Supporting Information

Bacterial Patterning Controlled by Light Exposure

Authors: Willem A. Velema,[a] Jan Pieter van der Berg,[b] Wiktor Szymanski,[a,c] Arnold J. M. Driessen[b] and Ben L. Feringa[a,c*]

Affiliations:
[a]Centre for Systems Chemistry, Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 4, 9747 AG, Groningen, The Netherlands.
[b]Molecular Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands.
[c]Department of Radiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands.

Corresponding author:
Ben L. Feringa, Phone: +31 50 363 4235. Fax: +31 50 363 4296. E-mail: b.l.feringa@rug.nl. Nijenbrogh 4, 9747 AG, Groningen, The Netherlands.

Table of Contents

Materials and Methods

Uncaging Experiments Irradiation experiments were performed with a Spectroline ENB-280C/FE UV lamp (312 nm) and Thor Labs OSL1-EC Fiber Illuminator (white light) (see Fig S1 for electromagnetic spectrum).

Quantum Yield Determination Quantum yields were determined with a JASCO FP-6200 spectrofluorometer by irradiating 2.5 μM solutions of **1** with 323 nm light. After each irradiation period the UV-Vis absorbance was measured. The percentage of photolysis was estimated by the decrease in absorbance at λ_{max}. Quantum yields (Q) of photolysis were calculated using the following equation: $Q = -\log(C_t/C_0)/(I\sigma t)$. C_t and C_0 are the concentrations of **1** at time t and time 0, respectively. σ is the decadic extinction coefficient in $cm^2 \cdot mol^{-1}$, t is the irradiation time in seconds and I is the light intensity in $einsteins \cdot cm^{-2} \cdot s^{-1}$ that was determined using ferrioxalate actinometry.[1]

Bacterial strains and growth conditions The bacterial strains used were *E. coli* CS1562 (*tolC6*::*tn10*)[2] and *M. luteus* ATCC 9341. *E. coli* were grown in LB medium (5 g/L yeast extract; 10 g/L tryptone; 0.5 g/L NaCl) at 37 °C. *M. luteus* ATCC 9341 were grown in 2x YP medium (16 g/L Peptone, 10 g/L yeast extract, 5 g/L NaCl pH 7.0) at 37 °C.

Solid medium Square LB agar plates containing 22 μM of compound **1** were partly covered with a sterile thin cardboard or plastic mask and irradiated with UV (312 nm) for various amounts of time. The plates were then streaked with approximately 10^7 CFUs of *E. coli* and/or *M. luteus* and incubated overnight at 37 °C.

Synthesis *General.* All chemicals for synthesis were obtained from commercial sources and used as received unless stated otherwise. Solvents were reagent grade. Thin-layer chromatography (TLC) was performed using commercial Kieselgel 60, F254 silica gel plates. Flash chromatography was performed on silica gel (Silicycle Siliaflash P60, 40-63 μ, 230-400 mesh). Drying of solutions was performed with $MgSO_4$ and solvents were removed with a rotary evaporator. Chemical shifts (ppm) for NMR measurements were determined relative to the residual solvent peaks (δ_H 7.26 for $CHCl_3$ and 2.50 for DMSO, δ_C 77.16 for $CDCl_3$ and 39.52 for DMSO). The following abbreviations are used to indicate signal multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad signal; appt, apparent triplet. HRMS (ESI) spectra were obtained on a Thermo scientific LTQ Orbitrap XL. Melting points were recorded using a Buchi melting point B-545 apparatus. UV/Vis absorption spectra were recorded on an Agilent 8453 UV-Visible Spectrophotometer using Uvasol grade solvents.

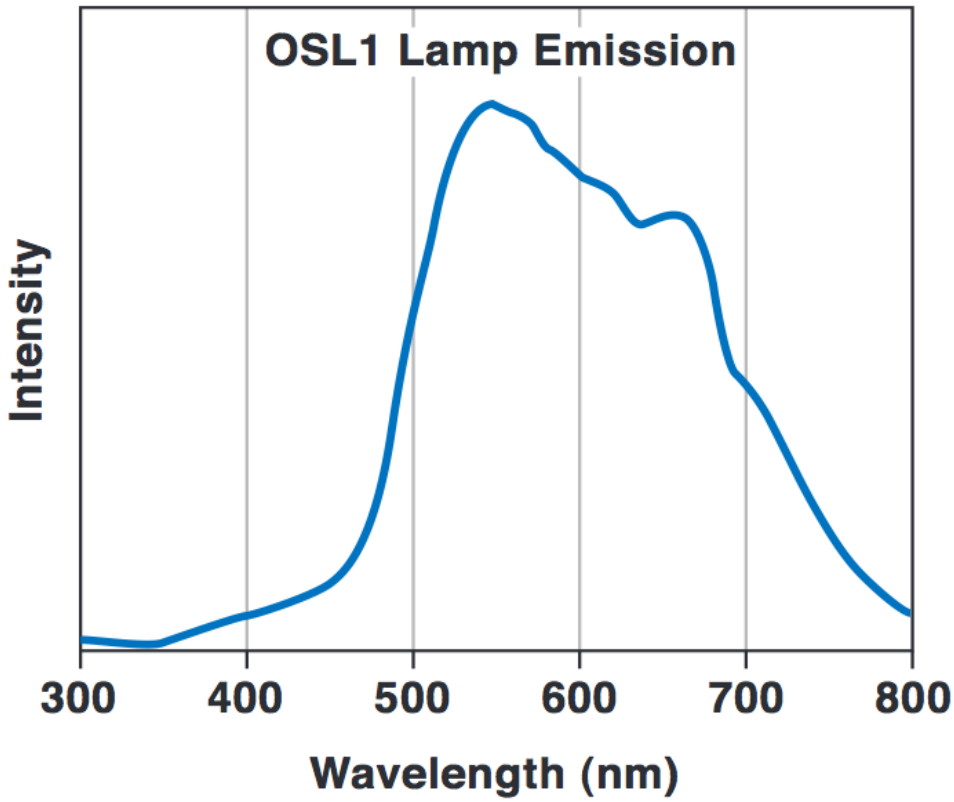
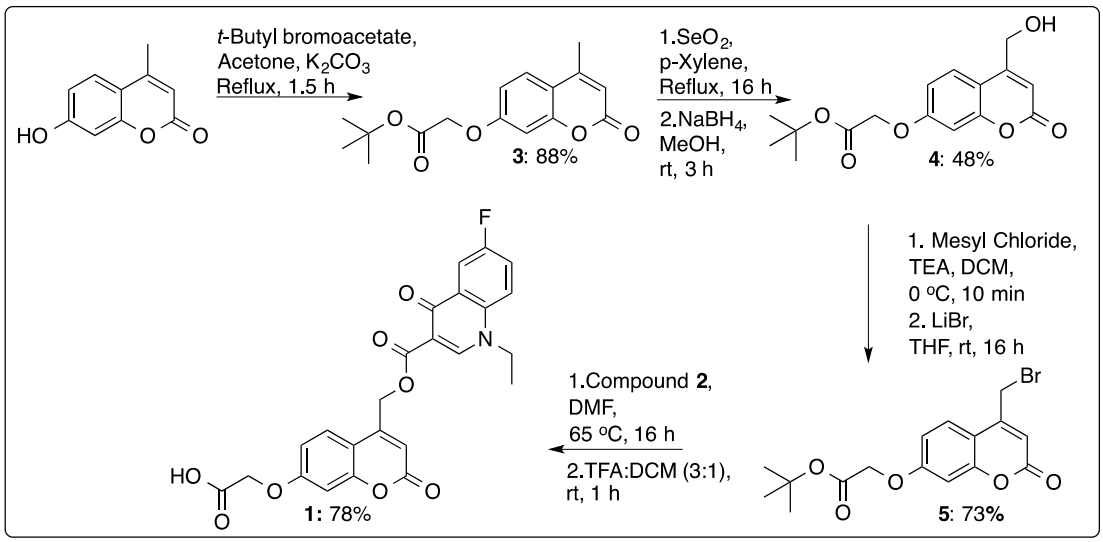


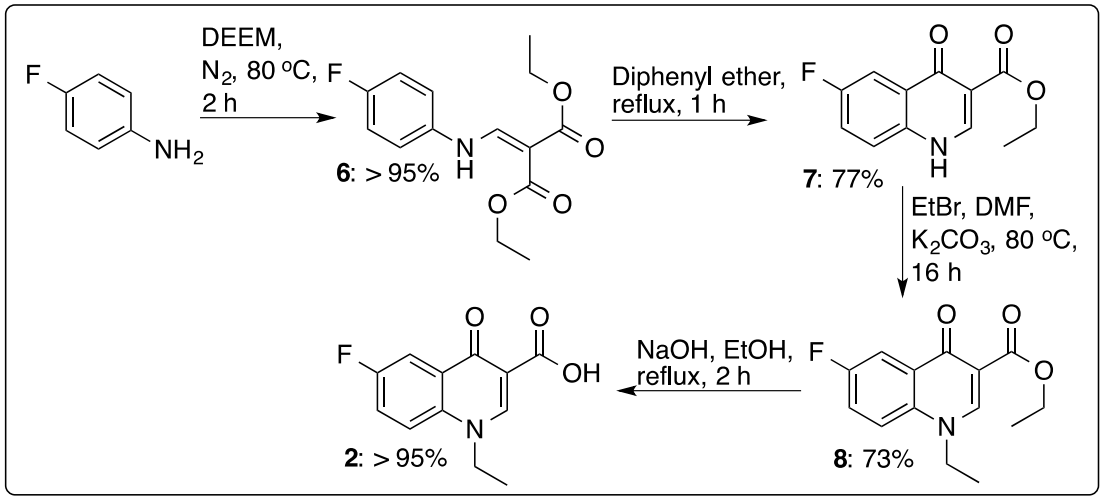
Figure S1. Electromagnetic spectrum of the white light source that was used for the white-light irradiation experiments (Thor Labs OSL1-EC Fiber Illuminator). *Source: www.thorlabs.de*

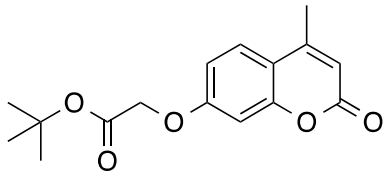
Synthesis of FQNC and BPOC

Scheme 1: Synthesis of compound **1**



Scheme 2: Synthesis of fluoroquinolone **2**



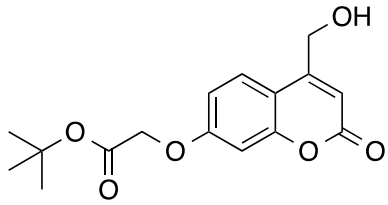


tert-*butyl 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetate* (**3**) 7-Hydroxy-4-methylcoumarin (2.84 mmol, 500 mg) and *t*-butyl bromoacetate (3.7 mmol, 720 mg) were dissolved in acetone (10 mL) to which K_2CO_3 (4.26 mmol, 588 mg) was added. The reaction mixture was stirred while heated at reflux for 2 h, after which the solvent was evaporated. The white residue was dissolved in DCM (20 mL) and the solution was washed with water (2 x 20 mL) and brine (20 mL) and dried ($MgSO_4$). After concentrating *in vacuo*, 718 mg (88%) of a white solid was obtained.

^{1}H NMR (400 MHz, DMSO-D$_6$): δ 7.68 (d, *J* = 8.6 Hz, 1H), 6.97 – 6.91 (m, 2H), 6.21 (s, 1H), 4.79 (s, 2H), 2.38 (s, 3H), 1.42 (s, 9H).

^{13}C NMR (100 MHz, DMSO-D$_6$): δ 167.7, 161.1, 160.5, 154.9, 153.8, 126.9, 114.0, 112.6, 111.8, 101.9, 82.1, 65.6, 28.1, 18.5.

HR-MS (ESI, [M+H]$^+$): Calcd. for $C_{16}H_{19}O_5$: 291.1227; Found: 291.1216



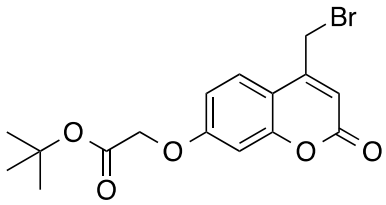
Di-tert-butyl 2,2'-((4-(hydroxymethyl)-2-oxo-2H-chromen-7-yl)azanediyl)diacetate (**4**) Selenium dioxide (3.45 mmol, 379 mg) was added to a solution of compound **3** (1.72 mmol, 500 mg) in *p*-xylene (10 mL). The resulting mixture was heated at reflux for 16 h after which it was filtered while hot. The filtrate was concentrated *in vacuo*, resulting in the aldehyde as a white solid (^{1}H NMR (400 MHz, DMSO-D$_6$): δ 10.09 (s, 1H), 8.38 (d, *J* = 8.6 Hz, 1H), 7.05 – 6.98 (m, 3H), 4.82 (s, 2H), 1.42 (s, 9H)), which was immediately reduced to the alcohol analogue without further purification.

The white solid was dissolved in methanol (10 mL) and cooled on ice. $NaBH_4$ (2.70 mmol, 86 mg) was added in small portions and the reaction mixture was stirred for 3 h. Next, the reaction was quenched by addition of aqueous 1 M HCl (10 mL) and the mixture was extracted twice with EtOAc (20 mL). The combined organic layers were washed with brine (20 mL) and dried ($MgSO_4$). Concentrating *in vacuo* resulted in 250 mg (48% over two steps) of a white solid.

^{1}H NMR (400 MHz, DMSO-D$_6$): δ 7.61 (d, *J* = 8.7 Hz, 1H), 6.97 – 6.89 (m, 2H), 6.30 (s, 1H), 5.60 (t, *J* = 4.4 Hz, 1H), 4.79 (s, 2H), 4.71 (d, *J* = 4.4 Hz, 2H), 1.42 (s, 9H).

^{13}C NMR (100 MHz, DMSO-D$_6$): δ 167.74, 160.9, 160.8, 157.0, 154.9, 125.8, 112.6, 111.6, 108.1, 102.0, 82.1, 65.6, 59.5, 28.2.

HR-MS (ESI, [M+H]$^+$): Calcd. for $C_{16}H_{19}O_6$: 307.1182; Found: 307.1165

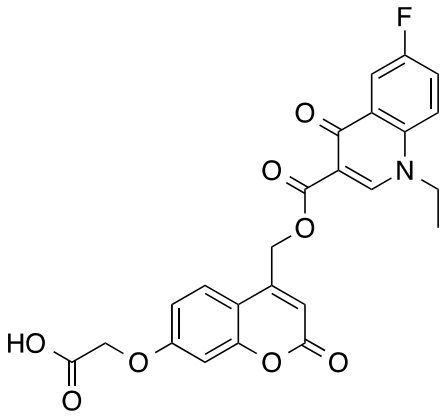


tert-*butyl 2-((4-(bromomethyl)-2-oxo-2H-chromen-7-yl)oxy)acetate* (**5**) A solution of compound **4** (0.69 mmol, 210 mg) in DCM (5 mL) was cooled on ice. To this was slowly added TEA (1.40 mmol, 141 mg) and methanesulfonic acid chloride (1.03 mmol, 118 mg) and the resulting mixture was stirred for 30 min. Next, the reaction mixture was washed with a saturated aqueous $NaHCO_3$ solution (5 mL) and brine (5 mL) and dried ($MgSO_4$). After concentrating *in vacuo*, the resulting solid was dissolved in THF (5 mL) and LiBr (2.76 mmol, 240 mg) was added. The obtained solution was stirred for 16 h at room temperature after which it was concentrated *in vacuo*. Next, the solid was dissolved in DCM (10 mL) and washed with water (10 mL) and brine (10 mL) and dried ($MgSO_4$). After concentrating in vacuo 186 mg (73%) of an orange solid was obtained.

^{1}H NMR (400 MHz, DMSO-D$_6$): δ 7.80 (d, *J* = 8.8 Hz, 1H), 7.04 – 6.95 (m, 2H), 6.55 (s, 1H), 4.84 (s, 2H), 4.81 (s, 2H), 1.42 (s, 9H).

^{13}C NMR (100 MHz, DMSO-D$_6$): δ 167.7, 161.4, 160.4, 155.5, 151.7, 127.0, 113.1, 112.8, 111.3, 102.3, 82.2, 65.6, 28.4, 28.1.

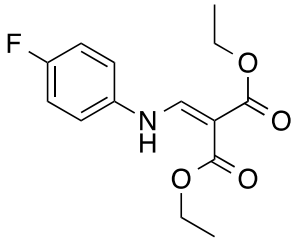
HR-MS (ESI, [M+H]$^+$): Calcd. for $C_{28}H_{27}N_2O_9S$: 369.0332; Found: 369.0331



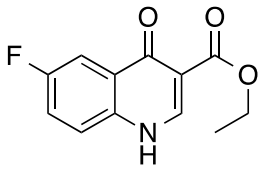
2-((4-(((1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carbonyl)oxy)methyl)-2-oxo-2H-chromen-7-yl)oxy)acetic acid (**1**) Compound **5** (0.27 mmol, 100 mg) and compound **2** (0.30 mmol, 70 mg) were dissolved in DMF (5 mL) to which K_2CO_3 (0.40 mmol, 56 mg) was added. The reaction mixture was stirred for 16 h at 65 ^{o}C after which it was poured into cold water (10 mL). The resulting precipitate was filtered off and dried yielding 132 mg of the condensation product as an orange solid (^{1}H NMR (400 MHz, DMSO-D$_6$): δ 8.85 (s, 1H), 8.00 - 7.95 (m, 2H), 7.76 - 7.69 (m, 2H), 7.01 - 6.95 (m, 2H), 6.74 (s, 1H), 5.57 (s, 2H), 4.82 (s, 2H), 4.47 (q, *J* = 6.9 Hz, 2H), 1.43 (s, 9H), 1.37 (t, *J* = 7.0 Hz, 3H)), which was immediately used in the subsequent deprotection step without further purification. The orange solid (0.19 mmol, 100 mg) was dissolved in TFA:DCM (3:1) (5 mL) and the mixture stirred for 1

h at room temperature after which the solvents were evaporated under vacuum. Diethylether (2 mL) was added to the obtained liquid, which resulted in the formation of an orange precipitate. The solid was filtered off and recrystallized from MeOH to obtain 74 mg (78% over two steps) of an orange solid. Mp: 179-182 °C

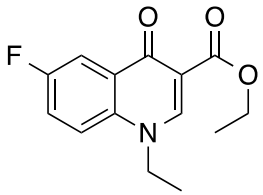
^{1}H NMR (400 MHz, DMSO-D$_6$): δ 8.84 (s, 1H), 7.99 - 7.92 (m, 2H), 7.75 - 7.68 (m, 2H), 7.03 - 6.95 (m, 2H), 6.74 (s, 1H), 5.56 (s, 2H), 4.83 (s, 2H), 4.46 (q, 2H), 1.37 (t, *J* = 7.0 Hz, 3H).
^{13}C NMR (100 MHz, DMSO-D$_6$): δ ^{13}C 172.5, 172.5, 170.0, 164.9, 161.3, 161.0, 160.5, 158.6, 155.1, 151.2, 150.3, 135.8, 130.8, 130.7, 126.2, 121.7, 121.5, 121.0, 120.9, 112.9, 111.7, 111.5, 111.1, 109.5, 108.7, 102.2, 65.3, 61.7, 48.9, 14.8.
^{19}F NMR (376 MHz, DMSO-D$_6$): δ -115.91.
HR-MS (ESI, [M+H]$^+$): Calcd. for $C_{24}H_{19}FNO_8$: 468.1089; Found: 468.1086



Diethyl 2-(((4-fluorophenyl)amino)methylene)malonate (**6**) A mixture of 4-fluoroaniline (18.0 mmol, 2.00 g) and diethyl-2-ethoxymethylenemalonate (DEEM) (18.0 mmol, 3.89 g) was stirred at 80 °C under a nitrogen atmosphere for 2 h. The reaction mixture was dissolved in DCM (50 mL), washed with aqueous 1M HCl (50 mL), brine (50 mL) and dried ($MgSO_4$). DCM was removed *in vacuo* resulting in 4.90 g (>95%) of a yellow solid.
^{1}H NMR (400 MHz, CDCl$_3$): δ 10.95 (d, *J* = 13.5 Hz, 1H), 8.39 (d, *J* = 13.9 Hz, 1H), 7.10 – 6.99 (m, 4H), 4.30 – 4.16 (m, 4H), 1.36-1.24 (m, 6H).
^{1}H NMR spectrum in agreement with published data.[3]



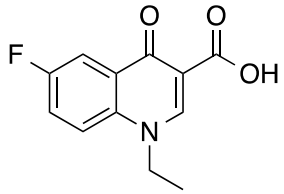
Ethyl 6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**7**) A solution of **6** (17.5 mmol, 4.90 g) in diphenylether (5 mL) was added dropwise to boiling diphenylether (60 mL). After heating under reflux for 1 h, the mixture was slowly cooled to room temperature and pentane (60 mL) was added. The solid was filtered off and the residue was recrystallized from DMF resulting in 3.40 g (83%) of a white solid.
^{1}H NMR (400 MHz, DMSO-D$_6$): δ 12.43 (s, 1H), 8.56 (s, 1H), 7.78 (dd, *J* = 9.3, 2.9 Hz, 1H), 7.69 (dd, *J* = 9.0, 4.6 Hz, 1H), 7.60 (td, *J* = 8.8, 3.0 Hz, 1H), 4.20 (q, *J* = 7.1 Hz, 2H), 1.26 (t, *J* = 7.1 Hz, 3H).
^{1}H NMR spectrum in agreement with published data.[4]



Ethyl 1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**8**) To a solution of **7** (14.5 mmol, 3.40 g) in DMF (50 mL) was added K_2CO_3 (15.0 mmol, 2.07 g) and the resulting suspension was heated to 80 °C. Ethyl bromide (20 mmol, 2.16 g) was added and the mixture was stirred overnight. The mixture was diluted with water (100 ml) and filtered over a glass filter. The solid was recrystallized from EtOH resulting in 1.6 g (42%) of a white powder.

^{1}H NMR (400 MHz, DMSO-D$_6$): δ 8.69 (s, 1H), 7.90 (m, 2H), 7.68 (m, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 4.21 (q, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.1 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 3H).

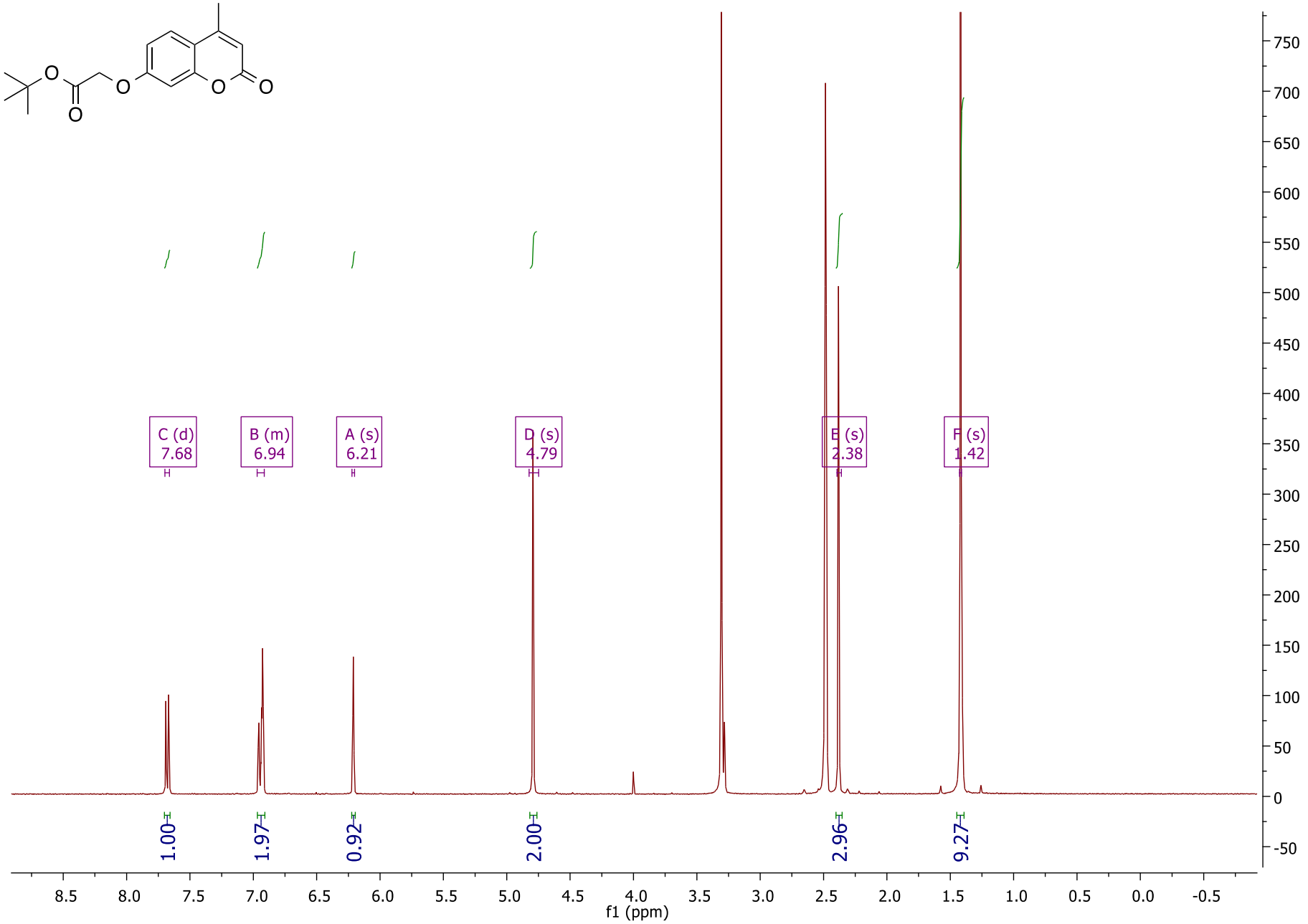
^{1}H NMR spectrum in agreement with published data.[4]

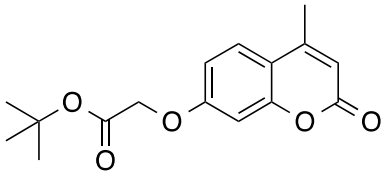


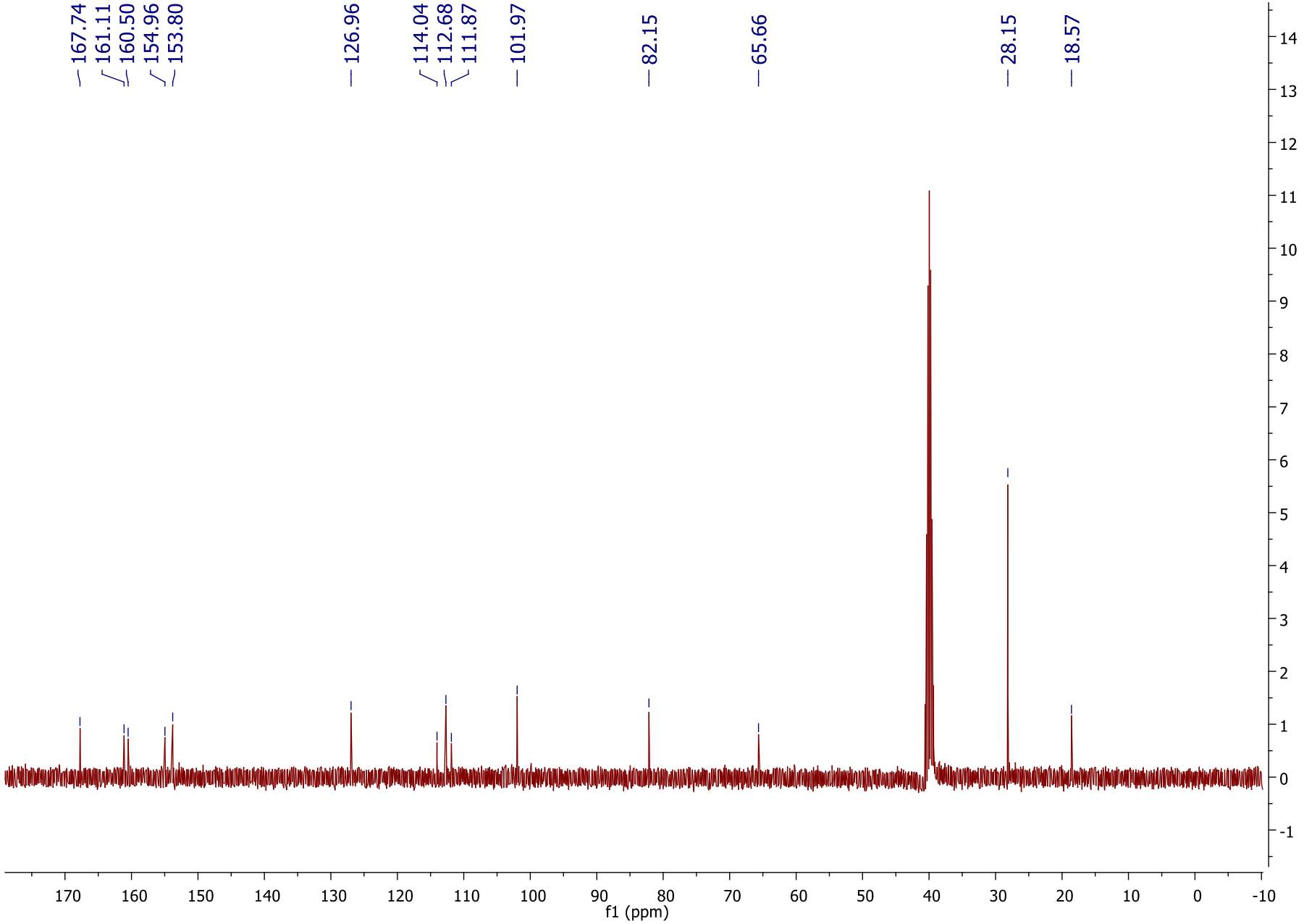
1-Ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**2**) Compound **8** (0.38 mmol, 100 mg) was added to EtOH (5 mL) on ice and a 2.5 M aq. NaOH solution (152 µL) was added dropwise. The reaction mixture was heated at 70 °C for 16 h. Subsequently, aqueous 1 M HCl (5 mL) was added and the precipitate was filtered off resulting in 85 mg (95%) of a white solid.

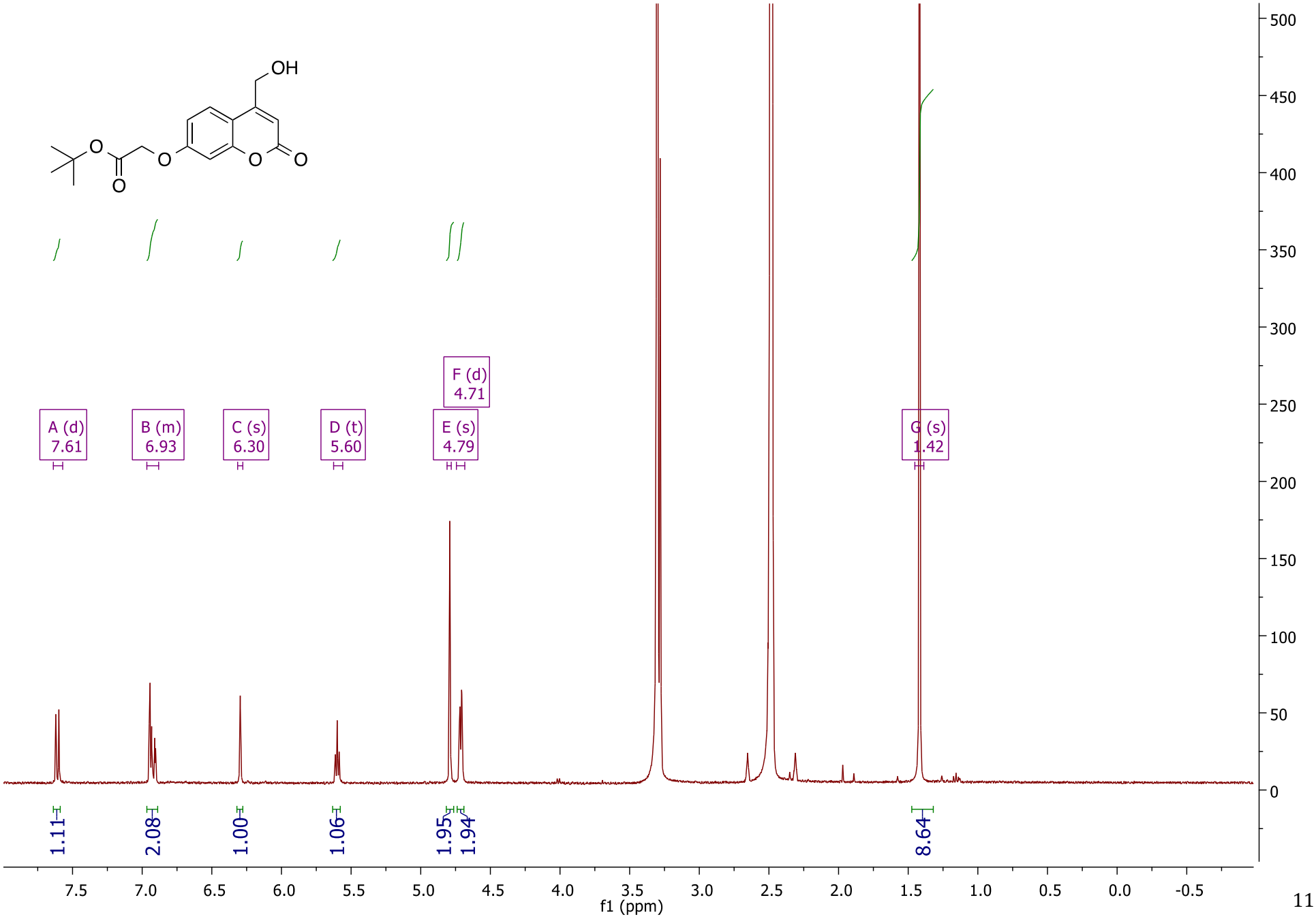
^{1}H NMR (400 MHz, DMSO-D$_6$): δ 15.00 (s, 1H), 9.06 (s, 1H), 8.16 (dd, *J* = 9.4, 4.4 Hz, 1H), 8.04 (dd, *J* = 8.8, 3.1 Hz, 1H), 7.88 (ddd, *J* = 9.4, 8.0, 3.1 Hz, 1H), 4.61 (q, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H).

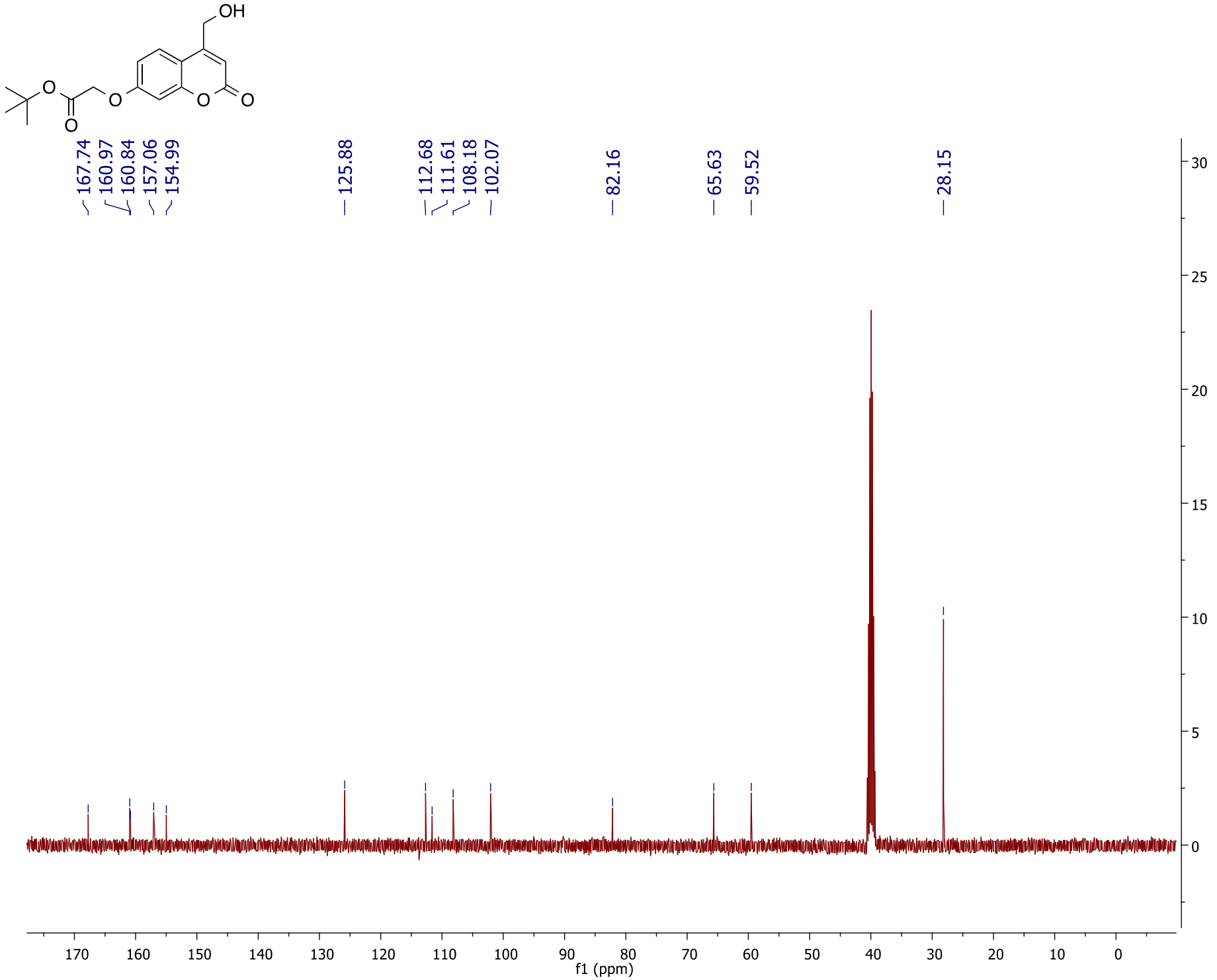
^{1}H NMR spectrum in agreement with published data.[4]

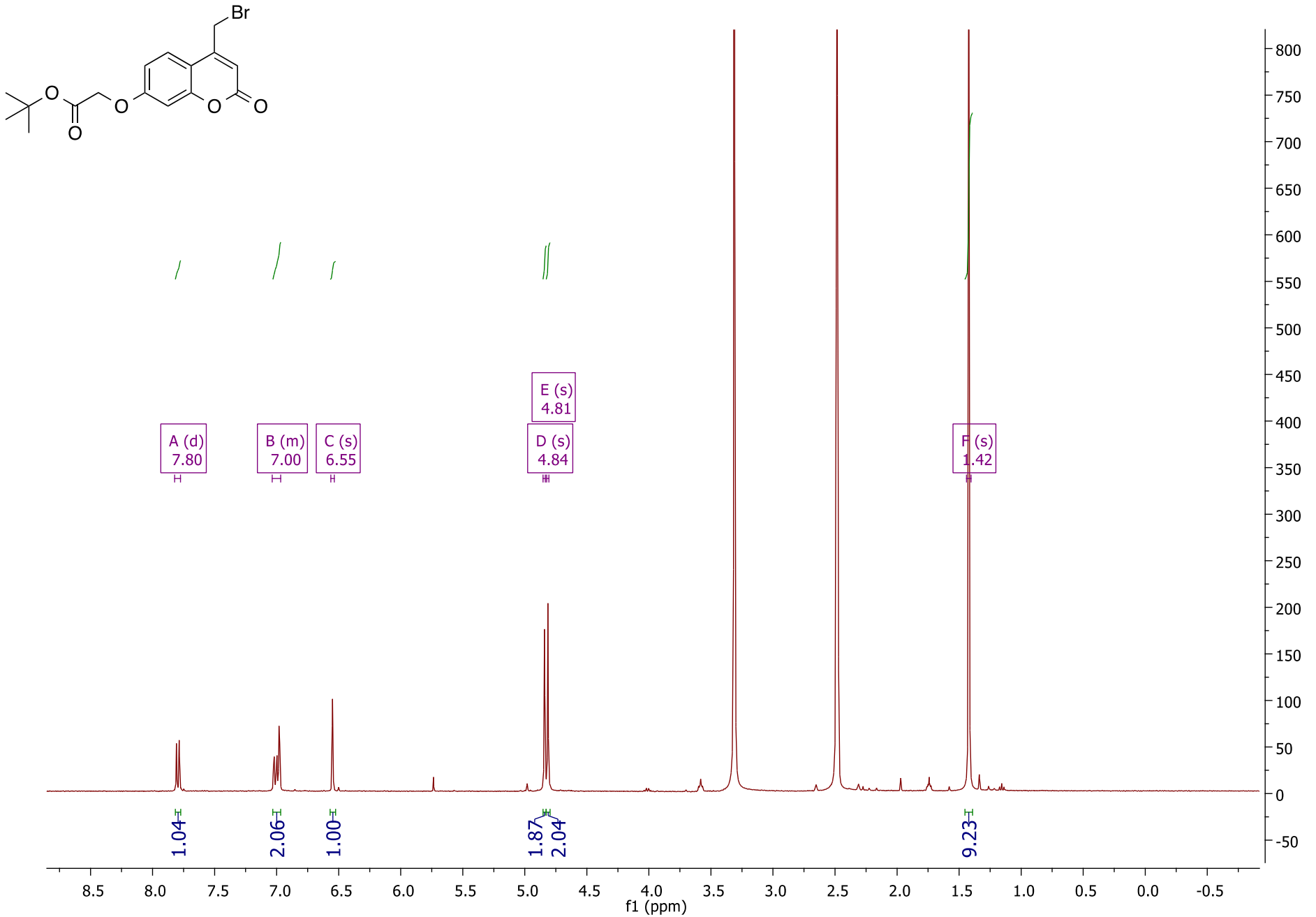

C (d)
7.68
B (m)
6.94
A (s)
6.21
D (s)
4.79
E (s)
2.38
F (s)
1.42
1.00
1.97
0.92
2.00
2.96
9.27
f1 (ppm)

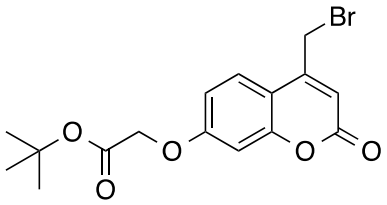


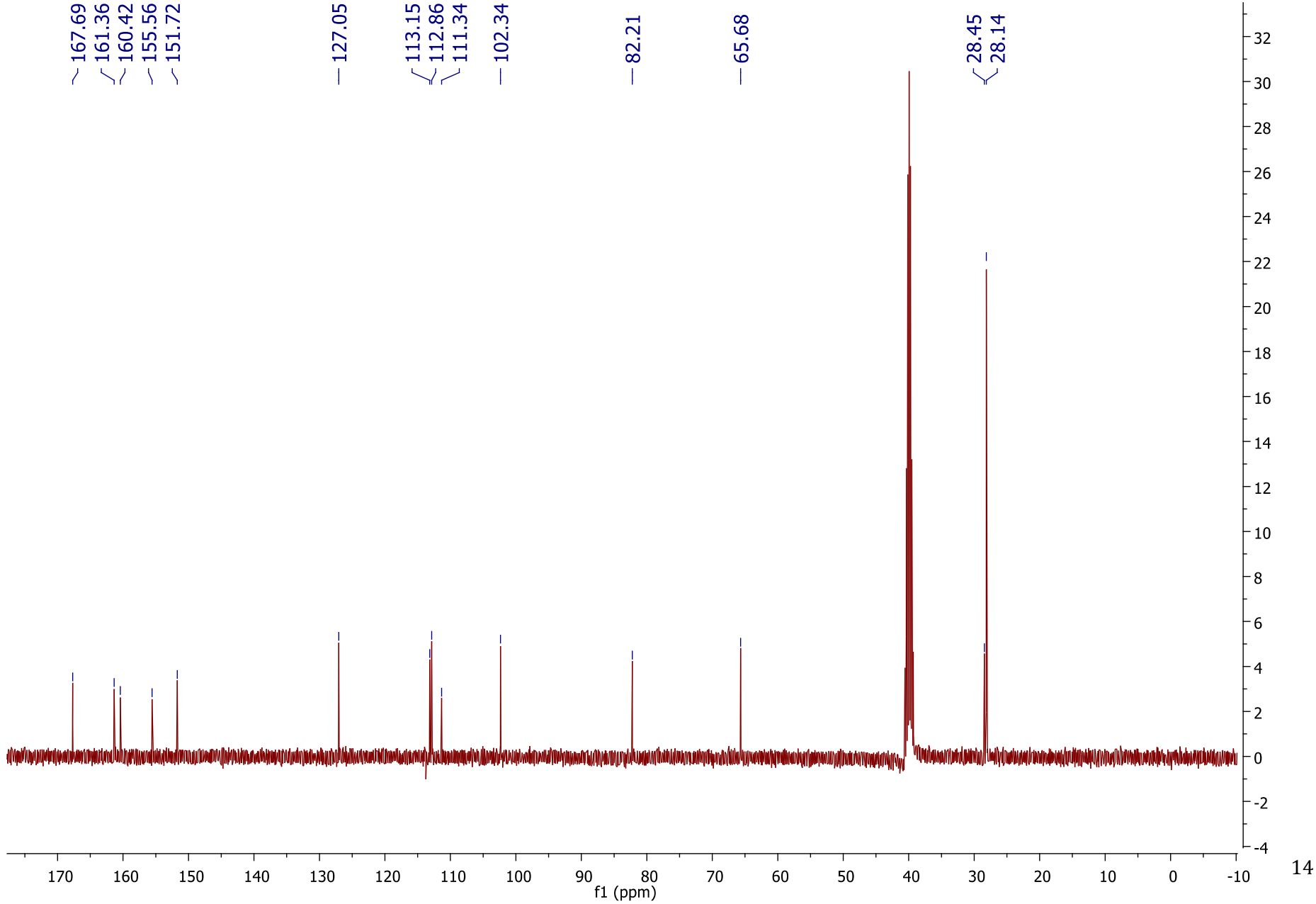

167.74
161.11
160.50
154.96
153.80
126.96
114.04
112.68
111.87
101.97
82.15
65.66
28.15
18.57
f1 (ppm)

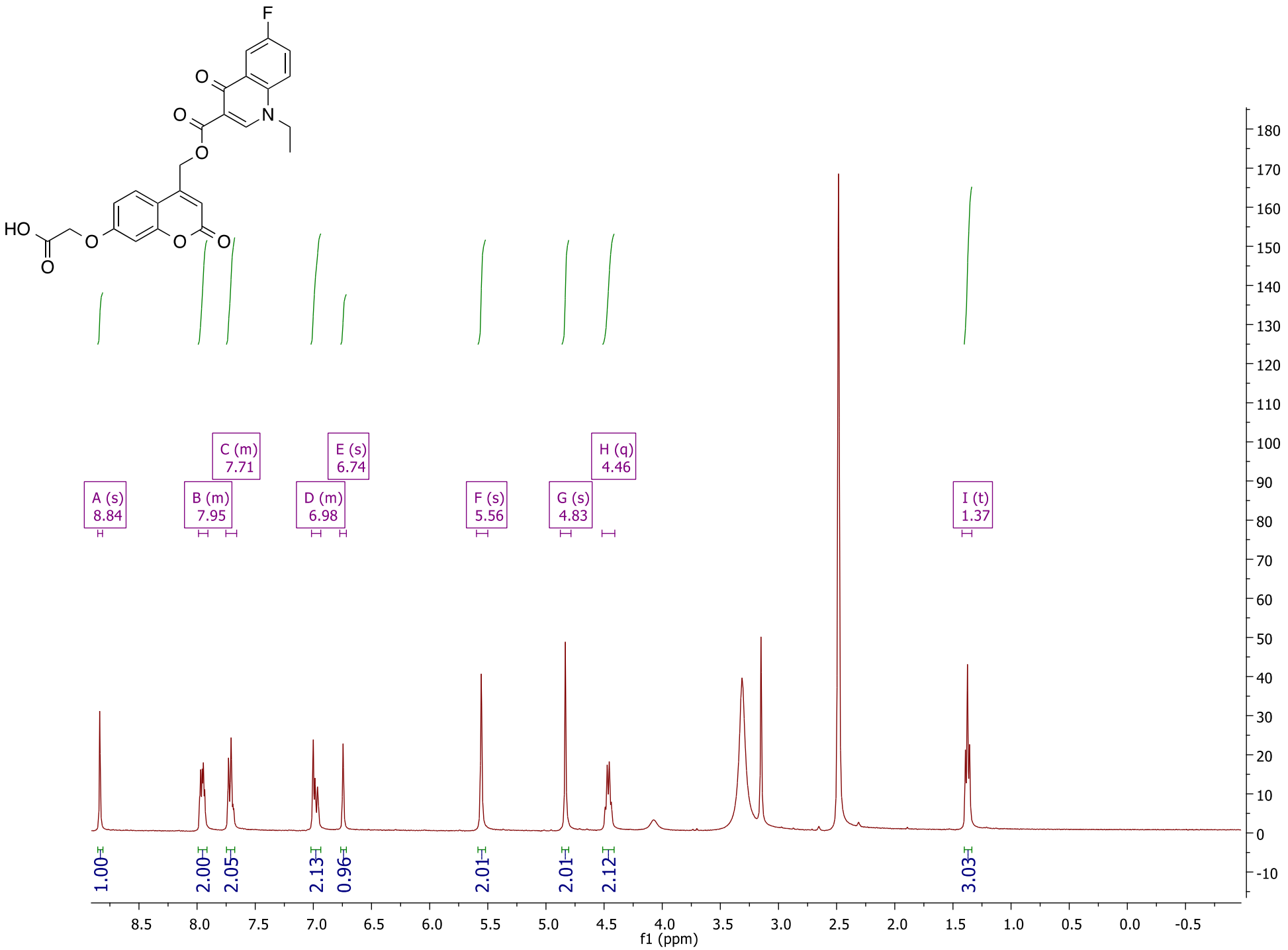

OH
O
O
O
O
O
A (d)
7.61
B (m)
6.93
C (s)
6.30
D (t)
5.60
F (d)
4.71
E (s)
4.79
G (s)
1.42
1.11
2.08
1.00
1.06
1.95
1.94
8.64
f1 (ppm)

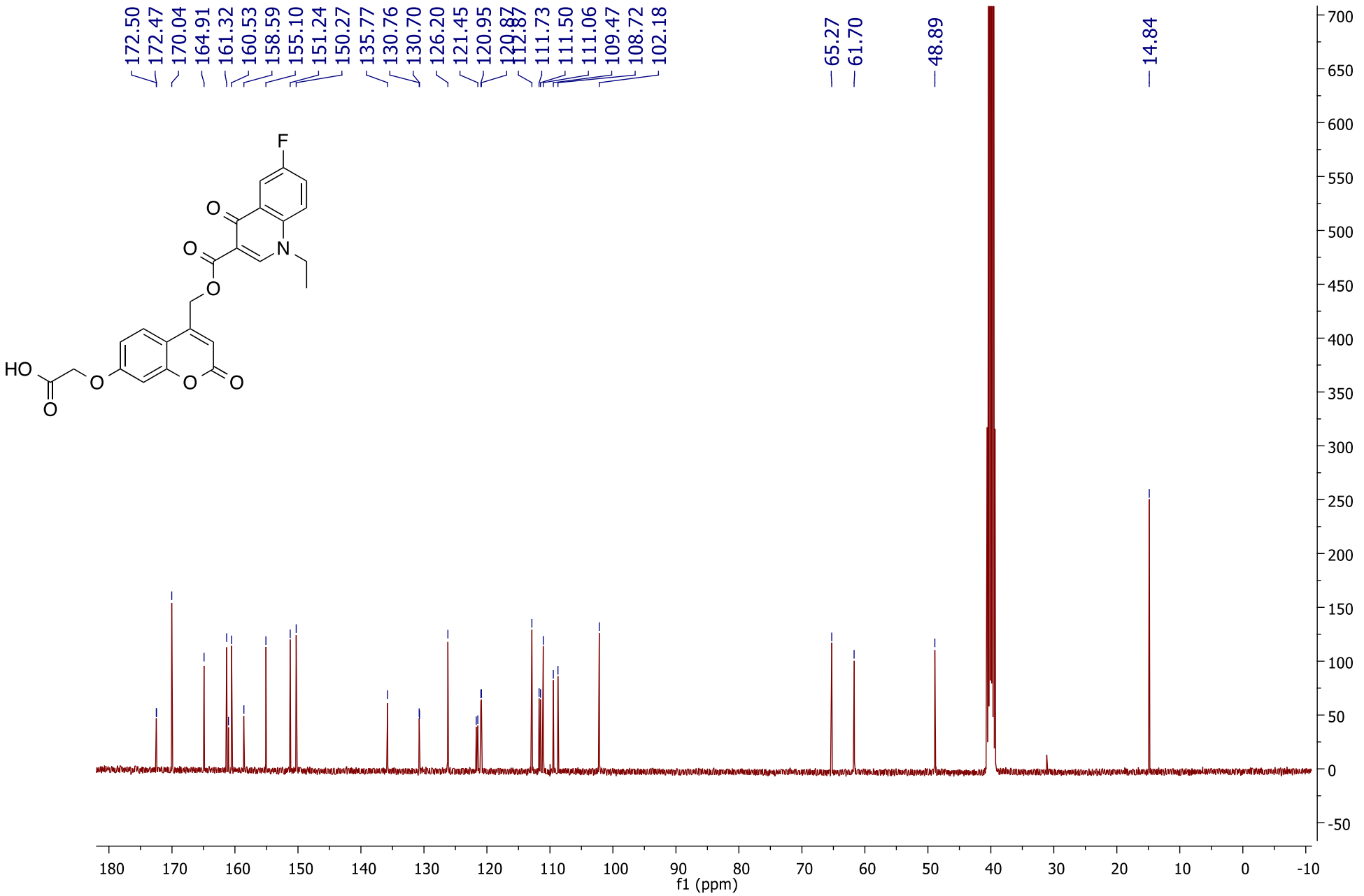

OH
O
O
O
O
O
167.74
160.97
160.84
157.06
154.99
125.88
112.68
111.61
108.18
102.07
82.16
65.63
59.52
28.15
30
25
20
15
10
5
0
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

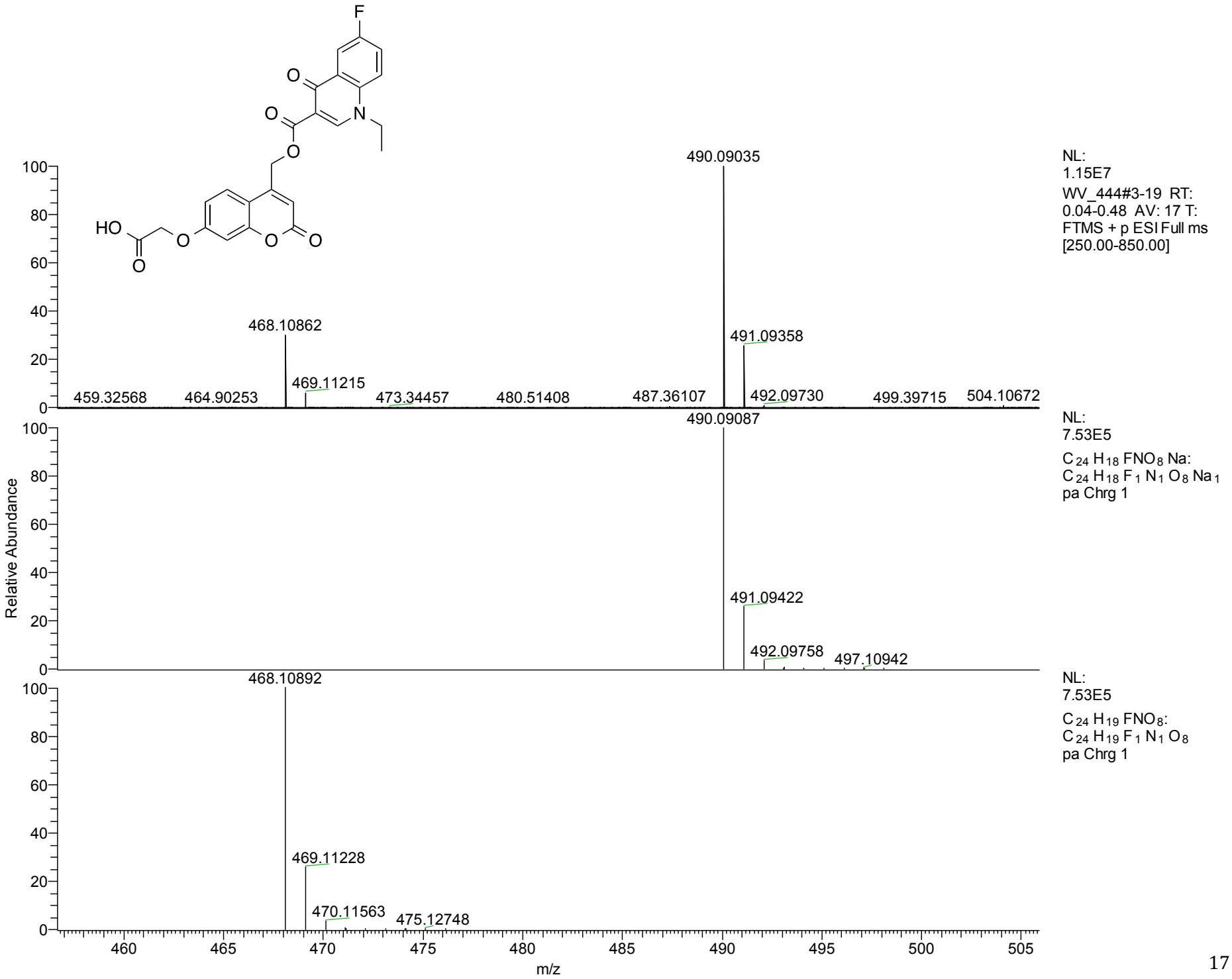

Br
O
O
O
O
O
A (d)
7.80
B (m)
7.00
C (s)
6.55
D (s)
4.84
E (s)
4.81
F (s)
1.42
1.04
2.06
1.00
1.87
2.04
9.23
f1 (ppm)


Br
O
O
O
O
O


167.69
161.36
160.42
155.56
151.72
127.05
113.15
112.86
111.34
102.34
82.21
65.68
28.45
28.14
f1 (ppm)


F
O
O
N
O
HO
O
O
O
O
A (s)
8.84
B (m)
7.95
C (m)
7.71
D (m)
6.98
E (s)
6.74
F (s)
5.56
G (s)
4.83
H (q)
4.46
I (t)
1.37
1.00
2.00
2.05
2.13
0.96
2.01
2.01
2.12
3.03
f1 (ppm)


172.50
172.47
170.04
164.91
161.32
160.53
158.59
155.10
151.24
150.27
135.77
130.76
130.70
126.20
121.45
120.95
120.87
112.87
111.73
111.50
111.06
109.47
108.72
102.18
65.27
61.70
48.89
14.84
F
O
O
N
O
HO
O
O
O
O
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
-50
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


F
O
O
N
O
HO
O
O
O
O
490.09035
NL:
1.15E7
WV_444#3-19 RT:
0.04-0.48 AV: 17 T:
FTMS + p ESI Full ms
[250.00-850.00]
468.10862
491.09358
469.11215
459.32568
464.90253
473.34457
480.51408
487.36107
492.09730
499.39715
504.10672
490.09087
NL:
7.53E5
C24 H18 FNO8 Na:
C24 H18 F1 N1 O8 Na1
pa Chrg 1
491.09422
492.09758
497.10942
Relative Abundance
468.10892
NL:
7.53E5
C24 H19 FNO8:
C24 H19 F1 N1 O8
pa Chrg 1
469.11228
470.11563
475.12748
m/z

References:

1. C. G. Hatchard and C. A. Parker, *Proc. R. Soc. London. Ser. A. Math. Phys. Sci.* , 1956, **235** , 518–536.

2. E. A. Austin, J. F. Graves, L. A. Hite, C. T. Parker, and C. A. Schnaitman, *J. Bacteriol.*, 1990, **172**, 5312–5325.

3. S. Eswaran, A. V. Adhikari, N. K. Pal, and I. H. Chowdhury, *Bioorganic Med. Chem. Lett.*, 2010, **20**, 1040–1044.

4. S. Niedermeier, K. Singethan, S. G. Rohrer, M. Matz, M. Kossner, S. Diederich, A. Maisner, J. Schmitz, G. Hiltensperger, K. Baumann, U. Holzgrabe, and J. Schneider-Schaulies, *J. Med. Chem.*, 2009, **52**, 4257–4265.