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Synthesis of [3',5'-$^3H_2$]-α-Fluoromethyl-Tyrosine as a Radioactive Specific Label of Rat Brain Tyrosine Hydroxylase

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Abstract—The [3',5']-ditritio-α-fluoromethyl-tyrosine **4** (specific activity 15.0 Ci/mmol) has been synthesized and used as a radioactive probe for rat neuronal tyrosine hydroxylase (TH). The route of synthesis for the preparation of **3** and **4** allowed us to not only introduce a fluorine atom into **3**/**4** using an inorganic source of fluorine (CsF), but also to take advantage of the high-yielding cyclization of (α,β)-acetamido alcohols mediated by diethylaminosulfur trifluoride (DAST) to give the corresponding oxazolines. The distribution and metabolism of **4** have been studied in control conditions within the rat locus caeruleus (LC). Intracisternal injection of 20 μCi of **4** was followed by a rapid disappearance of **4** ($t_{1/2}$ = 1.5 h) and by a specific accumulation of radioactivity into the LC anatomical limits. This was investigated each 140 μm along the caudo-rostral axis of the noradrenergic nucleus. In each anatomical interval, its distribution correlated nicely with already described caudo-rostral distribution of TH in noradrenergic cells. Thus, **4** may provide a reliable measure of TH activity in such catecholamine structures.

Introduction

Tyrosine hydroxylase (TH) is the rate-limiting enzyme involved in the biosynthesis of catecholamine neurotransmitters.[1] It is a cytosolic soluble protein, found only in catecholaminergic neurons, which specifically catalyzes the conversion of L-tyrosine **1** to L-3,4-dihydroxy phenylalanine **2** (DOPA).[1] An enzymatic decarboxylation of DOPA mediated by an aromatic amino acid decarboxylase (AADC) leads then to dopamine.[1] Therefore, knowledge of the properties of TH and even more interestingly, the precise location and the level of its enzymatic activity in neurons under various *in vivo* conditions would be of great interest. Conceptually, such a question can be answered since the α-fluoromethyl-tyrosine **3**, analogue of **1**, is a good substrate of TH[2] while its corresponding hydroxylated 3,4-dihydroxy-α-fluoromethyl-phenylalanine, analogue of **2**, is known to be a powerful time-dependent irreversible inhibitor of AADC.[3] So, the tritium labelled compound **4** used as a substrate for TH would afford a radioactivity accumulation in TH containing neurons proving that TH activity can be located and quantified very precisely. Moreover and more efficiently than classical immunological methodologies, the TH protein would be characterized in its in *vivo* active form.

In this context, the purpose of this work is to describe a new synthesis of **3**, tritium labelling of **3** to afford **4** and our first biochemical results involving **4**. Our synthetic sequence emphasizes on key-points such as (1) the formation of the quaternary centre present in **3** and **4** and (2) the way of introduction of the fluorine atom in the desired target. It is particularly relevant to note that this preparation uses CsF as an inorganic fluorine source[4,5] without recourse to hard to come by, toxic or expensive fluorinated reagents.

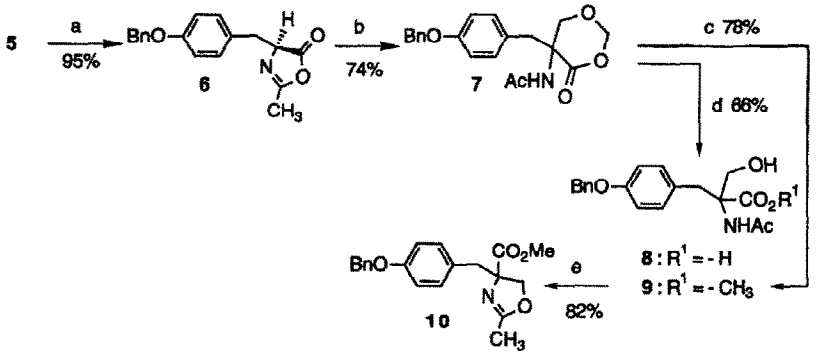
Chemistry

Cyclic dehydration[6] of L-*O*-benzyl-tyrosine **5** in acetic anhydride under sonication affords the azlactone **6** in a state of purity sufficient to be alkylated without purification by excess paraformaldehyde in the presence of pyridine (Scheme I). Particularly relevant is the beneficial use of ultrasonic waves both in terms of yield and purity of **6** when compared to a structurally related case.[6b] Saponification or transesterification of the obtained 1,3-dioxan-4-one **7** leads respectively to the α-hydroxymethyl acetamido-acid **8** and acetamido-methyl ester **9**. Compounds **8** and **9** are seemingly well suited for fluoride displacement using DAST[7] but many attempts at introducing the fluorine atom at this stage of the synthesis met with little success. Compound **8**, when reacted with DAST, leads to an intractable mixture of compounds which do not contain the -CH_2F moiety, while **9** affords the 1,3-oxazoline **10** in a very good yield (82 %).[8] Any tentative derivatization (MsCl or TsCl/Py, PPh_3/CBr_4) of the OH function in **9** always affords unreacted **9** accompanied by the oxazoline **10** (16–44 % yield).

This type of intramolecular cyclization, however, proved to be quite useful in a modified sequence with the intermediate diol **11** as a key intermediate (Scheme II). Compound **11** is easily obtained by reduction of **7** using $NaBH_4$ (78 % yield). The formation of the oxazoline **12** from **11** using Mitsunobu conditions[9] (70 %) leaves *one* of

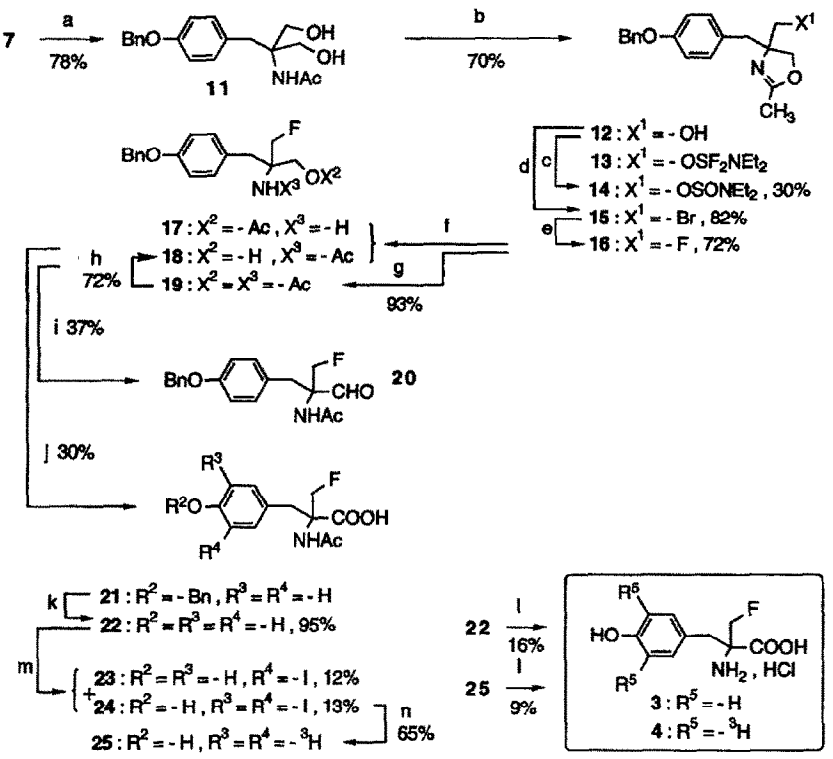
the two hydroxymethyl groups of **12** available for further chemical manipulations. The oxazoline **12**, when reacted with DAST, leads to starting material (60 %) and the relatively stable diethylaminosulfinate **14** (30 % yield). Compound **14** is a rare isolated structural probe of the formation of the activated intermediate **13**.[10] Its isolation confirms the extremely crowding nature of the quaternary carbon of the oxazoline alcohol **12** since the solvolysis of **14** is not complete under these reaction conditions. Fortunately, the fluorine atom in the α-fluoromethyl-oxazoline **16** can easily be introduced using a high-yielding fluorination sequence through the bromide **15** using an inorganic fluoride salt CsF as the source of the fluoride anion (59 % overall yield from **12**).

The aqueous acidic opening of **16** is not regioselective in favor of the desired acetamido-alcohol **18** since similar **17**/**18** ratios (**17**/**18** ~2/1) were obtained despite the various assays attempted. Therefore, a more efficient two step procedure has been successfully developed. This method



Reagents: (a) Ac_2O,))))), 1.5 h, 50 °C; (b) $(HCHO)_n$, AcOEt–pyridine, 1.3 h, 80 °C; (c) K_2CO_3, MeOH, 18 h, 20 °C; (d) NaOH, MeOH–H_2O,))))), 45 min, 50 °C; (e) DAST, CH_2Cl_2, 1.25 h, –78 °C.

Scheme I.



Reagents: (a) $NaBH_4$, THF, 3 h, from 0 °C to room temperature; (b) PPh_3, DEAD, THF, 2.5 h, 20 °C; (c) DAST, CH_2Cl_2, 3 h, –78 °C; (d) CBr_4/PPh_3, CH_2Cl_2, 48 h, 20 °C; (e) CsF, DMF, 40 h, reflux; (f) HF or CF_3COOH or AcOH, H_2O, overnight, room temperature, **17**/**18** ~ 2/1; (g) $AcOH/Ac_2O$, Et_2O, 19 h, room temperature; (h) K_2CO_3, $MeOH/H_2O$, 3 h, room temperature; (i) PDC, DMF, 70 h, room temperature; (j) CrO_3, H_2SO_4/H_2O, 3.5 h, from –15 °C to 0 °C; (k) NH_4CO_2H, 10 % Pd/C, MeOH, 50 min, reflux; (l) 3 N HCl, MeOH,))))), 4 h (**22** ⇒ **3**) or 2.5 h (**25** ⇒ **4**), 50 °C; (m) ICl, MeOH, 1.5 h, 0 °C; (n) $^3H_2$, 10 % Pd/C, MeOH, 1.5 h, 20 °C.

Scheme II.

combines a one-pot *in situ* hydrolysis–acetylation process affording **19** as a sole product followed by its alkaline hydrolysis to **18** (67 % overall yield from **16**). Compound **18** is then oxidized to **21** under Jones conditions. Several disfavorable factors like the steric hindrance of the quaternary atom of **18**, the electronic deactivation caused by the fluorine atom and the difficulty in removing chromium salts by repeated filtrations on silica gel, explain the modest yield (30 %) of this particular oxidation step. Interestingly, the PDC/DMF[11] oxidation system was found unusable in this case since the oxidation of **18** stops at the level of the aldehyde **20** without any detected traces of the expected fluorinated acid **21**.

The reductive debenzylation of **21** using ammonium formate as the source of hydrogen[12] proceeds uneventfully giving the acetylated amino acid **22**. The final acidic cleavage of the acetylamino group of **22** proved more troublesome than expected because of the sensitivity of the desired fluorinated amino acid **3** to the acidic medium. Although not fully characterized, the α-hydroxymethyl-tyrosine (loss of the -CH_2F moiety, 300 MHz, ^1H NMR, DMSO-d_6, -$\underline{CH}_2OH$, two doublets, δ = 3.82 and 3.72 ppm, J = 10.0 Hz) was obtained in variable amounts most likely through the hydrolysis of a putative aziridine intermediate.[13] Nevertheless, after much experimentation, special acidic conditions were found fruitful (hydrogen chloride in anhydrous methanol, 50 °C, ultrasonic waves). The crude amino acid was purified by HPLC on a C18 silanized TSK-ODS column affording pure **3** as a white crystalline solid (16 % yield). Attempts at resolving **3** enzymatically (hog kidney aminoacylase[14]) via the fluorinated acetamide **22** or chemically via the diastereoisomeric imidazolidines[15] prepared on the previously obtained fluorinated aldehyde **20** were unsuccessful.

The introduction of the tritium label was performed as follows: the electrophilic iodination[16] (ICl) of **22** furnished a mixture of the monoiodinated and diiodinated compounds **23** and **24** (**23/24** : 1/1 as seen by ^1H NMR). The highly unstable monoiodinated **23** was lost during the chromatographic purification step of **24**. Iodine tritium exchange[17] ($^3H_2$, Pd/C) performed on **24** gave the tritiated acetamide **25** (specific activity 24.0 Ci/mmol). Acidic deacetylation of **25** under our previously described conditions then furnishes the final tritium labelled fluorinated amino acid **4** (9 % radiochemical yield, specific activity 15.0 Ci/mmol) necessary for our biochemical studies.

Results and Discussion

A total of 20 Sprague Dawley rats (200 g body weight, IFA-CREDO, France), received between 9 and 9.30 a.m., were placed under slight anesthesia and administered an intracisternal injection of 20 μCi of a saline solution of **4** (1 μCi/μL). After a period, 0.5, 1, 3 and 6 h groups of 5 animals were sacrificed by decapitation. Their brains were quickly removed, frozen in isopentane at –80 °C until processing. Twenty micron serial coronal sections were performed with a cryomicrotome (Reichert–Jung) at –18 °C. Each 140 μm dried section of the brainstem region containing the locus caeruleus (LC) was apposed onto a ^3H sensitive film (RNP 12, Amersham) and exposed for 2 months at room temperature. The adjacent coronal section was stained with cresyl violet. On each film, radioactive tritiated microscales (Amersham) were used for calibration.

As shown in Figure 1, a highly specific radioautographic reaction was observed 6 h after the injection, in perfect correspondence with the presence of the noradrenergic cell bodies in which TH protein has been quantitatively identified following immunochemical detection.[18] The quantitative radioautography (Figure 2) indicated that, in the whole LC, the total radioactivity diffused from the cerebrospinal fluid into the ventricular gray and disappeared according to a monoexponential law with a mean K value of 0.45 ± 0.036 h^{-1} which corresponded to a half life of 1.55 h. During this time interval, a specific radioactive labelling was found accumulated within the limits of the LC. It reached a maximum between 0.5 and 1 h and remained remarkably constant until 6 h after administration. These results indicated that the half life of **4** in non catecholaminergic brain area was rather rapid and contrasted with the stability of the radioactivity accumulated into the LC after 30 min, a typical noradrenergic brain region. These results suggested (1) that there is poor evidence for a significant incorporation of **4**

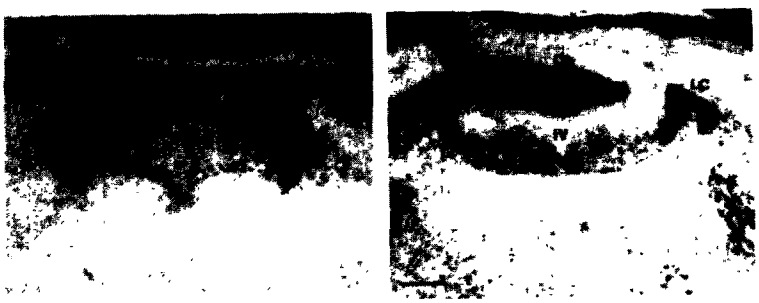


Figure 1. Specific accumulation of total radioactivity as revealed by radioautography in the rat brainstem, 6 h after intracisternal injection of 20 μCi of [3',5'-$^3H_2$]-α-fluoromethyl-tyrosine **4**. Note the high and selective autoradiographic reaction in (A) after 60 days of exposure upon a ^3H sensitive film. It corresponds to the localization of cell bodies within the locus caeruleus as shown in (B) in the adjacent coronal 20 μm section stained with cresyl violet. Cb: cerebellum; IV: fourth ventricule; LC: locus caeruleus. Scale: 1 cm to 0.4 cm.

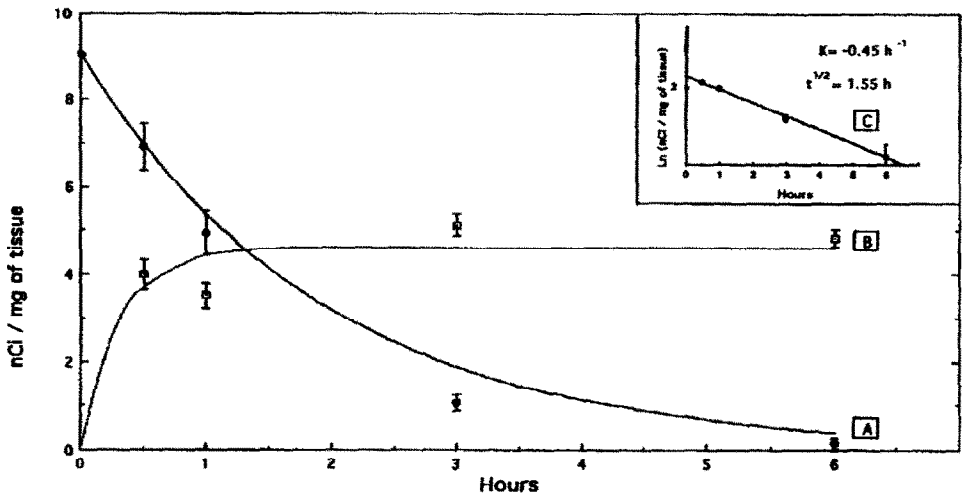


Figure 2. Time course of the accumulation of the radioactivity within the area delimited by the LC cell bodies. The total radioactivity is measured by quantitative densitometry each 140 μm along the caudo-rostral axis of the structure after a precise delineation of the LC on the adjacent Nissl-stained section. Two measurements are performed: (1) X, in the area of the LC, (2) Y (curve A), in the periventricular gray, in the area, immediately medial and delimited by a copy of the corresponding LC limits. Y is considered as a quantitative index of the diffusion of **4**; (X–Y) is chosen as the best radioautographic quantitative index of the transformation of **4** by TH activity (see curve B). One measurement is performed each 140 μm along the LC and each point represents the mean ± sem (n = 5 rats) calculated for the total LC. The variation of LnX versus time (see curve C) is best fitted by linear regression (LnX = –0.45t + 2.2, $p < 0.0001$). The variation of X–Y versus time is best fitted as X–Y = 4.6(± 0.15)[1–e(–3.28(± 0.06)t)].

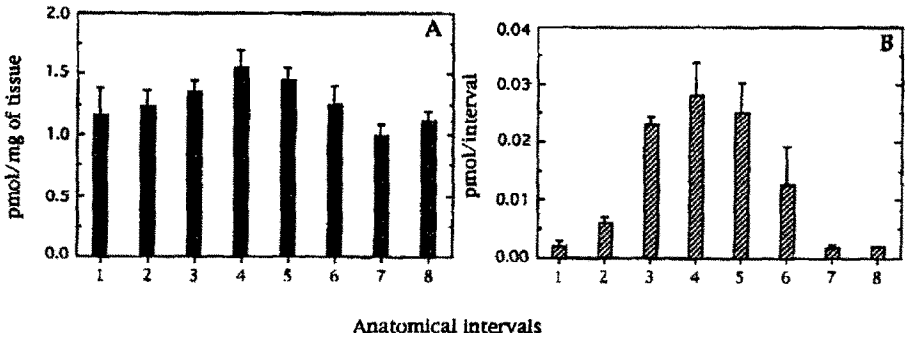


Figure 3. Caudo-rostral distribution of the calculated TH activity expressed as picomoles of transformed precursor **4** by mg of tissue as shown in A and as absolute value of activity corresponding to each anatomical interval in B. Each anatomical interval corresponded to 140 μm (levels 1 to 8). Each bar represents the mean ± sem (n = 5) of the value calculated in the corresponding anatomical interval.

in brain protein metabolism and (2) that the *in vivo* metabolite of **4** via TH activity would be, as expected from previous pharmacological results, a potent and irreversible substrate for AADC.[3] It is interesting to note that, when estimated at several levels of the caudo-rostral axis of the LC, the estimated activity calculated from the specific accumulation of radioactivity within the LC (Figure 3) was found correctly correlated with the already described distribution of the TH protein concentration, and that the absolute quantity of **4** transformed by time unit in each of the intervals of this anatomical distribution appeared finally in accordance with the absolute quantity of the enzymatic protein found in the same intervals.[18]

It may thus reasonably be concluded that the *in vivo* administration of **4** was followed by the accumulation of radioactivity, specifically localized within a typical area containing TH enzyme, and that the dynamics of such accumulation allowed an estimation of the TH activity within this area which is in good agreement with those already estimated following TH *in vivo* assay of this dissected area.[19] It remains necessary to demonstrate that the same dynamic and specific accumulation, directly related to TH activity, also occurs in other catecholaminergic sub-groups, especially dopaminergic, since these neurons would have been found less sensitive to peripheral administration of pharmacological doses of **4**.[3] Results involving the better peripherally absorbed esters of **4** will be reported in due course.

Experimental Section

Melting points were determined with a Koffler apparatus. ^1H, ^{13}C and ^3H NMR spectra were recorded at 300, 75 and 320 MHz, respectively, on a Bruker AM 3000 spectrometer (except for iodo and end products); ^{19}F NMR were recorded at 235 MHz. All chemical shifts are reported in δ units (ppm) relative to tetramethylsilane for ^1H, ^{13}C and ^3H NMR and to CCl_3F for ^{19}F NMR. Mass spectra

were recorded on a Mass Spectrometer Finnigan-Mat 4600 equipped with chemical ionization (CI), using methane or ammonia gases. Infrared spectra (IR) were recorded on a Beckman 4250 spectrophotometer. UV spectra were recorded on a Kontron Model Uvikon 860 spectrophotometer. Thin-layer chromatography (TLC) was performed on silica gel plates (Merck $60F_{254}$) or reverse-phase plates (Merck RP18) using the following detection methods: UV; I_2; 0.05 % aqueous $KMnO_4$; slightly alkaline alcoholic solution of bromocresol green (0.04 g in 100 mL of EtOH, trace of 0.1 N NaOH). Analytical HPLC was performed on Zorbax silica gel, Zorbax C18 reverse-phase or TSK C18 reverse-phase column by using Waters HPLC pump at 1 mL/min flow rate. Radioactive products were detected by scintillation. Flash chromatography was performed on Merck silica gel (25–40 μm) or Merck C18 reverse-phase (25–40 μm). Semi-preparative HPLC was performed on Waters semi-preparative column (250 mm × 10 mm). For ultrasound experiments a cleaning bath Bronson 1200 sonicator (95 W; 50 Hz; internal medium temperature 52 °C) was used.

(4S)-(4-Benzyloxybenzyl)-2-methyl-oxazolone (6)

(4*S*)-Benzyloxy-phenylalanine **5** (25.0 g, 127.0 mmol) was allowed to react, under nitrogen atmosphere, with 100 mL of acetic anhydride in an ultrasound cleaning bath for 1.5 h. The solvent was then evaporated under reduced pressure (95 % yield). The product, thus obtained, was of sufficient purity for the next step. Compound **6** can be purified, with some decomposition, by HPLC using EtOAc/hexane 60/40 as eluent (21.3 g of a green solid, 57 %, V_e = 73.0 mL). TLC (SiO_2) R_f = 0.56 (EtOAc/hexane, 9/1). HPLC (SiO_2, λ = 280 nm) V_e = 4.3 mL (EtOAc/hexane, 3/2). IR (KBr) ν: 1830 (C=O, azlactone), 1735 (C=O, ester), 1680 (C=N), 1505 (C=C), 1385, 1240, 1180 cm^{-1}. ^1H NMR (CD_2Cl_2) δ: 2.07 (s, 3H, CH_3), 2.97 (dd, 1H, *J* = 7.1 Hz, *J* = 14.0 Hz, CH_2Ar), 3.17 (dd, 1H, *J* = 4.9 Hz, *J* = 14.0 Hz, CH_2Ar), 4.38 (m, 1H, N–CH–CO), 5.04 (s, 2H, O-CH_2–Ph), 6.90 and 7.13 (2d, 4H, *J* = 8.5 Hz, Ar), 7.33–7.45 (m, 5H, Ph). MS (CI, NH_3) m/z: 296 ($[M + 1]^+$, 59 %), 313 ($[M + 18]^+$, 100 %).

5-Acetamido-5-(4-benzyloxybenzyl)-4-oxo-1,3-dioxane (7)

To a solution of **6** (33.6 g, 114.0 mmol) in EtOAc were successively added paraformaldehyde (10.2 g, 3.0 eq.) and pyridine (18.4 mL, 2.0 eq.). The reaction mixture was heated at 80 °C for 1.3 h, under nitrogen atmosphere, then cooled to room temperature and diluted with EtOAc. The organic layer was washed with 1 M HCl (3 × 30 mL), evaporated under reduced pressure, and the residue was recrystallized from EtOAc/hexane 3/7 to give 27.45 g (74 %) of **7** as a white solid. Mp 145 °C. TLC (SiO_2) R_f = 0.35 (EtOAc/hexane, 9/1). IR (KBr) ν: 3325 (N-H), 1725 (C=O, ester), 1655 (C=O, amide), 1610, 1510 (C=C), 1380, 1370, 1290, 1240, 1215, 1180 cm^{-1}. ^1H NMR (d_6-acetone) δ: 1.92 (s, 3H, CH_3), 2.98 and 3.26 (2d, 2H, *J* = 13.6 Hz, CH_2Ar), 4.07 and 4.09 (2d, 2H, *J* = 11.9 Hz, C–CH_2–O), 5.13 (s, 2H, O–CH_2Ph), 5.23 and 5.52 (2d, 2H, *J* = 5.08 Hz, CO_2CH_2O), 6.99 and 7.26 (d, 4H, *J* = 8.5 Hz, Ar), 7.30–7.50 (m, 5H, Ph). ^{13}C NMR (d_6-acetone) δ: 22.1 (CH_3), 42.2 (CH_2–Ar), 58.8 (C–N), 70.1 (CH_2–O), 70.8 (O–CH_2Ph), 94.5 (O–CH_2–O), [115.2, 127.2, 128.3, 129.0, 132.2, 138.1, 158.9 (phenyl groups)], 169.6 (NH–CO), 170.3 (COO). MS (CI, NH_3) m/z: 356 ($[M + 1]^+$, 12 %), 373 ($[M + 18]^+$, 100 %). UV (MeOH) λ_{max} (ε): 227.1 (15309), 275.2 (1506) nm. HRMS calcd for $C_{20}H_{21}NO_5$ 355.14196; found 355.143.

2-Acetamido-2-(4-benzyloxybenzyl)-3-hydroxypropanoic acid (8)

4-Oxo-1,3-dioxane **7** (465.0 mg, 1.3 mmol) was sonicated with 4 mL of a solution of NaOH (1 % in 1/1 MeOH/H_2O) in an ultrasound cleaning bath for 45 min. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography using MeOH/$CHCl_3$ 1/1 as eluent to give 294.3 mg (66 %) of **8** as a white solid. Mp 172–175 °C. TLC (SiO_2) R_f = 0.51 (Et_2O/iPrOH/AcOH/H_2O, 80/15/5/6). IR (KBr) ν: 3400 (N–H and O–H), 1715 (C=O, ester), 1660 (C=O, amide), 1610, 1510 (C=C), 1385, 1290, 1240, 1180 cm^{-1}. ^1H NMR (d_6-DMSO) δ: 1.77 (s, 3H, CH_3), 3.01 and 3.27 (2d, 2H, *J* = 12.9 Hz, CH_2Ar), 3.75 and 3.82 (2d, 2H, *J* = 9.9 Hz, CH_2–OH), 5.01 (s, 2H, O–CH_2Ph), 6.80 and 7.01 (d, 4H, *J* = 8.4 Hz, Ar), 7.13 (s, 1H, NH), 7.30–7.40 (m, 5H, Ph). ^{13}C NMR (d_6-acetone) δ: 26.5 (CH_3), 38.9 (CH_2–Ar), 55.0 (C–N), 67.5 (CH_2O), 73.2 ($PhCH_2$–O), [117.9, 131.1, 132.0, 134.6, 141.2 (phenyl groups)], 161.4 (NH–CO), 164.9 (COOH). MS (CI, NH_3) m/z: 326 ($[M - OH]^+$, 20 %), 344 ($[M + 1]^+$, 19 %), 361 ($[M + 18]^+$, 100 %). UV (MeOH) λ_{max} (ε): 225.3 (20257), 285.0 (15644) nm. Anal. calcd for $C_{19}H_{21}NO_5$: C, 66.47; H, 6.12; N, 4.08; found: C, 66.55; H, 6.10; N, 3.95.

Methyl 2-acetamido-2-(4-benzyloxybenzyl)-3-hydroxypropanoate (9)

To a suspension of 4-oxo-1,3-dioxane **7** (1.3 g, 3.7 mmol) in 20 mL of MeOH was added K_2CO_3 (1.27 g, 2.5 eq.). The reaction mixture was stirred 18 h under a nitrogen atmosphere at room temperature and was then poured into 0.25 N AcOH at 0 °C. The pH was adjusted to 6.0 with 0.1 N AcOH (10.0 mL). After extraction with CH_2Cl_2 (2 × 20 mL), the combined organic layers were washed with H_2O (5 × 20 mL), dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by flash chromatography using EtOAc/hexane 9/1 as eluent to give 1.02 g of **9** (78 %) as a white powder. Mp 161–164 °C. TLC (SiO_2) R_f = 0.34 (EtOAc/hexane 90/10). IR (KBr) ν: 3350 (N–H and O–H), 1720 (C=O, ester), 1645 (C=O, amide), 1610, 1550, 1510 (C=C), 1375, 1240, 1180 cm^{-1}. ^1H NMR (CD_2Cl_2) δ: 1.98 (s, 3H, CH_3–CO), 3.03 and 3.39 (2d, 2H, *J* = 13.5 Hz, CH_2–Ar), 3.77 (s, 3H, CH_3O), 3.89 (dd, 1H, *J* = 7.1 Hz, *J* = 11.1 Hz, $\underline{CH}_2$–OH), 4.23 (dd, 1H, *J* = 7.1 Hz, *J* = 11.0 Hz, $\underline{CH}_2$–OH), 5.03 (s, 2H, O–CH_2Ph), 6.24 (s, 1H, NH), 6.90 and 6.97 (2d, 4H, *J* = 8.6 Hz, Ar), 7.40–7.50 (m, 5H, Ph). ^{13}C NMR (CD_2Cl_2) δ: 24.1 (CH_3), 36.7 (CH_2–Ar), 55.0 (CH_3–O), 65.6 (C–N), 68.1 (CH_2–OH), 70.3 ($PhCH_2$–O), [115.1, 127.9, 128.0,

128.3, 128.9, 131.1, 137.5, 158.4 (phenyl groups)], 171.1 (NH–CO), 172.7 (COO). MS (CI, NH_3) m/z: 340 ([M–OH]$^+$, 48 %), 358 ([M + 1]$^+$, 43 %), 375 ([M + 18]$^+$, 100 %). UV (MeOH) λ_{max} (ε): 228.8, 274.7 (1467), 281.5 (1243) nm. HRMS calcd for $C_{20}H_{23}NO_5$ 357.1576; found 357.157.

*4-(4-Benzyloxybenzyl)-4-methoxycarbonyl-2-methyloxazoline (**10**)*

To a solution of the ester **9** (96.5 mg, 0.27 μmol) in 5 mL of CH_2Cl_2 at –78 °C was slowly added a solution of diethylaminosulfur trifluoride (DAST, 53.6 μL, 1.5 eq.) in 1 mL of CH_2Cl_2. The solution was stirred for 1.25 h at –78 °C and then the reaction was quenched with crushed ice. The solution was diluted with CH_2Cl_2 and the organic layer washed with saturated aqueous NH_4Cl. After extraction with CH_2Cl_2 (3 × 5 mL), the combined organic layers were washed with a 2 N aqueous solution of ammonia (1 × 3 mL), with water (5 × 3 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography using EtOAc/hexane 1/1 as eluent to afford 75.1 mg of **10** (82 %) as a colorless oil. TLC (SiO_2) R_f = 0.35 (EtOAc/hexane 50/50). ^{1}H NMR (CD_2Cl_2) δ: 1.9 (s, 3H), 3.0 (s, 2H, CH_2Ar), 3.7 (s, 3H, CH_3OOC), 4.1 and 4.5 (2d, 2H, J = 9.1 Hz, CH_2–O), 5.0 (s, 2H, O–CH_2Ph), 6.9 and 7.1 (2d, 4H, J = 8.5 Hz), 7.3–7.5 (m, 5H). ^{13}C NMR (CD_2Cl_2) δ: 13.9 (CH_3), 42.9 (CH_2Ar), 52.6 (C–N), 70.3 (CH_3–O), 73.0 (CH_2–O), 78.8 ($PhCH_2$–O), [114.8, 127.9, 128.2, 128.8, 131.7, 137.6, 158.2 (phenyl groups)], 166.1 (N=C–O), 173.5 (COO). MS (CI, NH_3) m/z: 340 ([M + 1]$^+$, 100 %), 357 ([M + 18]$^+$, 62 %). Anal. calcd for $C_{20}H_{21}NO_4$: C, 70.73; H, 6.23; N, 4.13; found: C, 70.28; H, 5.96; N, 4.14. HRMS calcd for $C_{20}H_{21}NO_4$ 339.1471; found 339.147.

*2-Acetamido-2-(4-benzyloxybenzyl)-1,3-propanediol (**11**)*

To a suspension of 4-oxo-1,3-dioxane **7** (7.4 g, 20.8 mmol) in 60 mL of THF was slowly added $NaBH_4$ (7.0 g, 8.9 eq.) at 0 °C. The reaction mixture was then stirred at room temperature for 3 h. The solution was then diluted with CH_2Cl_2 (150 mL) and added to a solution of 1 N HCl (22 mL) cooled to 0 °C. The aqueous layer was washed with CH_2Cl_2 (3 × 50 mL) and the combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by flash chromatography on reverse phase using MeOH/H_2O 65/35 as eluent gave 5.34 g of **11** (78 %) as a white solid. Mp 123–126 °C. TLC (SiO_2) R_f = 0.13 (EtOAc/hexane 95/5). TLC (C18 RP) R_f = 0.65 (MeOH/H_2O 80/20). IR (KBr) ν: 3400 (OH), 3280 (NH), 1645 (C=O, amide), 1615, 1580, 1505 (C=C), 1440, 1385, 1375 (CN), 1250, 1180 cm^{-1}. ^{1}H NMR (CD_2Cl_2) δ: 1.97 (s, 3H, CH_3), 2.87 (s, 2H, CH_2Ar), 3.49 and 3.66 (2d, 4H, J = 11.4 Hz, 2 × $\underline{CH_2}$–OH), 3.74 (s, 2H, 2 × OH), 5.05 (s, 2H, O–CH_2Ph), 5.83 (s, 1H, NH), 6.94 and 7.17 (2d, 4H, J = 8.5 Hz, Ar), 7.3–7.5 (m, 5H, Ph). ^{13}C NMR (CD_2Cl_2) δ: 24.2 (CH_3), 37.3 (CH_2Ar), 61.9 (C–N), 65.3 (CH_2–OH), 70.3 ($PhCH_2$–O), [115.3, 127.8, 128.2, 128.8, 131.7, 132.0, 137.5, 158.0 (phenyl groups)], 172.2 (NH–CO). MS (CI, NH_3) m/z: 330 ([M + 1]$^+$, 100 %), 347 ([M + 18]$^+$, 82 %). MS (CI, CH_4) m/z: 312 ([M – OH]$^+$, 45 %), 330 ([M + 1]$^+$, 100 %). HRMS calcd for $C_{19}H_{21}NO_3$ 311.1521; found 311.151. UV (MeOH) λ_{max} (ε): 235.0 (5876), 276.2 (1580) nm.

*4-(4-Benzyloxybenzyl)-4-hydroxymethyl-2-methyloxazoline (**12**)*

To a solution of the diol **11** (210.0 mg, 0.64 mmol) in 10 mL of THF was slowly added PPh_3 (251.1 mg, 0.96 mmol, 1.5 eq.), then DEAD (261.2 mg, 151.0 μL, 1.5 eq.) under nitrogen atmosphere at 20 °C. The yellow solution was stirred for 2.5 h at 20 °C. The reaction was quenched with water (10 mL). The aqueous layer was then extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by flash chromatography using acetone/hexane/TEA 70/30/5 as eluent gave 4.48 g of **12** (70 %) as a white solid. Mp 109–111 °C. TLC (SiO_2) R_f = 0.31 (acetone/hexane 80/20). TLC (C18 RP) R_f = 0.63 (MeOH/H_2O 80/20). IR (KBr) ν: 3200 (OH), 1650 (C=O, amide), 1600, 1495 (C=C), 1440, 1375 (CN), 1285, 1240, 1165 cm^{-1}. ^{1}H NMR (CD_2Cl_2) δ: 1.87 (s, 3H, CH_3), 2.69 and 2.82 (2d, 2H, J = 13.6 Hz, CH_2Ar), 3.45 and 3.67 (2d, 2H, J = 11.35 Hz, $\underline{CH_2}$–OH), 4.07 and 4.12 (2d, 2H, J = 8.40 Hz, CH_2–O), 5.06 (s, 2H, O–CH_2Ph), 6.93 and 7.10 (2d, 4H, J = 8.5 Hz, Ar), 7.3–7.5 (m, 5H, Ph). ^{13}C NMR (CD_2Cl_2) δ: 13.8 (CH_3), 41.3 (CH_2Ar), 66.9 (C–N), 70.3 (CH_2–OH), 72.4 (CH_2–O), 75.6 (O–CH_2Ph), [115.0, 127.9, 128.2, 129.2, 131.9, 132.5, 137.7, 158.0 (phenyl groups)], 166.1 (N=C–O). MS (CI, NH_3) m/z: 312 ([M + 1]$^+$, 100 %). HRMS calcd for $C_{19}H_{21}NO_3$ 311.152; found 311.151. Anal. calcd for $C_{19}H_{21}NO_3$: C, 73.29; H, 6.97; found: C, 72.5; H, 6.97.

*4-(4-Benzyloxybenzyl)-4-N,N-diethylaminosulfinyloxymethyl-2-methyloxazoline (**14**)*

To a solution of **12** (73.0 mg, 235.0 μmol) in 4 mL of CH_2Cl_2 was slowly added a solution of DAST (59.0 μL, 1.9 eq.) in 1 mL of CH_2Cl_2 under dry nitrogen at –78 °C. After stirring at –78 °C for 3 h, the reaction was quenched with crushed ice and then added to a 2 N aqueous solution of NH_4OH (1 mL) at 0 °C. After extraction with CH_2Cl_2 (2 × 4 mL), the combined organic layers were washed with H_2O (5 × 2 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by flash chromatography using EtOAc/hexane 8/2 as eluent gave 30.3 mg of **14** (30 %) as an oil. TLC (SiO_2) R_f = 0.39 (EtOAc/hexane 70/30). ^{1}H NMR (CD_2Cl_2) δ: 1.13 (2t, 6H, 2 × CH_3 (Et)), 1.84 (s, 3H, CH_3), 2.72 and 2.73 (2d, 1H, J = 13.7 Hz, CH_2Ar (2 diast.)), 2.83 (d, 1H, J = 13.7 Hz, CH_2Ar), 3.12–3.25 (m, 4H, 2 × CH_2 (Et)), 3.57 and 3.58 (2d, 1H, J = 9.9 Hz, CH_2–O (2 diast.)), 3.74 and 3.75 (2d, 1H, J = 9.9 Hz, CH_2–O (2 diast.)), 3.90 and 4.07 (2d, 2H, J = 8.7 Hz, CH_2–OSO), 5.04 (s, 2H, O–CH_2Ph), 6.88 and 7.12 (2d, 4H, J = 8.4 Hz, Ar), 7.30–7.45 (m, 5H, Ph). MS (CI, NH_3) m/z: 431 ([M + 1]$^+$, 100 %). HRMS molecular ion not observed [M – NEt_2] calcd for $C_{16}H_{20}NSO_4$ 359.1146; found 359.119.

4-(4-Benzyloxybenzyl)-4-bromomethyl-2-methyloxazoline (15)

To a solution of **12** (4.49 g, 14.4 mmol) in 100 mL of CH_2Cl_2 were successively added CBr_4 (6.24 g, 1.3 eq.) and PPh_3 (5.7 g, 1.5 eq.) under dry nitrogen at 0 °C. The reaction mixture was then stirred 48 h at room temperature and then concentrated under reduced pressure to give an orange residue. Purification by flash chromatography using EtOAc/hexane/TEA 25/75/1 as eluent gave 4.43 g of the bromomethylated oxazoline **15** (82 %) as a white solid. Mp 98–99 °C. TLC (SiO_2) R_f = 0.58 (EtOAc/hexane 1/1). IR (NaCl) ν: 1670 (N=C–O), 1505 (C=C), 1425, 1385 (C–N), 1265 cm^{-1}. ^1H NMR (CD_2Cl_2) δ: 1.87 (s, 3H, CH_3), 2.86 and 2.95 (2d, 2H, J = 13.7 Hz, CH_2Ar), 3.42 and 3.45 (2d, 2H, J = 10.3 Hz, CH_2O), 4.01 and 4.14 (2d, 2H, J = 8.9 Hz, CH_2–Br), 5.04 (s, 2H, O–CH_2Ph), 6.90 and 7.17 (2d, 4H, J = 8.7 Hz, Ar), 7.33–7.46 (m, 5H, Ph). ^{13}C NMR (CD_2Cl_2) δ: 14.8 (CH_3), 42.3 (CH_2Ar), 68.3 (C–N), 70.2 (CH_2–O), 73.7 (O–CH_2Ph), 74.5 (CH_2–Br), [115.7, 127.8, 128.9, 130.9, 137.6, 159.0 (phenyl groups)], 168.0 (O–C=N). MS (CI, NH_3) m/z: 374/376 ([M + 1]$^+$, 100 %/94 %), 391/393 ([M + 18]$^+$, 17 %/14 %). HRMS calcd for $C_{19}H_{20}NO_2{}^{79}Br$ 373.0677; found 373.067.

4-(4-Benzyloxybenzyl)-4-fluoromethyl-2-methyloxazoline (16)

To a solution of the bromomethylated oxazoline **15** (4.38 g, 11.7 mmol) in 120 mL of DMF was added dry CsF (7.3 g, 4.1 eq.). The mixture was refluxed for 40 h, then cooled to room temperature, diluted with CH_2Cl_2 (200 mL), poured into saturated aqueous NaOH (150 mL) and extracted with CH_2Cl_2 (5 × 100 mL). The combined organic layers were concentrated to 50 mL, washed with H_2O (5 × 20 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. Two successive purifications by flash chromatography using EtOAc/hexane/TEA 30/70/1 as eluent gave 2.64 g of **16** (72 %) as a white solid. Mp 83 °C. TLC (SiO_2) R_f = 0.49 (EtOAc/hexane 1/1). ^1H NMR (CD_2Cl_2) δ: 1.86 (s, 3H, CH_3), 2.72 and 2.84 (2d, 2H, J = 13.7 Hz, CH_2Ar), 3.99 (dd, 2H, J = 8.7 Hz, $^4J_{HF}$ = 2.5 Hz, $^4J_{HF}$ = 1.3 Hz, CH_2–O), 4.10 (dd, 2H, J = 8.7 Hz, $^4J_{HF}$ = 2.5 Hz, $^4J_{HF}$ = 1.3 Hz, CH_2–O), 4.37 (d, 2H, J_{HF} = 47.6 Hz, CH_2–F), 5.04 (s, 2H, O–CH_2Ph), 6.89 and 7.13 (2d, 4H, J = 8.5 Hz, Ar), 7.30–7.45 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$) δ: 14.0 (CH_3), 40.6 (CH_2Ar), 70.1 (CH_2–O), 72.3 (O–CH_2Ph), 74.0 (C–N, $^2J_{CF}$ = 20.6 Hz), 84.7 and 88.2 (CH_2–F, J = 175.5 Hz), [114.7, 127.6, 128.1, 128.7, 131.6, 138.0, 158.0 (phenyl groups)]. MS (CI/NH_3) m/z: 314 ([M + 1]$^+$, 100 %), 331 ([M + 18]$^+$, 7 %). Anal. calcd for $C_{19}H_{20}NO_2F$: C, 72.80; H, 6.43; N, 4.46; found: C, 72.61; H, 6.46; N, 4.27.

2-Acetamido-2-(4-benzyloxybenzyl)-3-fluoropropanol (18)

To a solution of the diacetate **19** (363.2 mg, 974.0 μmol) in 10 mL of MeOH was successively added K_2CO_3 and 5 mL of water. The solution, protected from the light, was stirred for 3 h at room temperature. After extraction with Et_2O (3 × 10 mL), the combined organic layers were washed with water (3 × 10 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by flash chromatography using EtOAc as eluent gave 232.0 mg of **18** (72 %) as a white solid. Mp 116–118 °C. TLC (SiO_2) R_f = 0.41 (EtOAc). TLC (C18 RP) R_f = 0.40 (MeOH/H_2O 75/25). HPLC (SiO_2, λ = 280 nm) V_e = 11.0 mL (EtOAc/hexane 7/3). FT-IR ($CHCl_3$) ν: 3424 (OH), 3322 (NH), 1665 (CO, amide), 1510 (C=C), 1244, 1178 cm^{-1}. ^1H NMR (CD_2Cl_2) δ: 1.97 (s, 3H, CH_3), 2.80 and 3.05 (2d, 2H, J = 13.9 Hz, CH_2Ar), 3.62 and 3.70 (2d, 2H, J = 11.9 Hz, CH_2–O), 4.29 (dd, 1H, J = 9.4 Hz, J_{HF} = 48.7 Hz, CH_2–F), 4.46 (dd, 1H, J = 9.4 Hz, J = 48.7 Hz, CH_2–F), 5.05 (s, 2H, CH_2Ar), 5.60 (s, 1H, NH), 6.93 and 7.15 (2d, 4H, J = 8.45 Hz, Ar), 7.3–7.5 (m, 5H, Ph). MS (CI, NH_3) m/z: 312 ([M-F]$^+$, 26 %), 332 ([M + 1]$^+$, 31 %), 349 ([M + 18]$^+$, 21 %). Anal. calcd for $C_{19}H_{21}NO_3F$: C, 68.86; H, 6.69; N, 4.23; found: C, 68.75; H, 6.65; N, 4.24.

2-Acetamido-2-(4-benzyloxybenzyl)-2-fluoromethyl-1-acetyloxyethane (19)

To a solution of **16** (295.0 mg, 942.5 μmol) in 6 mL of anhydrous Et_2O was successively added, under nitrogen atmosphere, 5 mL of acetic acid, 3 mL of acetic anhydride, and 360 μL of H_2O. The mixture was stirred for 19 h at room temperature. After extraction with Et_2O (1 x 25 mL), the organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 25 mL) and H_2O (2 × 25 mL), dried over $MgSO_4$, filtered on silica gel using Et_2O as eluent, and evaporated under reduced pressure. The residue was purified by flash chromatography using EtOAc/hexane 40/60 as eluent to give 327.0 mg of **19** (93 %) as a yellow oil. TLC (SiO_2) R_f = 0.76 (EtOAc). HPLC (SiO_2, λ = 280 nm) V_e = 7.0 mL (EtOAc/hexane 7/3). ^1H NMR (CD_2Cl_2) δ: 1.92 (s, 3H, CH_3CON), 2.07 (s, 3H, CH_3COO), 3.05 (dd, 1H, J = 13.7 Hz, $^4J_{HF}$ = 1.6 Hz, CH_2Ar), 3.21 (d, 1H, J = 13.7 Hz, CH_2Ar), 4.17 (dd, 1H, J = 13.7 Hz, $^4J_{HF}$ = 4.8 Hz, CH_2–O), 4.19 (dd, 1H, J = 13.7 Hz, $^4J_{HF}$ = 6.0 Hz, CH_2–O), 4.55 (dd, 1H, J = 9.4 Hz, J_{HF} = 47.2 Hz, CH_2–F), 4.71 (dd, 1H, J = 9.4 Hz, J_{HF} = 46.5 Hz, CH_2-F), 5.04 (s, 2H, O–CH_2Ph), 5.44 (s, 1H, NH), 6.92 and 7.08 (2d, 4H, J = 9.3 Hz, Ar), 7.3–7.45 (m, 5H, Ph). ^{13}C NMR (CD_2Cl_2) δ: 20.9 (CH_3, amide), 24.3 (CH_3, acetate), 35.4 (CH_2Ar), 54.1 (C–N), 63.7 (CH_2–OH), 70.3 (O–CH_2Ph), 81.5 and 83.8 (CH_2–F, J = 171.1 Hz), [115.1, 127.8, 128.2, 128.8, 131.7, 158.2 (phenyl groups)], 170.5 (CO–NH), 170.9 (O–CO). MS (CI, NH_3) m/z: 374 ([M + 1]$^+$, 100 %), 391 ([M + 18]$^+$, 47 %). HRMS calcd for $C_{21}H_{24}NO_4F$ 373.1690; found 373.169. Anal. calcd for $C_{21}H_{24}NO_4F$: C, 67.54; H, 6.48; N, 3.75; found: C, 66.8; H, 6.30; N, 3.68.

2-Acetamido-2-(4-benzyloxybenzyl)-3-fluoropropanal (20)

To a solution of the fluorinated alcohol **18** (100.0 mg, 302.1 μmol) in 2 mL of DMF was added pyridinium dichromate (454.7 mg, 4.0 eq.) under dry nitrogen. The reaction mixture was stirred for 70 h at room temperature and then filtered on a $MgSO_4$ column using Et_2O (30 mL)

as eluent. The solution was washed with water (5 × 10 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified using EtOAc/hexane 1/1 as eluent to give 36.2 mg of **20** (37 %) as a white solid. Mp 137–139 °C. TLC (SiO_2) R_f = 0.43 (EtOAc/hexane 1/1). ^1H NMR (CD_3OD) δ: 1.96 (s, 3H, CH_3), 2.99 and 3.09 (2d, 2H, J = 14.1 Hz, CH_2Ar), 4.41 (dd, 2H, J = 9.7 Hz, J_{HF} = 47.1 Hz, CH_2–F), 4.57 (dd, 2H, J = 9.7 Hz, J_{HF} = 46.6 Hz, CH_2–F), 4.82 (s, 2H, O–CH_2Ph), 6.88 and 6.99 (2d, 4H, J = 8.5 Hz, Ar), 7.24–7.38 (m, 5H, Ph), 9.38 (d, 1H, J = 2.6 Hz, CHO). MS (CI, NH_3) m /z: 330 ([M + 1]$^+$, 79 %), 347 ([M + 18]$^+$, 100 %).

2-Acetamido-2-(4-benzyloxybenzyl)-3-fluoro-propanoic acid (21)

To a solution of the fluorinated alcohol **18** (60.8 mg, 183.7 μmol) in 1.5 mL of acetone was added dropwise, within 1 h, Jones reagent (141.3 μL of a suspension of 2.9 g of CrO_3 partly dissolved in a mixture of H_2SO_4 (2.5 mL) and H_2O (5 mL)). The temperature was maintained between –15 °C and –5 °C. The reaction mixture was stirred for 3.5 h at 0 °C. The medium was quenched with 1 mL of i-PrOH, then filtered twice on silica gel using acetone as eluent. The solvent was evaporated under reduced pressure and the residue purified on silica gel using EtOAc/hexane (3/7 then 2/3 then 1/1) and acetone as eluents to give 19.0 mg of the acid **21** (30 %) as a white powder. Mp 146 °C. TLC (C18 RP) R_f = 0.64 (MeOH/H_2O 60/40). FT-IR (KBr) ν: 3450 (OH), 3355 (NH), 1718 (CO, acid), 1616 (CO, amide), 1558, 1515 (C=C), 1385, 1245 (CO, arom. ether) cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.98 (s, 3H, CH_3), 3.00 and 3.45 (2d, 2H, J = 13.4 Hz, CH_2Ar), 4.73 (dd, 1H, J = 9.0 Hz, J = 46.3 Hz, CH_2–F), 4.97 (s, 2H, O–CH_2Ph), 5.09 (dd, 1H, J = 9.0 Hz, J = 47.5 Hz, CH_2–F), 6.29 (s, 1H, NH), 6.86 and 7.06 (2d, 4H, J = 8.2 Hz, Ar), 7.30–7.37 (m, 5H, Ph). ^{13}C NMR (d_6-acetone) δ: 23.2 (CH_3), 36.1 (CH_2Ar), 66.9 (C–N, $^2J_{CF}$ = 18.5 Hz), 71.0 (O–CH_2Ph), 83.0 and 85.5 (CH_2–F, J = 173.9 Hz), [115.1, 128.0, 128.5, 129.0, 129.2, 131.8, 152.0, 159.0 (phenyl groups)], 172.5 (NH–CO), 173.0 (COOH). MS (CI, NH_3) m/z: 326 (M – F, 62 %), 346 ([M + 1]$^+$, 46 %), 363 ([M + 18]$^+$, 83 %). MS (CI, CH_4) m/z: 326 ([M – F]$^+$, 63 %). HRMS calcd for $C_{19}H_{20}NO_4F$ 345.1376; found 345.136; calcd for $C_{19}H_{19}NO_4$ 325.1314; found 325.132. Anal. calcd for $C_{19}H_{20}NO_4F$: C, 67.54; H, 6.48; N, 3.75; found: C, 66.85; H, 6.30; N, 3.68.

N-*Acetyl-α-fluoromethyl-tyrosine (22)*

The acid **21** (54.7 mg, 158.0 μmol) was dissolved in MeOH (3 mL) and heated at reflux in the presence of 10 % Pd/C (10.6 mg) and ammonium formate (50.0 mg, 5.0 eq.) for 50 min. The mixture was filtered through Celite and the filtrate concentrated under reduced pressure. Purification by HPLC (TSK-C18 RP) using H_2O/CH_3CN/TFA 85/15/0.1 as eluent gave 38.4 mg of **21** (95 % yield, V_e = 44.0 mL) as a colorless oil. HPLC (TSK-C18 RP, l = 270 nm) V_e = 6.4 mL (H_2O/CH_3CN/TFA, 8/2/0.01). ^1H NMR (CD_3OD) δ: 1.87 (s, 3H, CH_3), 2.86 and 3.15 (2d, 2H, J_{HF} = 12.9 Hz, CH_2Ar), 4.64 (dd, 1H, J = 8.4 Hz, J_{HF} = 46.0 Hz, CH_2–F), 5.00 (dd, 1H, J = 8.4 Hz, J_{HF} = 48.5 Hz, CH_2–F), 6.58 and 6.93 (2d, 4H, J = 8.1 Hz, Ar). ^{13}C NMR (CD_3OD) δ: 22.7 (CH_3), 34.9 (CH_2–Ar), 66.6 and 66.7 (d, C–N), 82.2 and 84.5 (CH_2–F, J_{CF} = 136.0 Hz), [114.8, 127.2, 131.0, 156.0 (phenyl group)], 171.4 (CO–N), 175.2 (COOH). ^{19}F NMR (CD_3OD) δ: 227.7 (t, J_{FH} = 47.8 Hz). MS (CI, NH_3) m/z: 192 ([M – F – COOH + 1]$^+$, 92 %), 236 ([M – F]$^+$, 33 %). UV (MeOH) λ_{max} (ε) 224.0 (5452), 276.2 (1049) nm. HRMS molecular ion not observed [M – HF] calcd for $C_{12}H_{13}NO_4$ 235.0845; found 235.085.

N-*Acetyl-3',5'-diiodo-α-fluoromethyl-tyrosine (24)*

A 3.11 mM methanolic solution of ICl (125.0 μL, 2.1 eq.) was added dropwise to a stirred solution of **22** (47.2 mg, 185.1 μmol) in anhydrous MeOH (2 mL) at –5 °C. The reaction mixture is protected from light and stirred for 1.5 h at 0 °C. Unreacted ICl was destroyed with 0.1 N Na_2SO_4 (1 mL) and the mixture neutralized with 2 N NH_4OH (1 mL). The red suspension was filtered and the filtrate concentrated under reduced pressure. Purification by HPLC (TSK-C18 RP) using H_2O/CH_3CN/TFA 80/20/0.1 as eluent gave 8.6 mg of the unstable monoiodide **23** (12 %) as a light yellow oil and a second oil which is then filtered again over a C18 reverse phase Sep-pack using the same eluent to afford 12.3 mg of **24** (13 %) as an orange oil. HPLC (TSK-C18 RP, λ = 270 nm) V_e = 26.0 mL for **23** and V_e = 80.0 mL for **24** (H_2O/CH_3CN/TFA, 8/2/0.01). ^1H NMR (CD_3OD, 250 MHz) δ: 1.90 (s, 3H, CH_3), 2.94 and 3.02 (2d, 2H, J = 14.0 Hz, CH_2Ar), 4.53 (dd, 1H, J = 12.0 Hz, J_{HF} = 48.0 Hz, CH_2–F), 4.72 (dd, 1H, J = 12.0 Hz, J_{HF} = 45.5 Hz, CH_2–F), 7.41 (s, 2H, Ph). ^{13}C NMR (CD_3OD, 62 MHz) δ: 22.8 (CH_3), 35.5 (CH_2–Ar), 62.3 (C–N), 81.1 and 83.4 (CH_2–F, J_{CF} = 143.0 Hz), 85.1 (C–I), 131.9 (C–H, Ph), 148.0 (C–C, Ph), 149.9 (C–OH, Ph), 172.9 (NH–CO), 173.2 (COOH). MS (CI, NH_3) m/z: 488 ([M – F]$^+$, 20 %), 508 ([M + 1]$^+$, 65 %), 525 ([M + 18]$^+$, 100 %). HRMS molecular ion not observed [M – HF] calcd for $C_{12}H_{11}NO_4I_2$ 486.8779; found 486.889.

N-*Acetyl-3',5'-ditritio-α-fluoromethyl-tyrosine (25)*

The mixture of iodinated products **23** (8.6 mg, 22.6 μmol) and **24** (12.3 mg, 24.2 μmol) were dissolved in anhydrous degassed MeOH (3.5 mL) and hydrogenated with tritium gas in the presence of 10 % Pd/C (30.0 mg) and TEA (19.5 μL, 3.0 eq.) for 1.5 h at 20 °C using a Toeppler apparatus. The mixture was then diluted with MeOH, filtered, and concentrated under reduced pressure. The residue was diluted again (× 3) and the solution concentrated to eliminate any trace of solvent. 1.4 Ci of crude **25** was obtained. Purification by HPLC (TSK-C18 RP) using H_2O/CH_3CN/TFA 80/20/0.1 as eluent gave 1.342 Ci of **25** (65 % radiochemical yield, V_e = 44.0 mL). ^3H NMR (CD_3OD) δ: 6.64 (s). UV (MeOH) λ_{max} (ε) 224.0 (5452), 276.2 (1049) nm. Specific activity 24.0 Ci/mmol.

α-Fluoromethyl-tyrosine Hydrochloride (3)

A solution of the amido acid **22** (27.0 mg, 105.9 μmol) in a 3 N solution of anhydrous hydrogen chloride in methanol

(2 mL) was sonicated for 4 h in an ultrasonic cleaning bath at 52 °C. The solvent was then evaporated under reduced pressure and the residue diluted with water. Purification by HPLC (TSK-C18 RP) using 2×10^{-4} M HCl (pH = 2.7) as eluent gave 3.6 mg of the amino acid **3** (16 %, V_e = 53.0 mL) as a white powder. Mp 239 °C. HPLC (TSK-C18 RP, λ = 270 nm) V_e = 13.0 mL (2×10^{-4} M HCl, pH = 2.7). FT-IR (KBr) ν: 2500–3550 (OH, NH, COOH), 1615 (CO, acid), 1580, 1516 and 1455 (C=C) cm^{-1}. ^1H NMR (D_2O, 400 MHz) δ: 2.93 and 3.21 (2d, 2H, J = 14.4 Hz, CH_2–Ph), 4.64 (dd, 1H, J = 10.5 Hz, J_{HF} = 41.9 Hz, CH_2–F), 4.91 (dd, 1H, J = 10.5 Hz, J_{HF} = 42.1 Hz, CH_2–F), 6.87 and 7.12 (2d, 4H, J = 8.6 Hz, Ph). ^1H NMR (CD_3SOCD_3 + DCl, 300 MHz) δ: 2.82 and 2.92 (2d, 2H, J = 14.3 Hz, CH_2–Ph), 4.53 (dd, 1H, J = 10.3 Hz, J_{HF} = 49.4 Hz, CH_2–F), 4.70 (dd, 1H, J = 10.3 Hz, J_{HF} = 49.4 Hz, CH_2–F), 6.57 and 6.85 (2d, 4H, J = 8.2 Hz, Ph). ^{13}C NMR (CD_3SOCD_3 + DCl, 75.47 MHz) δ: 36.9 (CH_2–Ar), 64.6 and 64.9 (C–N, J_{CF} = 17.3 Hz), 83.4 and 85.7 (CH_2–F, J_{CF} = 175.5 Hz), 116.8 (C–H, Ph), 123.5 (C–C, Ph), 132.5 (C–H, Ph), 157.4 (C–OH, Ph), 169.8 (COOH). ^{19}F NMR (D_2O) δ: 227.6 (t, J_{HF} = 47.0 Hz). MS (CI, NH_3) m/z: 214 ($[M + 1]^+$, 100 %), 231 ($[M + 18]^+$, 5 %). HRMS molecular ion not observed [M – HF] calcd for $C_{10}H_{11}NO_3$ 193.0739; found 193.074. UV (H_2O) λ_{max} (ε) 222.0 (3249), 273.2 nm.

α-Fluoromethyl-3',5'-ditritio-tyrosine hydrochloride (4)

The tritiated amido acid **25** (100.0 mCi, 4.1 μmol) dissolved in a 3 N solution of anhydrous hydrogen chloride in methanol (900 μL) was hydrolyzed for 2.5 h in an ultrasonic cleaning bath at 52 °C, then the solvent was evaporated under vacuum, and the residue diluted with water. Purification by HPLC (TSK-C18 RP) as described before using 2×10^{-4} M HCl (pH = 2.7) as eluent gives 9.0 mCi of **4** (9 %, V_e = 53.0 mL). ^3H NMR (D_2O) δ: 6.8 (s). UV (H_2O) λ_{max} (ε) 222.0 (3249), 273.2 nm. Specific activity 15.0 Ci/mmol.

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