# Synthesis of a tetrafluorinated analogue of the quinazoline antifolate 2-desamino-2-methyl-$N^{10}$-propargyl-5,8-dideazafolic acid

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## Abstract

A synthesis of a tetrafluoro derivative of the quinazoline antifolate 2-desamino-2-methyl-$N^{10}$-propargyl-5,8-dideazafolic acid is described. 2-Desamino-2-methyl-$N^{10}$-propargyl-2′,3′,5′,6′-tetrafluoro-5,8-dideazafolic acid (**1**) has been made in four stages from 4-amino-2,3,5,6-tetrafluorobenzoic acid (**2**). The reaction of **2** with thionyl chloride followed by diethyl L-glutamate gave *N*-(4-amino-2,3,5,6-tetrafluorobenzoyl)-L-glutamate (**3**). Condensation of **3** with propargyl bromide using caesium carbonate as base gave diethyl *N*-[2,3,5,6-tetrafluoro-4-(prop-2-ynylamino)benzoyl]-L-glutamate (**4**) which in turn reacted with 6-bromomethyl-3,4-dihydro-2-methyl-4-oxoquinazoline using the same base to give the diethyl ester **5** of 1. Finally, base-catalysed hydrolysis of **5** gave the target product. The sequence differs from other reported syntheses of quinazoline antifolates most notably in the use of an aminobenzoic acid derivative rather than a nitrobenzoic acid derivative as starting material but also in the need for the relatively strongly basic caesium carbonate to effect the two subsequent steps. Evidence for restricted rotation in the intermediate **4** is also presented.

## Introduction

The discovery that the quinazoline-based analogue of folic acid $N^{10}$-propargyl-5,8-dideazafolic acid has experimental [1] and clinical antitumour activity [2] and the later finding that replacement of the 2-amino substituent in the quinazoline residue by 2-methyl conferred further advantageous properties [3] has prompted an extensive programme of analogue synthesis. Among numerous analogues of 2-desamino-2-methyl-$N^{10}$-propargyl-5,8-dideazafolic acid reported have been the 2′-fluoro derivative [4] and its 3′-fluorinated counterpart [5]. The synthesis of these derivatives followed generally the route used in the synthesis of non-fluorinated counterparts, namely the condensation of the appropriate 4-nitrobenzoic acid with diethyl L-glutamate, reduction of the nitro function, alkylation of the resulting amino function using propargyl bromide and, finally, condensation with the appropriate 6-bromomethyl quinazoline followed by deprotection.

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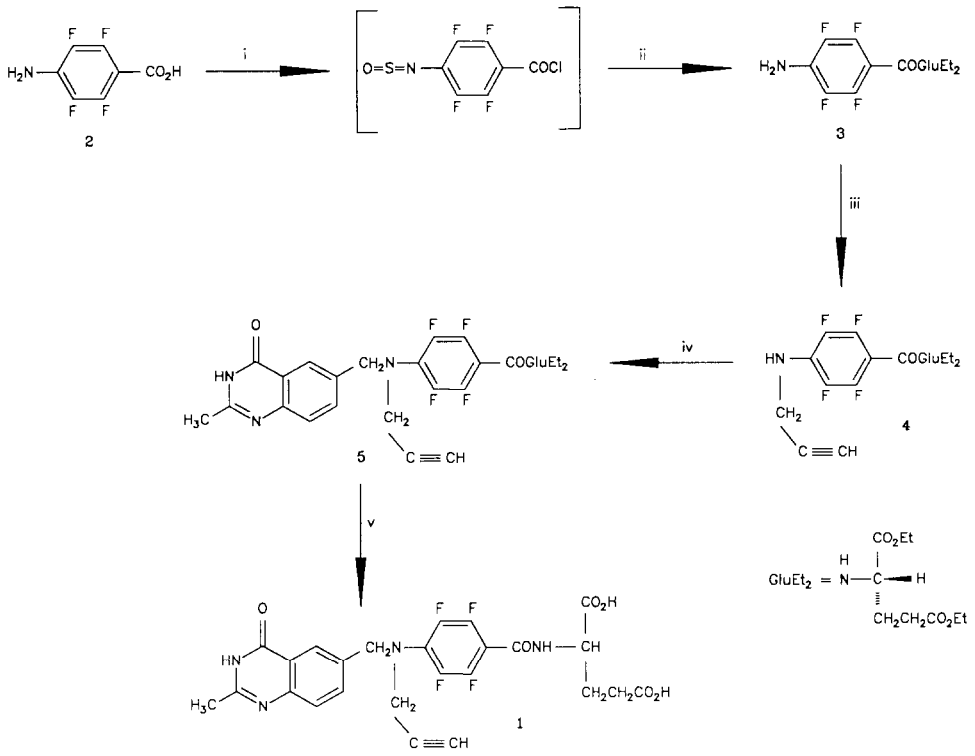

Scheme 1. Reagents: i, $SOCl_2$; ii, diethyl L-glutamate, $Et_3N$; iii, $HC{\equiv}CCH_2Br$, $Cs_2CO_3$; iv, 6-bromomethyl-3,4-dihydro-2-methyl-4-oxoquinazoline, $Cs_2CO_3$; v, NaOH and then HCl.

2,3,5,6-Tetrafluoro-4-nitrobenzoic acid, the precursor for an analogous approach to the corresponding tetrafluorinated analogue **1** was not commercially available, whereas 4-amino-2,3,5,6-tetrafluorobenzoic acid (**2**) was, as it is readily prepared [6] by lithiation of 2,3,5,6-tetrafluoroaniline followed by reaction with carbon dioxide. Further, the use of a nitro derivative as starting material can give rise to partial reduction of C–F bonds during the subsequent hydrogenation step, which had been a problem [4] in the reduction of diethyl *N*-(2-fluoro-4-nitrobenzoyl)-L-glutamate. For these reasons, we used **2** in a synthesis of **1** (Scheme 1), which we here describe.

## Results and discussion

Treatment of **2** with thionyl chloride (*cf.* refs. 4 and 5) followed by reaction of the product with diethyl L-glutamate gave a 77% yield of *N*-(4-amino-2,3,5,6-tetrafluorobenzoyl)-L-glutamate (**3**). There was evidence that thionyl chloride reacted with both the amino and carboxyl functions in **2** to form 2,3,5,6-tetrafluoro-4-sulphinylaminobenzoyl chloride (Scheme 1), and that it is this intermediate which reacts with diethyl glutamate. The unstable

intermediate resisted attempts at purification but evidence for its identity was provided by mass spectrometry and IR spectroscopy. Thus the IR spectrum of the yellow intermediate contained bands ascribable to the 4-sulphinylamino substituent at $\nu = 1310$ and 1171 $cm^{-1}$. The mass spectrum contained signals consistent with a mixture of this compound ($M^+$ at $m/z = 273$) and 4-amino-2,3,5,6-tetrafluorobenzoyl chloride ($M^+$ at $m/z = 227$) (for details see Experimental details). There is a precedent in the formation of 4-sulphinylaminobenzoyl chloride by reaction of 4-aminobenzoic acid with thionyl chloride [7]; it reacts readily with hydrogen chloride to form 4-aminobenzoyl chloride, and acylates alcohols and amines in high yield with concomitant loss of the sulphinyl group to generate aminobenzoyl derivatives [8]. Indeed the mass spectrum as well as the IR spectrum of 4-sulphinylaminobenzoyl chloride (synthesized by the procedure in the literature) contained features corresponding to those recorded for the present intermediate (see Experimental details). This explanation also accounts for the failure of attempts to use an alternative condensing agent, oxalyl chloride, to convert **2** into **3** [9].

Potassium carbonate had been used as base in the condensation of diethyl *N*-(4-aminobenzoyl)-L-glutamate [1] as well as the 2-fluorobenzoyl [4] and 3-fluorinated [5] analogues with propargyl bromide. However, the corresponding reaction with **3** was not successful. Caesium carbonate has previously shown advantages over potassium carbonate, and other alkali metal carbonates, as a reagent [10]. In the present reaction it afforded a modest (31%) yield of diethyl *N*-[2,3,5,6-tetrafluoro-4-(prop-2-ynylamino)benzoyl]-L-glutamate (**4**). In view of the powerful effect of multiple fluorine substitution in weakening the basic strength of the amino function, reflected for example in the reduction of $pK_a$ from 4.58 for aniline to $-2.2$ in pentafluoroaniline [11], the amino group in **3** could be functioning as a weak acid rather than as a base in this condensation reaction. Hence, a stronger base may be needed to effect its condensation with propargyl bromide than was the case for unsubstituted or monofluorinated counterparts, where the base served mainly to scavenge liberated HBr. The same argument applies to the subsequent reaction of **4** with 6-bromomethyl-3,4-dihydro-2-methyl-4-oxoquinazoline. Again, caesium carbonate was needed (cf. refs. 1, 3 and 5) to effect the condensation to give the protected glutamate **5** (yield, 20%). The final step, basic hydrolysis of the ester function, was conventionally accomplished [5] to give the title compound **1** in 59% yield.

The nuclear magnetic resonance (NMR) spectra of the new compounds described here contain features of interest. In the $^1H$ NMR spectra of diethyl *N*-(4-amino-2-fluorobenzoyl)-L-glutamate, the 2′-monofluorinated counterpart of **3**, and of 2′-fluorobenzoyl derivatives corresponding to **4**, **5** and **1**, the amide proton signals were double doublets [4]. This was ascribed to an interaction between the 2′-fluorine substituent and the amide hydrogen atom, in addition to the expected coupling between the latter and the adjacent CH proton. However, this additional H–F coupling was not seen in **1** or **5**, the amide NH signals appearing as doublets. Presumably the presence of two C–F dipoles *ortho* to carbonyl sets up a mutual repulsion, thereby preventing

the near coplanarity between the amide N–H and a C–F residue which is presumed necessary for the additional coupling to occur.

In contrast, there was evidence for an attractive interaction between the 3(5)-fluorine substituent and the adjacent amine N–H function in the intermediate **4**. Thus, whereas the $^{19}F$ NMR spectra for **1**, **3** and **5** comprised the expected two $A_2X_2$ signals characteristic of 1,4-disubstituted tetrafluorobenzenes, that of **4**, at 305 K, was complex (Fig. 1(a)). It was consistent with the presence of four non-equivalent, mutually coupled fluorine substituents giving four multiplets, the upfield pair of which overlapped. On heating to 323 K the signals remained distinct but less well resolved. At 353 K, the upfield and the downfield signals had each coalesced, the spectrum now simplifying to two broad signals. At 383 K (Fig. 1(b)) the upfield signal had resolved to a doublet, the downfield signal, which had sharpened considerably at that temperature, itself also resolving to a doublet at 408 K.

The hindered rotation in **4** at the lower temperatures was also reflected in certain features of the $^1H$ NMR spectra (see Experimental details). Thus the glutamate CH signal in the spectrum taken at 305 K appeared as two distinct multiplets (double doublets) which remained distinct at 323 K but which coalesced to a single broad signal at 353 K which sharpened at 383

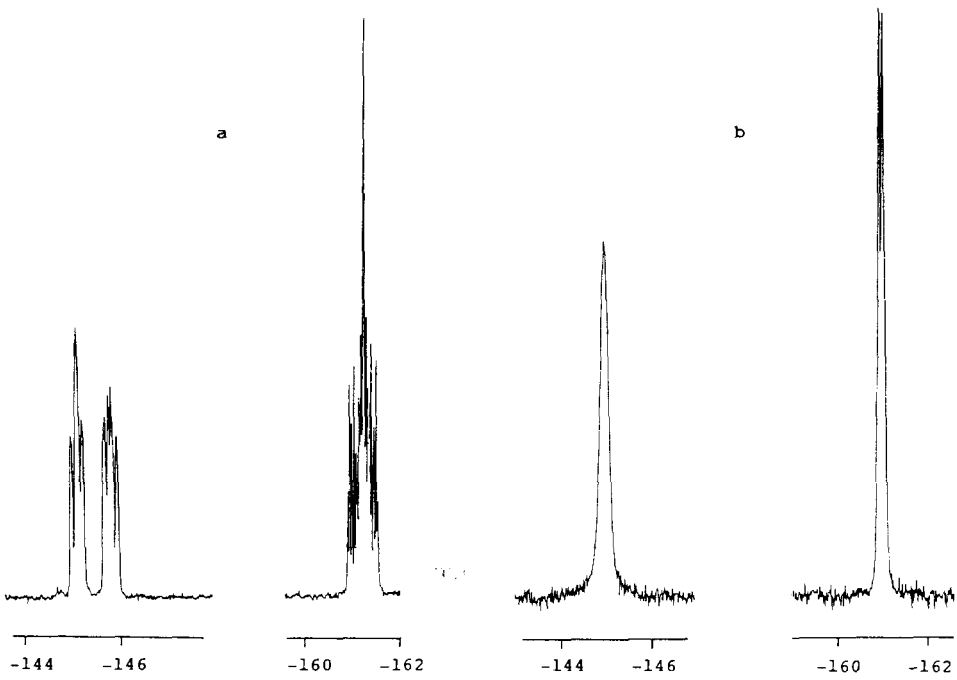


Fig. 1. Appearance of signals in the $^{19}F$ NMR spectra of diethyl *N*-[4-(prop-2-ynylamino)-2,3,5,6-tetrafluorobenzoyl]-L-glutamate (**4**) taken in dimethyl sulphoxide-$d_6$ at (a) 305 K and (b) 383 K.

K. Two distinct doublets ascribable to amide NH in the spectrum at 305 K behaved likewise, coalescing to a broad singlet at these higher temperatures.

These observations have a precedent in a study by $^{19}F$ NMR spectroscopy of restricted rotation in 2,6-difluoro-*N*-methylaniline [12]. However, the energy barrier to free rotation was much smaller in that case, the coalescence temperature being near 120 K as opposed to the value of near 353 K estimated in the present case.

## Experimental details

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra (samples as Nujol mulls unless otherwise indicated) were recorded on a Perkin–Elmer 1310 spectrometer. NMR spectra were recorded on a Bruker AC250 spectrometer ($^1H$ at 250 MHz, and $^{19}F$ at 230 MHz) using $SiMe_4$ and $C_6F_6$ ($\delta = -163.0$ ppm) respectively as internal standards. Mass spectra were obtained on a VG 7070H spectrometer with VG 2235 data system using the direct insertion method by electron impact with an ionizing voltage of 70 eV unless otherwise stated. The fast atom bombardment (FAB) spectrum was carried out on the same instrument using an Iontech saddle field gun which directed fast atoms of xenon (6 kV) at the matrix (glycerol) on a stainless steel probe tip. Only selected ions in the mass spectra are recorded. Column chromatography was carried out on silica gel Kieselgel 60, Art. No. 9385. 4-Amino-2,3,5,6-tetrafluorobenzoic acid was purchased from Fluorochem Ltd., Old Glossop, Derbyshire, UK, and *N*-sulphinylaniline from Aldrich Chemical Company Ltd., The Old Brickyard, New Road, Gillingham, Dorset SP8 4JL, UK. Elemental analyses were determined by C.H.N. Analysis, South Wigston, Leicester, UK.

*Diethyl* N-*(4-amino-2,3,5,6-tetrafluorobenzoyl)-*L*-glutamate (**3**)*

A stirred solution of 4-amino-2,3,5,6-tetrafluorobenzoic acid (**2**) (10.46 g, 0.05 mol) and thionyl chloride (4.6 ml) in dry toluene (400 ml) was heated under reflux for 3 h and then concentrated under vacuum to give crude 2,3,5,6-tetrafluoro-4-sulphinylaminobenzoyl chloride as a yellow oil which solidified when cool: $\nu_{max}$ ($CH_2Cl_2$): 3417 (NH str), 1763 (CO), 1664 (NH bend), 1505 (CF), 1310, 1171 (N=S=O) $cm^{-1}$; 4-sulphinylaminobenzoyl chloride [7] gave *inter alia* 1289, 1183 $cm^{-1}$ and *N*-sulphinylaniline 1284, 1164 $cm^{-1}$ (cf. ref. 13). *m/z*: 273 ($M^+$, 7), 238 ($M^+$–Cl, 96), 227 ($M^+$ of $H_2NC_6F_4COCl$, 46), 192 (227 – Cl, 100), 164 (192 – CO, 33); 4-sulphinylaminobenzoyl chloride gave 201 ($M^+$, 21), 166 ($M^+$–Cl, 100), 138 (166 – CO, 14). The crushed solid was added to a stirred solution of diethyl L-glutamate hydrochloride (12.0 g, 0.05 mmol) and triethylamine (20.8 ml) in dichloromethane (200 ml) at such a rate that the temperature was kept below 25 °C and the mixture was then stirred for a further 1 h at room temperature. After washing twice with water (250 ml), the organic phase was dried ($MgSO_4$) and concentrated to give a solid residue which after recrystallization produced

white crystals of the title compound (15.11 g, 77%) from toluene (m.p., 123–125 °C). Found: C, 48.32; F, 19.34; H, 4.65; N, 7.12%. $C_{16}H_{18}F_4N_2O_5$ requires: C, 48.73; F, 19.27; H, 4.60; N, 7.11%. $\nu_{max}$:1725 (ester CO), 1650 $cm^{-1}$ (amide CO). $^1H$ NMR ($CDCl_3$) $\delta$: 1.26, 1.29 (2t, each 3H, $J=7.1$ Hz, $CH_2CH_3$); 2.12, 2.31 (2m, each 1H, $CHCH_2CH_2CO_2H$), 2.44 (m, 2H, $CHCH_2CH_2CO_2H$), 4.13, 4.24 (2q, each 2H, $CH_2CH_3$), 4.27 (br s, 2H, $NH_2$), 4.80 (m, 1H, $CHCH_2CH_2CO_2H$), 6.75 (br d, 1H, $J=6.8$ Hz, CONH). $^{19}F$ NMR $\delta$: −145.6 ($A_2X_2$, 2F, 2-F, 6-F), −162.2 ($A_2X_2$, 2F, 3-F, 5-F). *m/z*: 394 ($M^+$, 9), 321 ($M^+-CO_2Et$, 59), 192 ($H_2NC_6F_4CO^+$, 100), 164 ($H_2NC_6F_4^+$, 72).

*Diethyl* N-*[4-(prop-2-ynylamino)-2,3,5,6-tetrafluorobenzoyl]-*L*-glutamate (4)*

A mixture of diethyl *N*-(4-amino-2,3,5,6-tetrafluorobenzoyl)-L-glutamate (**3**) (0.985 g, 2.5 mmol), caesium carbonate (0.815 g, 2.5 mmol), propargyl bromide (80% w/w in toluene, 1.12 ml, 10 mmol) and acetonitrile (20 ml) was stirred at 80 °C for 3 h. After cooling, the pale-brown slurry was filtered through Celite and the filtrate concentrated. The residual gum was dissolved in ethyl acetate (100 ml) and the solution washed with water (200 ml), then dried ($MgSO_4$) and finally concentrated to give an orange oil. Column chromatography with ethyl acetate–light petroleum (b.p., 60–80 °C) (2:3) as eluent gave the title compound as a pale-yellow oil (0.34 g, 31.5%) which later solidified (m.p., 106.5–107.5 °C). Found: C, 53.01; F, 17.57; H, 4.69; N, 6.38%. $C_{19}H_{20}F_4N_2O_5$ requires: C, 52.78; F, 17.58; H, 4.66; N, 6.48%. $\nu_{max}$: 2120 (monosubstituted C≡C), 1745, 1725 (ester CO), 1640 $cm^{-1}$ (amide CO). $^1H$ NMR ($Me_2SO$-$d_6$) $\delta$ (305 K): 1.67, 1.94 (2t, each 3H, $J=6.9$ Hz, $CH_2CH_3$), 2.0–2.15 (m, 1H, one of $CHCH_2CH_2CO_2Et$), 2.15–2.55 (2m, 3H, other of $CHCH_2CH_2CO_2Et$ + $CHCH_2CH_2CO_2Et$), 3.17 (br t, 1H, C≡CH), 3.95–4.2 (2×q + 2×d, 4H, $CH_2CH_3$ + $HC{\equiv}CCH_2$), 4.32, 4.39 (2d, $J_{H,F}=2.3$ Hz, amide NH) becomes br s, 4.25 at 383 K, 4.47, 4.74 (2dd, 1H, $J=5.5$, 9.6 Hz, $CHCH_2CH_2CO_2Et$) becomes br s, 4.65 at 383 K, 6.29 (s, 1H, amine NH). $^{19}F$ NMR $\delta$ (305 K): −145.3, −146.0 (2m, 2F, 2-F, 6-F), −161.3 (2m, 2F, 3-F, 5-F): $^{19}F$ NMR (383 K): −145.2 (br s, 2F, 2-F, 6-F), −161.0 (br d, 2F, 3-F, 5-F). *m/z*: 432 ($M^+$, 16), 387 ($M^+$–OEt, 13), 359 ($M^+-CO_2Et$, 49), 285 (44), 230 ($M^+$–glu(Et)$_2$, 50), 192 (100).

*Diethyl* N-*[4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2,3,5,6-tetrafluorobenzoyl]-*L*-glutamate (5)*

A mixture of diethyl *N*-[4-(prop-2-ynylamino)-2,3,5,6-tetrafluorobenzoyl]-L-glutamate (**4**) (1.73 g, 4 mmol), 6-bromomethyl-3,4-dihydro-2-methyl-4-oxoquinazoline [3] (1.012 g, 4 mmol) and caesium carbonate (1.273 g, 8 mmol) in acetonitrile (33 ml) was stirred at 80 °C for 16 h. The cooled mixture was filtered through Celite and the filtrate concentrated under vacuum. A solution of the residual oil in ethyl acetate (200 ml) was washed twice with water (250 ml) and then concentrated to a gum. Column chromatography with dichloromethane–ethanol (19:1) as eluent afforded the title compound **5** (0.476 g, 20%) (m.p., 155–158 °C). Found: C, 57.85; F, 12.16; H, 4.78;

N, 9.20%. $C_{29}H_{28}F_4N_4O_6$ requires: C, 57.61; F, 12.57; H, 4.68; N, 9.27%. $^1$H NMR ($Me_2SO$-$d_6$) $\delta$: 1.17, 1.20 (2t, each 3H, $J$=7.1 Hz, $CH_2CH_3$), 1.98, 2.06 (2m, each 1H, $CHCH_2CH_2CO_2H$), 2.33 (s, 3H, quinazoline $CH_3$), 2.42 (m, 2H, $CHCH_2CH_2CO_2H$), 3.94 (s, 2H, $HC{\equiv}CCH_2$), 4.04, 4.09 2q, each 2H, $CH_2CH_3$), 4.44 (m, 1H, $CHCH_2CH_2CO_2H$), 4.58 (s, 2H, $ArCH_2N<$), 7.56 (d, 1H, $J$=8.3 Hz, quinazoline 8-H), 7.71 (dd, 1H, quinazoline 7-H), 8.03 (d, 1H, $J$=1.8 Hz, quinazoline 5-H), 9.31 (d, 1H, $J$=7.6 Hz, CONH), 12.24 (s, 1H, quinazoline NH). $^{19}$F NMR $\delta$: −144.0 ($A_2X_2$, 2F, $J$=8.7, 23.9 Hz, 2-F, 6-F), −147.8 ($A_2X_2$, 2F, 3-F, 5-F). *m/z* 604 ($M^+$, 2), 432 ($M^+$–quinazolinyl $CH_2$, 16), 359 (55), 285 (49), 192 (100).

N-*[4-[N-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]*-N-*prop-2-ynylamino]-2,3,5,6-tetrafluorobenzoyl]*-L-*glutamic acid* (**1**)

The diethyl glutamate **5** (0.23 g, 0.38 mmol) was stirred for 4 h at room temperature in a mixture of 1 N aqueous NaOH (1.52 ml), ethanol (1 ml) and water (6 ml). The resulting solution was filtered through Celite and brought to pH 3.0 with 1 N aqueous HCl. The precipitate was recovered by filtration, washed with water and vacuum dried first at room temperature for 18 h and then at 55 °C for 3 h to yield **1** as a white powder (0.126 g, 59%) (m.p., 156–160 °C). Found: C, 53.20; F, 13.10; H, 3.91; N, 9.95%. $C_{25}H_{20}F_4N_4O_6 \cdot H_2O$ requires: C, 53.00; F, 13.42; H, 3.92; N, 9.89%. $^1$H NMR ($Me_2SO$-$d_6$) 1.87, 2.01 (2m, each 1H, $CHCH_2CH_2CO_2H$), 2.33 (m+s, 2H+3H, $CHCH_2CH_2CO_2H$ + quinazoline $CH_3$), 3.35 (s, 1H, C≡CH), 3.95 (s, 2H, $HC{\equiv}CCH_2$), 4.40 (m, 1H, $CHCH_2CH_2CO_2H$), 4.57 (s, 2H, $ArCH_2N<$), 7.55 (d, 1H, $J$=8.3 Hz, quinazoline 8-H), 7.71 (dd, 1H, quinazoline 7-H), 8.04 (d, 1H, $J$=1.8 Hz, quinazoline 5-H), 9.07 (d, 1H, $J$=7.9 Hz, CONH), 12.17 (s, 1H, quinazoline NH), 12.65 (br s, 2H, $2CO_2H$). $^{19}$F NMR $\delta$: −143.9 ($A_2X_2$, 2F, 2-F, 6-F), −147.9 ($A_2X_2$, 2F, 3-F, 5-F). *m/z* (FAB): 549 ($[M+H]^+$).

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