JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2003; **46**: 613–622.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.701

Research Article

A convenient synthesis of $^{13}C_4$-Leflunomide and its primary metabolite $^{13}C_4$-A77 1726

Robert J. Faragher[1], John. M. Motto[1], Maciej A. Kaminski[2] and Adrian L. Schwan[1,*]

[1] *Department of Chemistry and Biochemistry, University of Guelph, Guelph, ON, Canada, N1G 2W1*

[2] *Health and Pharmaceutical Services International, P.O. Box 21012 R.P.O. Meadowvale, Mississauga, ON, Canada, L5N 6A2*

Summary

A convenient synthesis of leflunomide and its primary pharmacologically active metabolite A77 1726, each labeled with four ^{13}C atoms has been achieved. Starting from $^{13}C_4$-ethyl acetoacetate, each step of the synthesis proceeds in 60–82% yield. Analysis of the mass spectra of the labeled and unlabeled materials demonstrates that the compounds prepared herein will be suitable as analytical standards for bioavailability studies. Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: leflunomide; metabolite; A77 1726; stable isotopes; synthesis; ^{13}C labeling

Introduction

Leflunomide is a recently approved disease modifying antirheumatic drug (DMARD) for the treatment of rheumatoid arthritis.[1–6] The drug's primary metabolite A77 1726 is believed to be the active ingredient[7–9] which possesses a mode of action involving the inhibition of pyrimidine synthesis and the consequent suppression of inflamma-

*Correspondence to: A. L. Schwan, Department of Chemistry and Biochemistry, University of Guelph, Guelph, ON, Canada, N1G 2W1. E-mail: schwan@uoguelph.ca

Received 14 October 2002
Revised 8 January 2003
Accepted 31 January 2003

tion. Leflunomide's clinical efficacy rivals that of methotrexate and salazopyrene yet elicits a faster onset of response.[10] Furthermore, unlike methotrexate,[11,12] leflunomide does not result in bone marrow toxicity.[2,4] The adverse effects of leflunomide have been summarized as mild to moderate and can be easily resolved.[3,13]

This paper outlines the preparation of leflunomide and its active metabolite A77 1726, each labeled with four ^{13}C atoms. The use of ^{13}C labeled drugs is well established as a reliable method for establishing drug pharmacokinetics.[14–16] Moreover, in this particular instance, the incorporation of an additional four mass units in analytical standards of the labeled drug and its metabolite ensures sufficient mass differentiation when compared with unlabeled material, thus establishing its usefulness for HPLC/MS analysis in bioavailability studies.

Results and discussion

Given the common synthesis of leflunomide,[17,18] the choice of ^{13}C atoms as the stable isotope labels is the preferred option as many of the available sites for deuterium labels are enolizable at one or more steps during the synthesis. The use of ^{13}C atoms precludes this problematic issue and since ethyl acetoacetate, a common precursor to the isoxazole ring of leflunomide[17,18] is commercially available with four ^{13}C atoms, the principal requirement for the chosen synthesis is conveniently in place.

As shown in Scheme 1, the $^{13}C_4$-ethyl acetoacetate (**1**) was treated with ethyl orthoformate to install the ethoxymethylene unit of **2** (79%). The distilled material (**2**) was then reacted with hydroxylamine hydrochloride which was neutralized *in situ*. Instead of using crude isoxazole **3** directly in the subsequent step, purification by distillation was performed. Even though this distillation was performed only on a small (3–4 g) scale using a short-path apparatus equipped with a 10 cm Vigreux column, isoxazole **3** was still isolated in 77% yield. Acidic hydrolysis of the ester component of **3** was essentially quantitative affording crude acid **4**. Toluene recrystallization gave pure **4** in 60% yield; some decomposition of the material during the recrystallization process was observed.

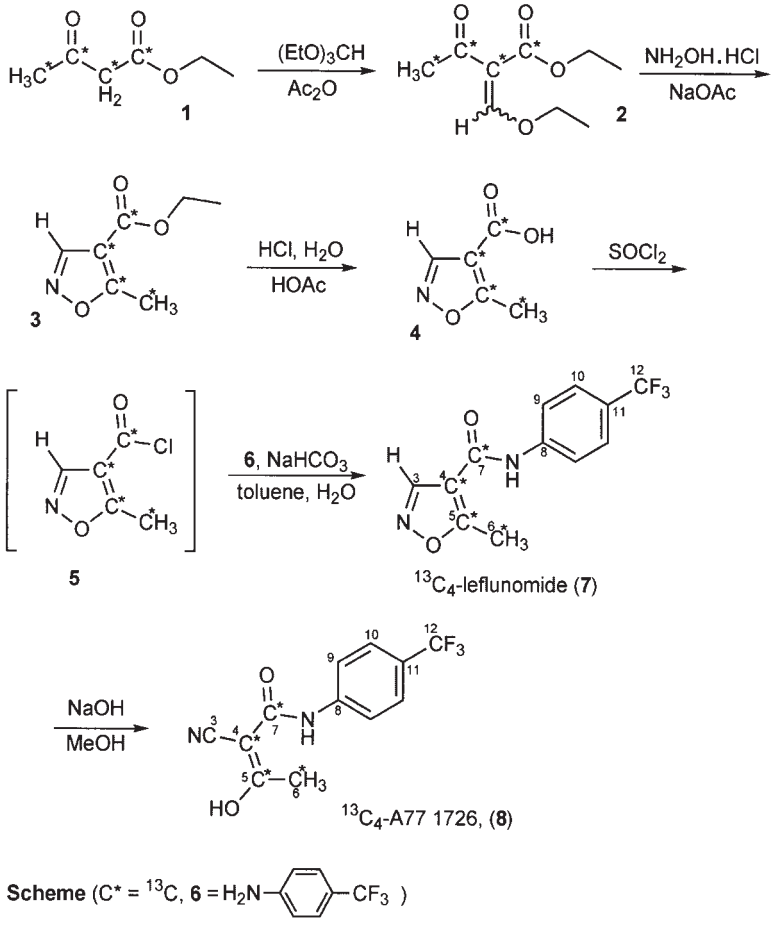
Conversion of acid **4** to its acid chloride was performed in a typical manner by reaction with thionyl chloride. At this stage the acid chloride is usually isolated and purified.[19, 20] In this study however, it was found

that direct reaction with 4-trifluoromethylaniline (**6**) proved most efficient. Thus the reaction of crude acid chloride **5** with aniline **6** and sodium bicarbonate in a two-phase mixture of toluene and water[21] was carried out. The wet, white solid which precipitated from the reaction mixture was filtered out and dissolved in ethyl acetate. The resulting solution was dried with magnesium sulfate and evaporated to dryness. Recrystallization from toluene afforded labeled leflunomide (**7**) in 60% yield starting from the acid. The purity of the labeled leflunomide was established by HPLC to be 99.8%.

Conversion of leflunomide to its metabolite A77 1726 was performed by treating leflunomide **7** with methanolic sodium hydroxide solution. Stirring the reaction mixture for 2 h at room temperature resulted in the complete conversion of **7** to the ring opened metabolite **8**. Acidification of the mixture yielded a white precipitate. Recrystallization of this material from toluene afforded labeled A77 1726 in 82% yield. The metabolite was established to be 99.8% pure by HPLC analysis.

The identity of all the compounds in the reaction scheme was confirmed by spectral analysis and comparison of their physical properties with published data. ^{13}C NMR spectroscopy proved to be particularly diagnostic. For instance, carbon chemical shifts were fully consistent with expectations. Most telling was the chemical shift migration of carbon-4 of **7** at 112 ppm to 81 ppm in **8**, a change consistent with the loss of isoxazole aromaticity and the resulting increase of electron donation from the hydroxy group to shield carbon-4. The $^{13}C,^{13}C$ coupling constants were also consistent with predictions. The $^{13}C_{sp3}$, $^{13}C_{sp2}$ coupling constant involving the labeled methyl group was consistently in the 43–50 Hz range. Also, once the isoxazole ring was formed, the two internal carbons of the four carbon isotope linkage uniformly exhibited a $^{13}C_{sp2}$, $^{13}C_{sp2}$ coupling constant of 70–71 Hz. Overall the consistency of the ^{13}C–^{13}C coupling constants for the whole set of compounds proves that the backbone structure of the four labeled carbon atoms remained intact through the entire series of reactions, as presented in Scheme 1. It should be noted that the four ^{13}C labels and particularly the presence of one of the labels on a methyl group opens the possibility that compounds **7** and **8** may prove suitable for the investigation of pharmacokinetic problems using NMR spectroscopy.[22]

To establish the value of the isotopically labeled compounds for bioavailability studies, their mass spectra must be compared to those of



Scheme 1.

the unlabeled analogues. Commercial leflunomide was converted to its active metabolite, i.e. the unlabeled A77 1726 using conditions identical to those described for the synthesis of the labeled material. The mass spectra of each of these materials were recorded and are presented in the experimental section. A close inspection of the mass spectra of the unlabeled compounds revealed that there are no significant peaks present at $m/z > 272$. To complement this, compounds **7** and **8** do not exhibit peaks in the $m/z = 268$–272 range that are distinguishable from the spectrum noise. Thus the labeled compounds **7** and **8** meet all the necessary criteria to be excellent candidates for bioavailability studies.

Conclusions

A simple, multistep synthesis of $^{13}C_4$-leflunomide (**7**) is presented. Furthermore, labeled leflunomide **7** can readily be converted to its labeled metabolite $^{13}C_4$-A77 1726 (**8**). The procedure affords gram quantities of these substances in >99.8% purity. The mass spectra of the labeled standards demonstrate that the introduction of the additional four mass units is sufficient to ensure their proper differentiation from their unlabeled analogs during the HPLC/MS analysis stage of bioavailability studies.

Experimental

Melting points were measured with a Thomas Hoover melting point apparatus and are uncorrected. IR spectra were recorded on a Bomem FT-IR spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded on a Bruker Avance 400 MHz NMR Spectrometer. ^{1}H NMR spectra are reported relative to TMS. ^{13}C NMR spectra which were run in $CDCl_3$ were referenced to the solvent peak at 77.0 ppm. ^{13}C NMR spectra which were run in DMSO-d_6 were referenced to the solvent peak at 39.7 ppm. Mass spectra were obtained with a VG ZAB-R instrument located at the McMaster Regional Center for Mass Spectrometry. HPLC analyses were performed on a Waters 400 instrument equipped with a Jones Chromatography Ltd. Genesis 4.6 × 250 mm C18 reversed phase column. The mobile phase consisting of methanol:water in the ratio of 80:20 was pumped at a rate of 1 ml/min. A UV detector set at 254 nm was used. [1,2,3,4-$^{13}C_4$]-Ethyl acetoacetate (99% ^{13}C) was purchased from Isotec, Inc. All other chemicals and reagents were purchased from Fisher or Aldrich. Reactions were carried out under dry nitrogen.

$^{13}C_4$-*Ethyl 2-(ethoxymethylene)-3-oxo-butanoate* (**2**)

To a flame dried, 25 ml round-bottomed flask equipped with a reflux condenser, were added ethyl acetoacetate (5.08 g, 37.9 mmol), ethyl orthoformate (6.30 ml, 37.9 mmol) and freshly distilled acetic anhydride (7.14 ml, 75.7 mmol) via syringe. The flask was placed into an oil bath and refluxed for 60 min. After cooling to r.t., the solution was concentrated under vacuum and the product was distilled to afford

5.68 g (79%) of a mixture of *E*/*Z* isomers of **2** as an orange oil possessing ca. 90% purity (by NMR); bp: 60–66°C (0.3 mm). Spectral data for **2**: ^{1}H NMR (400 MHz, $CDCl_3$), mixture of isomers, δ: 7.50, (m, 1 H), 4.13–4.02 (m, 4 H), 2.38–1.95 (m, 3 H), 1.24–1.09 (m, 6 H); ^{13}C NMR (100.6 MHz, $CDCl_3$), major isomer, δ: 194.3 (ddd, $^{1}J_{^{13}C,^{13}C} = 56.4$ Hz, $^{1}J_{^{13}C,^{13}C} = 43.8$ Hz, $^{2}J_{^{13}C,^{13}C} = 3.4$ Hz, ketone C), 164.5 (dd, $^{1}J_{^{13}C,^{13}C} = 76.2$ Hz, $^{2}J_{^{13}C,^{13}C} = 3.4$ Hz, ester C), 114.3 (ddd, $^{1}J_{^{13}C,^{13}C} = 76.2$ Hz, $^{1}J_{^{13}C,^{13}C} = 56.4$ Hz, $^{2}J_{^{13}C,^{13}C} = 13.8$ Hz, C between carbonyls), 72.2 (s, $\underline{C}H_2$), 59.8 (s, $\underline{C}H_2$), 27.6 (dd, $^{1}J_{^{13}C,^{13}C} = 43.8$ Hz, $^{2}J_{^{13}C,^{13}C} = 13.8$ Hz, $\underline{C}H_3C(O)$), 14.8 (s, $CH_2\underline{C}H_3$), 13.7 (s, $CH_2\underline{C}H_3$), anticipated peak near 164 ppm obscured by labeled carbons; ^{13}C NMR (100.6 MHz, $CDCl_3$), minor isomer, δ: 196.1 (dd, $^{1}J_{^{13}C,^{13}C} = 54.6$ Hz, $^{1}J_{^{13}C,^{13}C} = 43.1$ Hz, (anticipated $^{2}J_{^{13}C,^{13}C} = 1.9$ Hz not resolved), ketone C), 164.8 (dd, $^{1}J_{^{13}C,^{13}C} = 77.6$ Hz, $^{2}J_{^{13}C,^{13}C} = 1.9$ Hz, ester C), 113.1 (ddd, $^{1}J_{^{13}C,^{13}C} = 77.6$ Hz, $^{1}J_{^{13}C,^{13}C} = 54.6$ Hz, $^{2}J_{^{13}C,^{13}C} = 13.7$ Hz, C between carbonyls), 72.2 (s, $\underline{C}H_2$), 59.9 (s, $\underline{C}H_2$), 31.1 (dd, $^{1}J_{^{13}C,^{13}C} = 43.1$ Hz, $^{2}J_{^{13}C,^{13}C} = 13.7$ Hz, $\underline{C}H_3C(O)$), 14.7 (s, $CH_2\underline{C}H_3$), 13.7 (s, $CH_2\underline{C}H_3$); anticipated peak near 164 ppm obscured by labeled carbons.

$^{13}C_4$-*Ethyl 5-methyl-4-isoxazolecarboxylate* (**4**)

To a 25 ml round-bottomed flask were added ester **2** (5.40 g, 28.4 mmol) and ethanol (6 ml) and the mixture was cooled to 0°C. To the reaction mixture was added, via a dropping funnel a solution of hydroxylamine hydrochloride (2.17 g, 31.2 mmol) and sodium acetate trihydrate (4.31 g, 31.2 mmol) dissolved in water (8 ml). The mixture was stirred overnight at 0°C and was extracted with EtOAc (50 ml, 2×25 ml) and the combined organic extracts were dried over Na_2SO_4. After filtration and concentration under vacuum, the residue was fractionally distilled (10 cm Vigreux column) to afford 3.46 g (77%) of ester **3** as a colourless oil; bp: 35–36°C (0.2 mm). Spectral data for **3**: ^{1}H NMR (400 MHz, $CDCl_3$), δ: 8.38 (dd, $^{2}J_{^{13}C,H}$ & $^{3}J_{^{13}C,H} = 7.7$ & 3.7 Hz, ring H), 4.24 (dq, $^{3}J_{^{13}C,H} = 3.2$ Hz; $^{3}J_{H,H} = 7.1$ Hz, $\underline{C}H_2CH_3$), 2.61 (ddd, $^{1}J_{^{13}C,H} = 131.3$ Hz; $^{2}J_{^{13}C,H}$ & $^{3}J_{^{13}C,H} = 7.1$ & 2.3 Hz, ring CH_3), 1.28 (t, $^{3}J_{H,H} = 7.1$ Hz, CH_2CH_3); ^{13}C NMR (100.6 MHz), δ:172.6 (ddd, $^{1}J_{^{13}C,^{13}C} = 71.4$ Hz; $^{1}J_{^{13}C,^{13}C} = 49.9$ Hz; $^{2}J_{^{13}C,^{13}C} = 7.4$ Hz, ring C α to O), 160.9 (dd, $^{1}J_{^{13}C,^{13}C} = 89.3$ Hz; $^{2}J_{^{13}C,^{13}C} = 7.4$ Hz, ester C), 149.4 (d, $^{1}J_{^{13}C,^{13}C} = 52.4$ Hz), 109.0 (ddd, $^{1}J_{^{13}C,^{13}C} = 89.3$ Hz; $^{1}J_{^{13}C,^{13}C} = 71.4$ Hz; $^{2}J_{^{13}C,^{13}C} = 4.8$ Hz, ring C bearing carbonyl), 60.1 (s, $\underline{C}H_2CH_3$), 14.0 (s,

$CH_2\underline{C}H_3$), 11.7 (dd, $^1J_{^{13}C,^{13}C} = 49.9$ Hz; $^2J_{^{13}C,^{13}C} = 4.8$ Hz, CH_3 on ring).

$^{13}C_4$-5-Methylisoxazole-4-carboxylic acid (**4**)

To a 25 ml round-bottomed flask equipped with a reflux condenser, were added ester **3** (3.02 g, 19.0 mmol) and 1:1:1 mixture of concentrated HCl, water and acetic acid (10 ml) and the solution was refluxed for 9.5 h. After cooling, acetone (10 ml) was added and the mixture was concentrated under vacuum to give a yellow solid. Recrystallization from toluene yielded 1.49 g (60%) of pure acid **4**, mp: 143.5–145°C; lit.[23] mp of unlabeled analog, 147–149°C. Spectral Data for **4**: ^{1}H NMR (400 MHz, DMSO-d_6), δ: 9.41 (br s, acid H), 8.76 (dd, $^2J_{^{13}C,H}$ & $^3J_{^{13}C,H} = 7.8$ & 3.7 Hz, ring H), 2.62 (ddd, $^1J_{^{13}C,H} = 131.2$ Hz; $^2J_{^{13}C,H}$ & $^3J_{^{13}C,H} = 7.1$ & 2.4 Hz, CH_3); ^{13}C NMR (100.6 MHz, DMSO-d_6), δ: 174.0 (ddd, $^1J_{^{13}C,^{13}C} = 70.3$ Hz; $^1J_{^{13}C,^{13}C} = 49.4$ Hz; $^2J_{^{13}C,^{13}C} = 7.3$ Hz, ring C α to O), 162.9 (dd, $^1J_{^{13}C,^{13}C} = 85.2$ Hz; $^2J_{^{13}C,^{13}C} = 7.3$ Hz, acid C), 151.0 (d, $^1J_{^{13}C,^{13}C} = 50.3$ Hz), 110.1 (ddd, $^1J_{^{13}C,^{13}C} = 85.3$ Hz; $^1J_{^{13}C,^{13}C} = 70.3$ Hz; $^2J_{^{13}C,^{13}C} = 4.7$ Hz, ring C bearing carbonyl), 12.3 (dd, $^1J_{^{13}C,^{13}C} = 49.4$ Hz; $^1J_{^{13}C,^{13}C} = 4.7$ Hz, CH_3).

Preparation of $^{13}C_4$-5-methyl-N-[4-(trifluoromethyl)phenyl]-4-Isoxazolecarboxamide ($^{13}C_4$-leflunomide, **7**)

Following a method previously published,[21] to a flame dried 10 ml round-bottomed flask was added acid **4** (1.46 g, 11.2 mmol) and an excess of freshly distilled thionyl chloride (4.23 ml) and the mixture was stirred at 50°C for 4 h. After cooling the mixture was concentrated *in vacuo* (50 mm) until constant mass was achieved, to afford crude acid chloride **5**. To a vigorously stirred mixture of $NaHCO_3$ (0.984 g, 11.7 mmol) and *p*-trifluoromethylaniline (**6**) (1.89 g, 11.7 mmol), toluene (20 ml) and water (4.1 ml) stirring vigorously at 60°C was added over a period of 20 min. neat acid chloride **5**. After stirring for 2 h at 60°C the mixture was allowed to cool overnight. The white solid which precipitated was filtered and was washed with water. The material was dissolved in EtOAc (50 ml) to make a solution which was washed with water (15 ml) and dried over $MgSO_4$. Filtration and concentration under vacuum afforded a crude solid which was recrystallized from toluene to afford 2.07 g (60%) of **7** as a white solid; mp. 165–166°C; lit.[24]mp of unlabeled analog, 166–167°C. Spectral data for **7**: ^{1}H NMR

(400 MHz, $CDCl_3$), δ: 10.32 (s, NH), 9.07 (dd, $^2J_{^{13}C,H}$ & $^3J_{^{13}C,H}$ = 8.5 & 3.8 Hz, ring H), 7.90 (d, $^3J_{H,H}$ = 8.6 Hz, Ar H), 7.68 (d, $^3J_{H,H}$ = 8.6 Hz, Ar H), 2.62 (ddd, $^1J_{^{13}C,H}$ = 131.2 Hz; $^2J_{^{13}C,H}$ & $^3J_{^{13}C,H}$ = 7.1 & 2.1 Hz, CH_3); ^{13}C NMR (100.6 MHz, DMSO-d_6), δ: 173.6 (ddd, $^1J_{^{13}C,^{13}C}$ = 71.4 Hz; $^1J_{^{13}C,^{13}C}$ = 49.5 Hz; $^2J_{^{13}C,^{13}C}$ = 6.6 Hz, C-5), 159.8 (dd, $^1J_{^{13}C,^{13}C}$ = 77.5 Hz; $^2J_{^{13}C,^{13}C}$ = 6.6 Hz, C-7), 149.2 (d, $^1J_{^{13}C,^{13}C}$ = 49.5 Hz, C-3), 142.3 (s, C-8)), 126.2 (q, $^3J_{^{13}C,F}$ = 3.7 Hz, C-10)), 124.6 (q, $^1J_{^{13}C,F}$ = 271.3 Hz, C-12)), 124.1 (q, $^2J_{^{13}C,F}$ = 22.0 Hz, C-11), 120.2 (q, $^2J_{^{13}C,F}$ = 1.8 Hz, C-9)), 112.0 (ddd, $^1J_{^{13}C,^{13}C}$ = 77.5 Hz; $^1J_{^{13}C,^{13}C}$ = 71.4 Hz; $^1J_{^{13}C,^{13}C}$ = 4.2 Hz, C-4), 12.4 (dd, $^1J_{^{13}C,^{13}C}$ = 49.5 Hz; $^2J_{^{13}C,^{13}C}$ = 4.2 Hz, C-6); IR (nujol), cm^{-1}: 3329, 3290, 3069, 1652, 1604, 1538, 1532, 1456, 1329, 1318, 1257, 1163, 1123, 1111, 1063, 854; MS (EI), *m/z*: 274 (M^+, 22), 273 ((M-1)$^+$, 8), 161 (5), 114 (1 0 0), 113 (28), 70 (7). HRMS: Calculated for $C_8^{13}C_4H_9N_2O_2F_3$, 274.0750; Found, 274.0730. HPLC retention time: 6.85 min.

Mass Spectrum of unlabelled leflunomide. MS (EI), *m/z*: 271 ((M + 1)$^+$, 14), 270 (M^+, 26), 161 (5), 110 (1 0 0), 109 (19), 85 (14).

Conversion of **7** *to* $^{13}C_4$*-2-cyano-3-hydroxy-N-[4-(trifluoromethyl)phenyl]-2-butenamide (*$^{13}C_4$*-A77 1726,* **8***)*

To a 25 ml round bottomed flask were added labeled leflunomide **7** (1.30 g, 4.81 mmol) and MeOH (50 ml). The stirred solution was cooled to 10°C and an aqueous 1.1 M NaOH solution (5 ml) was added. Stirring continued for 2 h at which time water (5 ml) was added. The solution was slowly brought to acidic pH through the slow addition of concentrated HCl resulting in a formation of a white precipitate. The precipitate was collected via suction filtration and was recrystallized from toluene to afford 1.07 g (82%) as a 15:1 (approximately) mixture of tautomers, mp. 216.5–218°C; lit.[25]mp of unlabeled analog, 230–233°C. Spectral data for **8**: ^{1}H NMR (400 MHz, DMSO-d_6, major (enol) tautomer), δ: 11.00 (s, v br, 1 H), 10.69 (s, 1 H), 7.76 (d, $^3J_{H,H}$ = 8.6 Hz, 1 H), 7.65 (d, $^3J_{H,H}$ = 8.6 Hz, 1 H), 2.25 (ddd, $^1J_{^{13}C,H}$ = 129.0 Hz; $^2J_{^{13}C,H}$ & $^3J_{^{13}C,H}$ = 5.9 Hz & 3.3 Hz, CH_3); ^{13}C NMR (100.6 MHz, DMSO-d_6), δ: 187.1 (dd, $^1J_{^{13}C,^{13}C}$ = 73.9 Hz; $^1J_{^{13}C,^{13}C}$ = 46.2 Hz, C-5), 166.6 (dd, $^1J_{^{13}C,^{13}C}$ = 72.1 Hz; $^3J_{^{13}C,^{13}C}$ = 2.8 Hz, C-7), 141.7 (s, C-8), 126.0 (q, $^3J_{^{13}C,F}$ = 3.6 Hz, C-10), 124.5 (q, $^1J_{^{13}C,F}$ = 271.3 Hz, C-12), (q, $^2J_{^{13}C,F}$ = 32.0 Hz, C11), 121.3 (q, $^2J_{^{13}C,F}$ = 1.7 Hz, C-9), 118.3 (d , $^1J_{^{13}C,^{13}C}$ = 87.7 Hz, C-3), 81.1 (ddd (apparent dt), $^1J_{^{13}C,^{13}C}$ = 73.9 Hz; $^1J_{^{13}C,^{13}C}$ = 72.1 Hz; $^2J_{^{13}C,^{13}C}$ = 6.9 Hz, C-4), 23.1 (ddd, $^1J_{^{13}C,^{13}C}$ =

46.2 Hz; $^{2}J_{^{13}C,^{13}C} = 6.9$ Hz; $^{3}J_{^{13}C,^{13}C} = 2.8$ Hz, C-6); IR (DMSO-d_6, mixture of tautomers), cm^{-1}: 3459, 3334, 3193, 3041, 2979, 2179, 1596, 1523, 1496, 1411, 1329, 1310, 1258, 1165; MS (EI), *m/z*: 274 (M^+, 21), 273 ((M-1)$^+$, 5), 161 (100), 160 (26), 114 (20), 111 (12), 70 (27). HRMS: Calculated for $C_8^{13}C_4H_9N_2O_2F_3$, 274.0750; Found, 274.0717. HPLC retention time: 3.18 min.

Mass Spectrum of unlabelled A77 1726. MS (EI), *m/z*: 272 ((M + 2)$^+$, 4) 271 ((M + 1)$^+$, 21), 270 (M^+, 33), 161 (100), 160 (24), 142 (10), 110 (7), 68(8).

References

1. Schattenkirchner M. *Immunopharmacology* 2000; **47**: 291–298.
2. Schuna AA, Megeff C. *Am J Health Syst Pharm* 2000; **57**: 225–234.
3. Alldred A, Emery P. *Exp Opin Pharmacother* 2001; **2**: 125–137.
4. El Desoky ES. *Curr Ther Res* 2001; **62**: 92–112.
5. Sanders S, Harisdangkul V. *Am J Med Sci* 2002; **323**: 190–193.
6. McCarey David W, Capell Hilary A, Madhok R. *Lancet* 2002; **359**: 1158.
7. Rozman B. *Clin Pharmacokinet* 2002; **41**: 421–430.
8. Herrmann ML, Schleyerbach R, Kirschbaum BJ. *Immunopharmacology* 2000; **47**: 273–289.
9. Prakash A, Jarvis B. *Drugs* 1999; **58**: 1137–1164.
10. Wendling D. *Ann Med Interne* 2002; **153**: 21–24.
11. Douglas IDC, Price LA. *Brit J Haematol* 1973; **24**: 625–631.
12. Bertino JR, Banerjee D, Zhao SC, Mineishi S, Ercikan-Abali E, Takebe N, Sadelain M, Moore MAS. *Haematol Blood Transfus* 1998; **39**: 483–490.
13. Schiff MH, Strand V, Oed C, Loew-Friedrich I. *Drugs Today* 2000; **36**: 383–394.
14. Baillie TA, Rettenmeier AW. *J Clin Pharmacol* 1986; **26**: 481–484.
15. Haskins NJ. *Biomed Mass Spectrom* 1982; **9**: 269–277.
16. Browne TR, Van Langenhove A, Costello CE, Biemann K, Greenblatt DJ. *J Clin Pharmacol* 1982; **22**: 309–315.
17. Zhou J-P, Zhang H-B, Huang W-L. *Zhongguo Yaoke Daxue Xuebao* 2000; **31**: 330-331. CA **134**: 252284.
18. Huang H, Wu S. *Zhongguo Yaowu Huaxue Zazhi* 2000; **10**: 132-133. CA **134**: 100792.
19. Fossa P, Menozzi G, Schenone P, Filippelli W, Russo S, Lucarelli C, Marmo E. *Farmaco* 1991; **46**: 789–802.
20. Doleschall G, Seres P. *J Chem Soc Perkin I* 1988; 1875–1879.
21. Avrutov I, Gershon N, Liberman A. WO *Patent* 0160363, 2001. CA **135**: 195553.

22. Baba S, Akira K, Suzuki H, Imachi M. *Biol Pharm Bull* 1995; **18**: 643–647.
23. Schenone P, Fossa P, Menozzi G. *J Heterocycl Chem* 1991; **28**: 453–457.
24. Hirth KP, Mann E, Shawyer LK, Ullrich A, Szekely I, Bajor T, Haimichael J, Orfi L, Levitzki A, Gazit A, Tang PC, Lammers R. *US Patent* 6331555, 2001. CA **136**: 53764.
25. Uckun FM, Zheng Y, Ghosh S. *WO Patent* 0056703, 2000. CA **133**: 252171.

