The synthesis of 5-fluorokynurenine and 6-fluorokynurenic acid as metabolic probes

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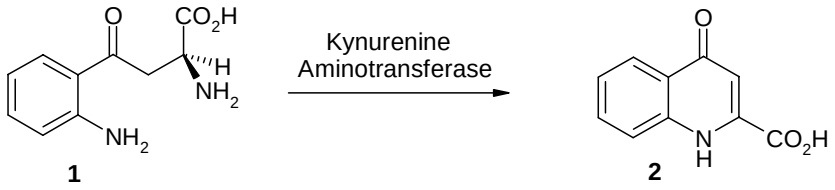
Dedicated to Douglas Lloyd to mark the occasion of his 80th birthday

Abstract
(2S)-5-Fluorokynurenine **9** and 6-fluorokynurenic acid **14** were synthesised as fluorinated probes to enable the metabolism of kynurenine to be monitored *in vivo* by ^{19}F NMR spectroscopy. The (2S)-5-fluorokynurenine **9** was prepared using a Friedel-Crafts acylation of *N*-(^{t}butoxycarbonyl)-4-fluoroaniline with a chiral oxazolidine derivative **6**, derived from 2s-aspartic acid. This represents a novel, and simple, method for the synthesis of kynurenine derivatives. The 6-fluorokynurenic acid **14** was synthesised using a Conrad-Limpach type synthesis. Preliminary biological studies are described.

Keywords: 5-Fluorokynurenine, 6-fluorokynurenic acid, metabolic probes, Conrad-Limpach type synthesis

Introduction

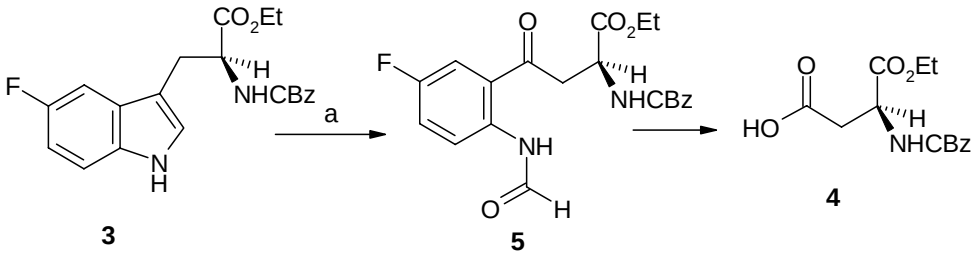
NMR spectroscopy offers the possibility of non-invasive measurement of metabolism *in vivo*[1] and ^{19}F-NMR spectroscopy has particular advantages. The sensitivity of ^{19}F-NMR spectroscopy is high, the chemical shift range is wider than ^{1}H-NMR spectroscopy and there are few background signals as there is little or no dietary source of fluorine. The aim of this work was to synthesise a fluorinated derivative of 2*S*-kynurenine **1** so that its *in vivo* conversion into kynurenic acid **2** (Scheme 1), as catalysed by the PLP (pyridoxal 5'-phosphate) dependent enzyme kynurenine aminotransferase,[2] could be monitored. This reaction lies on the kynurenine pathway of tryptophan metabolism[3] which produces a number of important neuroactive metabolites including quinolinic acid and kynurenic acid. Recently it has been postulated by Schwarcz *et al.* that kynurenic acid is an important marker for excitatory brain damage.[4] They showed that after a certain level of kynurenic acid had been released into the brain the damage became irreversible. The eventual aim is to use the fluorinated derivatives to produce a non-invasive assay to monitor brain damage using this hypothesis.



Scheme 1. The reaction catalysed by kynurenine aminotransferase.

Results and Discussion

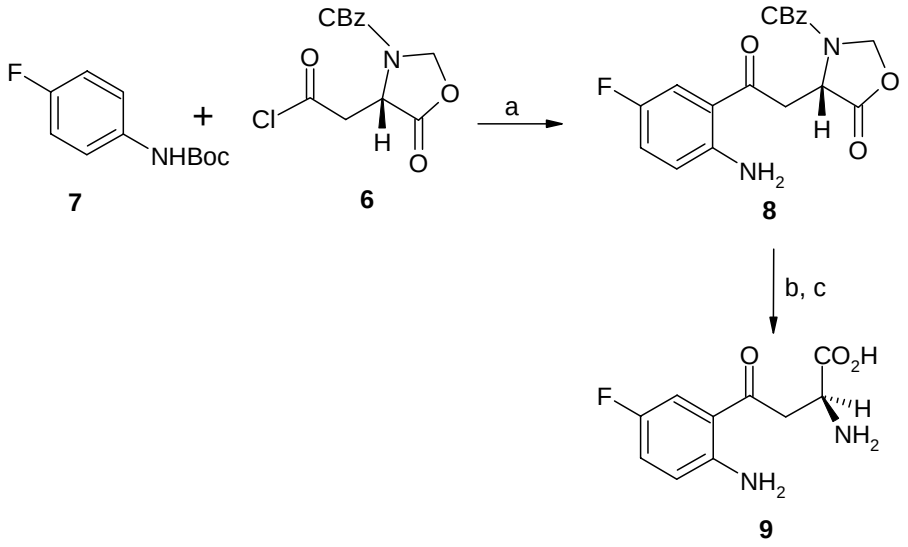
The original and simplest synthesis[5,6]of kynurenine involves the ozonolysis of *N*-protected tryptophan derivatives and has been successfully employed in our laboratory to produce 2S-[2-^{2}H]-kynurenine.[7] It was thus decided to prepare 5-fluorokynurenine from commercially available (2S)-5-fluorotryptophan, which was protected as the ethyl ester and *N*-carbobenzyloxy (CBz) derivative **3**. However, ozonolysis of this compound, using previously established conditions[7] did not produce the expected product. ^{19}F NMR spectroscopy showed the loss of the fluorine-containing ring and the only isolated product was an aspartic acid derivative **4**. It appeared that ozonolysis had taken place but then excess ozone brought about a Baeyer–Villiger type insertion reaction followed by hydrolysis during work-up (Scheme 2). The Baeyer–Villiger reaction appeared to be faster than oxidative cleavage as it was not possible to stop reaction after the first step by using shorter reaction times. As this was not observed in the ozonolysis of other tryptophans, it is likely that reaction takes place because of the increased reactivity of the ketone **5** formed by ozonolysis, as a result of the electron withdrawing effect of the fluorine. Alternatives to ozonolysis were investigated including oxidative cleavage using 4-tbutyliodoxybenzene[8] but none were successful.



Scheme 2. (a) O_3, MeOH, -78 ˚C, then Me_2S.

An alternative synthesis was thus sought. A number of synthetic routes to kynurenine derivatives have employed a protected oxazolidine derivative **6** prepared from 2S-aspartic acid, which is then coupled to an aromatic fragment using palladium (II) chemistry.[9,10] In this case it was decided to investigate a simple Friedel Crafts type acylation reaction using a protected

fluoroaniline derivative (Scheme 3). Thus 4-fluoroaniline was protected as the *N*-^{t}butoxycarbonyl (Boc) derivative **7** and then reacted with oxazolidine **6**, prepared *via* standard literature methodology,[11,12] using boron trifluoride as the Lewis acid catalyst. This gave the protected (2S)-5-fluorokynurenine **8** in 63% yield. The ^{t}butoxycarbonyl protection was found to be cleaved from the aniline during reaction. Further deprotection was then carried out in 6N HCl under reflux to give the final product, which was isolated as the free amino acid in 33% yield following neutralisation using propylene oxide.



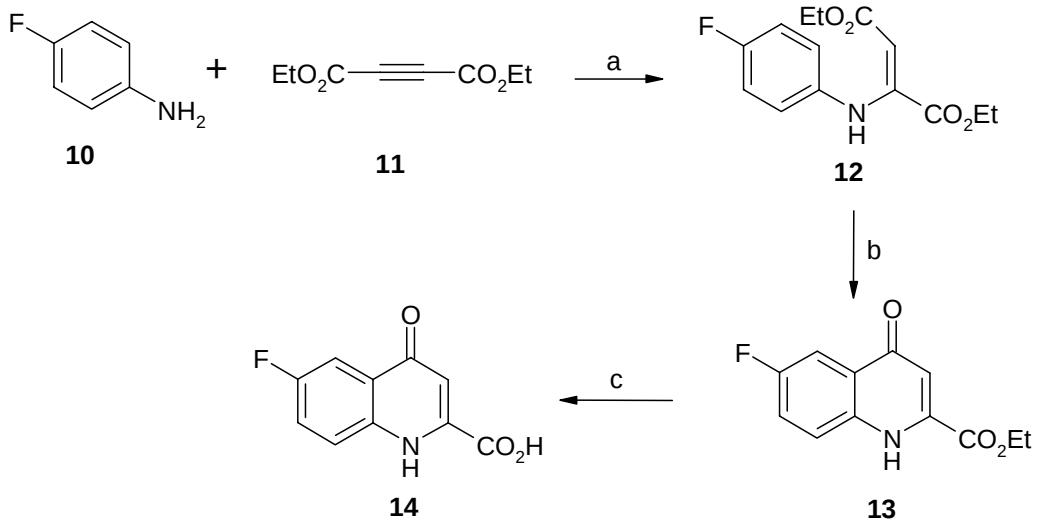
Scheme 3. (a) $BF_3.Et_2O$, DCM, rt, 12 h; b) 6N HCl, THF, reflux, 3 h; c) ^{i}PrOH, propylene oxide.

The Mosher's amide[13,14] was prepared from the (2S)-5-fluorokynurenine **9** to ensure that no racemisation had taken place during the synthesis. A single peak was observed in the ^{19}F NMR spectrum at -78.1 ppm for the amide implying that only one diastereomer was present and that there had indeed been no racemisation. The fluorine at the 5-position of the product gave a peak at -113.6 ppm under these conditions.

The synthesis of kynurenic acid analogues is well documented. There are established routes such as the Conrad–Limpach synthesis and related procedures.[15,16] The desired fluorinated derivative was thus synthesised from 4-fluoroaniline **10** and diethyl acetylenedicarboxylate **11** as shown in Scheme 4. The two starting materials were heated under reflux in methanol to give the butenedioate **12** in 91% yield. However, as this compound was difficult to purify further it was carried straight through into the second step. Cyclisation was achieved by heating to reflux in diphenyl ether (250 ˚C) giving the product **13** in 72% yield. ^{1}H NMR spectroscopy confirmed the structure, including a characteristic signal at 6.97 ppm for the hydrogen at the 3-position. The mass spectrum gave a strong signal at the correct M^{+} of 235 and microanalysis established the purity.

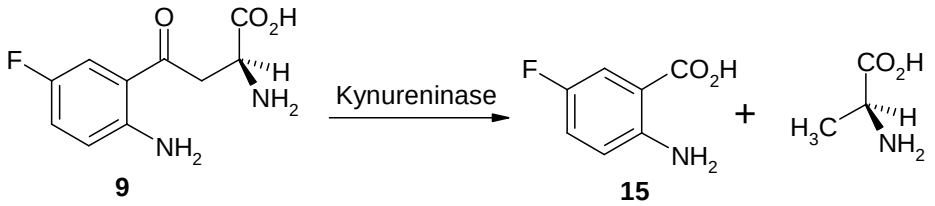
In the final step the ethyl ester was hydrolysed under basic conditions to yield the 6-fluorokynurenic acid **14** in 84% yields. The ^{1}H NMR spectrum confirmed the structure and the ^{19}F NMR spectrum showed a single peak -117.4 ppm.

It can thus be seen that using the methods described the two target compounds were successfully synthesised. The (2S)-5-fluorokynurenine **9** was prepared using a novel, but simple, modification of previous procedures. This Friedel-Crafts route may prove useful for other kynurenine derivatives and its applications are being further explored.



Scheme 4. (a) MeOH, reflux, 4.5 h; (b) Ph_2O, reflux, 75 min; (c) MeOH, 1M NaOH, rt, 48 h.

In order to check that the compounds could be used for metabolic studies the ^{19}F NMR spectrum of a solution containing both compounds in aqueous was measured and two singlets were readily observed with a separation of 1.6 ppm. The metabolism of the (2S)-5-fluorokynurenine **9** by a related enzyme, kynureninase, was also examined. Kynureninase is also a PLP-dependent enzyme and catalyses the hydrolytic β,γ-cleavage of kynurenine to give anthranilic acid and alanine (Scheme 5).[17]



Scheme 5. The reaction catalysed by kynureninase.

When (2S)-5-fluorokynurenine **9** was incubated with bacterial kynureninase (isolated from *Pseudomonas fluorescens*[18]) in 10 mM potassium phosphate buffer (pH 7.4) and the reaction

followed by ^{19}F NMR spectroscopy, a clean reaction was observed showing the decrease in the signal due to (2S)-5-fluorokynurenine **9** at -125.15 ppm and the appearance of a new signal at 128.0 ppm. This was shown to be due to the expected product, 5-fluoroanthranilic acid **15**, by comparison with the ^{19}F NMR spectrum of a commercial sample under identical conditions. It can thus be seen that the (2S)-5-fluorokynurenine **9** is a substrate for kynureninase. Bearing in mind the mechanistic similarities between the two PLP-dependent enzymes it is likely that the compound will also be a substrate for kynurenine aminotransferase. These results are in good agreement with some previous studies by Harada, who examined the metabolism of 5-fluorotryptophan in rat kidney and observed its transformation to 5-fluorokynurenine by ^{19}F NMR spectroscopy.[19] Future studies will assess the utility of (2S)-5-fluorokynurenine **9** for monitoring the kynurenine aminotransferase reaction *in vivo*.

Experimental Section

General procedures. Analytical TLC was performed using aluminium plates precoated with silica gel 60 F_{254} (Merck) and visualized using UV light. Flash chromatography was carried out on silica gel 60 (35–70 μ, Fluorochem). Melting points (uncorrected) were determined on a Gallenkamp Melting Point apparatus. All NMR spectra were recorded on a Varian Gemini f.t. spectrometer (^{1}H, 300 MHz; ^{19}F, 298 MHz; ^{13}C, 74.76 MHz) or a Varian Gemini f.t. spectrometer (^{1}H, 200 MHz; ^{13}C, 50.31 MHz). ^{1}H- and ^{13}C-NMR spectra were referenced on chloroform, TMS, methanol or DMSO. ^{19}F NMR spectra were referenced on fluorotrichloromethane. Coupling constants are given in Hz and without sign. The IR-spectra were recorded as nujol mulls on a Perkin-Elmer series 1420 IR instrument. Optical rotations were measured at room temperature using an Optical Activity Ltd. AA 1000 polarimeter with 10 cm path-length cells. The measurements are given in 10^{-1} ˚ cm^2 g^{-1}. Mass spectra were recorded on an A. E. I./Kratos MS-50 instrument or a Micromass TofSpec 2E for MALDI-TOF spectra. HPLC purifications were carried out on a Cecil CE1200 series chromatograph using Luna 5u C-18 silica-gel on a 150 x 4.60 mm 5 micron sized column. Ozonolysis was carried out using a Fischer Ozon Ozon-generator 500.

Materials. Unless otherwise stated, these were commercial samples. Solvents were dried and purified according to the methods of Perrin and Armarego.[20] Solvent mixtures are defined by volume ratios (v/v).

Ozonolysis of (2*S*)-*N*-CBz-5-fluorotryptophan ethyl ester (3). (2*S*)-*N*-CBz-5-Fluorotryptophan ethyl ester (0.974 g, 2.54 mmol) was dissolved in dry distilled methanol (110 cm^3). The solution was cooled to -78 ˚C with stirring and then treated with ozone for one hour. The reaction was then quenched with dimethyl sulfide (10 cm^3) and left to stir at -78 ˚C for 1 h. The solution was then evaporated at reduced pressure to give a brown/orange oil, which was dissolved in dichloromethane (20 cm^3), washed with water (3 x 50 cm^3) and brine (2 x 50 cm^3),

dried ($MgSO_4$) and the solvent removed at reduced pressure. Purification was carried out by column chromatography (silica, ethyl acetate: petroleum ether, 1:2). This gave ethyl *N*-CBz-aspartate as an oil (0.46 g, 62%). δ_H (200 MHz; $CDCl_3$) 1.25 (3 H, t, *J* 4.9, $CO_2CH_2C\underline{H}_3$), 2.93 - 3.07 (2 H, m, 3-$C\underline{H}_2$), 4.23 (2 H, q, *J* 4.9, $CO_2C\underline{H}_2CH_3$), 4.60 - 4.67 (1 H, m, 2-C<u>H</u>), 5.15 (2 H, s, $C\underline{H}_2Ph$), 7.27 - 7.36 (5 H, m, CBz aromatic); δ_C (50.31 MHz; $CDCl_3$) 14.45 ($OCH_2\underline{C}H_3$), 36.78 (3-<u>C</u>H_2), 50.79 (2-<u>C</u>H), 62.41 ($O\underline{C}H_2CH_3$), 67.57 (<u>C</u>H_2Ph), 128.54-128.97 (aromatic), 136.6 (quat. aromatic), 156.67 (NH<u>C</u>O), 171.28 (<u>C</u>O_2Et), 174.78 (<u>C</u>O_2H); *m/z* (CI) 296 ([*M*+H], 43%), 278 (9, [*M*-H_2O]$^+$), 252 (54, [*M*-CO_2]$^+$), 147 (89, [CBz]$^+$), 91 (100, [$PhCH_2$]$^+$).

(4*S*)-*N*-Carbobenzyloxy-5-oxo-4-oxazolidine acetic acid. A mixture of *N*-carbobenzyloxy-L-aspartic acid (5.35 g, 0.02 mol), paraformaldehyde (1.8 g), acetic anhydride (4.0 g, 0.04 mol) and thionyl chloride (0.3 cm^3) was added to glacial acetic acid (80 cm^3). This solution was heated at 100 ˚C on an oil bath for 4 h. The solvent was then removed under reduced pressure to give a yellow/orange syrup. This was taken up in ethyl acetate (50 cm^3) and extracted into a 5% solution of sodium hydrogen carbonate (95 cm^3). The aqueous layer was acidified, whilst cooled in an ice bath, with 6 M HCl to pH 1 to give a yellow precipitate. This was then extracted with ethyl acetate (100 cm^3) and washed with water (2 x 100 cm^3) and brine (100 cm^3). The organic layers were dried ($MgSO_4$), filtered and the solvent removed under reduced pressure to give a pale yellow syrup. Purification by column chromatography (silica; DCM: ethyl acetate; 3:1), gave the *title compound* as a yellow syrup (3.77 g, 67%) $[\alpha]_{20D}$ +186.3 ˚ (c = 1.1, $CHCl_3$), [lit.,[201] not given]; ν_{max} (neat)/cm^{-1} 3000 (br. OH), 1700 (carbamate CO); δ_H (200 MHz; $CDCl_3$) 2.95 - 3.19 (2 H, m, β-$C\underline{H}_2$), 4.44 - 4.52 (1 H, m, 4-C<u>H</u>), 5.17 (2 H, s, $OC\underline{H}_2$Ph), 5.22 (1 H, s, 2-$\underline{H}_A$), 5.47 (1 H, br s, 2-$\underline{H}_B$), 7.39 (5 H, s, Ph), 9.66 (1 H, br s, CO_2H); δ_C (50.31 MHz; $CDCl_3$) 51.76 (β-<u>C</u>H_2), 67.01 (4-<u>C</u>), 78.32 ($O\underline{C}H_2$Ph), 86.82 (2-<u>C</u>), 127.89 (3' & 5'-<u>C</u>), 128.28 (4'-<u>C</u>), 128.67 (2' & 6'-<u>C</u>), 136.31 (1'-<u>C</u>), 152.90 (N<u>C</u>O), 170.55 (<u>C</u>O_2H), 171.72 ($CH_2\underline{C}O_2$); *m/z* (EI) 279 [*M*$^+$, 4.9%], 235 (22, [*M*-CO_2]$^+$), 144 (11, [*M*-CO_2-$PhCH_2$]$^+$), 126 (9, [*M*-CO_2-$PhCH_2$-H_2O]$^+$), 107 (36, [$PhCH_2O$]$^+$), 91 (100, [$PhCH_2$]$^+$).

(4*S*)-*N*-Carbobenzyloxy-5-oxo-4-oxazolidineacetyl chloride (7). (4*S*)-*N*-Carbobenzyloxy-5-oxo-4-oxazolidine acetic acid (0.937 g, 3.35 mmol) was dissolved in toluene (5 cm^3) and oxalyl chloride (10 cm^3) added. The solution was heated under reflux for 2 h and the solvent then removed at reduced pressure. The residue was washed several times with toluene to remove traces of oxalyl chloride and used without further purification. The *title compound* was obtained as an orange oil (0.72 g, 72%) δ_H (200 MHz, $CDCl_3$) 3.55 - 3.93 (2 H, m, β-$C\underline{H}_2$), 4.37 (1 H, s, α-C<u>H</u>), 5.23 (2 H, s, $OC\underline{H}_2$Ph), 5.36 (1 H, t, *J* 5.4, H_A of 2-$C\underline{H}_2$), 5.52 (1 H, br s, H_B of 2-$C\underline{H}_2$), 7.41 (5 H, s, $OCH_2\underline{Ph}$); δ_C (50.31 MHz, $CDCl_3$) 52.9 (β-<u>C</u>H_2), 69.4 (α-<u>C</u>H), 79.7 ($O\underline{C}H_2$Ph), 88.2 (2-<u>C</u>H_2), 129.7 (3' & 5'-<u>C</u>), 130.3 (4'-<u>C</u>), 130.6 (2' & 6'-<u>C</u>), 136.2 (1'-<u>C</u>), 153.8 (N<u>C</u>O), 171.5 ($CH_2\underline{C}O_2$).

4-Fluoro-2-[(4*S*)-*N*-carbobenzyloxy-5'-oxo-4'-oxazolidinoacetyl]-aniline (8). (*4S*)-*N*-Carbobenzyloxy-5-oxo-4-oxazolidineacetyl chloride (1.747 g, 7.85 mmol, 1.1 eq) was dissolved in dry dichloromethane (30 cm^3) and boron trifluoride etherate ($BF_3.Et_2O$), (1.3 cm^3, 10.5 mmol, 1.8 eq wrt acid chloride) added. The solution was stirred at room temperature for 20 minutes

before adding *N*-(tbutoxycarbonyl)-4-fluoroaniline (1.011 g, 5.26 mmol). The reaction was stirred at room temperature overnight, poured into 1 M hydrochloric acid (30 cm^3), extracted into dichloromethane (3 x 20 cm^3), and the organic fractions dried ($MgSO_4$) and concentrated under reduced pressure to give a red-brown oil. Purification by column chromatography (silica; pet. ether: ethyl acetate; 2:1 graduating to 1:1 and collected in the ethyl acetate wash) gave the product as an orange oil (1.23 g, 63%) (Found: M^+, 373.120473. $C_{18}H_{18}N_2O_5F$ requires M^+, 373.119975); $[\alpha]_{20D}$ +157.1 ˚ (c = 0.27, $CHCl_3$); δ_H (200 MHz, $CDCl_3$) 3.05 (2 H, d, *J* 10.7, β-C<u>H</u>$_2$), 4.31 (1 H, br s, α-C<u>H</u>), 5.02 - 5.18 (2 H, m, 2-C<u>H</u>$_2$) 5.25 (2 H, s, OC<u>H</u>$_2$Ph), 6.88 (1 H, t, $J_{3',4'}$ 8.9, 4'-C<u>H</u>), 7.03 -7.21 (2 H, m, 3' & 6'-C<u>H</u>) 7.32 (5 H, s, OCH$_2$<u>Ph</u>); δ_F (282.3 MHz, $CDCl_3$) -118.53; δ_C (50.31 MHz, $CDCl_3$) 52.3 (β-<u>C</u>H$_2$), 68.4 (4-<u>C</u>H), 78.8 (O<u>C</u>H$_2$Ph), 88.1 (2-<u>C</u>H$_2$), 127.9 (3" & 5"-<u>C</u>), 128.3 (4"-<u>C</u>), 128.6 (2" & 6"-<u>C</u>), 128.8 (4'-<u>C</u>), 128.9 (6'-<u>C</u>), 129.1 (1'-<u>C</u>), 129.2 (3'-<u>C</u>), 133.9 (1"-<u>C</u>), 153.3 (N<u>C</u>O), 157.9 (2'-<u>C</u>), 163.2 (5'-<u>C</u>), 167.8 (5-<u>C</u>O), 191.9 (<u>C</u>O); *m/z* (CI) 373 ([*M*+H], 13%), 343 (72, [*M*+H-CH$_2$O]$^+$), 236 (49, [*M*+H-PhCH$_2$OCO]$^+$), 192 (53, [*M*+H-PhCH$_2$OCO-NCH$_2$O]$^+$), 91 (100, [PhCH$_2$]$^+$).

(2*R*)-5-Fluorokynurenine (9). To 4-fluoro-2-[(*4S*)-*N*-carbobenzyloxy-5'-oxo-4'-oxazolidinoacetyl]-aniline (0.3 g, 0.8 mmol) dissolved in THF (5 cm3), was added 6M hydrochloric acid (15 cm^3) and the solution heated under reflux for 3 h. After cooling, the reaction mixture was washed with diethyl ether (2 x 10 cm^3) and the aqueous phases concentrated under reduced pressure to give a brown/orange oil. Purification by column chromatography (C18 reverse phase silica; acetonitrile:water; 1:1) followed by reverse phase HPLC separation of the resulting oil (water: acetonitrile; 90:10; flow rate: 1.8 cm^3 min^{-1}) gave the *title compound* (60 mg, 33%) (Found: M^+, 228.0911 $C_{10}H_{13}N_2O_3F$ requires M^+, 228.2227; $[\alpha]_{20D}$ + 6 ˚ (c = 0.2, MeOH); ν_{max} (neat/cm^{-1}) 3420 - 3315 (OH), 1720 (CO), 1650 (acid CO); δ_H (300 MHz, CD_3OD) 3.08 (2 H, d, *J* 10.7, β-C<u>H</u>$_2$), 4.52 - 4.61 (1 H, m, α-C<u>H</u>), 7.30 - 7.38 (3 H, m, 3, 4, & 6-<u>H</u>); δ_F (282.3 MHz, CD_3OD) -115.8; δ_C (75.4 MHz, CD_3OD) 45.4 (β-<u>C</u>H$_2$), 66.0 (α-CH), 129.0 (4-<u>C</u>), 129.2 (6-<u>C</u>), 129.7 (3-<u>C</u>), 130.1 (1-<u>C</u>), 137.9 (2-C), 158.5 (5-<u>C</u>), 171.7 (<u>C</u>O$_2$H), 191.2 (<u>C</u>O); *m/z* (MALDI-TOF) 268 ([*M*+2Na], 48%), 250 (24, [*M*+Na+H]), 228 (27, [*M*+2H]).

Diethyl (2*E*)-2-(4-fluoroanilino)-2-butenedioate (12). Diethyl acetylenedicarboxylate (1.886 g, 11 mmol) was added slowly to a stirred solution of 4-fluoroaniline (1.114 g, 10 mmol) in dry redistilled methanol (15 cm^3). The solution was heated under reflux for 2 hours and then cooled to room temperature. The reaction was followed by tlc (silica; dichloromethane) and heated at reflux for a further 2.5 h with no further change in the chromatogram. The resulting solution was evaporated at reduced pressure to give the *title compound* as an orange oil (2.57 g, 91%) which was used without further purification in the next step. δ_H (200 MHz; $CDCl_3$) 1.10 (3 H, t. *J* 7.1, OCH$_2$C<u>H</u>$_3$), 1.29 (3 H, t, *J* 7.1, OCH$_2$C<u>H</u>$_3$), 4.08-4.16 (2 H, m, OC<u>H</u>$_2$CH$_3$), 4.18-4.24 (2 H, m, OC<u>H</u>$_2$CH$_3$), 5.39 (1 H, s, C<u>H</u>), 6.86-7.03 (4 H, m, aromatic), 9.59 (1 H, br s, N<u>H</u>);

Ethyl 6-fluoro-4-oxo-1,4-dihydro-2-quinoline carboxylate (13). Diethyl (2*E*)-2-(4-fluoroanilino)-2-butenedioate (191, 3.112 g, 11 mmol) was added to warm diphenyl ether (100 cm^3). The solution was stirred continuously and heated to reflux (250 ˚C). The solution darkened from pale orange to brown. After 45 minutes at reflux the condenser water supply was

switched off to aid the removal of ethanol. After 30 minutes the solution was allowed to cool. The beige product was filtered off, washed several times with diethyl ether and recrystallised from ethyl acetate to give the *title compound* (1.55 g, 72%) mp 235-8 ˚C (lit.,[21] 239-240 ˚C); (Found: C, 61.46; H, 4.50; N, 5.96. $C_{12}H_{10}FNO_3$ requires C, 61.28; H, 4.29; N, 5.95%); ν_{max} (nujol)/cm^{-1} 2920 (NH), 1720 (ester CO); δ_H (200 MHz; $CDCl_3$) 1.44 (3 H, t, *J* 7.1, $OCH_2C\underline{H}_3$), 4.48 (2 H, q, *J* 7.1, $OC\underline{H}_2CH_3$), 6.97 (1 H, s, 3-H), 7.46 (1 H, dt, $J_{6,8}$ 9.1, $J_{5,8}$ 2.2, 8-H), 7.54 (1 H, dd, $J_{5,6}$ 9.1, $J_{5,7}$ 3.5, 5-H), 8.00 (1 H, dd, $J_{6,7}$ 8.7, $J_{5,7}$ 3.5, 7-H); δ_F (298 MHz; $CDCl_3$) -116.2; δ_C (50.31 MHz; d$_6$-DMSO) 19.56 ($CO_2CH_2\underline{C}H_3$), 68.35 ($CO_2\underline{C}H_2CH_3$), 114.35 (8-C), 127.07 (5-C), 127.58 (7-C), 128.83 ($J_{C,F}$ 8.5, 6-C), 142.95 (3-C), 144.39 (2-C), 162.15 ($\underline{C}O_2Et$), 166.99 (4a-C), 167.86 (1a-C), 181.56 (4-C); *m/z* (EI) 235 (*M*$^+$, 67%), 189 (34, [*M*-EtOH]$^+$), 161 (100, [*M*-$EtCO_2H$]$^+$).

6-Fluorokynurenic acid (14). Ethyl 6-fluoro-4-oxo-1,4-dihydro-2-quinoline carboxylate (1 g, 4.25 mmol) was dissolved in methanol (70 cm^3) and 1 M sodium hydroxide (12 cm^3) added. The solution was stirred at room temperature for 48 h. The resulting solution was then filtered to remove insoluble materials and the solvent removed at reduced pressure to give an off white solid. The residue was taken up in distilled water (45 cm^3) and stirred. 2 M Hydrochloric acid solution was then added dropwise and the resulting white solid was filtered, dried in a desiccator (P_2O_5) and then crushed to a fine powder. Recrystallisation from water gave the *title compound* as white flaky crystals (0.74 g, 84%) mp 280 ˚C (Found: M$^+$, 208.045193, $C_{10}H_7FNO_3$ requires M$^+$, 208.040996); ν_{max} (nujol)/cm^{-1} 2920 (NH), 1720 (ketone CO), 1590 (acid CO); δ_H (200 MHz; d$_6$-DMSO) 6.72 (1 H, s, C$\underline{H}$), 7.61-7.77 (2 H, m, 8-C$\underline{H}$ and 5-C$\underline{H}$), 8.04 (1 H, dd, $J_{6,7}$ 9.1, $J_{5,7}$ 4.7, 7-C$\underline{H}$); δ_F (298 MHz; d$_6$-DMSO) -117.4; δ_C (50.31 MHz; D_2O) 110.05 (8-C), 124.58 (5-C), 125.09 (7-C), 128.06 ($J_{C,F}$ 8.5, 6-C), 148.14 (3-C), 159.59 (2-C), 164.46 ($\underline{C}O_2H$), 167.12 (4a-C), 169.41 (1a-C), 181.89 (4-C); *m/z* (CI): 208 (*M*+H, 18%), 164 (17, [*M*+H-CO_2]$^+$), 57 (100, [CCO_2H]$^+$).

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