Electronic Supplementary information

to accompany the manuscript entitled:

Synthesis and *in vivo* evaluation of a radiofluorinated ketone body derivative

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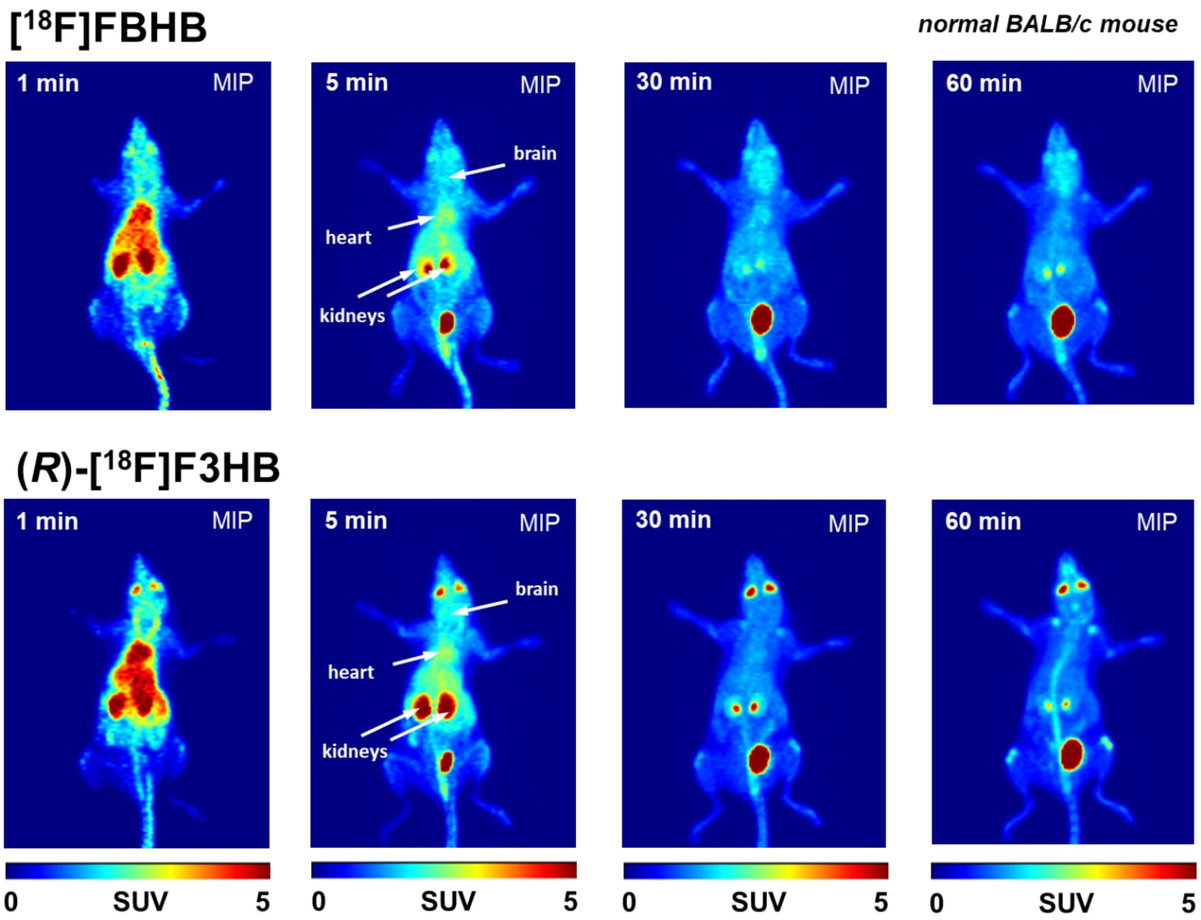


Figure S1 PET images in normal BALB/c mice post tail vein injection of [^{18}F]FBHB (upper) and (*R*)-[^{18}F]F3HB (lower) over 60 min. MIP, maximum intensity projection; SUV, standard uptake value.

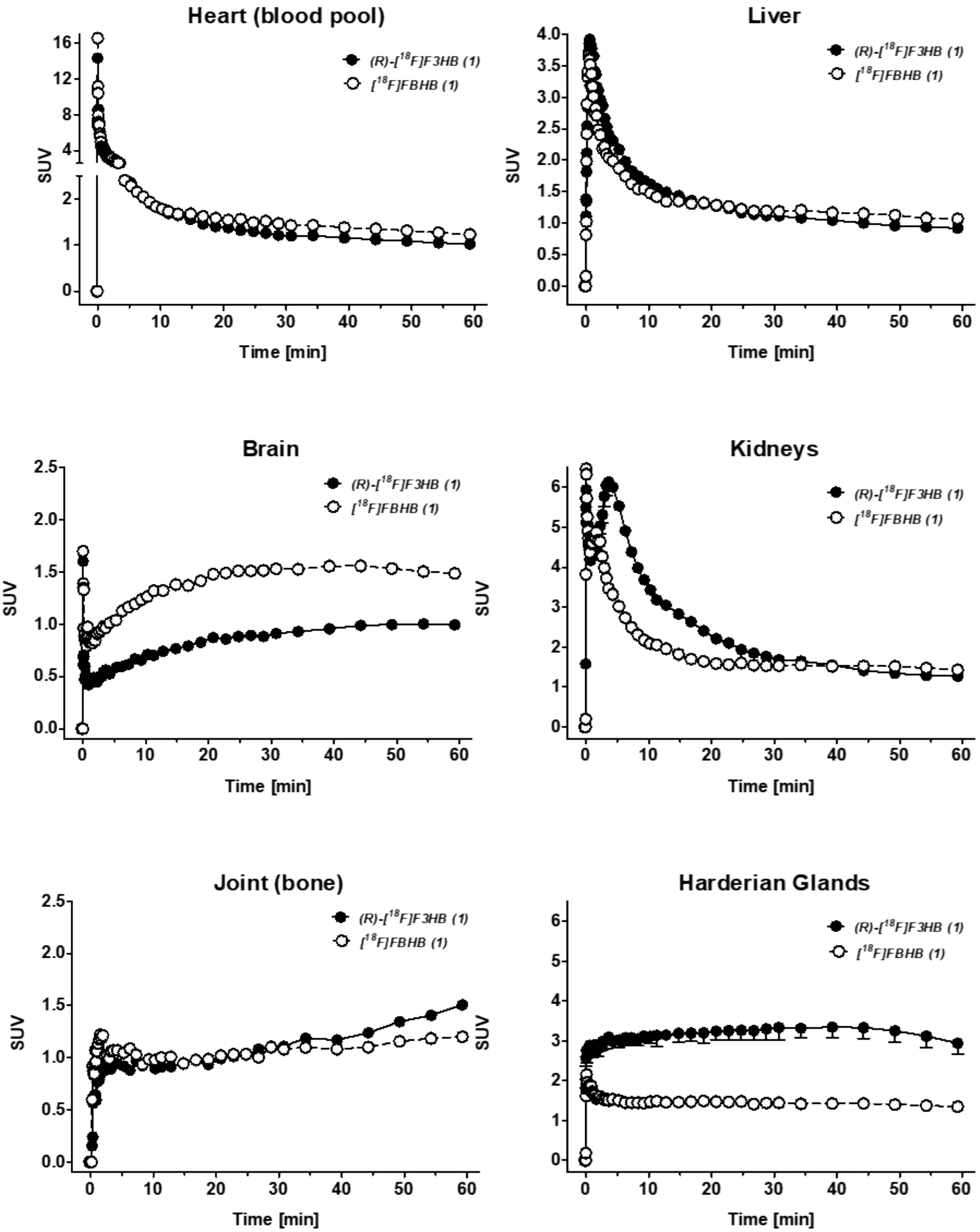


Figure S2 Time-activity curves of select organs in normal BALB/c mice post tail vein injection of [^{18}F]FBHB or (*R*)-[^{18}F]F3HB over 60 min.

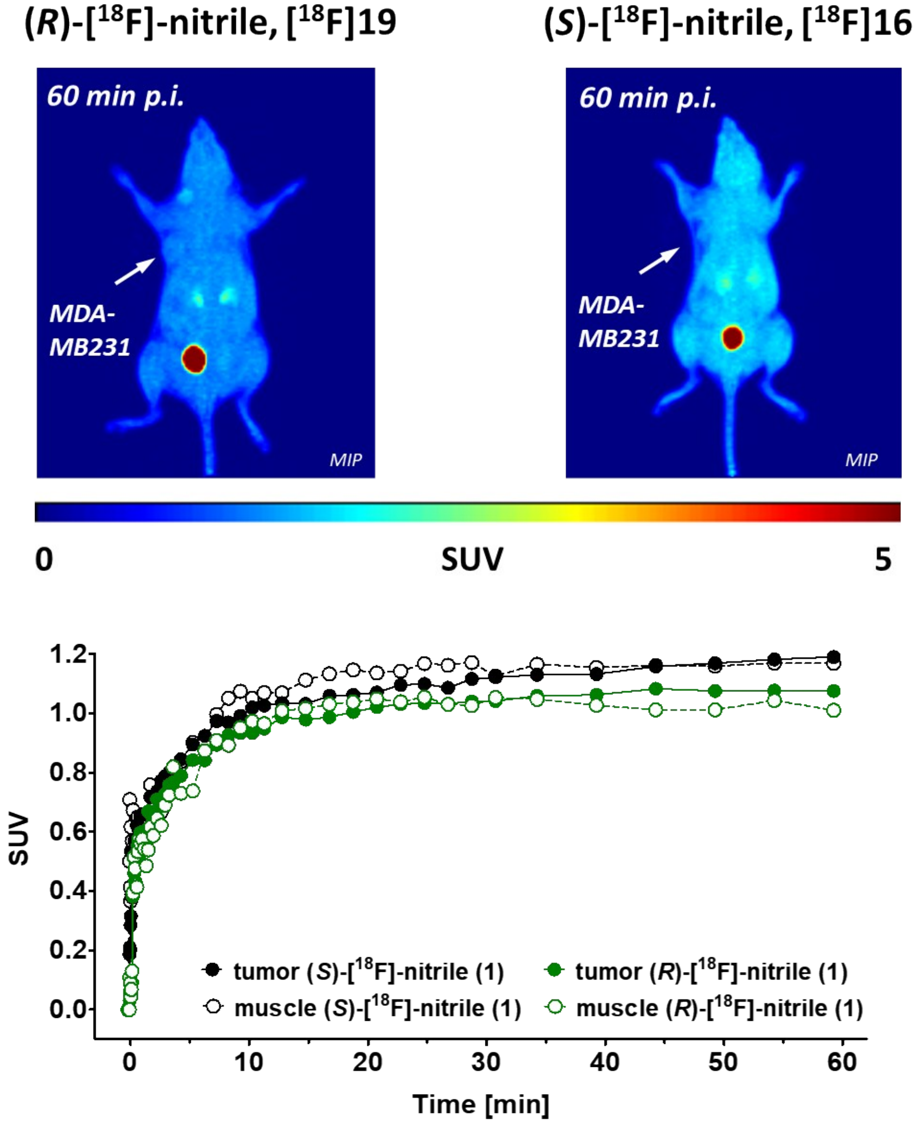


Figure S3 PET images of MDA-MB231 xenograft mice with nitrile intermediates compounds [^{18}F]**19** ((*R*)-[^{18}F]-nitrile) or **[^{18}F]16** ((S)-[^{18}F]-nitrile) 1h post injection (*upper*); corresponding time-activity curves of tumor versus muscle uptake (*lower*)

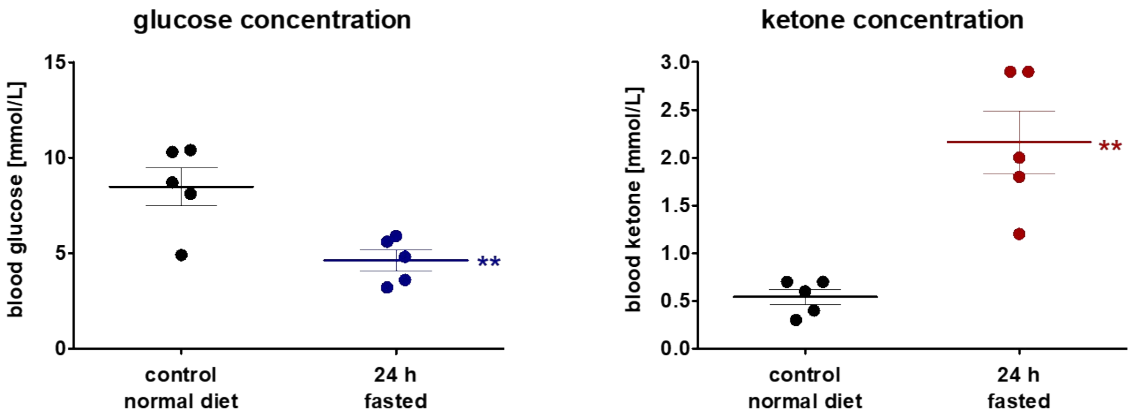
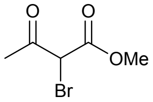


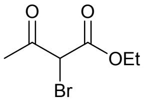
Figure S4 Changes in blood glucose and blood ketone body concentrations prior to and post a 24 h fast

Preparation of compound **3a**



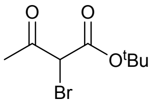
Compound **3a** was prepared according to Li et al.[1] To a solution of methyl 3-oxobutanoate (0.538 g, 4.63 mmol) in benzene (23 mL) was added N-bromosuccinimide (1.016 g, 5.71 mmol) and catalytic AIBN (0.316 g of a 12% solution in acetone, 0.2316 mmol). The mixture was stirred at rt for 24h. The solvents were removed by rotary evaporation. The crude product was purified by silica column chromatography (7:3 CH_2Cl_2:hexanes isocratic) and the purified product was isolated as a yellow oil (0.5634 g, 90.3%) (R_f = 0.59 1:1 hexanes/EtOAc, KMnO4 stain). The spectral properties differed slightly from Li et al. but were in agreement with the same compound prepared by Khan et al.[2] ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.78 (s, 1H), 3.85 (s, 3H), 2.46 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 196.2 (*C*), 165.6 (*C*), 53.8 (*C*H), 48.6 (*C*H_3), 26.4 (*C*H_3). LCMS: *m/z* $[M]^+$ calcd for $C_5H_8BrO_3$: 194.9656, 196.9636; found: 195.0, 197.0.

Preparation of compound **3b**



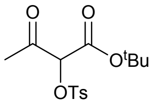
Compound **3b** was prepared using the same methodology as for **3a**. Spectral properties were in agreement with the compound as prepared by Wu et al.[3] ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.76 (s, 1H), 4.29 (q, *J* = 6.40 Hz, 2H), 2.45 (s, 3H), 1.32 (t, *J* = 7.15, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 196.4 (*C*), 165.1 (*C*), 63.2 (*C*H_2), 49.1 (*C*H), 26.4 (*C*H_3), 13.9 (*C*H_3).

Preparation of compound **3c**



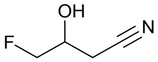
Compound **3c** was prepared using the same methodology as for **3a**. Spectral properties were in agreement with the same compound prepared by Khan et al.[2] ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.68 (s, 1H), 2.42 (s, 3H), 1.50 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 196.7 (*C*), 164.0 (*C*), 84.5 (*C*), 50.7 (*C*H), 27.7 (*C*H_3), 26.3 (*C*H_3).

Preparation of compound **4**



Compound **4** was prepared according to Coats et al.[4] To *tert*-butyl acetoacetate (0.477 g, 3.01 mmol) in CH_3CN (15 mL) was added Koser's reagent ([hydroxy(tosyloxy)iodo]benzene) (1.42 g, 3.62 mmol). The slurry was heated to 70 °C until a colorless homogenous solution was obtained (<2h). The crude product was purified by silica column chromatography, gradient elution 1:0 to 7:3 hexanes:EtOAc (R_f = 0.31 4:1 hexanes/EtOAc). Compound **4** was isolated as a white solid (0.7418 g, 99%). Spectral properties compare well with those reported for the methyl ester derivative by Yamamoto et al.[5] ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.84 (d, *J* = 8.28 Hz, 2H), 7.37 (d, *J* = 8.66 Hz, 2H), 5.31 (s, 1H), 2.46 (s, 3H), 2.29 (s, 3H), 1.40 (s, 9H).

Preparation of nitrile reference standard $FCH_2CHOHCH_2CN$

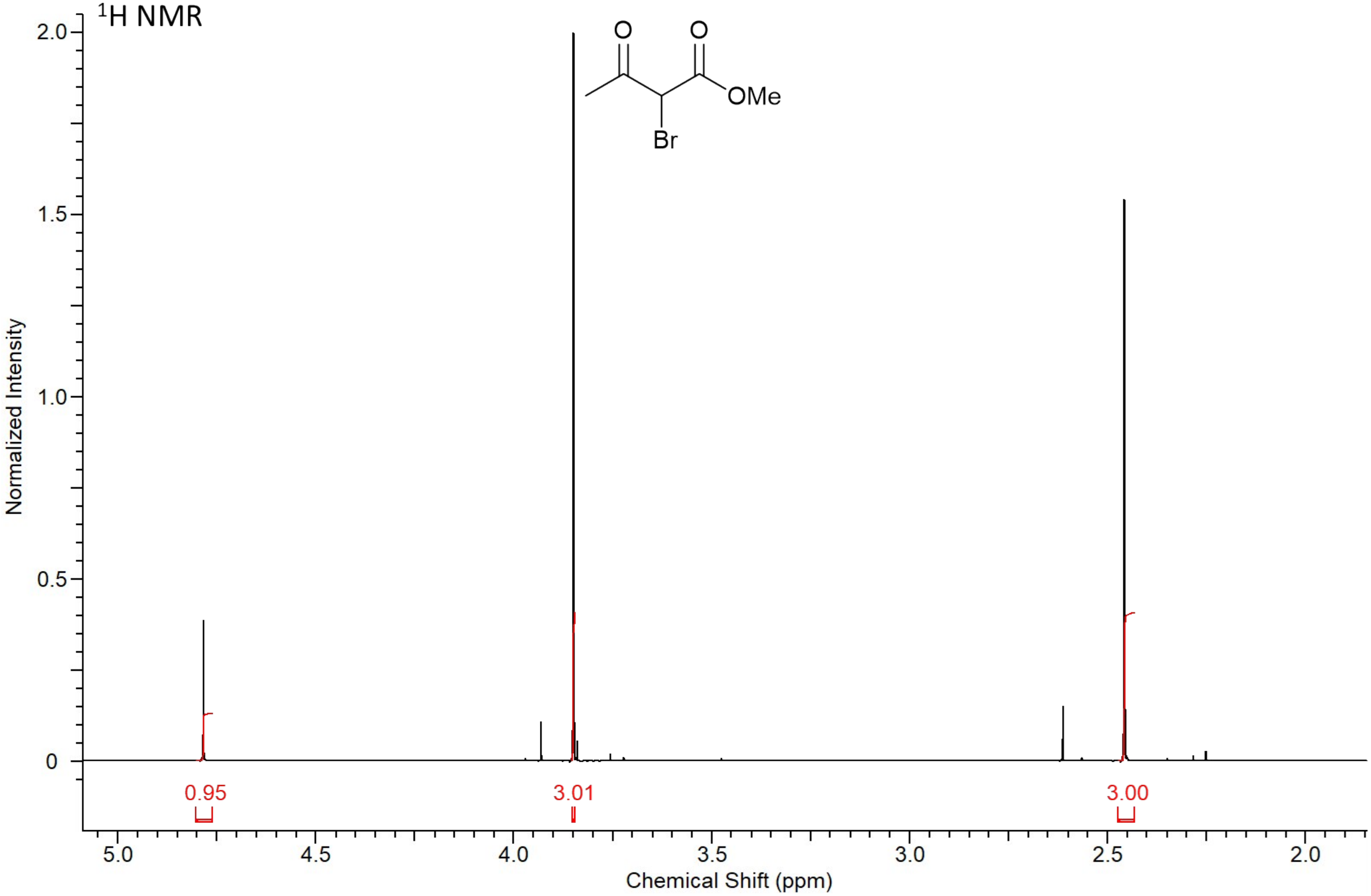


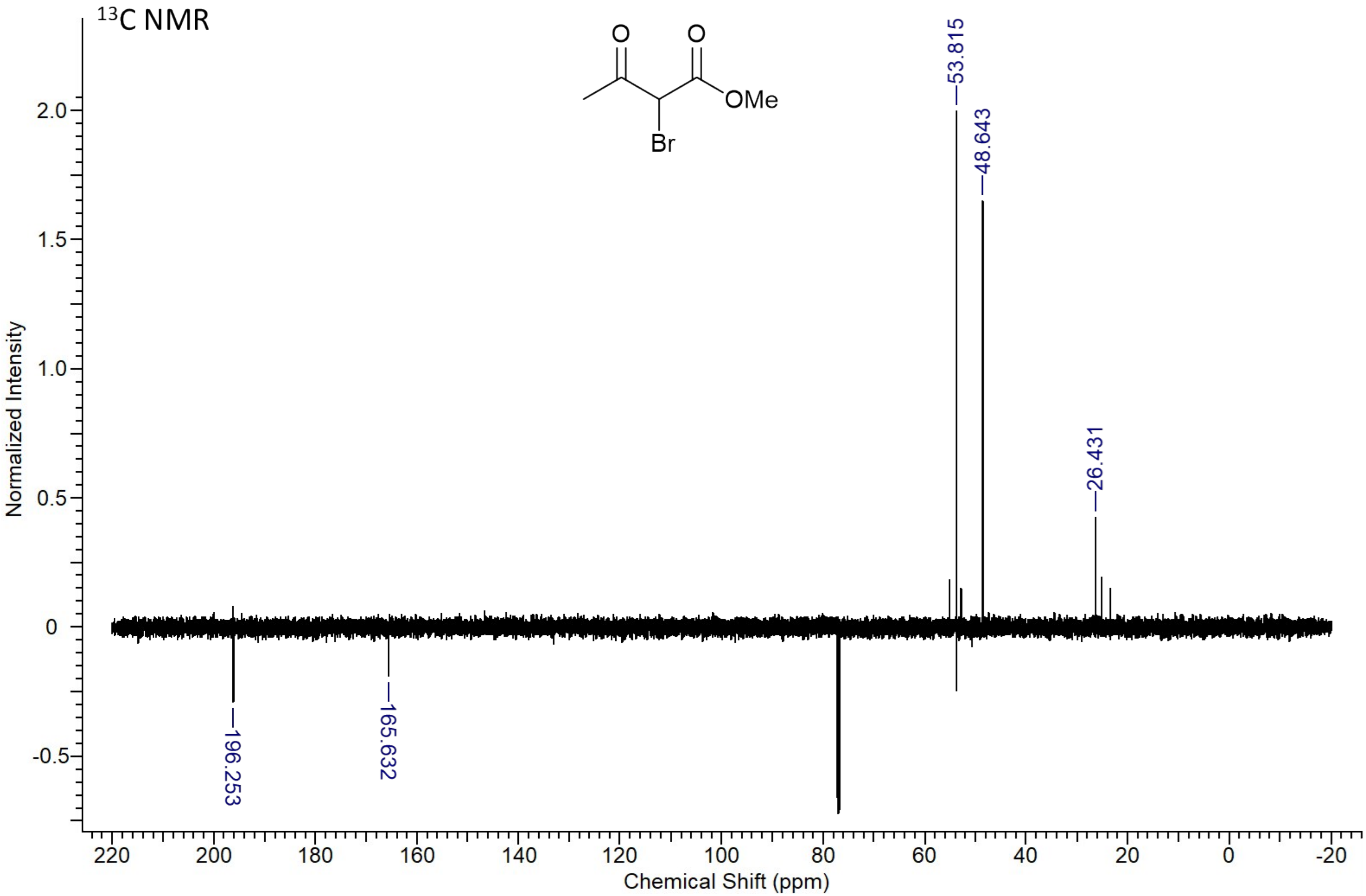
Nitrile reference standard $FCH_2CHOHCH_2CN$ was prepared according to Liu et al.[6] CAUTION: HCN (b.p. 25.6 °C) and epifluorohydrin (b.p. 85-86 °C) are toxic. H_2SO_4 (0.967 g, 9.86 mmol) was dissolved in water (33 mL) and chilled in an ice bath. To this solution was added epifluorohydrin (0.250 g, 3.29 mmol) followed by KCN (1.712 g, 26.3 mmol). The reaction mixture was allowed to gradually come to rt and was stirred for 16 h. The reaction mixture was extracted with EtOAc (3x30 mL), washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried over Na_2SO_4, and the solvent evaporated by rotary evaporation. The crude product was purified by silica column chromatography eluting with hexanes/EtOAc (R_f = 0.28 1:1 hexanes/EtOAc, $KMnO_4$ stain). The solvents were evaporated yielding $FCH_2CHOHCH_2CN$ (0.3097 g, 91.3%) as a bright yellow oil. ^{19}F NMR (565 MHz, $CDCl_3$): δ = –230.94 (td, *J* = 47.0, 17.8 Hz). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 4.47 (dd, $^{2}J_{H\text{-}F}$ = 47.1 Hz, $^{3}J_{H\text{-}H}$ = 4.89 Hz, 2H), 4.17-4.27 (m, 1H),3.08 (brs, 1H), 2.71-2.61 (m, 2H). ^{13}C NMR (151 MHz, $CDCl_3$): δ = 116.8 (*C*), 85.6 (d, $^{1}J_{C\text{-}F}$ = 172.6 Hz, *C*H_2F), 66.1 (d, $^{2}J_{C\text{-}F}$ = 22.1 Hz, *C*H), 21.7 (d, $^{3}J_{C\text{-}F}$ = 7.7 Hz, *C*H_2).

Notes: nitrilase conversion step

The enzymatic conversion step resulted in lower yields at temperatures above 30 °C and at pH below 9. Yields improved when PBS buffer was added; however, in this instance the presence of buffer salts hindered QMA cartridge capture of the final product acids. Note that for HPLC-based verifications of acids [^{18}F]FBHB and (R)-[^{18}F]F3HB, elution times vary depending on solvent pH and ionic strength (i.e. the presence of buffer salts).

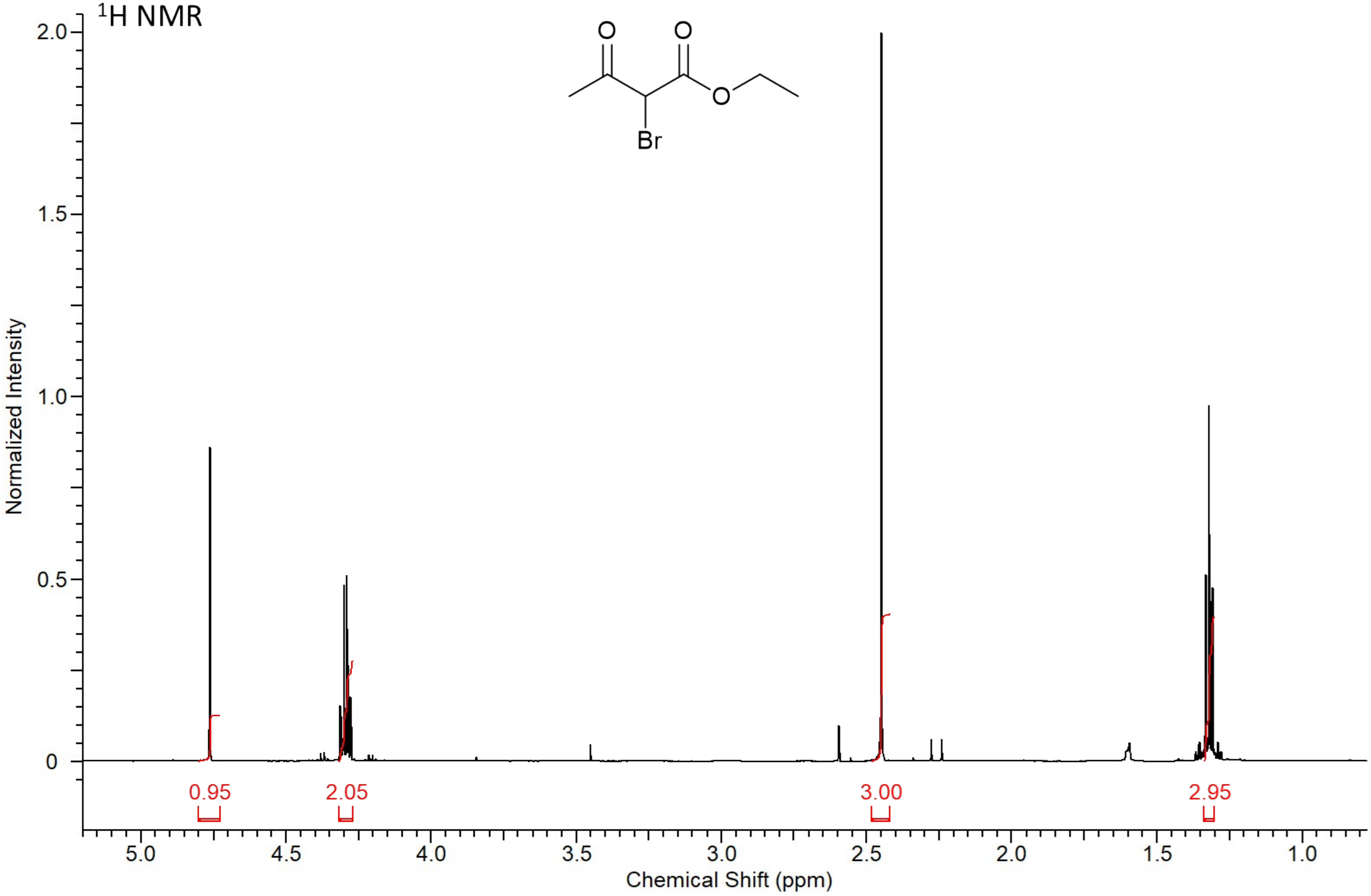
NMR spectra: compound **3a**

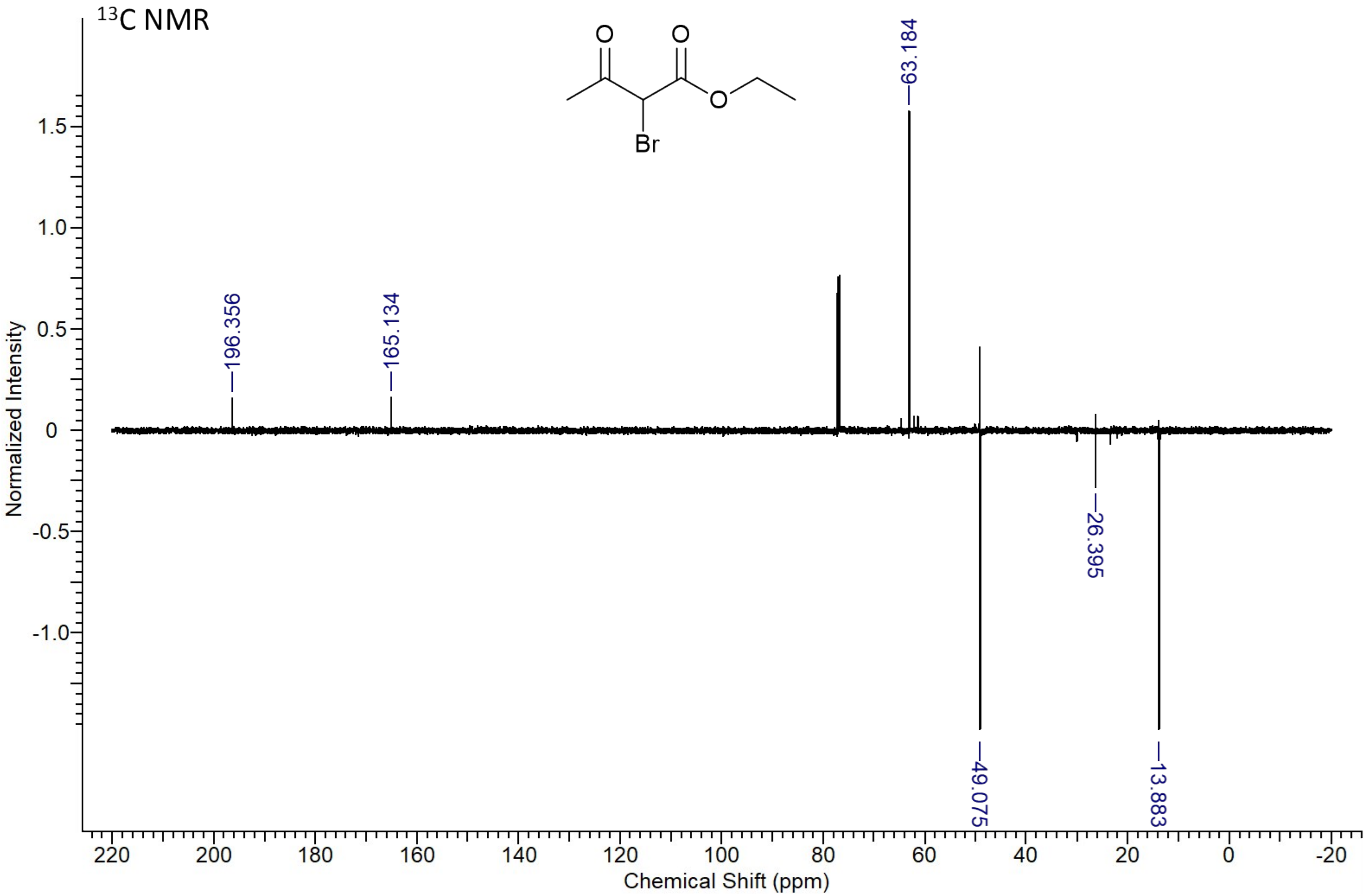




^{13}C NMR
O
O
OMe
Br
53.815
48.643
26.431
196.253
165.632
Normalized Intensity
2.0
1.5
1.0
0.5
0
-0.5
220
200
180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)

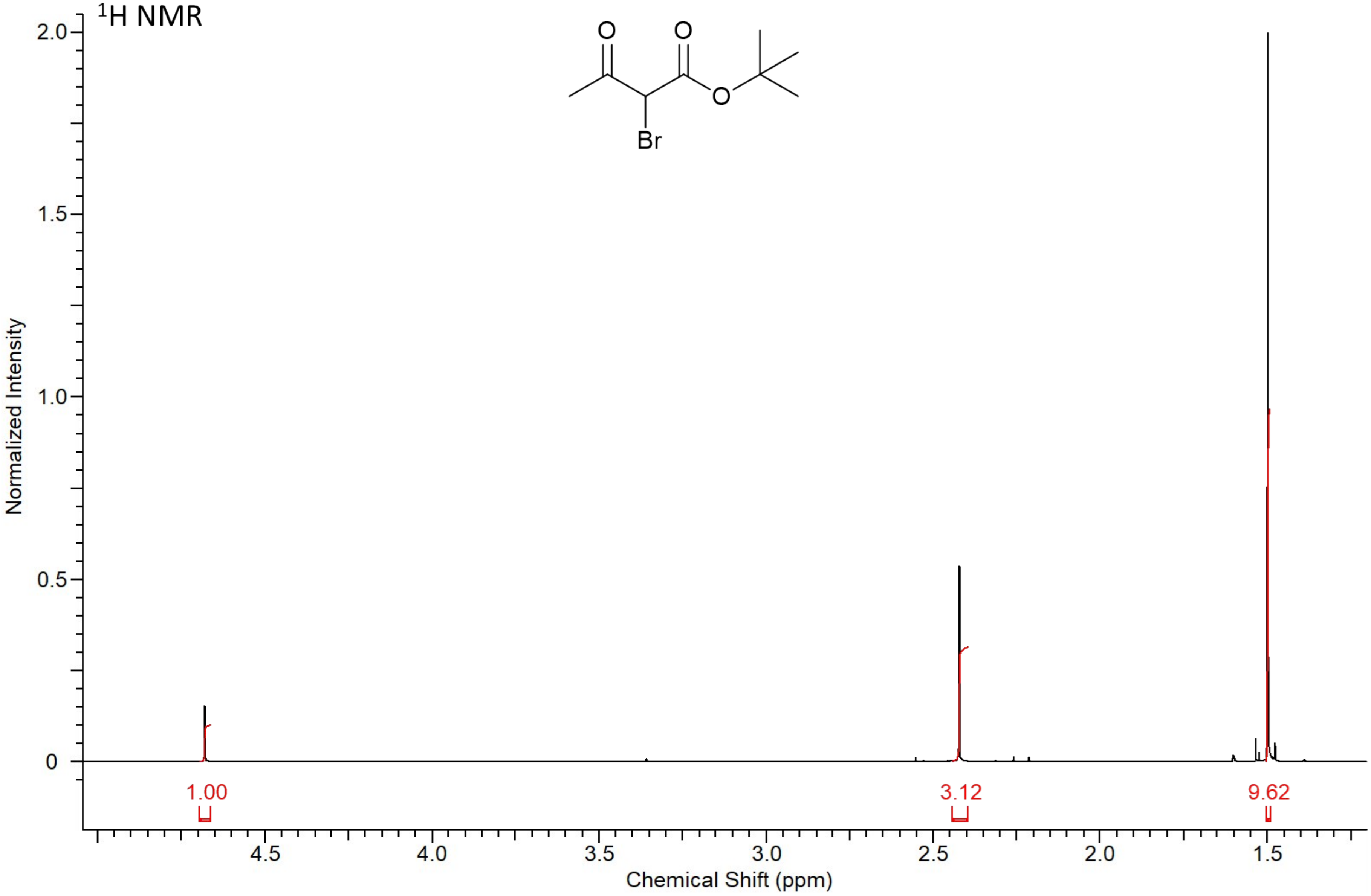
NMR spectra: compound **3b**

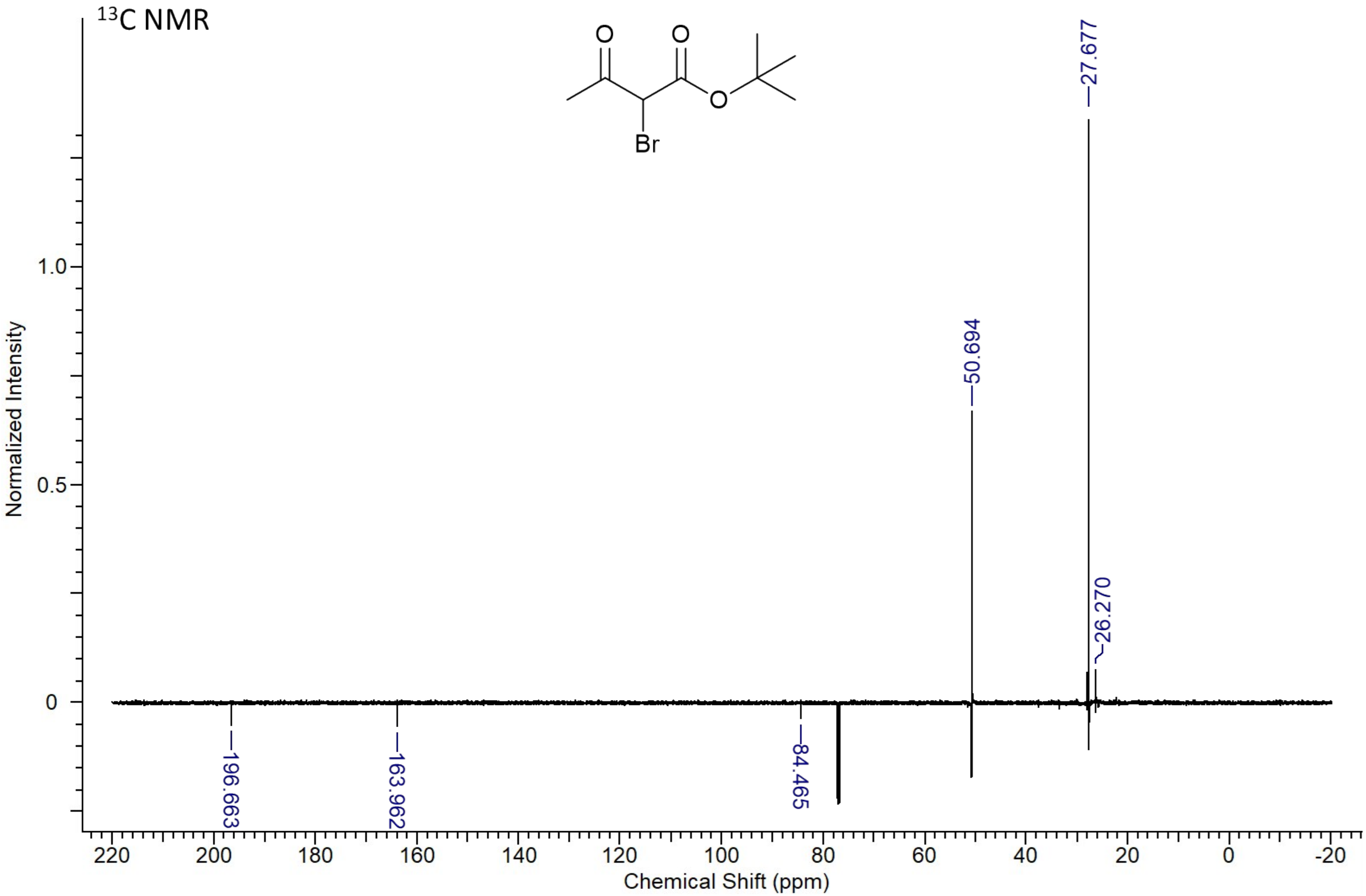




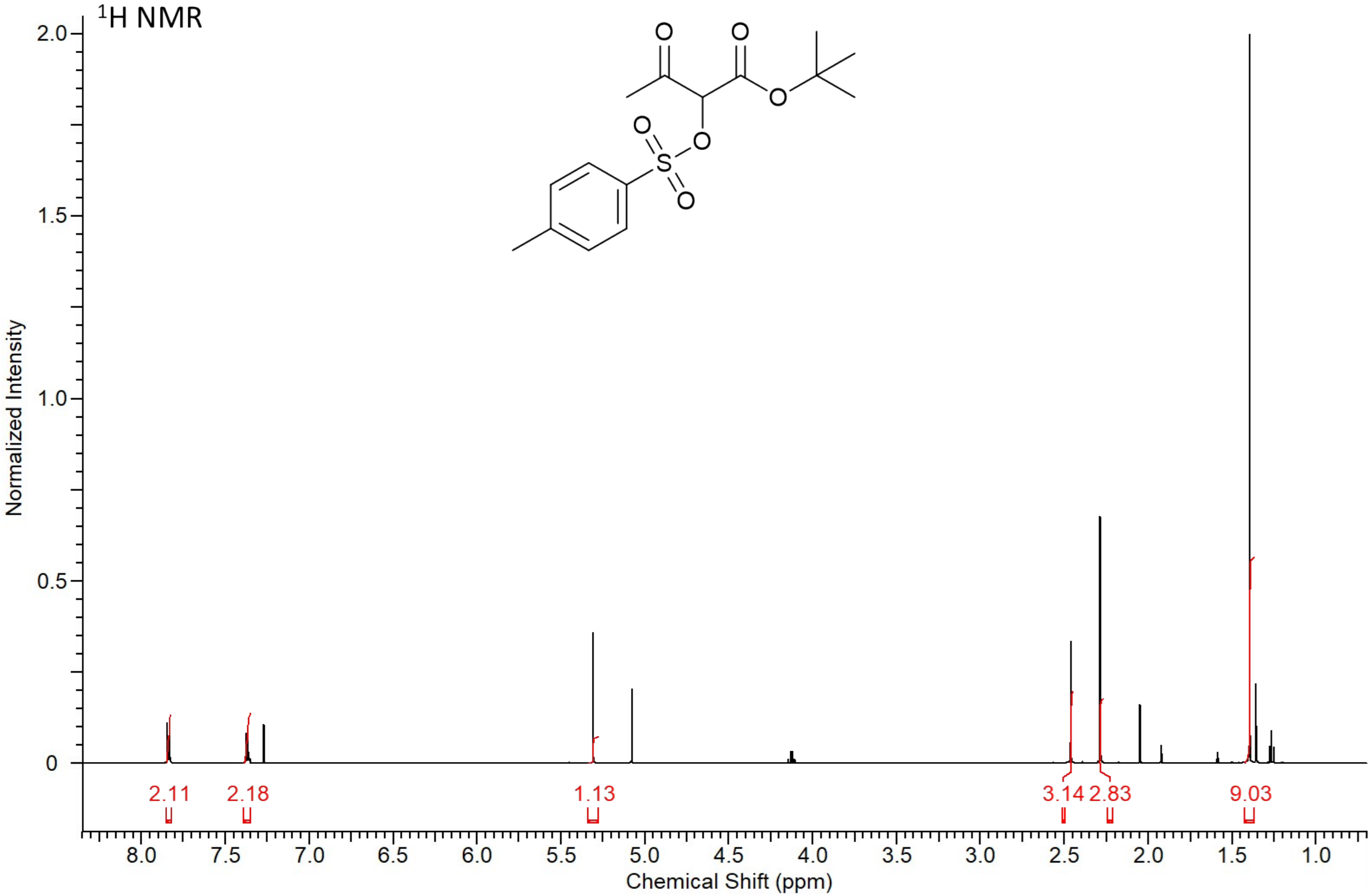
^{13}C NMR
O
O
O
Br
196.356
165.134
63.184
49.075
26.395
13.883
Normalized Intensity
1.5
1.0
0.5
0
-0.5
-1.0
220
200
180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)

NMR spectra: compound **3c**



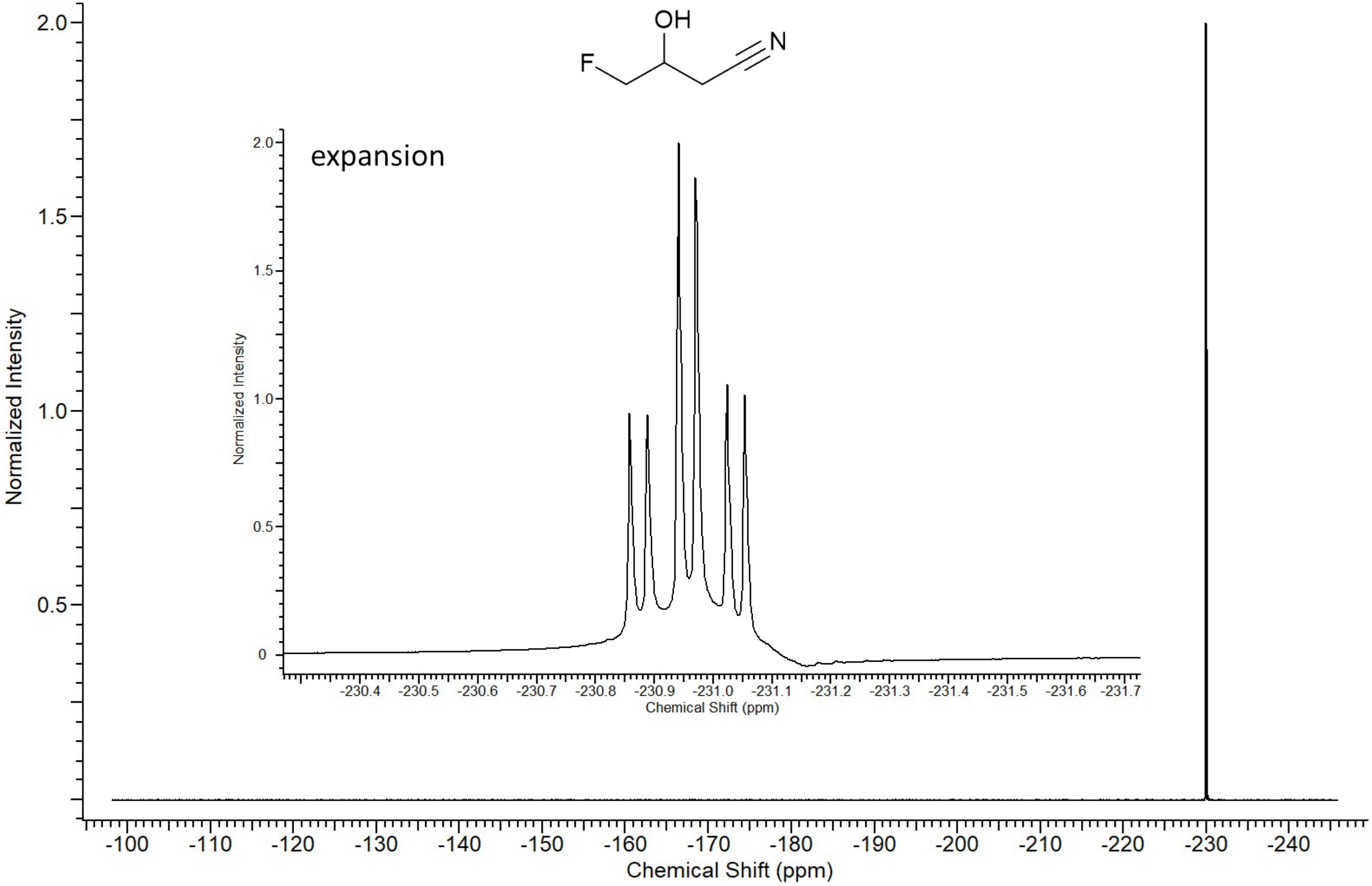

^{13}C NMR
O
O
O
Br
27.677
50.694
26.270
196.663
163.962
84.465
1.0
0.5
0
Normalized Intensity
220
200
180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)

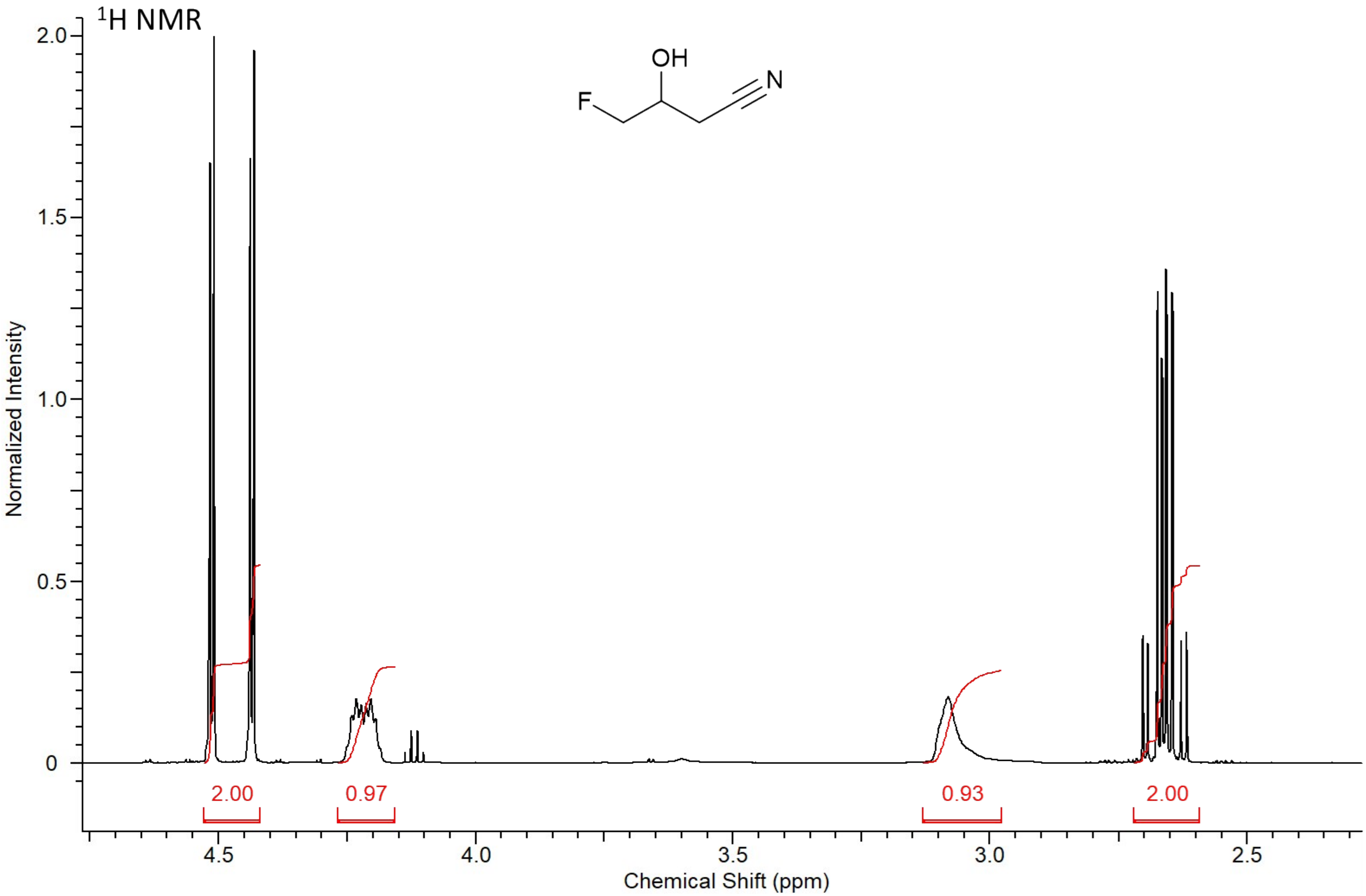
NMR spectra: compound **4**



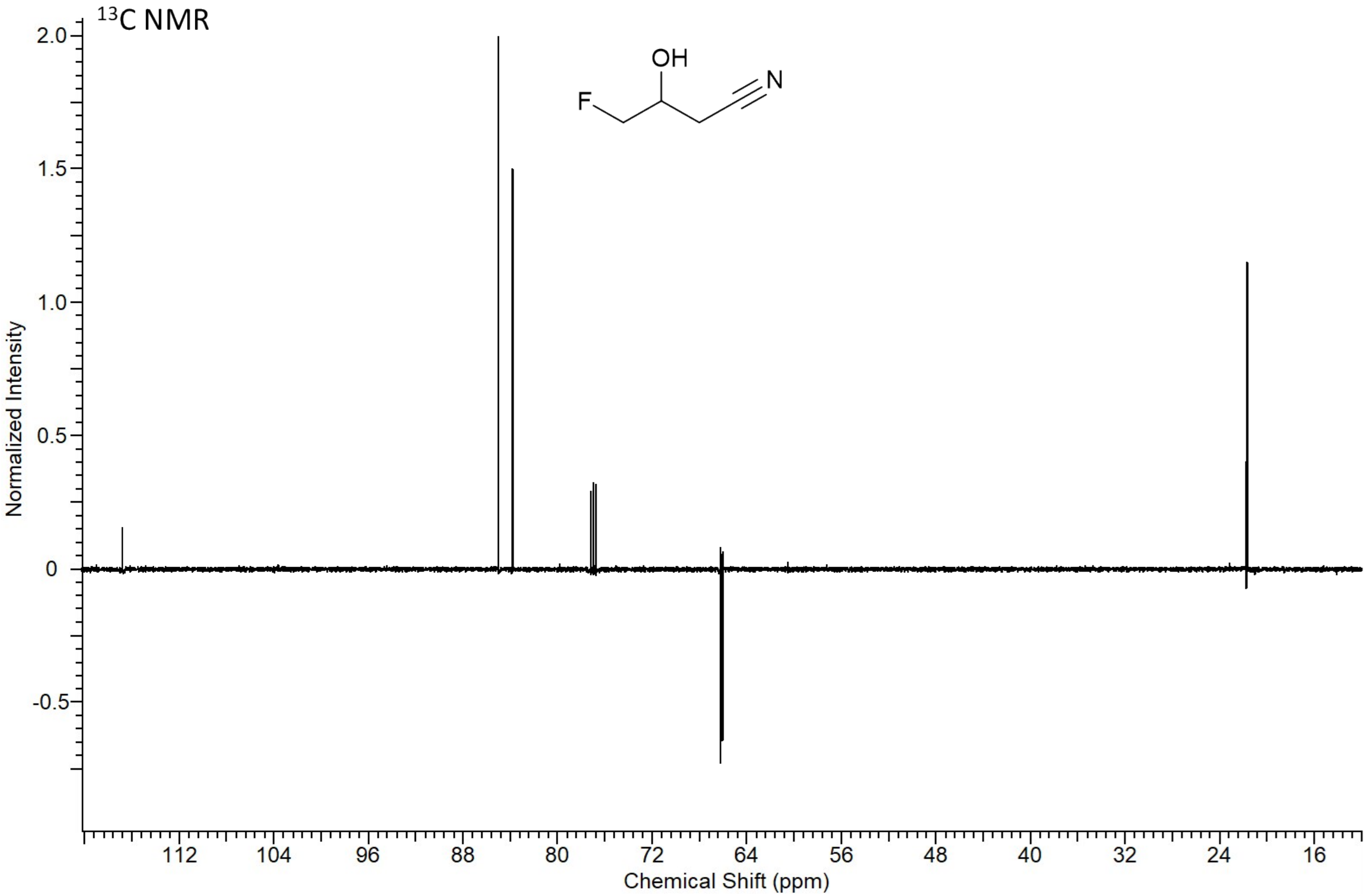
NMR spectra: nitrile reference standard $FCH_2CHOHCH_2CN$

^{19}F NMR





^{1}H NMR
OH
F
N
2.0
1.5
1.0
0.5
0
Normalized Intensity
2.00
0.97
0.93
2.00
4.5
4.0
3.5
3.0
2.5
Chemical Shift (ppm)



^{13}C NMR
OH
F
N
Normalized Intensity
2.0
1.5
1.0
0.5
0
-0.5
112
104
96
88
80
72
64
56
48
40
32
24
16
Chemical Shift (ppm)

1. Z. Li, M. Khaliq, Z. Zhou, C. B. Post, R. J. Kuhn and M. Cushman, *J. Med. Chem.*, 2008, **51**, 4660-4671.
2. A. T. Khan, M. A. Ali, P. Goswami and L. H. Choudhury, *J. Org. Chem.*, 2006, **71**, 8961-8963.
3. L. Wu and Z. Yin, *European Journal of Inorganic Chemistry*, 2013, **2013**, 6156-6163.
4. S. J. Coats and H. H. Wasserman, *Tetrahedron Lett.*, 1995, **36**, 7735-7738.
5. Y. Yamamoto, Y. Kawano, P. H. Toy and H. Togo, *Tetrahedron*, 2007, **63**, 4680-4687.
6. Y. Liu, C. Hazzard, A. S. Eustaquio, K. A. Reynolds and B. S. Moore, *J. Am. Chem. Soc.*, 2009, **131**, 10376-10377.