}H NMR ($CDCl_3$) δ 1.31 (m, 3H), 4.31 (m, 2H), 5.31 (d, 1H, *J* = 7.0 Hz), 5.63 (s, 1H), 6.60 (d, 1H, *J* = 7.4 Hz), 6.99 (d, 1H, *J* = 8.0 Hz), 7.02 (d, 1H, $^2J_{H,F}$ = 79.4 Hz), 7.06 (broad d, 1H), 7.24 (t, 1H, *J* = 7.7 Hz); ^{19}F NMR ($CDCl_3$) δ −75.99 [s (67%)], −76.21 [s (33%)], −115.79 [d, $^2J_{H,F}$ = 79.4 Hz (33%)], −116.96 [d, $^2J_{H,F}$ = 79.4 Hz (67%)]. Anal. Calcd for $C_{14}H_{12}NO_4F_4I$: C, 36.46; H, 2.62; N, 3.04. Found: C, 36.11; H, 2.59; N, 3.03.

Ethyl 2-[(Trifluoroacetyl)amino]-3-(3-hydroxy-2,4-diiodophenyl)-4-fluoro-3-butenoate (6c). The product **6c** (0.14 g, 12%), mp 158−159 °C (petroleum ether), was obtained as another minor fraction from the preparation of **6a**: UV (96% EtOH) λ_{max} 320 nm (ϵ = 2500), λ_{max} 298 nm (ϵ = 3670), λ_{sh} 248 nm (ϵ = 10 000), λ_{max} 215 nm (ϵ = 27000); ^{1}H NMR ($CDCl_3$) δ 1.33 (m, 3H), 4.32 (m, 2H), 5.28 (d, 1H, *J* = 7.2 Hz), 5.98 (s, 1H), 6.35 (d, 1H, *J* = 8.2 Hz), 6.99 (d, 1H, $^2J_{H,F}$ = 79.0 Hz), 7.03 (broad d, 1H), 7.69 (d, 1H, *J* = 8.3 Hz); ^{19}F NMR ($CDCl_3$) δ −76.0 [s (63%)], −76.2 [s (37%)], −115.0 [d, $^2J_{H,F}$ = 79.0 Hz (35%)], −116.3 [d, $^2J_{H,F}$ = 79.0 Hz (65%)]. Anal. Calcd for $C_{14}H_{11}NO_4F_4I_2$: C, 28.64; H, 1.89; N, 2.39. Found: C, 28.68; H, 1.83; N, 2.41.

Ethyl 2-[(Trifluoroacetyl)amino]-3-(4-iodo-3-methoxyphenyl)-4-fluoro-3-butenoate (7a). A solution of diazomethane (prepared from 4.3 g *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide) in ether (30 mL) was added to a solution of **6a** (0.43 g, 0.92 mmol) in ether (5 mL) and left at room tempera-

ture for 4 h. The reaction was quenched with 5 N HOAc (20 mL), the ether layer was washed with saturated $NaHCO_3$ solution and dried, and the solvent was evaporated. Flash chromatography of the product (CH_2Cl_2) afforded crystalline **7a** (0.37 g, 84%), mp 86–87°C (petroleum ether): UV (96% EtOH) λ_{max} 289 nm (ϵ = 5165), λ_{max} 248 (ϵ = 10990), λ_{max} 207 (ϵ = 28500); ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, J = 7.3 Hz), 3.85 (s, 3H), 4.26 (q, 2H, J = 7.3 Hz), 5.17 (d, 1H, J = 7.0 Hz), 6.52 (dd, 1H, J = 7.6 and 2.1 Hz), 6.64 (s, 1H), 7.06 (d, 1H, $^2J_{H,F}$ = 80.4 Hz), 7.16 (broad d, 1H), 7.77 (d, 1H, J = 7.6 Hz); ^{19}F NMR ($CDCl_3$) δ −76.29 (s), −121.12 (d, $^2J_{H,F}$ = 80.4 Hz). Anal. Calcd for $C_{15}H_{14}NO_4F_4I$: C, 37.92; H, 2.97; N, 2.95. Found: C, 37.91; H, 2.93; N, 2.93.

Ethyl 2-[(Trifluoroacetyl)amino]-3-(3-acetoxy-4-iodophenyl)-4-fluoro-3-butenoate (7b). Acetic anhydride (2 mL) was added to a solution of **6a** (0.12 g, 0.25 mmol) in dry pyridine (2 mL) at 0 °C and stirred at room temperature for 5 h. The solvent was evaporated and the residual oil was dissolved in CH_2Cl_2 (20 mL). The solution was washed with water (2 × 20 mL) and saturated $NaHCO_3$ solution (20 mL), and the organic layer was dried. Evaporation of the solvent gave the crude product which was purified by flash chromatography (CH_2Cl_2) to give pure **7b** (0.12 g, 92%, oil): ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.0 Hz), 2.36 (s, 3H), 4.24 (q, 2H, J = 7.0 Hz), 5.18 (d, 1H, J = 6.8 Hz), 6.84 (dd, 1H, J = 7.7 and 2.1 Hz), 6.99 (d, 1H, J = 2.1 Hz), 7.03 (d, 1H, $^2J_{H,F}$ = 79.5 Hz), 7.25 (broad d, 1H), 7.83 (d, 1H, J = 7.4 Hz); ^{19}F NMR ($CDCl_3$) δ −76.22 (s), −120.48 (d, $^2J_{H,F}$ = 79.5 Hz). Anal. Calcd for $C_{16}H_{14}NO_3F_4I$: C, 38.19; H, 2.80; N, 2.78. Found: C, 37.88; H, 2.72; N, 2.68.

Ethyl 2-[(Trifluoroacetyl)amino]-3-[3-methoxy-4-(trimethylstannyl)phenyl]-4-fluoro-3-butenoate (8a). Hexamethylditin (0.27 g, 0.82 mmol) was added to a solution of **7a** (0.40 g, 0.82 mmol) and tetrakis(triphenylphosphine)palladium-(0) (51 mg, 0.041 mmol) in anhydrous 1,4-dioxane (15 mL). The reaction mixture was heated under reflux in a nitrogen atmosphere for 5 h. The mixture was cooled and the precipitated solid was filtered off. The filtrate was evaporated to give an oil which was chromatographed (petroleum ether–ether, 4:1) to give **8a** as a colorless oil (0.27 g, 64%): ^{1}H NMR ($CDCl_3$) δ 0.26 (s with Sn satellites, 9H, $^2J_{Sn,H}$ = 55.1 Hz), 1.28 (t, 3H, J = 7.0 Hz), 3.75 (s, 3H), 4.26 (q, 2H, J = 7.0 Hz), 5.20 (d, 1H, J = 7.2 Hz), 6.61 (s with characteristic side bands, 1H, $^4J_{Sn,H}$ = 16.5 Hz), 6.74 (d, 1H, J = 7.3 Hz), 7.01 (d, 1H, $^2J_{H,F}$ = 80.6 Hz), 7.17 (broad d, 1H), 7.36 (d with characteristic side bands, 1H, J = 7.3 Hz, $^3J_{Sn,H}$ = 47.9 Hz); ^{13}C NMR ($CDCl_3$) δ −9.23 (s with Sn satellites, $^1J_{Sn,C}$ = 353.8 Hz), 14.05, 53.95, 55.29, 62.98, 109.42 (s with Sn satellites, $^3J_{Sn,C}$ = 26.3 Hz), 115.49 (q, $^1J_{C,F}$ = 287.0 Hz), 118.98, 120.86 (s with Sn satellites, $^2J_{Sn,C}$ = 44.7 Hz), 131.74, 32.04, 136.73 (s with Sn satellites, $^3J_{Sn,C}$ = 26.3 Hz), 150.07 (d, $^1J_{C,F}$ = 268.7 Hz), 156.44 (q, $^2J_{C,F}$ = 36.6 Hz), 164.09, 168.64; ^{19}F NMR ($CDCl_3$) δ −76.29 (s), −122.01 (d, $^2J_{H,F}$ = 80.6 Hz); ^{119}Sn NMR ($CDCl_3$) δ −32.04 (s). Anal. Calcd for $C_{18}H_{23}NO_4F_4Sn$: C, 42.22; H, 4.53; N, 2.74. Found: C, 42.88; H, 4.77; N, 2.28.

Ethyl 2-[(Trifluoroacetyl)amino]-3-[3-acetoxy-4-(trimethylstannyl)phenyl]-4-fluoro-3-butenoate (8b). This tin derivative **8b** was prepared from **7b** in 74% yield, as a colorless oil, using the procedure described above: ^{1}H NMR ($CDCl_3$) δ 0.31 (s with Sn satellites, 9H, $^2J_{Sn,H}$ = 55.0 Hz), 1.25 (t, 3H, J = 7.2 Hz), 2.28 (s, 3H), 4.26 (q, 2H, J = 7.2 Hz), 5.20 (d, 1H, J = 7.2 Hz), 7.00 (s with characteristic side bands, 1H, $^4J_{Sn,H}$ = 14.6 Hz), 7.02 (d, 1H, $^2J_{H,F}$ = 79.7 Hz), 7.04 (d, 1H, J = 7.2 Hz), 7.20 (broad d, 1H), 7.47 (d with characteristic side bands, 1H, J = 7.2 Hz, $^3J_{Sn,H}$ = 43.9 Hz); ^{13}C NMR ($CDCl_3$) δ −9.23 (s with Sn satellites, $^1J_{Sn,C}$ = 366.3 and 351.0 Hz), 13.82, 21.10, 53.75, 63.02, 115.36 (q, $^1J_{C,F}$ = 287.0 Hz), 118.09, 121.58 (s with Sn satellites, $^3J_{Sn,C}$ = 23.6 Hz), 125.53 (s with Sn satellites, $^2J_{Sn,C}$ = 37.9 Hz), 131.91, 134.81, 136.83 (s with Sn satellites, $^3J_{Sn,C}$ = 23.6 Hz), 150.36 (d, $^1J_{C,F}$ = 271.4 Hz), 155.87, 156.47 (q, $^2J_{C,F}$ = 37.5 Hz), 168.42, 168.96; ^{19}F NMR ($CDCl_3$) δ −76.29 (s), −121.24 (d, $^2J_{H,F}$ = 79.7 Hz); ^{119}Sn NMR ($CDCl_3$) δ −27.47 (s). Anal. Calcd for $C_{19}H_{23}NO_5F_4Sn$: C, 42.25; H, 4.29; N, 2.59. Found: C, 42.52; H, 4.61; N, 2.68.

Fluorination of 8a. Fluorine (1% in argon, 0.25 mmol) was bubbled into a solution of **8a** (0.12 g, 0.234 mmol) in freon-11 (15 mL) at room temperature over a period of 45 min. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and the white precipitate was filtered through Celite. Evaporation of the filtrate gave an oily residue which was flash chromatographed (ether–petroleum ether, 1:4). Four fractions, two major (fractions A and C) and two minor (fractions B and D), were collected. Fraction A contained essentially the starting material **8a** (55 mg, 46%), fraction B comprised the 2,4-difluoro derivative **10b** (6 mg, 12%), fraction C constituted the 4-fluoro derivative **9a** (20 mg, 43%), and fraction D consisted of a mixture of **9a** (3 mg, 6%) and the 4,6-difluoro derivative **10a** (7 mg, 14%) (the yields of the fluoro derivatives were calculated based on the amount of the starting material recovered).

Recrystallization of fraction C from petroleum ether gave an analytical sample of ethyl 2-[(trifluoroacetyl)amino]-3-(3-methoxy-4-fluorophenyl)-4-fluoro-3-butenoate (**9a**): mp 90–92 °C; UV (96% EtOH) λ_{max} 278 nm (ϵ = 1970), λ_{max} 238 (ϵ = 5770), λ_{max} 212 (ϵ = 16150); ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, J = 7.2 Hz), 3.86 (s, 3H), 4.27 (q, 2H, J = 7.2 Hz), 5.16 (d, 1H, J = 7.0 Hz), 6.70 (ddd, 1H, J = 8.3 Hz, J = 2.0 Hz, $^4J_{H,F}$ = 4.2 Hz), 6.81 (dd, 1H, J = 2.0 Hz, $^4J_{H,F}$ = 8.0 Hz), 7.01 (d, 1H, $^2J_{H,F}$ = 80.4 Hz), 7.07 (dd, 1H, J = 8.4 Hz, $^3J_{H,F}$ = 11.09 Hz), 7.11 (broad s, 1H); ^{19}F NMR ($CDCl_3$) δ −76.29(s), −121.94 (d, $^2J_{H,F}$ = 80.4 Hz), −134.56 (s). Anal. Calcd for $C_{15}H_{14}NO_4F_5$: C, 49.05; H, 3.84; N, 3.81. Found: C, 48.87; H, 3.66, N, 3.64.

4-Fluoro-(*E*)-β-(fluoromethylene)-*m*-tyrosine (2c). The 4-fluoro derivative **9a** (40 mg, 0.11 mmol) (fraction C from above) was stirred under reflux with 48% HBr (2.0 mL), protected from light, for 1 h. Evaporation of HBr under reduced pressure gave a light pink solid product which was dissolved in water (5 mL) and purified with an Amberlite IRA-400 resin (acetate form) column (3 mL). The column was eluted with water (10 × 5 mL) followed by 0.1% HOAc (10 × 1.0 mL). The fractions containing the free amino acid (ninhydrin test) were lyophilized to yield **2c** (20 mg, 80%): mp 210–212°C (d) (water); UV (H_2O) λ_{max} 275 nm (ϵ = 1610), λ_{sh} 239 nm (ϵ = 3210); ^{1}H NMR (D_2O) δ 4.46 (s, 1H), 6.81 (ddd, 1H, J = 2.2 Hz, J = 8.2 Hz, $^4J_{H,F}$ = 4.3 Hz), 6.94 (dd, 1H, J = 2.2 Hz, $^4J_{H,F}$ = 8.4 Hz), 7.10 (d, 1H, $^2J_{H,F}$ = 81.0 Hz), 7.18 (dd, 1H, J = 8.4 Hz, $^3J_{H,F}$ = 11.2 Hz). FAB HRMS M$^+$ + H calcd for $C_{10}H_{10}NO_3F_2$: 230.0629. Found: 230.0628.

4-Fluoro-(*Z*)-β-methylene-*m*-tyrosine (11). The fraction D (see above) (10 mg total weight) was heated under reflux with 48% HBr (2.0 mL) under ambient light conditions for 1 h. Removal of HBr under reduced pressure gave a solid product which was dissolved in water (5 mL) and passed through Amberlite IRA-400 (acetate form) resin column (2 mL). The resin column was eluted with 0.1% HOAc (30 mL). The eluate was evaporated and the residue was separated on an Alltech Econosil C18 semipreparative HPLC column (500 × 10 mm) with a mobile phase of 3% CH_3OH in 0.1% HOAc (flow rate 5 mL/min; UV detection at 279 nm). The 4-fluoro-(*Z*)-amino acid **11** with a retention time of 18 min was collected and evaporated to dryness at room temperature. The residue was redissolved in water and lyophilized to give **11** as a white solid (1 mg, 53% based on the amount of **9a** present in fraction D): mp 180–182 °C (d); UV (H_2O) λ_{max} 275 nm (ϵ = 1740), λ_{sh} 240 nm (ϵ = 3350); ^{1}H NMR (D_2O) δ 4.96 (s 1H), 6.82 (ddd, 1H, J = 8.4 Hz, J = 2.3 Hz, $^4J_{H,F}$ = 4.4 Hz), 6.94 (dd, 1H, J = 2.2 Hz, $^4J_{H,F}$ = 8.3 Hz), 7.03 (d, 1H, $^2J_{H,F}$ = 82.3 Hz), 7.16 (dd, 1H, $^3J_{H,F}$ = 11.2 Hz, J = 8.5 Hz); ^{13}C NMR (D_2O) δ 52.4, 117.3, 117.5, 119.1, 120.8, 129.1, 146.2, 151.2 (d, $^1J_{C,F}$ = 268.1 Hz), 152.1 (d, $^1J_{C,F}$ = 232.1 Hz), 172.9; ^{19}F NMR (D_2O) δ −119.29 (d, $^2J_{H,F}$ = 82.3 Hz), −134.78 (s); CI HRMS (ammonia) M$^+$ + H calcd for $C_{10}H_{10}NO_3F_2$ 230.0629, found 230.0637.

4,6-Difluoro-(*E*)-β-(fluoromethylene)-*m*-tyrosine (12a). 4,6-Difluoro derivative **12a** was obtained from the above described HPLC separation. The fraction with t_R 34 min was collected and processed as described for **11** to give **12a** as a white solid (2 mg, 45% based on the amount of **10a** present in fraction D): mp 158–160°C (d); UV (H_2O) λ_{max} 278 nm (ϵ = 2020); ^{1}H NMR (D_2O) δ 4.53 (s 1H), 6.87 (dd, 1H, $^4J_{H,F}$ = 8.0 Hz, $^4J_{H,F}$ = 10.0 Hz), 7.09 (dd, 1H, $^3J_{H,F}$ = 10.9 Hz, $^3J_{H,F}$ = 9.8 Hz), 7.19 (d, 1H, $^2J_{H,F}$ = 81.0 Hz); CI HRMS (ammonia) M$^+$ + H calcd for $C_{10}H_9NO_3F_3$ 248.0536, found 248.0535.

Table 3. Crystal Data, Data Collection, and Refinement Parameters

	2a	2b (*S*)	2b (*R*)	2c
formula	$C_{10}H_{11}F_2NO_4$	$C_{10}H_{13}O_5F_2N$	$C_{10}H_{13}O_5F_2N$	$C_{10}H_{11}NO_4F_2$
fw	247.20	265.21	265.21	247.20
cryst syst	monoclinic	orthorhombic	orthorhombic	monoclinic
space group	$P2_1$	$P_12_12_1$	$P2_12_12_1$	$P2_1/c$
cryst dimens, mm	0.45 × 0.30 × 0.25	0.30 × 0.25 × 0.18	0.50 × 0.30 × 0.25	0.10 × 0.15 × 0.35
a, Å	8.613(1)	10.475(2)	10.487(1)	12.798(1)
b, Å	5.419(1)	18.572(5)	18.626(1)	5.272(1)
c, Å	11.719(1)	5.912(2)	5.919(1)	15.743(2)
Z	2	4	4	4
V, Å^3	517.5(1)	1150.1(5)	1156.2(1)	1060.3(3)
ϱ (calcd), g cm^{-3}	1.59	1.53	1.52	1.55
radiation	Mo Kα	Mo Kα	Mo Kα	Cu Kα
abs coeff (μ), cm^{-1}	1.37	1.34	1.33	12.05
F(000), e	256	552	552	512
temp, K	156	156	156	296
diffractometer	Picker (Crystal Logic)	Picker (Crystal Logic)	Picker (Crystal Logic)	AFC5R (Rigaku)
index ranges	h (0 to 12)	h (0 to 13)	h (0 to 14)	h (0 to 14)
	k (0 to 7)	k (0 to 24)	k (0 to 26)	k (0 to 6)
	l (−16 to 16)	l (0 to 7)	l (0 to 8)	l (−17 to 17)
scan mode, speed (deg/min)	θ−2θ, 6.0	θ−2θ, 3.0	θ−2θ, 6.0	2θ−ω, 16.0
2θ range, deg	1−60	1−55	1−60	1−115
total data collected	1752	1581	1984	1795
unique data	1669	1581	1984	1646
observed data used	1124 ($F > 3\sigma(F)$)	860 ($I > 3\sigma(I)$)	1290 ($I > 3\sigma(I)$)	1031 ($F > 6\sigma(F)$)
no. of parms refined	151	163	163	154
final shift/error, max and avg	0.012, 0.003	0.004, 0.001	0.006, 0.001	0.004, 0.001
max resid density, e/Å^3	0.66	0.62	0.52	0.52
$R = \Sigma\|\|F_o\|-\|F_c\|\|/\Sigma\|F_o\|$	0.055	0.059	0.058	0.048
$R_w = (\Sigma\omega(\|F_o\|-\|F_c\|)^2/\Sigma\omega(\|F_o\|)^2)^{1/2}$	0.069	0.068	0.068	0.064
GOF = $(\Sigma\omega(\|F_o\|-\|F_c\|)^2/(N_o - N_v))^{1/2}$	1.980	1.803	1.848	1.702

2,4-Difluoro-(*E*)-β-(fluoromethylene)-*m*-tyrosine (12b). The fraction B containing **10b** (6 mg, 16 μmol) was hydrolyzed with HBr and purified by preparative HPLC as described for **11**, to give **12b** as an amorphous solid (1 mg, 26%): ^{1}H NMR (D_2O) δ 4.51 (s, 1H), 6.73 (ddd, 1H, J = 8.8 Hz, $^4J_{H,F}$ = 7.5 Hz, $^4J_{H,F}$ = 5.8 Hz), 7.01 (dt, 1H, $J_{H,H} = {}^3J_{H,F}$ = 9.5 Hz, $^5J_{H,F}$ = 2.0 Hz), 7.18 (d, 1H, $^2J_{H,F}$ = 80.4 Hz); FAB (*m*-nitrobenzyl alcohol + NaI) HRMS M + Na$^+$ calcd for $C_{10}H_8NO_3F_3Na$ 270.0354, found 270.0367.

Ethyl 2-[(Trifluoroacetyl)amino]-3-(3-acetoxy-4-fluorophenyl)-4-fluoro-3-butenoate (9b). Fluorine (1% in argon, 0.28 mmol) was bubbled into a solution of **8b** (60.0 mg, 0.11 mmol) in freon-11 (15 mL) at room temperature over a period of 2 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and filtered. The filtrate was evaporated and the residue chromatographed on a preparative TLC silica plate with ether−petroleum ether (1:4) mixture. From the zone with R_f 0.6, the starting material **8b** was recovered (20 mg). The product **9b** was recovered from the zone with R_f 0.5 (6 mg, 21% yield based on the amount of starting material recovered from the reaction mixture): mp 94 − 95°C (petroleum ether); ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, J = 6.9 Hz), 2.34 (s, 3H), 4.25 (q, 2H, J = 6.9 Hz), 5.16 (d, 1H, J = 7.0 Hz), 7.02 (d, 1H, $^2J_{H,F}$ = 80.3 Hz), 7.06 (m 2H), 7.17 (t, 1H, J = 9.1 Hz), 7.21 (broad d, 1H); ^{19}F NMR ($CDCl_3$) δ −76.28 (s), −121.48 (d, $^2J_{H,F}$ = 80.3 Hz), −127.39 (s); EI HRMS calcd for $C_{16}H_{14}NO_5F_5$ 395.0792, found 395.0799.

X-ray Structure Determination.[30] The colorless crystal used for the X-ray study was grown by slowly cooling aqueous solutions of **2a**, **2b** (*R* and *S*) and **2c**, respectively. The crystal data, parameters of data collection and refinement results are given in Table 3. The unit cell dimensions were determined by least squares using 25 centered reflections using graphite monochromated Cu Kα or Mo Kα radiation. Three standard reflections were recorded every 97 reflections to monitor the intensity and they showed no significant variations. The intensity data were corrected for Lorentz and polarization effects. No absorption correction was applied for **2a** and **2b**. The data set for **2c** was corrected for absorption effects with an empirical absorption factor ranging from 0.81 to 1.00. The structures were solved using SHELXS86 program[31] and refined by full matrix least squares method. The non-hydrogen atoms were refined anisotropically and the hydrogens (excluding water molecules) were localized by the intermediate difference maps using the fixed thermal parameter and a "riding" model.

Acknowledgment. We are grateful to Dr. M. G. Palfreyman of Merrell Dow Research Institute (Cincinnati, OH) for kindly providing us with an authentic sample of racemic (*E*)-FMMT for chromatographic reference. We thank Dr. Saeed Khan, UCLA Department of Chemistry and Biochemistry, for performing X-ray analysis and Dr. Pak Poon, for his help with CD spectral studies. We are grateful to Prof. Joseph Horwitz, Jules Stein Eye Institute, UCLA for helpful discussions. This work was supported in part by Department of Energy Grant DE-FC0387-ER60615, NIH Grant PO1-NS-15654, and donations from the Jennifer Jones Simon and Ahmanson Foundations.

JO941493R

(30) The atomic coordinates for all the X-ray structures 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, UK.

(31) Sheldrick, G. M. SHELXS86: Program for the Solution of Crystal Structures from X-ray Data, University of Gottingen, Germany, 1986.