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(Z)-β-Fluoromethylene-m-tyrosine: synthesis, crystal structure and fluorination

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Abstract

(Z)-β-Fluoromethylene-*m*-tyrosine (**2**) has been prepared by acid hydrolysis of ethyl 2-trifluoroacetylamino-3-(3-methoxyphenyl)-4-fluoro-3-butenoate (**1**) and its structure established by single crystal X-ray diffraction analysis. Fluorination of the amino acid **2** with acetyl hypofluorite yielded a mixture of products containing fluorine substituted on the ring (**3a–c**) as well as added across the vinylic double bond (**4a,b**). The mechanism for the formation of the diastereomeric adducts **4a** and **4b** is discussed based on tight ion pair intermediates. All major products of fluorination of **2** were isolated by semipreparative HPLC and their structures determined by spectral analyses.

Keywords: Mechanism-based inhibition; Fluoromethylene amino acid; Monoamine oxidase; Fluorination; Crystal structure

1. Introduction

Mechanism-based irreversible inhibition of specific enzymes has been an active area of research for more than twenty years [1]. A variation of this approach is the use of a prodrug (or a promechanism-based inactivator) to produce, in situ, an inhibitor that selectively inactivates an enzyme [2]. An interesting example of this approach was developed by MacDonald et al. [3] using the drug (E)-β-fluoromethylene-*m*-tyrosine (**6**). In the central nervous system, the amino acid **6** first undergoes a decarboxylation reaction mediated by the enzyme aromatic amino acid decarboxylase (AAAD) and the resulting product, (E)-2-(3-hydroxyphenyl)-3-fluoroallylamine, selectively and irreversibly inhibits a second enzyme, monoamine oxidase (MAO). Hence, this process of MAO inactivation is termed dual enzyme-activated irreversible inhibition. Further, this drug **6** when coadministered with peripheral AAAD inhibitors preferentially suppresses the MAO activity in the central monoamine neurons, with little or no MAO inhibition in peripheral tissues [4]. Such properties differentiate **6** and related derivatives from previous-generation MAO inhibitors which occassionally exhibit severe side effects [5].

Among the number of β-fluoromethylene amino acids thus far tested, (E)-β-fluoromethylene-*m*-tyrosine (**6**) has emerged as a useful dual enzyme-activated inhibitor of MAO [6]. Also, this amino acid has been recently ring fluorinated with the ^{18}F isotope [7] for investigating the brain MAO system using positron emission tomography (PET) [8]. However, only limited biochemical/chemical information exists on the geometrical isomer of **6**, namely (Z)-β-fluoromethylene-*m*-tyrosine (**2**). Thus, to investigate the suitability of **2** and its ring fluorinated analogs as potential markers for central MAO, we have now prepared (Z)-β-fluoromethylene-*m*-tyrosine [6] and established its structure using single crystal X-ray crystallographic analysis. Fluorination of the amino acid **2** with acetyl hypofluorite was also investigated as a prelude to preparing the ring fluorinated derivatives, the ^{18}F-labeled counterparts of which may be useful as PET biochemical probes.

2. Results and discussion

The synthesis of (E)- and (Z)-fluoromethylene-*m*-tyrosines (**2** and **6**) has previously been achieved via two different synthetic routes — a Wittig-type reaction or with intermediates involving an oxazoline moiety [6]. We have slightly modified the Wittig-type reaction to yield a mixture of (Z)-β-fluoromethylene derivative **1** and the corresponding (E)-isomer, from which the pure (Z)-isomer was isolated [9]. In this investigation, pure (Z)-β-fluoromethylene-*m*-tyrosine (**2**) was obtained by acid hydrolysis of the protected derivative **1** with HBr followed by ion exchange chromatographic

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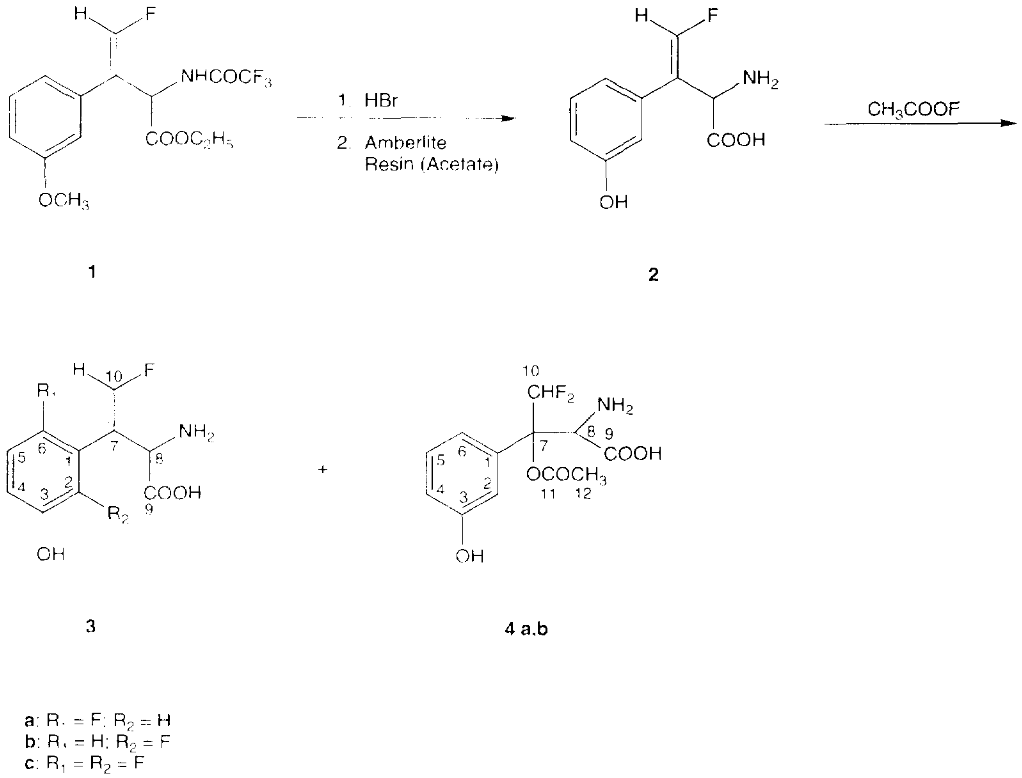


Scheme 1.

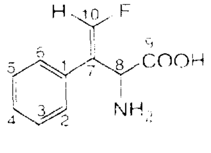
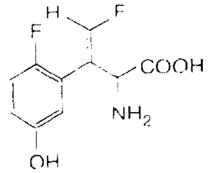
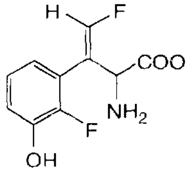
purification (Scheme 1). The amino acid **2** was fully characterized by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis (see Experimental and Table 1). Unequivocal evidence for its structure was obtained from X-ray crystallographic analysis.

The ORTEP plot of the amino acid **2** is shown in Fig. 1. The final fractional atomic coordinates are given in Table 2. Selected interatomic distances and bond angles are provided in Tables 3 and 4. The X-ray structure determination of **2** showed half a molecule of water of crystallization (Fig. 1). The oxygen atom of water (O1W) was located at 2.44 Å and 2.79 Å from H8 and O3, respectively. The X-ray analysis also confirmed the (*Z*)-geometry at the vinylic double bond in **2**. The dihedral angle between the plane of the benzene ring and of the vinylic double bond was found to be 131° (Fig. 2). Interestingly, this angle is close to the corresponding angle (134.5°) observed in the (*E*)-isomer **6** [9]. Overall, the geometry of **2** in the solid state resembled that of the (*E*)-isomer **6** [9]. However, as expected, the non-bonded distances between the fluorine atom and the oxygen atoms of the carboxylic group in **2** were 1.1–1.3 Å shorter than the corresponding values determined in **6**. Similarly, the distance between fluorine and nitrogen atom in the (*Z*)-amino acid **2** was shorter by 0.5 Å.

Fluorination of the amino acid **2** was investigated with a view to preparing the ring fluorinated derivatives. In general, fluoroaryl analogs of aromatic amino acids exhibit interesting biochemical properties [10]. Recently, it was reported that (*E*)-*β*-fluoromethylene-*m*-tyrosine (**6**) reacts with acetyl hypofluorite to give only the ring fluorinated analogs of **6** [7]. On the other hand, in this reaction, we have isolated, along with the ring fluorinated derivatives, products due to the addition of acetyl hypofluorite across the vinylic double bond [9]. A similar fluorination of the (*Z*)-amino acid **2** in glacial acetic acid-trifluoroacetic acid (1:1) at 0 °C with a small excess of acetyl hypofluorite yielded a mixture of products (Scheme 1) which was separated completely using two successive semipreparative HPLC systems. The first system was used to get a baseline separation of the unreacted starting material (**2**, 50%), ring fluorinated products (**3a–c**, 45%) and the vinylic double bond addition products (**4a,b**, 5%) in three distinct fractions. The ring fluorinated derivatives (**3a–c**) were further separated into their individual components with two semipreparative HPLC columns connected in series (see Experimental). The diastereomeric addition products **4a** and **4b** were also separated using the HPLC system.

The structure of the fluoro derivatives **3a** and **3b** was established by ^{1}H, ^{13}C (Table 1), ^{19}F (Table 5) NMR and mass spectroscopy. The position of the fluorine atom on the aromatic ring in **3a** and **3b** was discerned based on the ^{1}H NMR signal splitting patterns and their comparison with the proton signals in the corresponding (*E*)-isomers whose structures have previously been established [7,9]. For example, the ^{1}H NMR signals for H(5) and H(2) in **3a** appeared as doublets of doublets due to their coupling with the fluorine on C(6) whereas the signal due to H(4) emerged as a doublet of doublet of doublets (ddd), consistent with its structure. In contrast, all aromatic protons in the 2-fluoro isomer (**3b**)

Table 1
^{13}C NMR chemical shift values for (Z)-β-fluoromethylene-*m*-tyrosine and its ring fluorinated products [a,b]

Carbon number	 **2**		 **3a**		 **3b**	
C-1	133.8 ($J_{C,F}$ = 5.8 Hz)		120.5 ($J_{C,F}$ = 15.8 Hz)	(120.9)	120.7 ($J_{C,F}$ = 11.7 Hz)	(120.9)
C-2	115.8		118.3	(117.2)	150.7 ($J_{C,F}$ = 239.5 Hz)	(150.6)
C-3	156.7		152.6	(152.2)	145.5 ($J_{C,F}$ = 9.9 Hz)	(143.8)
C-4	116.8		118.4	(118.2)	119.8	(118.2)
C-5	131.3		117.5	(118.4)	125.6	(126.8)
C-6	121.1		155.4 ($J_{C,F}$ = 236.1 Hz)	(155.9)	122.5	(122.5)
C-7	119.6 ($J_{C,F}$ = 6.5 Hz)		114.6 ($J_{C,F}$ = 6.5 Hz)		114.5 ($J_{C,F}$ = 9.0 Hz)	
C-8	52.1 ($J_{C,F}$ = 3.7 Hz)		52.2		52.3	
C-9	172.4		172.1		172.2	
C-10	151.4 ($J_{C,F}$ = 270.0 Hz)		153.0 ($J_{C,F}$ = 271.7 Hz)		152.8 ($J_{C,F}$ = 272.1 Hz)	

[a] In D_2O with 1,4-dioxane at 67.4 ppm as an external standard.
[b] Chemical shifts given in parentheses are calculated values.

appeared as multiplets. These signal patterns are similar to those observed in the closely related 6- and 2-fluoro-(*E*)-β-fluoromethylene-*m*-tyrosines, respectively [7,9]. Further, the aromatic signal patterns in **3a** and **3b** are quite different from those reported for 4-fluoro-(*Z*)-β-fluoromethylene-*m*-tyrosine [9]. The measured ^{13}C chemical shift values for **3a** and **3b** are in good agreement with the calculated values (Table 1), which strongly supports the identities of these

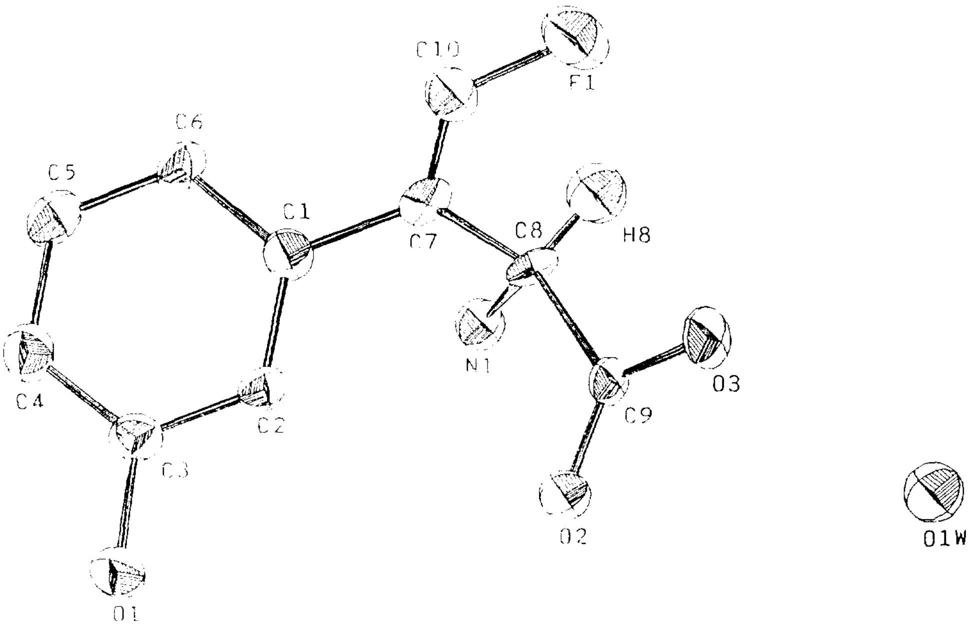


Fig. 1. ORTEP diagram of (Z)-β-fluoromethylene-*m*-tyrosine (2). Ellipsoids drawn at 50% probability level except for H-8 atom.

Table 2
Final fractional atomic coordinates [a]

Atom	x	y	z
C1	0.1484(4)	−0.2836(12)	0.5520(4)
C2	0.1212(4)	−0.1249(12)	0.4897(4)
C3	0.1218(4)	−0.1786(13)	0.4137(4)
O1	0.0908(3)	−0.0181(9)	0.3538(3)
C4	0.1511(4)	−0.3909(13)	0.3964(4)
C5	0.1792(4)	−0.5469(13)	0.4586(4)
C6	0.1774(4)	−0.5009(13)	0.5359(4)
C7	0.1449(3)	−0.2338(11)	0.6343(4)
C8	0.0788(4)	−0.1619(11)	0.6498(4)
C9	0.0675(4)	0.1228(12)	0.6467(4)
N1	0.0180(3)	−0.2817(10)	0.5935(3)
O2	0.0374(2)	0.2132(8)	0.5816(3)
O3	0.0916(3)	0.2223(8)	0.7117(3)
C10	0.2006(4)	−0.2582(12)	0.6935(4)
F1	0.1989(2)	−0.2281(8)	0.7710(2)
O1W	0.5000	0.0439(13)	0.7500
H2	0.1008	0.0311	0.5008
H10	0.0788	−0.0420	0.2926
H4	0.1519	−0.4279	0.3405
H5	0.2018	−0.6979	0.4471
H6	0.1965	−0.6194	0.5798
H8	0.0833	−0.2162	0.7057
H10	0.2448	−0.2999	0.6823
H1N	0.0015	0.2484	0.5385
H2N	−0.0172	−0.2623	0.6141
H1W	0.5463	−0.0105	0.7503

[a] Values in parentheses are the e.s.d.

Table 3
Interatomic distances (Å) involving non-H atoms [a]

From	To	Distance
C2	C1	1.386(9)
C6	C1	1.402(9)
C3	C2	1.363(9)
C4	C3	1.389(10)
C3	O1	1.381(8)
C5	C4	1.379(10)
C6	C5	1.384(9)
C1	C7	1.484(9)
C7	C10	1.307(9)
C7	C8	1.498(9)
C8	N1	1.500(8)
C8	C9	1.591(8)
C9	O3	1.232(8)
C9	O2	1.235(7)
C10	F1	1.373(8)

[a] E.S.D. are given in parentheses.

products. The ^{19}F NMR chemical shifts (Table 2) for the aromatic fluorine in **3a** and **3b** were interestingly close to those recorded for the corresponding (*E*)-isomers [7,9]. The trace amounts of the difluoro product (**3c**) obtained was tentatively identified by ^{19}F NMR only (Table 2).

Generally, a preponderance of products of addition has been noted for the reaction of acetyl hypofluorite with substituted styrenes and stilbenes [11]. Only in special cases has a minor contribution of the substitution of fluorine on the aromatic ring been detected [11]. However, the reaction of acetyl hypofluorite with the (*Z*)-amino acid **2** yielded a mixture in which products due to aromatic substitution predominated over those of addition across the vinylic double bond (substitution:addition ~10:1). A similar product ratio of substitution vs. addition was also obtained in the case of the (*E*)-amino acid **6** [9]. The lower proportion of products of addition realized with **2** and **6** is most probably due to the deactivation of the vinylic double bond by fluorine. Analo-

Table 4
Bond angles (°) involving non-H atoms [a]

From	Through	To	Angle
C2	C1	C6	118.9(6)
C2	C1	C7	121.6(6)
C6	C1	C7	119.4(6)
C3	C2	C1	121.0(6)
C2	C3	O1	118.0(6)
C2	C3	C4	121.2(6)
O1	C3	C4	120.8(6)
C5	C4	C3	117.8(7)
C4	C5	C6	122.2(7)
C5	C6	C1	118.8(7)
C10	C7	C1	118.6(6)
C10	C7	C8	120.4(6)
C1	C7	C8	121.0(6)
C7	C8	N1	112.4(5)
C7	C8	C9	112.7(5)
N1	C8	C9	109.3(5)
O3	C9	O2	129.2(7)
O3	C9	C8	113.3(6)
O2	C9	C8	117.5(6)
C7	C10	F1	120.6(7)

[a] Values in parentheses are the E.S.D.

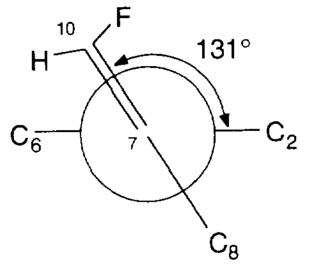
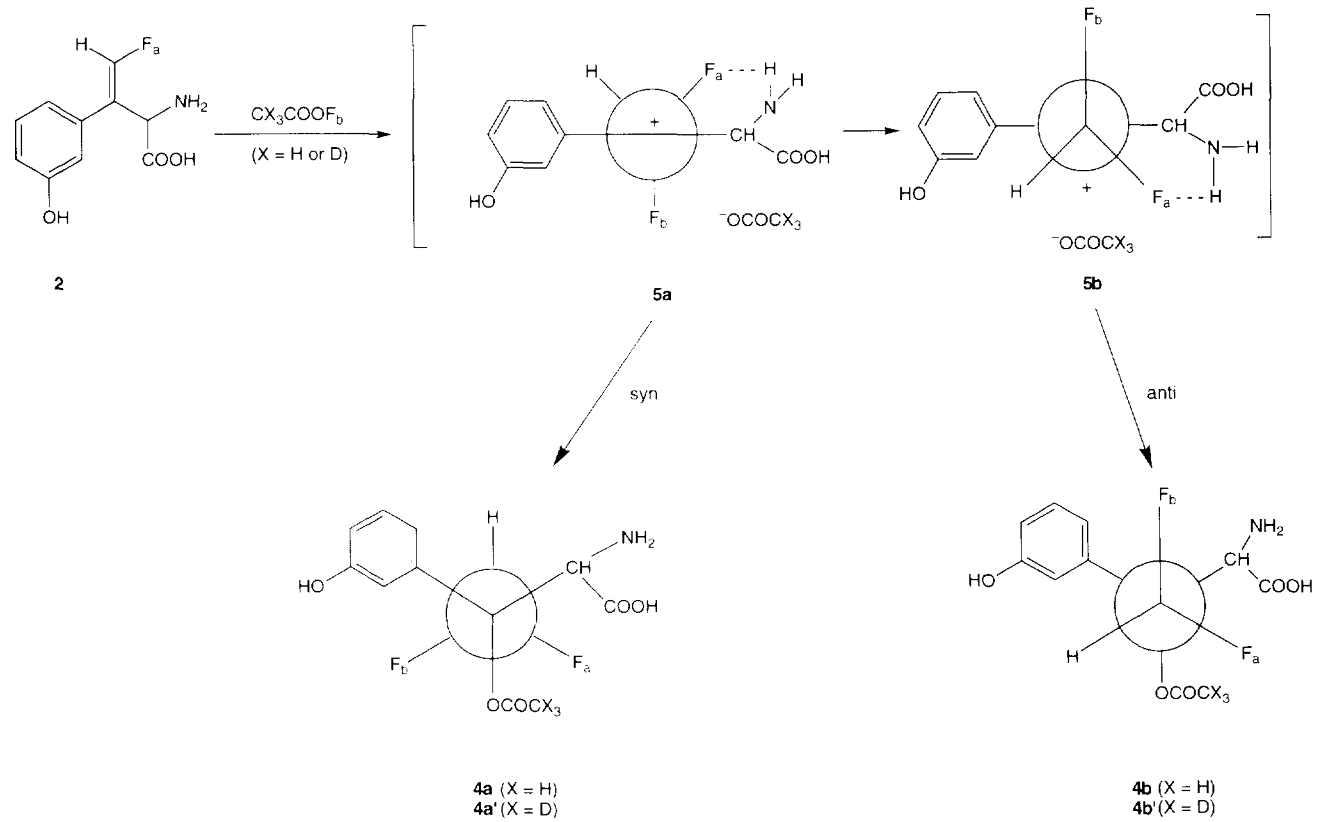


Fig. 2. The dihedral angle between the plane of the phenyl ring and of the fluorovinyl group.

Table 5
^{19}F NMR data

Compound	δ (ppm)	
	CHF	ArF
2	−119.3 ($J_{F,H}$=82.5 Hz)	
3a	−114.7 ($J_{F,H}$=81.7 Hz)	−123.5
3b	−114.6 ($J_{F,H}$=81.7 Hz)	−136.5
3c	−110.4 ($J_{F,H}$=81.0 Hz)	−120.6 (F-6) −132.0 (F-2)



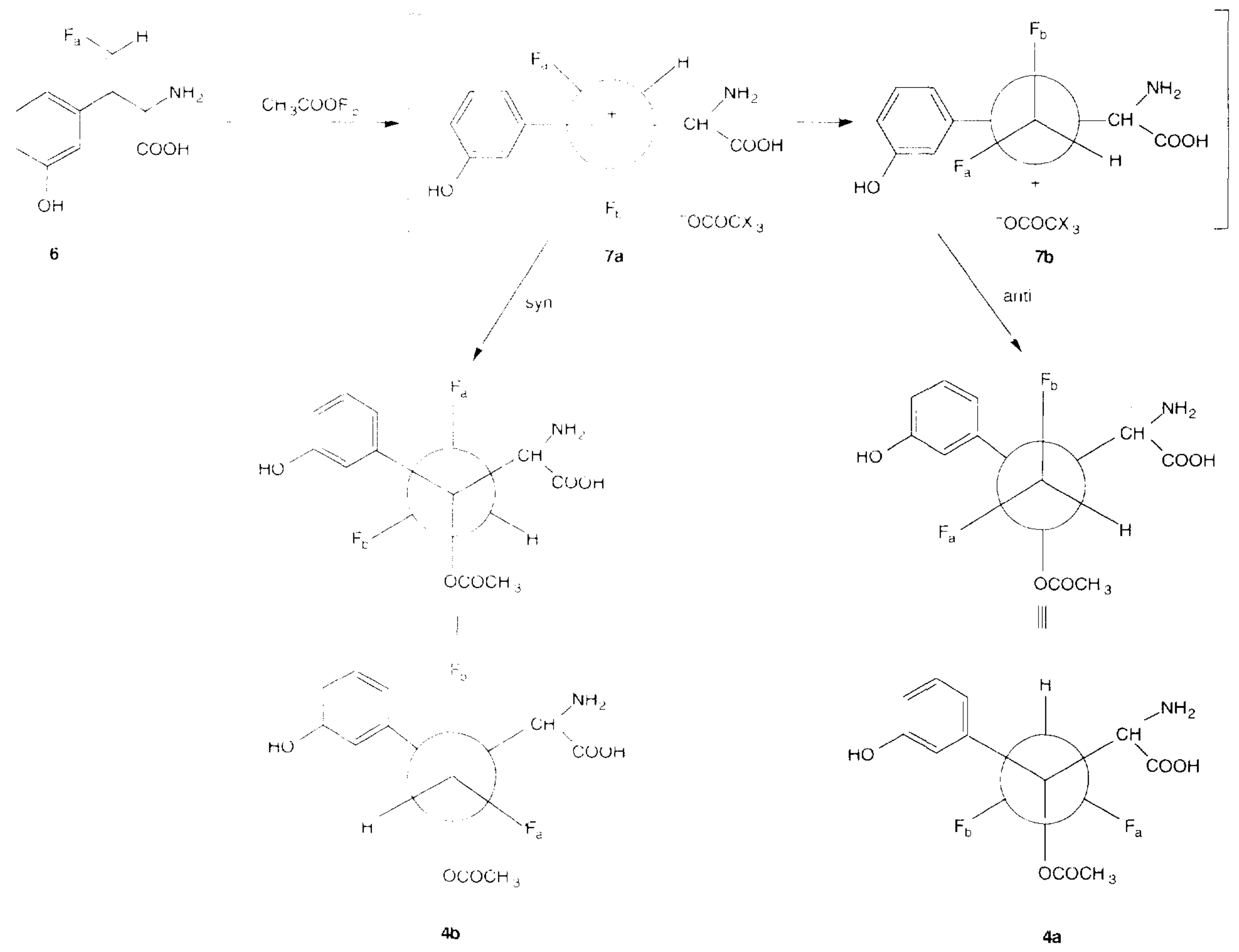
Scheme 2.

gous suppression of electrophilic addition to sp^2 carbons attached to fluorine has been recognized [12].

The diastereomeric addition products **4a** and **4b** were obtained in a ratio of 1:1 from the (*Z*)-amino acid **2** (Scheme 2). Interestingly, the same two adducts were also derived from the (*E*)-isomer **6** (Scheme 3) but in a ratio of 1:9. It has been shown that the addition of acetyl hypofluorite to double bonds regioselectively follows Markownikoff's rule with a predominant stereoselective *syn* mode of addition [11]. Further, tight ion pair intermediates [13] have been invoked for this reaction. Thus, the *syn* addition of acetyl hypofluorite to the (*E*)-amino acid **6** leads to the tight-ion pair **7a** which predominantly collapses to the *syn* adduct **4b** (Scheme 3). However, the C(7) carbenium ion in the tight-ion pair **7a** has some benzylic character and hence may have a lifetime sufficiently long enough to allow the rotation of the C(1)–C(7) bond for the formation of the ion pair **7b**. The *anti* addition of the acetate ion to **7b** then leads to the *anti* adduct **4a**. This predominant formation of the *syn* adduct over the *anti* product (**4b**:**4a** = 9:1) is in accordance with the previous observations of the reaction of acetyl hypofluorite with styrenes and stilbenes [11].

The *syn* addition of acetyl hypofluorite to the vinylic double bond in the (*Z*)-amino acid would lead to the tight-ion pair **5a** (Scheme 2). Intramolecular hydrogen bonding of the (*Z*)-fluorine (designated F_a) with a hydrogen on the amino group (or $\overset{+}{N}H_3$ as the case may be) could stabilize the six-membered cyclic tight-ion pair **5a**. Analogous hydrogen bondings are also known in β-fluoroamino acids [14]. It has been noted that a six-membered alicyclic carbenium ion is more stable than the corresponding open chain analog [15]. Thus, it could be reasoned that the cyclic tight-ion pair **5a** would have a relatively longer lifetime than its linear counterpart **7a**. This would perhaps permit the rotation of C(1)–C(7) bond in **5a**, leading to a more efficient formation of **5b** than the corresponding process in the tight-ion pair **7a**. Further, the ion pair **5b** could also be stabilized by the hydrogen bonding. The collapse of the tight-ion pair **5a** and the *anti* addition of the acetate to the ion pair **5b** would then lead to the diastereomeric adducts **4a** and **4b**, respectively. The formation of **4a** and **4b** in a ratio of 1:1 strongly suggests the formation of equal amounts of the ion pairs **5a** and **5b**.

It has been reported that in the reaction of acetyl hypofluorite with olefins, *syn* addition products are derived from tight ion pair intermediates and *anti* adducts from open carbenium ions [11]. Generation of such open carbenium ion intermediates during the addition of acetyl hypofluorite to glycals and pyrimidines has been supported by trapping experiments with extraneous nucleophiles such as water and deuterated acetic acid [16,17]. On the other hand, in the reaction of uracil with acetyl hypofluorite in the presence of deuterated acetate ion, it has been observed that the incorporated acetate moiety stems entirely from the added acetate and not from acetyl hypofluorite [18]. Since the fluorination reaction of



Scheme 3.

the amino acids **2** and **6** was conducted in acetic acid/trifluoroacetic acid medium the origin of the acetate group in the products **4a** and **4b** was not clear. Thus, to further understand the involvement of the open carbenium ion in the formation of *anti* adducts a few additional experiments were carried out.

Fluorination of the (*Z*)-amino acid **2** in CH_3COOH/CF_3COOH with CD3COOF gave the adducts **4a′** and **4b′** also in a ratio of 1:1. The presence of only small amounts (<5%) of **4a** and **4b** (products that stem from the diffusion of the tight ion pair to form open carbenium ion which is trapped by the solvent acetic acid) in the product mixture was observed by ^{1}H NMR and high resolution mass spectroscopy. While the formation of the *syn* adduct with a near exclusive deuterated acetoxy group is not surprising, a similar disposition with the *anti* adduct is rather interesting. If the *anti* adducts are derived from open carbenium ions [11], the *anti* product **4b′** in this case should have been contaminated significantly with **4a** since the large excess of acetic acid present as the solvent would overwhelmingly compete with the deuterated acetate for the carbenium ion. Thus, it is probable that the formation of the *anti* product **4b′** (or **4b**), at least in these amino acids, does not involve an open carbenium ion. Instead, there is a rotation of carbon–carbon bond [C(1)–C(7)] in the tight ion pair **5a** leading to another ion pair **5b** which is responsible for the formation of the *anti* adduct. Such an explanation has been proposed before for the halogenation of olefins [19–21]. Further, the reaction of the (*Z*)-amino acid **2** in CD_3COOD/CF_3COOD with CH_3COOF gave the addition products that contained only **4a** and **4b**, supporting strongly the above rationale. A similar conclusion could be drawn based on the reaction of the (*E*)-amino acid **6** with acetyl hypofluorite with added deuterated acetate salt to the reaction medium, which yielded the products **4a** and **4b** devoid of the deuterated derivatives **4a′** and **4b′**. As expected, the trifluoroacetoxy group from the solvent was never incorporated into the adducts in any of the reactions due to the extremely low nucleophilicity of CF_3COOH [22].

The diastereomeric products **4a** and **4b** exhibited similar ^{1}H and ^{13}C NMR spectra. However, ^{19}F NMR differentiated the diastereotopic fluorine nuclei (designated F_a and F_b in Schemes 2 and 3) within each diastereomer. Analogous anisochronism of geminal fluorine nuclei in asymmetric difluoroethanes has long been known [23,24]. The CHF_2 group in **4a** and **4b** formed ABX systems in the ^{19}F NMR that displayed perceptible chemical shift differences in the two pseudo quartets [25].

In summary, fluorination of (Z)-β-fluoromethylene-*m*-tyrosine yields a mixture of ring fluorinated products along with a pair of diastereomeric addition products. The product mixture has been completely separated and identified by NMR spectroscopy. The fluorination procedure adopted herein can easily be applied to preparation of ^{18}F-labeled analogs of **3a** and **3b** for potential use with positron emission tomography [8].

3. Experimental

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. NMR spectra were recorded with Bruker AM-360 WB (operating at 360.14, 90.57 and 338.87 MHz for ^1H, ^{13}C and ^{19}F nuclei, respectively) and Bruker AM-500 (operating at 125.77 MHz for ^{13}C nucleus) spectrometers. The chemical shifts were referenced to internal (2,2-dimethyl-2-silapentane-5-sulfonate, DSS, for ^1H) and external ($CFCl_3$ for ^{19}F and 1,4-dioxane or TMS for ^{13}C) standards. Ultraviolet spectra were recorded with a Beckman DU-50 spectrophotometer. High resolution electron impact and chemical ionization (ammonia) mass spectral data were collected on a VG Analytical AutoSpec mass spectrometer, and high resolution fast atom bombardment (FAB) mass spectral data were collected on a ZAB SE mass spectrometer. Elemental analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, TN.

The semipreparative HPLC separations and purifications of fluorinated amino acid products were performed with two different column systems. System I consisted of an Alltech Econosil C18 semipreparative 10μ column (500 × 10 mm; loop volume: 500 μl) and system II consisted of an Econosil C18 (Alltech) semipreparative 10μ column (250 × 10 mm) and Spherisorb 5 ODS (Phenomenex) semipreparative 5μ column (250 × 10 mm) connected in series (loop volume: 100 μl). Two different mobile phases (mobile phase A, 3% methanol in 0.1% acetic acid; mobile phase B, 0.02 M NaOAc, pH = 3.5 adjusted with HOAc) were used each at a flow rate of 5.0 m min^{-1}. The column effluents were monitored with a UV detector (279 nm). The analytical HPLC was carried out with column system II and mobile phase A.

3.1. (Z)-β-Fluoromethylene-m-tyrosine (2)

A mixture of the (Z)-isomer of ethyl 2-trifluoroacetylamino-3-(3-methoxyphenyl)-4-fluoro-3-butenoate (**1**) [9] (0.51 g, 1.46 mmol) and 48% HBr (2.5 ml) was refluxed for 30 min protected from light. The acid was evaporated under reduced pressure, the light pink residue dissolved in water (5 ml) and the residue chromatographed (Amberlite IRA-400 resin-acetate form). The column was eluted with water (3 × 5 ml) and 0.1% acetic acid (2 × 5 ml). The ninhydrin-positive fractions were pooled and lyophilized to obtain 0.29 g (94%) of **2**. The product was recrystallized from water, m.p. 183–184 °C (d), (lit. [6] 178–180 °C). UV (H_2O): λ_{max} 280 nm (ϵ = 1630); λ_{sh} 239 nm (ϵ = 3640); λ_{max} 214 nm (ϵ = 7610). ^1H NMR (D_2O) δ: 5.00 (s, 1H, CH); 6.64 (t, 1H, J = 2.0 Hz, H-2); 6.91 (dd, 1H, J = 1.9 and 8.0 Hz, H-6); 6.94 (dd, 1H, J = 8.6 and 2.0 Hz, H-4); 7.08 (d, 1H, $J_{H,F}$ = 82.5 Hz, CHF); 7.13 (t, 1H, J = 8.0 Hz, H-5) ppm. Anal. calcd for $C_{10}H_{10}NO_3F \cdot 1/2H_2O$: C, 54.54; H, 5.04; N, 6.36. Found: C, 54.39; H, 5.09; N, 6.32%.

3.2. Fluorination of (Z)-β-Fluoromethylene-m-tyrosine (2)

Into a solution of **2** (63.3 mg, 0.3 mmol) in 1:1 glacial acetic acid and trifluoroacetic acid (15 ml) at 0 °C, acetyl hypofluorite (0.46 mmol, prepared by passing 1% F_2 in argon through a $NaOAc \cdot 3H_2O$ (0.40 g) cartridge [26]) was bubbled over a period of 60 min. The solvents were evaporated under reduced pressure at room temperature. The residue was dissolved in water (1.5 ml) and chromatographed; column: system I, mobile phase B. This chromatographic system enabled the separation of the starting material **2** and the fluorinated products into three major fractions. The elution sequence with the increasing retention times was **2** < **3a–c** < **4a,b**. The collected fractions were lyophilized and rechromatographed on the same HPLC column with mobile phase A to remove salts. The last fraction containing the products of addition upon rechromatography on the HPLC column system I again but with mobile phase A allowed the separation of **4a** and **4b**. The middle fraction containing a mixture of the ring fluorinated products **3a–c** was dissolved in water (1.5 ml) and rechromatographed on column system II with mobile phase A. A baseline separation of this mixture was achieved; the elution sequence with increasing retention times was **3a** < **3c** < **3b**. The individual products thus isolated from HPLC were lyophilized to get the following pure products: unreacted starting material **2** (30.1 mg, 47.6%), **3a** (10.1 mg, 27.9%), **3b** (6.1 mg, 16.8%), **3c** (0.2 mg, 0.52%), **4a** (1.5 mg, 3.3%) and **4b** (1.5 mg, 3.3%). The yields of the products **3a–c** and **4a,b** were based on the amount of **2** recovered.

3.3. 6-Fluoro-(Z)-β-fluoromethylene-m-tyrosine (3a)

White solid, m.p. 157–159 °C (d). UV (H_2O): λ_{max} 284 nm (ϵ = 2430); λ_{sh} 233 nm (ϵ = 3450). ^1H NMR (D_2O) δ: 4.91 (s, 1H, CH); 6.73 (dd, 1H, J = 3.4 and 5.9 Hz, H-2); 6.86 (ddd, 1H, J = 8.8 and 3.5 Hz and $J_{H,F}$ = 3.5 Hz); 7.01 (d, 1H, $J_{H,F}$ = 81.7 Hz, CHF); 7.04 (dd, 1H, J = 9.5 and 9.5 Hz, H-5) ppm. FAB-HRMS calcd for $C_{10}H_{10}NO_3F_2$ (M^+ + H): 230.0629. Found: 230.0623.

3.4. 2-Fluoro-(Z)-β-fluoromethylene-m-tyrosine (3b)

White solid, m.p. 163–165 °C (d). UV (H_2O): λ_{max} 275 nm (ϵ = 1340); λ_{sh} 236 nm (ϵ = 3540). ^1H NMR (D_2O) δ: 4.93 (s, 1H, CH); 6.79 (m, 1H, H-6); 7.03 (d, 1H, $J_{H,F}$ = 81.7 Hz, CHF); 7.05 (m, 2H, H-4 and H-5) ppm. FAB-HRMS calcd for $C_{10}H_{10}NO_3F_2$ (M^+ + H): 230.0629. Found: 230.0626.

3.5. 2-Amino-3-acetoxy-3-(3-hydroxyphenyl)-4,4-difluorobutyric acid (4a)

The product that eluted from the semipreparative HPLC column system I with mobile phase A, $R_T \sim 33$ min, white solid, m.p. 172-175 °C (d). UV (H_2O): λ_{sh} 279 nm (ϵ = 1480); λ_{max} 273 nm (ϵ = 1693); λ_{sh} 215 nm (ϵ = 7550). ^{1}H NMR (D_2O) δ: 2.02 (s, 3H, CH3); 4.94 (s, 1H, CH); 6.36 (t, 1H, $J_{H,F}$ = 55.0 Hz, CHF_2); 6.89 (dd, 1H, J = 2.3 and 8.0 Hz, H-6); 7.03 (d, 1H, J = 2.3 Hz, H-2); 7.07 (d, 1H, J = 8.0 Hz, H-4); 7.32 (t, 1H, J = 8.0 Hz, H-5) ppm. ^{13}C NMR (D_2O/external TMS) δ: 21.66 (C-12); 57.42 (C-8); 76.64 (t, $J_{C,F}$ = 19.2 Hz, C-7); 113.11 (C-4); 115.26 (t, $J_{C,F}$ = 245.4 Hz, C-10); 115.42 (C-2); 118.06 (C-6); 129.75 (C-5); 137.99 (C-1); 155.38 (C-3); 173.03 (C-9); 173.77 (C-11) ppm. ^{19}F NMR (D_2O) δ: −131.95 (pseudo q, $J_{F,F}$ = 272.1 Hz, $J_{F,H}$ = 44.2 Hz, A part of ABX system in CHF_2); −127.16 (pseudo q, $J_{F,F}$ = 290.3 Hz, $J_{F,H}$ = 44.3 Hz, B part of ABX system in CHF_2) ppm. HRMS calcd for $C_{12}H_{14}NO_5F_2$ (M^+ + H): 290.0840. Found: 290.0844.

3.6. 2-Amino-3-acetoxy-3-(3-hydroxyphenyl)-4,4-difluorobutyric acid (4b)

The product with $R_T \sim 35$ min in the semipreparative HPLC (see above), white solid, m.p. 172–175 °C (d). UV (H_2O): λ_{sh} 279 nm (ϵ = 1500); λ_{max} 273 nm (ϵ = 1670); λ_{sh} 215 nm (ϵ = 6950). ^{1}H NMR (D_2O) d: 1.78 (s, 3H, CH_3); 4.89 (s, 1H, CH); 6.35 (t, 1H, $J_{H,F}$ = 55.1 Hz, CHF_2); 6.87 (dd, 1H, J = 1.9 and 7.7 Hz, H-6); 7.01 (d, 1H, J = 2.3 Hz, H-2); 7.05 (d, 1H, J = 7.7 Hz, H-4); 7.30 (t, 1H, J = 7.7 Hz, H-5) ppm. ^{13}C NMR (D_2O/external TMS) δ: 21.34 (C-12); 57.16 (C-8); 77.22 (t, $J_{C,F}$ = 19.0 Hz, C-7); 113.28 (C-4); 115.20 (C-2); 115.51 (t, $J_{C,F}$ = 246.5 Hz, C-10); 118.21 (C-6); 129.53 (C-5); 138.00 (C-1); 155.21 (C-3); 173.09 (C-11); 173.69 (C-9) ppm. ^{19}F NMR (D_2O) δ: −129.23 (pseudo q, $J_{F,F}$ = 289.2 Hz, $J_{F,H}$ = 44.5 Hz, A part of ABX system in CHF_2); −125.31 (pseudo q, $J_{F,F}$ = 274.7 Hz, $J_{F,H}$ = 44.2 Hz, B part of ABX system in CHF_2) ppm. FAB-HRMS calcd for $C_{12}H_{14}NO_5F_2$ (M^+ + H): 290.0840. Found: 290.0845.

3.7. Fluorination of (Z)-β-fluoromethylene-m-tyrosine (2) with CD_3COOF

CD_3COOF (0.15 mmol) was produced in the gas phase using 1% F_2 and a cartridge of $NaOCOCD_3 \cdot 3D_2O$ (0.40 g) (prepared from anhydrous $NaOCOCD_3$ and D_2O) [26] and bubbled into a solution of 2 (0.021 g, 0.1 mmol) in acetic acid: trifluoroacetic acid (1:1, 5 ml) at 0°C. The product was worked up and separated by HPLC as described above to provide the deuterated diastereomeric products **4a′** (3%) and **4b′** (3%) (yields based on the amount of **2** recovered). The ^{1}H NMR spectra of these products were identical to those of **4a** and **4b** given above except for the absence of signals for the methyl in the acetoxy groups. The ^{19}F NMR spectra of **4a′** and **4b′** were also identical to the corresponding normal acetoxy derivatives **4a** and **4b**, respectively. High resolution mass spectra showed >95% deuterated acetoxy groups in both **4a′** and **4b′**. FAB-HRMS for **4a′** calcd for $C_{12}H_{11}D_3NO_5F_2$ (M^+ + H): 293.1028. Found: 293.1027. HRMS for **4b′** calcd for $C_{12}H_{10}D_3NO_5F_2$ (M^+): 292.0950. Found: 292.0961.

3.8. Fluorination of (Z)-β-fluoromethylene-m-tyrosine (2) with CH_3COOF in CD_3COOD/CF_3COOD

CH_3COOF (0.25 mmol) [26] was bubbled into a solution of **2** (0.030 g, 0.14 mmol) in CD_3COOD/CF_3COOD (1:1, 5 ml) at 0 °C over a period of 30 min. The products of addition **4a** (1.5 mg, 3.6%) and **4b** (1.5 mg, 3.6%) (yields based on the amount of **2** recovered) were isolated using the semipreparative HPLC as described above. Fast atom bombardment high resolution mass spectra of the isolated derivatives showed the presence of **4a** and **4b** and indicated a near absence of the deuterated derivatives **4a′** and **4b′**.

3.9. Fluorination of (E)-β-fluoromethylene-m-tyrosine (6) with CH_3COOF

Into a solution of the (*E*)-amino acid (0.042 g, 0.2 mmol) **6** [3] and anhydrous $NaOCOCD_3$ (0.051 g, 0.6 mmol) in 1:1 glacial acetic acid and trifluoroacetic acid (5 ml) at 0 °C acetyl hypofluorite (0.25 mmol) [26] was bubbled in over a period of 40 min. The addition products **4a** (0.3 mg, 0.8%) and **4b** (2.2 mg, 6.2%) (yields based on the amount of **6** recovered) were isolated by the preparative HPLC technique as described earlier. The fast atom bombardment high resolution mass spectra of both these products (**4a** and **4b**) showed the complete absence of the deuterated analogs **4a′** and **4b′**.

3.10. X-ray crystallography

Colorless and needle shaped crystals of the (Z)-amino acid were obtained from an aqueous solution. Crystal data: $C_{10}H_{10}O_3NF \cdot 1/2H_2O$; f_w = 223.49; monoclinic; space group *C2/c*; a = 20.336 (6) Å; b = 5.535 (1) Å; c = 17.454 (9) Å; β = 106.37 (3)°; Z = 8 (1/2 molecule water of crystallization); V = 1884 (2) Å^3, ρ_{calc} = 1.58 g cm^{-3}, absorption coefficient = 11.41 cm^{-1}; F(000) = 932e. The dimensions of the crystal used for data collection: 0.40 × 0.30 × 0.20 mm. The data were collected on an AFC5R Rigaku Rotating Anode diffractometer (graphite crystal monochromator), λ (Cu Kα) = 1.5418 Å, at T = 298 K, to a maximum 2θ = 1–115° for the range $0 \le h \le 22$, $0 \le k \le 6$, $-19 \le l \le 19$, giving a total of 1262 unique reflections, of which 723 reflections with $|F| > 6\sigma(F)$ were retained for structural analysis. The intensity data were corrected for Lorentz polarization and absorption corrections were applied with the absorption factor ranging from 0.81 to 1.00. The structure was solved and refined with SHELXS86 program [27]. All non-hydrogen atoms were refined anisotropically; H atoms were localized (excluding water molecules) by difference electron density determination using the fixed temperature factor and a ''riding'' model. The refinements converged at $R = 0.065$, $R_w = 0.078$ [$w = 1/\sigma^2_{(|Fo|)}$]. The maximum residual density in the final difference Fourier map was 0.36 e $Å^{-3}$. The X-ray structure atomic coordinates for **2** have been deposited with the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge, CB2 1EZ, UK.

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