Synthesis of Copper Catalysts for Click Chemistry from Distillery Wastewater Using Magnetically Recoverable Bionanoparticles

Richard L. Kimber,*[,#,a] Fabio Parmeggiani,[#,b] Nimisha Joshi,[a] Alexander Rakowski,[c] Sarah J. Haigh,[c] Nicholas J. Turner,[b] Jonathan R. Lloyd [a]

[a] School of Earth and Environmental Sciences and Williamson Research Centre for Molecular Environmental Science, University of Manchester, Manchester, UK
*E-mail: richard.kimber@manchester.ac.uk

[b] School of Chemistry, Manchester Institute of Biotechnology, University of Manchester, Manchester, UK

[c] School of Materials, University of Manchester, Manchester, UK

[#] These authors contributed equally.

SUPPORTING INFORMATION

Table of contents **Page**

Supplementary Tables

Table S1. Metals detected in spent lees by ICP-AES.

	Concentration [mg/L]
Cu	49 ± 1.00
Zn	0.26 ± 0.007
Fe	0.01 ± 0.00
Mg	4.9 ± 0.13

Table S2. Characterisation of VFAs and anions present in spent lees by IC.

VFA / anion	Concentration in spent lees [mg/L]	Experimental error [± mg/L]
Lactate	33.7	1.01
Acetate	2.24	0.05
Propionate	1.75	0.02
Formate	1.23	0.02
Isobutyrate	4.03	0.06
Pyruvate	5.02	0.04
Isovalerate	3.86	0.04
Hexanoate	1.12	0.03
Heptanoate	17.19	0.54
Glutarate	2.92	0.06
Succinate	40.30	0.57
Oxalate	6.75	0.02
Citrate	9.68	0.15
Chloride	8.13	0.16
Sulphate	6.54	0.13
Phosphate	47.50	0.24

Supplementary Figures

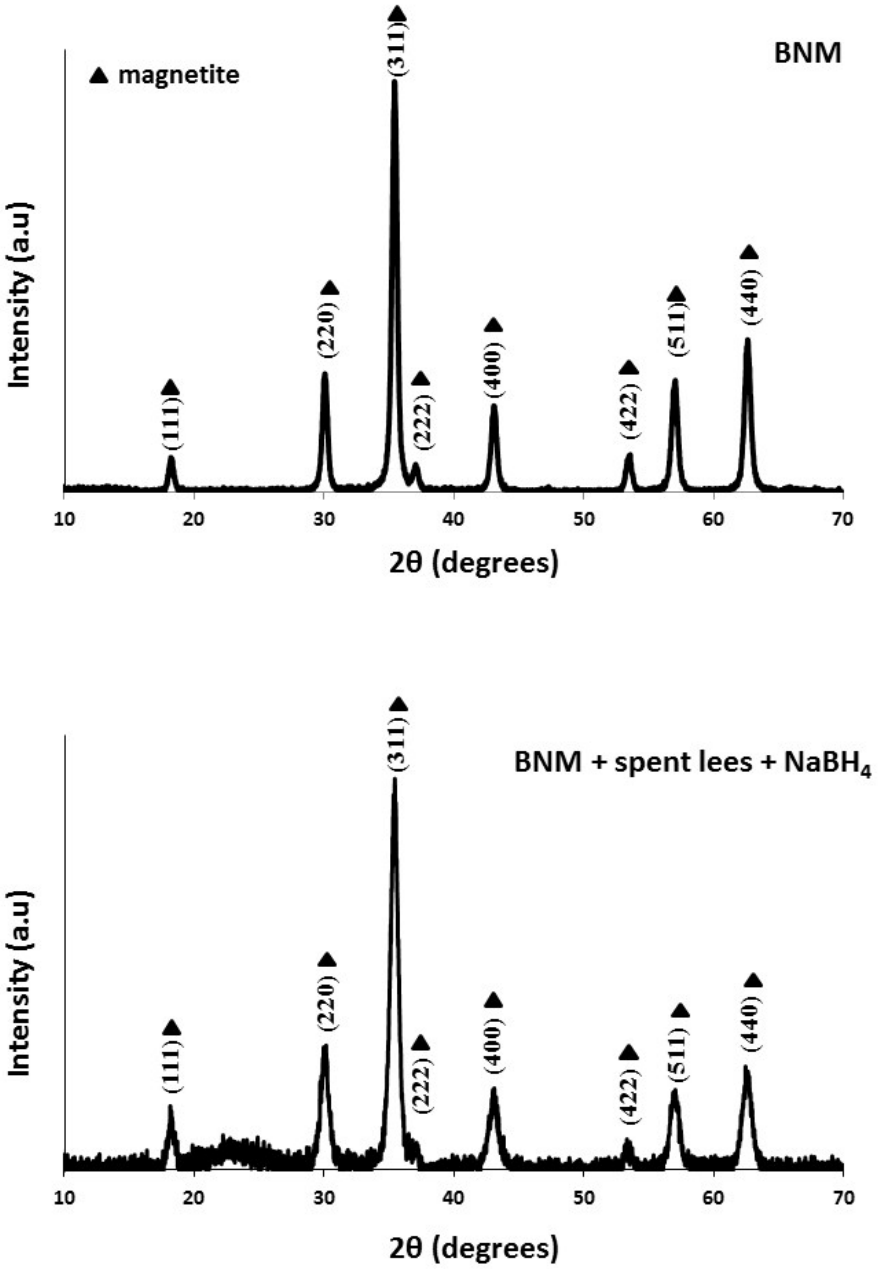


Figure S1. XRD of BNM (top) and BNM after Cu recovery from spent lees and reduction with $NaBH_4$ (bottom).

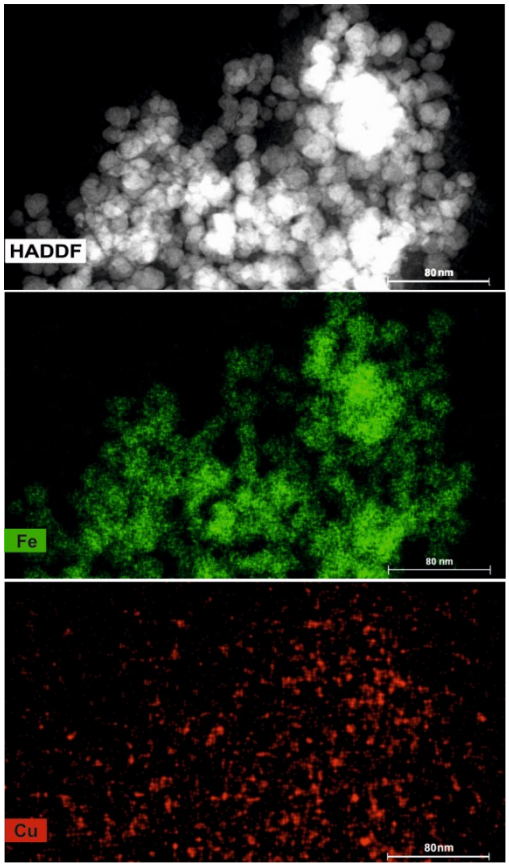


Figure S2. High-angle annular dark field (HAADF) images and corresponding elemental maps of Fe and Cu of the Cu_{salt}BNM catalyst.

Experimental methods

General methods

All reagents and solvents were purchased from Sigma Aldrich, Alfa Aesar or Fluorochem and used as received.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz) at 298 K. Chemical shifts are reported as δ in parts per million (ppm) and are calibrated against residual solvent signal.

HRMS analyses were performed using an Agilent 1200 series LC system, coupled to an Agilent 6520 QTOF mass spectrometer, ESI positive mode. The sample (2 µL) was flow-injected into 0.3 mL min^{-1} MeCN/H_2O 1:1 + formic acid 0.1% v/v. The data was analyzed using Agilent MassHunter software.

Preparation of BNM and Cu-BNM catalysts

BNM was synthesised by dissimilatory reduction of ferrihydrite by the subsurface bacterium *Geobacter sulfurreducens*[S1] following a previously reported method.[S2] Ferrihydrite was prepared by alkaline hydrolysis of a $FeCl_3 \cdot 6H_2O$ solution as described previously.[S3,S4] BNM production was verified by XRD (see Figure S1). Following synthesis, the BNM was washed twice using N_2 purged 18.2 MΩ water under anoxic conditions and stored as a stock suspension with a Fe_3O_4 (BNM) concentration of 26 g/L as confirmed by ICP-AES. The spent lees was analysed by IC and ICP-AES which confirmed a copper concentration of 49 mg/L (see Table S1). The pH and total organic carbon (TOC) were measured as pH 4.5 and 938 mg/L TOC. To prepare the Cu_{lees}BNM catalyst, the spent lees was first purged with N_2. A BNM concentration of 2.8 mg/mL spent lees was then added under anoxic conditions to a volume of up to 30 mL of spent lees. The container was sealed and placed on a rotary shaker for 1 h. Except where stated, $NaBH_4$ (10 mM) was then added to reduce the Cu. The Cu_{lees}BNM catalyst was then washed twice and resuspended in N_2 purged 18.2 MΩ water. A similar method was used to prepare the Cu_{salt}BNM catalyst, except a $CuSO_4$ solution was used instead of spent lees.

Characterization of Cu-BNM catalysts

HAADF STEM images were collected using a probe corrected FEI Titan 80-200 X-FEG super twin fitted with a super-X EDX detector system with a solid angle of ~0.8 sr. Operated with a 200 keV acceleration potential, a beam current of 90 pA, a 21 mrad convergence semi-angle, and a 54 mrad HAADF inner angle.

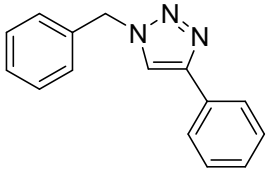
Preparative scale synthesis of triazoles with Cu-BNM

For the preparative scale CuAAC reactions, the suitable azide **1a-c** (0.25 mmol) and alkyne **2a-h** (0.25 mmol) were dissolved in *t*-BuOH (1.25 mL) and water (4.5 mL) was added. An aliquot of Cu-BNM suspension (0.5 mL, corresponding to 6.5 mg BNM and 0.16 mg Cu) was added and the mixture was incubated on a rotary shaker at room temperature for 12 h. Brine (1 mL) was added to the suspension and the product was extracted with EtOAc (3 × 5 mL). The combined organic phase was washed with brine (5 mL), dried over $MgSO_4$ and concentrated under reduced pressure. Where required, the crude product was purified by recrystallysation (from EtOH/H_2O) or by passing it through a short silica pad (eluting with cyclohexane/EtOAc 8:2). The corresponding triazoles **3a-l** were obtained in 81-97% yield (characterisation data are reported below).

A gram-scale synthesis of **3a** was performed according to the same protocol, using benzyl azide **1a** (666 mg, 5 mmol) and phenylacetylene **2a** (510 mg, 5 mmol) dissolved in *t*-BuOH (20 mL). Water (75 mL) was added, followed by Cu_{lees}BNM suspension (5 mL, corresponding to 65 mg BNM and 0.8 mg Cu) and the mixture was stirred vigorously on a magnetic stirrer at room temperature for 12 h. The product was isolated as reported in the procedure above, yielding 960 mg (82% yield) of **3a**.

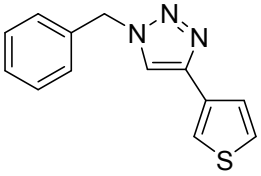
Characterisation data of triazoles 3a-l

1-Benzyl-4-phenyl-1*H*-1,2,3-triazole (3a)



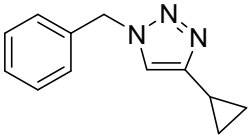
White crystals, 53 mg (91% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73-7.68 (m, 2H), 7.59 (s, 1H), 7.33-7.26 (m, 5H), 7.24-7.17 (m, 3H), 5.47 (s, 2H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 148.2, 134.8, 130.6, 129.2, 128.9, 128.2, 128.1, 125.8, 119.6, 54.3.
HRMS (ESI): m/z for $C_{15}H_{14}N_3^+$ $[M+H]^+$ calcd. 236.1182, found 236.1168.

1-Benzyl-4-(thiophen-3-yl)-1*H*-1,2,3-triazole (3b)



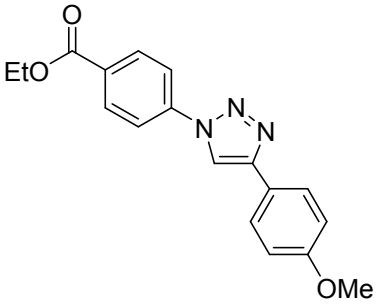
Pale brown crystals, 56 mg (94% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (dd, J = 2.9, 1.2 Hz, 1H), 7.56 (s, 1H), 7.41 (dd, J = 5.0, 1.2 Hz, 1H), 7.40-7.33 (m, 4H), 7.32-7.27 (m, 2H), 5.56 (s, 2H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 144.5, 134.8, 131.9, 129.7, 128.9, 128.2, 126.4, 125.9, 121.2, 119.4, 54.3.
HRMS (ESI): m/z for $C_{13}H_{12}N_3S^+$ $[M+H]^+$ calcd. 242.0746, found 242.0711.

1-Benzyl-4-cyclopropyl-1*H*-1,2,3-triazole (3c)



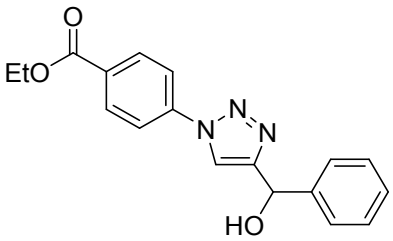
White solid, 40 mg (81% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32-7.23 (m, 3H), 7.21-7.14 (m, 2H), 7.06 (s, 1H), 5.38 (s, 2H), 1.88-1.79 (m, 1H), 0.88-0.80 (m, 2H), 0.77-0.71 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 150.7, 134.9, 129.0, 128.6, 128.0, 119.6, 54.0, 7.7, 6.7.
HRMS (ESI): m/z for $C_{12}H_{14}N_3^+$ $[M+H]^+$ calcd. 200.1182, found 200.1166.

Ethyl 4-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzoate (3d)



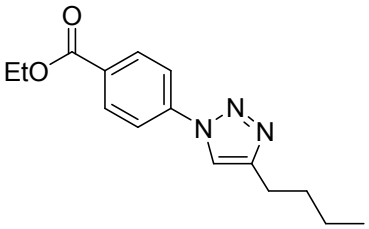
Pale yellow crystals, 70 mg (87% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25-8.19 (m, 2H), 8.18 (s, 1H), 7.92-7.87 (m, 2H), 7.86-7.81 (m, 2H), 7.02-6.97 (m, 2H), 4.42 (q, J = 7.1 Hz, 2H), 3.86 (s, 3H), 1.43 (t, J = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 165.6, 160.1, 148.8, 140.2, 131.4, 130.6, 127.4, 122.7, 119.9, 116.5, 114.5, 61.6, 55.5, 14.5.
HRMS (ESI): m/z for $C_{18}H_{18}N_3O_3^+$ $[M+H]^+$ calcd. 324.1343, found 324.1361.

Ethyl 4-(4-(hydroxy(phenyl)methyl)-1*H*-1,2,3-triazol-1-yl)-benzoate (3e)



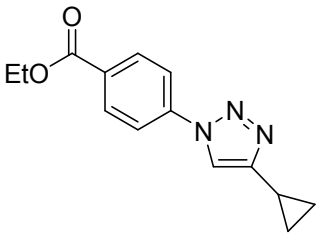
Pale yellow crystals, 69 mg (85% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17-8.13 (m, 2H), 7.79 (s, 1H), 7.78-7.74 (m, 2H), 7.52-7.49 (m, 2H), 7.41-7.36 (m, 2H), 7.35-7.30 (m, 1H), 6.12 (s, 1H), 4.40 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 165.5, 152.6, 141.7, 140.0, 131.3, 130.7, 128.8, 128.3, 126.5, 120.0, 119.4, 69.3, 61.6, 14.4.
HRMS (ESI): m/z for $C_{18}H_{18}N_3O_3^+$ $[M+H]^+$ calcd. 324.1343, found 324.1377.

Ethyl 4-(4-butyl-1*H*-1,2,3-triazol-1-yl)benzoate (3f)



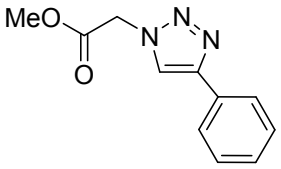
Colourless crystals, 56 mg (82% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21-8.15 (m, 2H), 7.84-7.80 (m, 2H), 7.78 (s, 1H), 4.40 (q, J = 7.1 Hz, 2H), 2.80 (t, J = 7.6 Hz, 2H), 1.78-1.67 (m, 2H), 1.48-1.37 (m, 2H), 1.41, (t, J = 7.2 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 165.6, 149.7, 140.4, 131.5, 130.3, 119.7, 118.7, 61.5, 31.5, 25.4, 22.4, 14.4, 13.9.
HRMS (ESI): m/z for $C_{15}H_{20}N_3O_2^+$ $[M+H]^+$ calcd. 274.1550, found 274.1540.

Ethyl 4-(4-cyclopropyl-1*H*-1,2,3-triazol-1-yl)benzoate (3g)



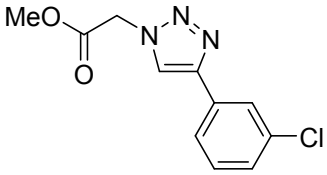
Pale yellow crystals, 62 mg (96% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17-8.13 (m, 2H), 7.81-7.76 (m, 2H), 7.76 (s, 1H), 4.38 (q, J = 7.1 Hz, 2H), 2.05-1.97 (m, 1H), 1.39 (t, J = 7.1 Hz, 3H), 1.03-0.95 (m, 2H), 0.94-0.88 (m, 2H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 165.6, 151.5, 140.2, 131.3, 130.3, 119.6, 117.8, 61.5, 14.4, 8.0, 6.8.
HRMS (ESI): m/z for $C_{14}H_{16}N_3O_2^+$ $[M+H]^+$ calcd. 258.1237, found 258.1243.

Methyl 2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetate (3h)



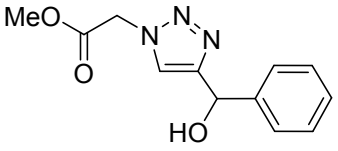
White solid, 46 mg (84% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (s, 1H), 7.86-7.81 (m, 2H), 7.45-7.40 (m, 2H), 7.37-7.31 (m, 1H), 5.22 (s, 2H), 3.81 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 166.7, 148.3, 130.3, 128.9, 128.3, 125.8, 121.0, 53.1, 50.8.
HRMS (ESI): m/z for $C_{11}H_{12}N_3O_2^+$ $[M+H]^+$ calcd. 218.0924, found 218.0940.

Methyl 2-(4-(3-chlorophenyl)-1*H*-1,2,3-triazol-1-yl)acetate (3i)



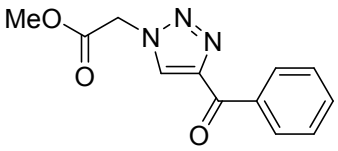
Colourless crystals, 58 mg (91% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H), 7.84 (t, J = 1.7 Hz, 1H), 7.72 (dt, J = 7.5, 1.5 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 7.31 (dt, J = 7.9, 1.5 Hz, 1H), 5.23 (s, 2H), 3.83 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 166.7, 147.2, 134.9, 132.2, 130.3, 128.4, 126.0, 124.0, 121.5, 53.3, 51.0.
HRMS (ESI): m/z for $C_{11}H_{11}ClN_3O_2^+$ $[M+H]^+$ calcd. 252.0534, found 252.0543.

Methyl 2-(4-(hydroxy(phenyl)methyl)-1*H*-1,2,3-triazol-1-yl)-acetate (3j)



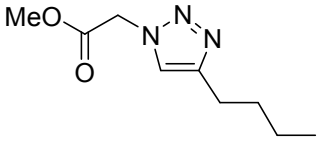
Pale yellow oil, 60 mg (97% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.33 (m, 2H), 7.32 (s, 1H), 7.30-7.24 (m, 2H), 7.24-7.19 (m, 1H), 5.93 (s, 1H), 5.00 (s, 2H), 3.68 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 166.8, 151.9, 141.9, 128.6, 128.0, 125.6, 122.9, 69.1, 53.1, 50.8.
HRMS (ESI): m/z for $C_{12}H_{14}N_3O_3^+$ $[M+H]^+$ calcd. 248.1030, found 248.1015.

Methyl 2-(4-benzoyl-1*H*-1,2,3-triazol-1-yl)acetate (3k)



White crystals, 52 mg (84% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43-8.93 (m, 2H), 8.42 (s, 1H), 7.64-7.59 (m, 2H), 7.55-7.49 (m, 1H), 5.28 (s, 2H), 3.83 (s, 3H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 185.6, 166.2, 136.6, 133.5, 130.7, 130.0, 128.5, 53.4, 51.0.
HRMS (ESI): m/z for $C_{12}H_{12}N_3O_3^+$ $[M+H]^+$ calcd. 246.0873, found 246.0894.

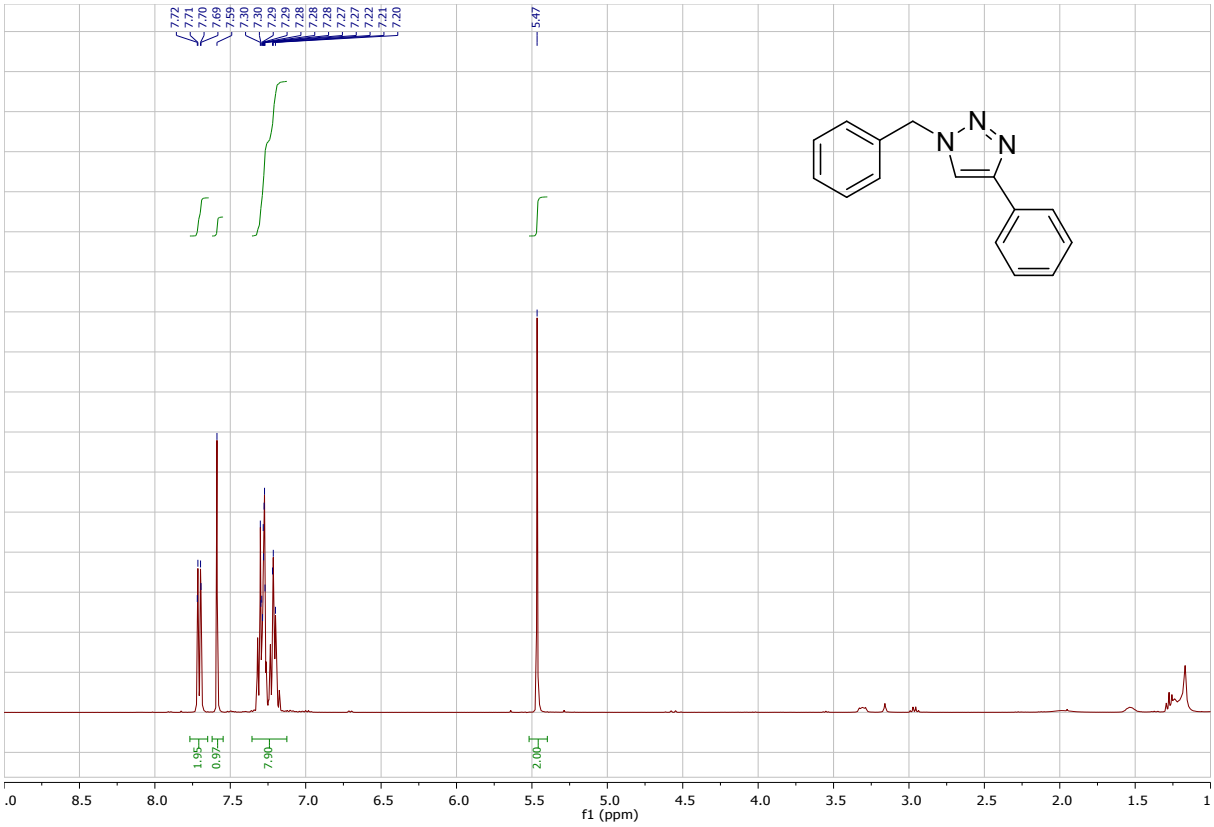
Methyl 2-(4-butyl-1*H*-1,2,3-triazol-1-yl)acetate (3l)

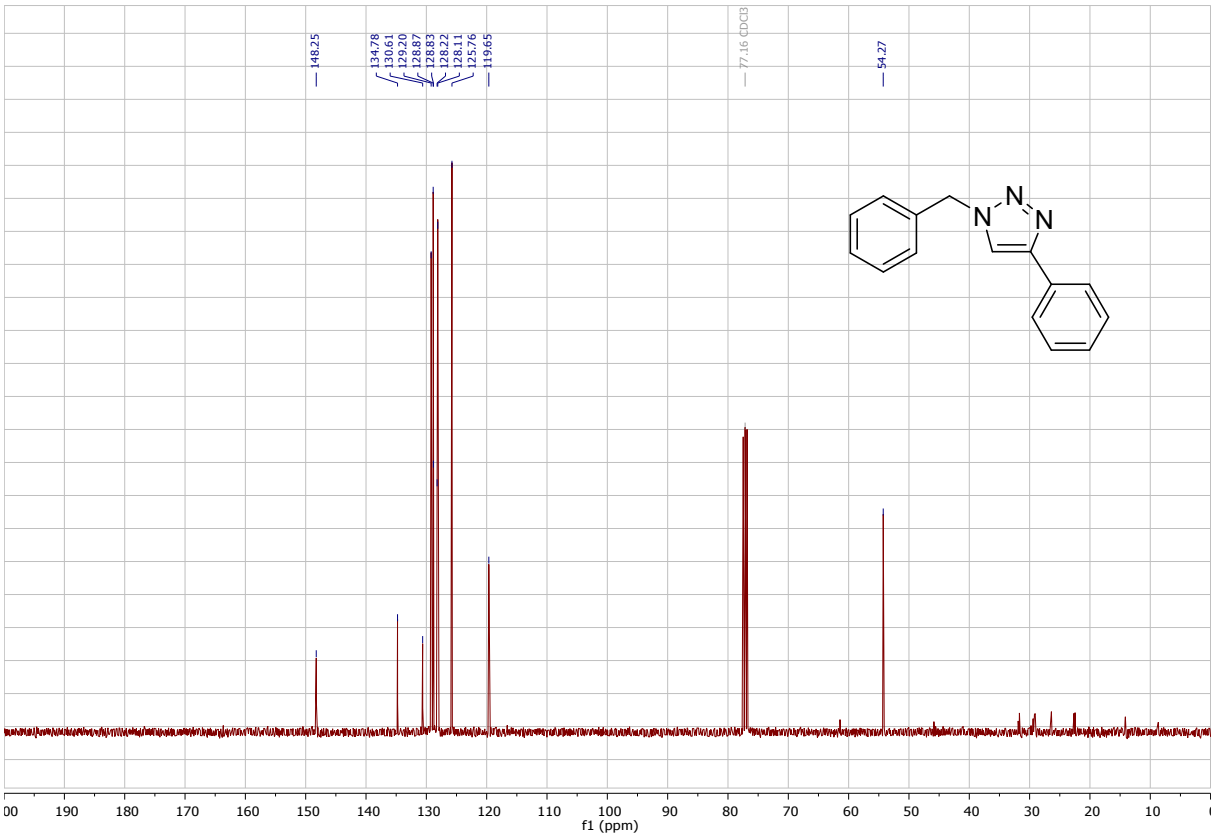


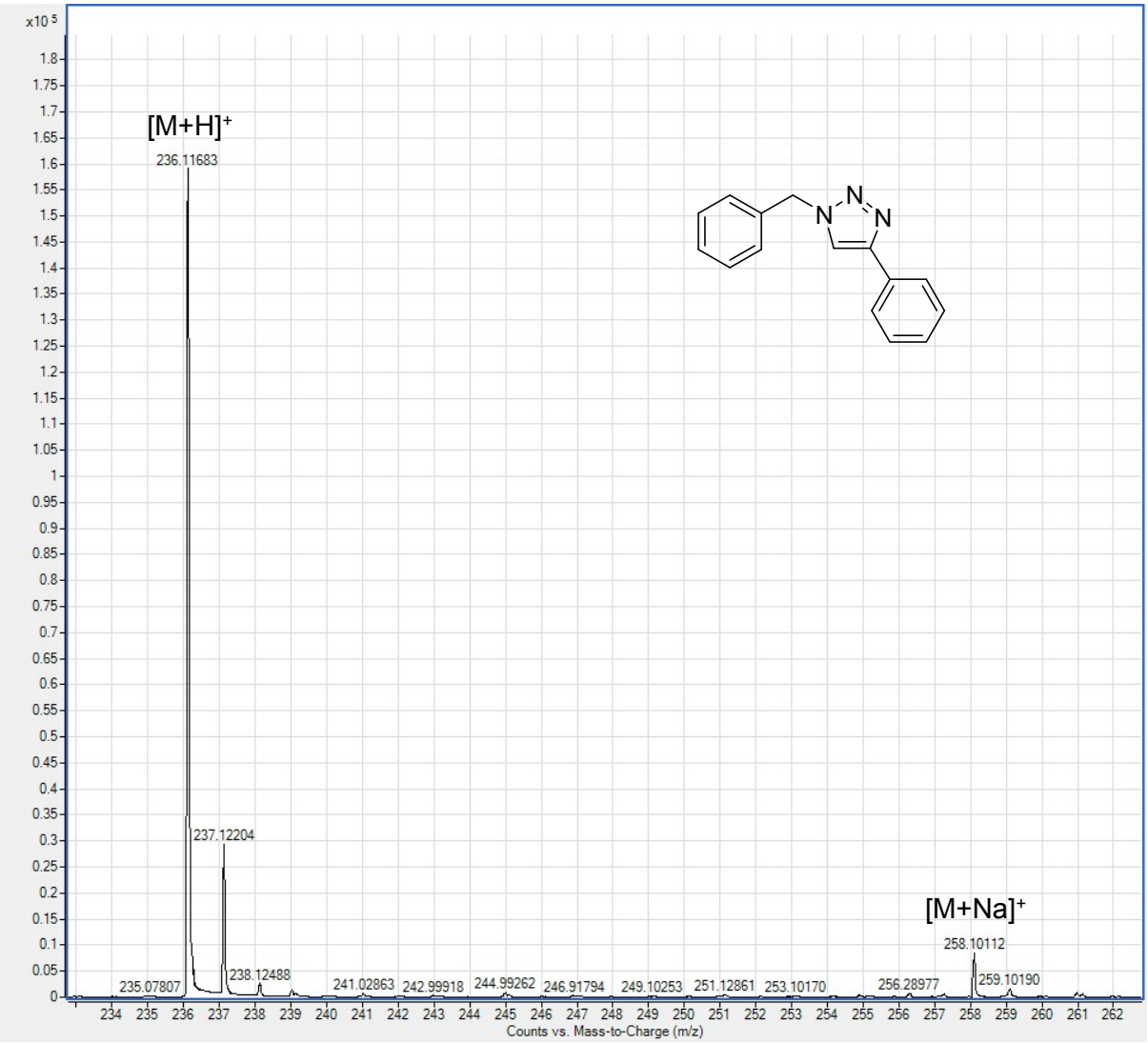
Colourless oil, 46 mg (93% isol. yield).
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (s, 1H), 5.13 (s, 2H), 3.80 (s, 3H), 2.73 (t, J = 7.7 Hz, 2H), 1.71-1.61 (m, 2H), 1.43-1.33 (m, 2H), 0.92 (t, J = 7.3 Hz, 3H).
^{13}C NMR (101 MHz, $CDCl_3$): δ 167.1, 149.1, 122.0, 53.1, 50.7, 31.5, 25.4, 22.4, 13.9.
HRMS (ESI): m/z for $C_9H_{16}N_3O_2^+$ $[M+H]^+$ calcd. 198.1237, found 198.1212.

Copies of NMR and HRMS spectra

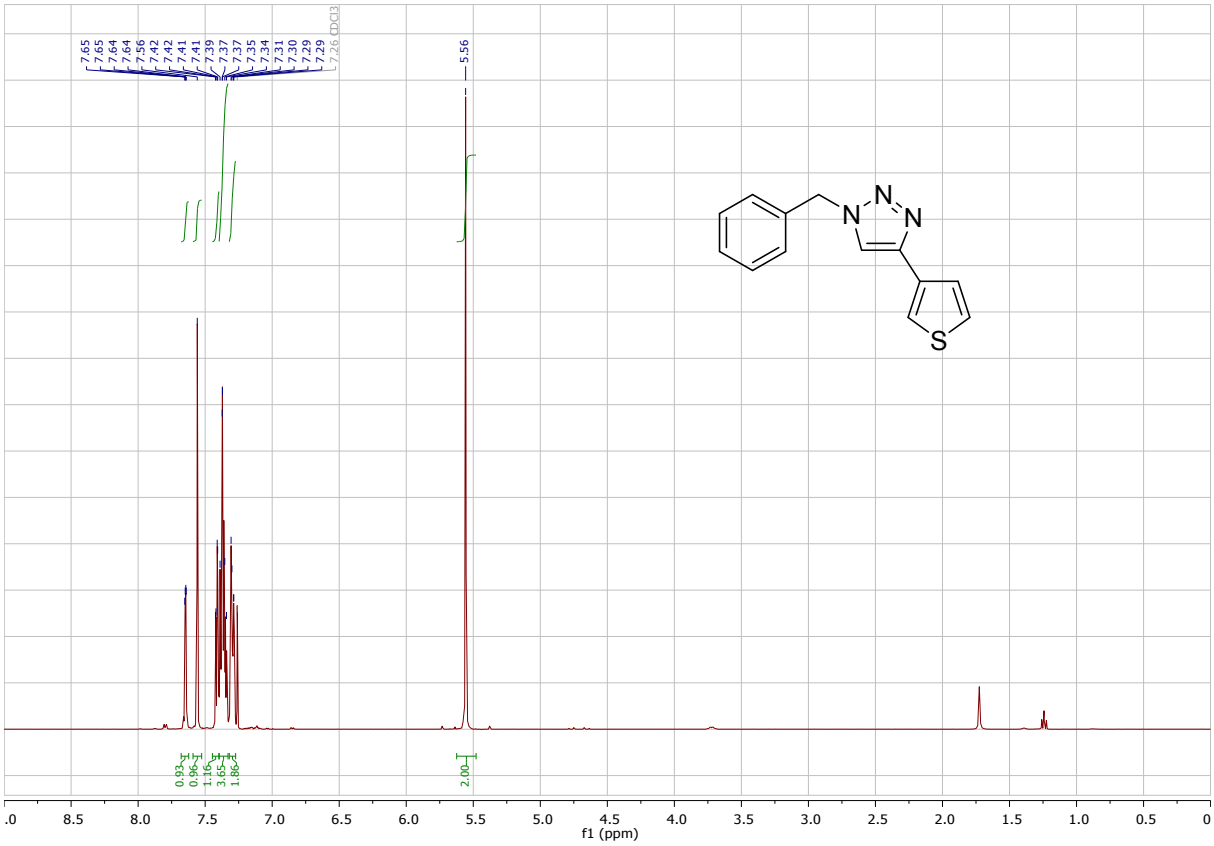
1-Benzyl-4-phenyl-1*H*-1,2,3-triazole (3a)

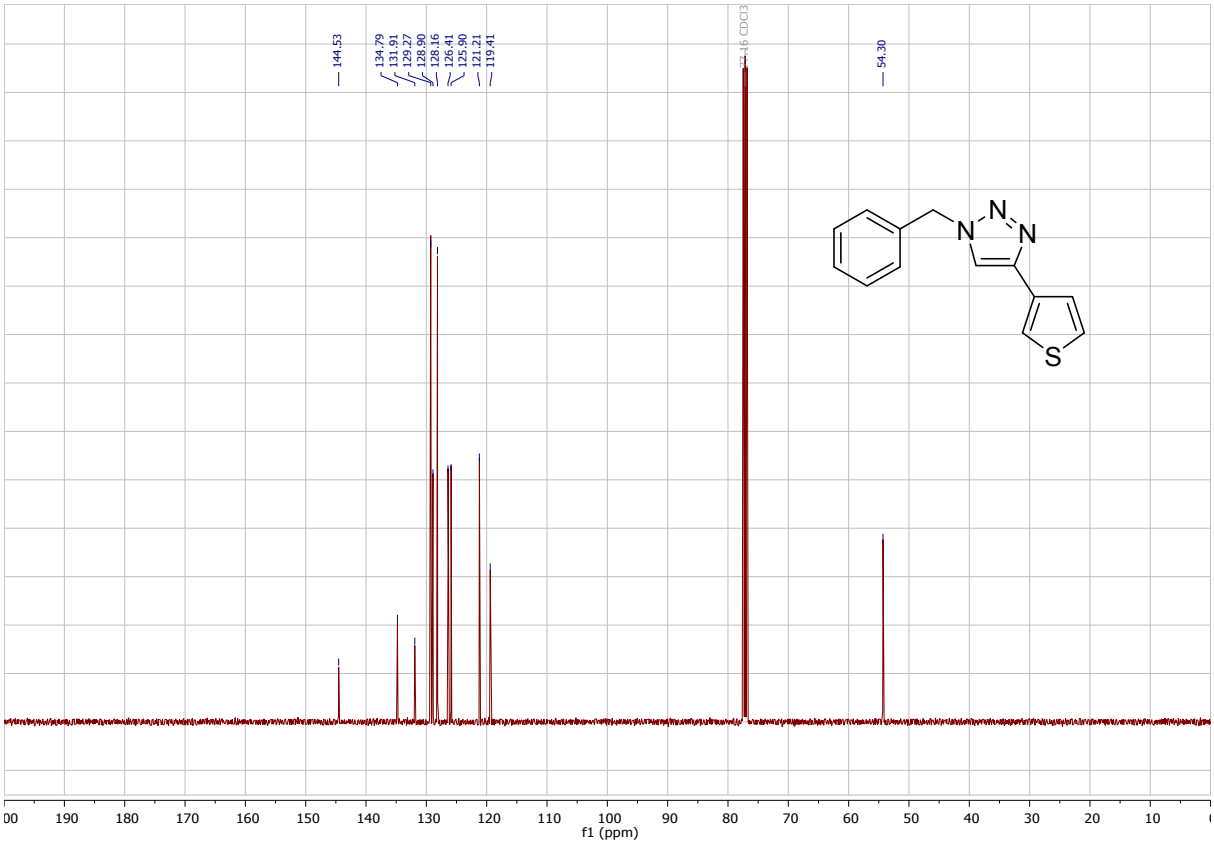


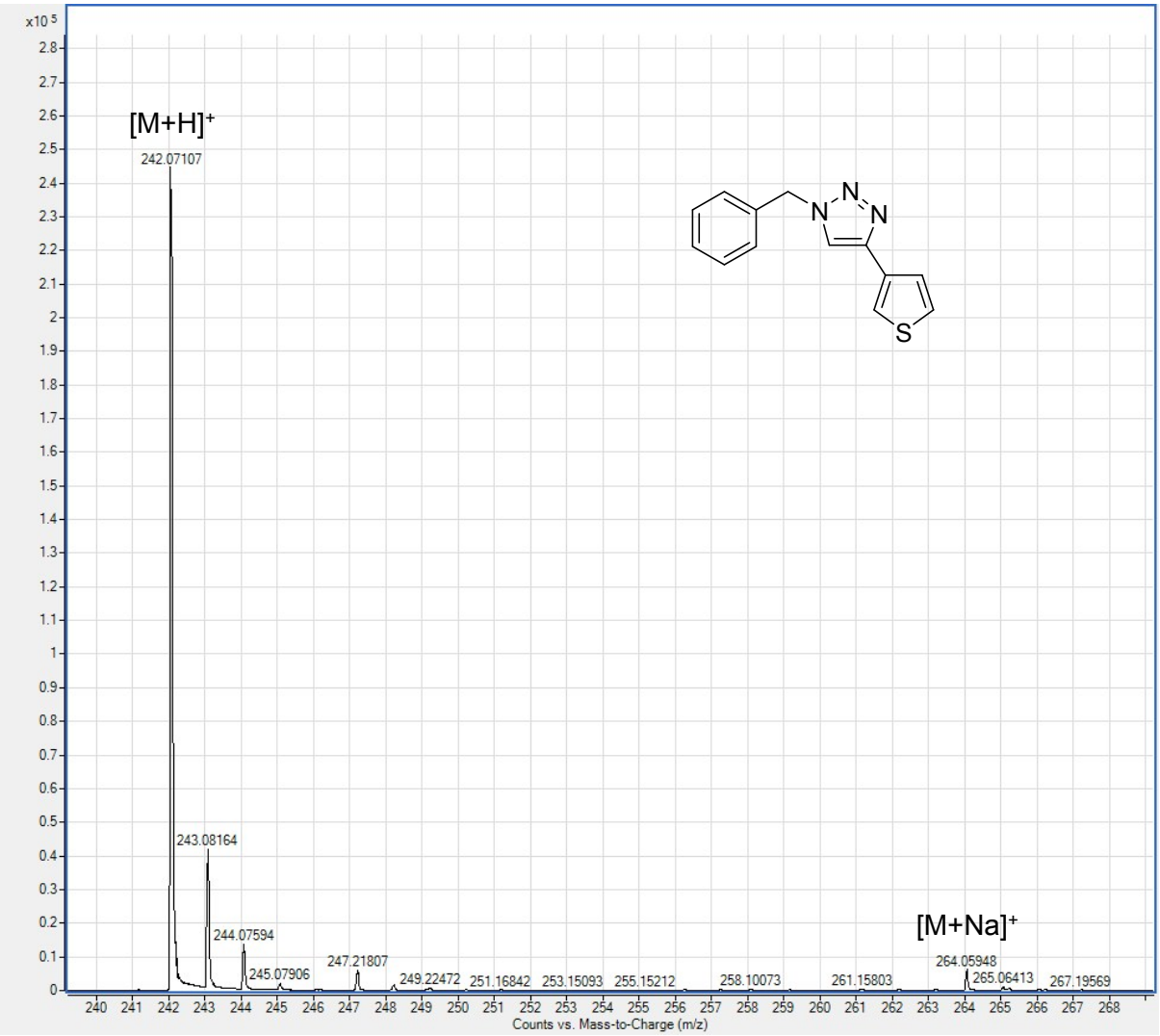



x10 5
[M+H]+
236.11683
237.12204
238.12488
235.07807
241.02863
242.99918
244.99262
246.91794
249.10253
251.12861
253.10170
256.28977
[M+Na]+
258.10112
259.10190
Counts vs. Mass-to-Charge (m/z)

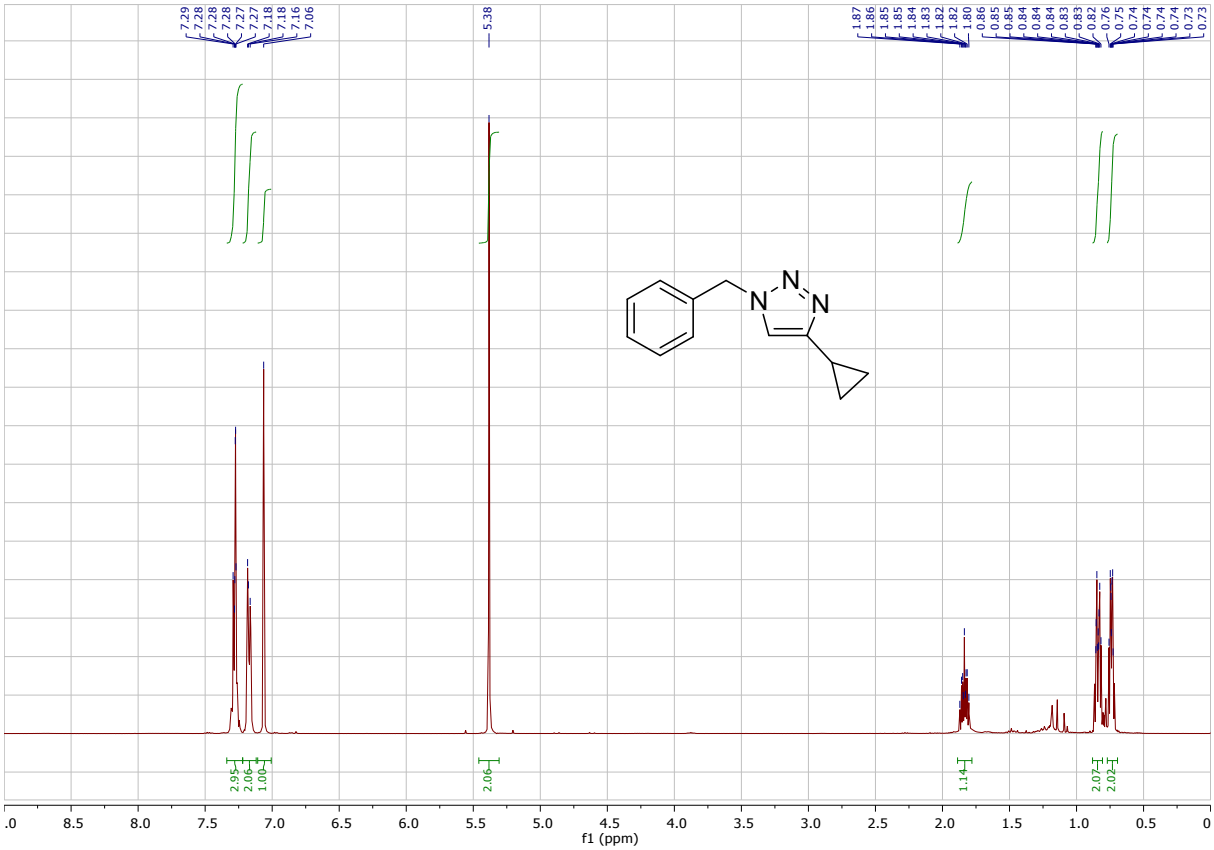
1-Benzyl-4-(thiophen-3-yl)-1*H*-1,2,3-triazole (3b)

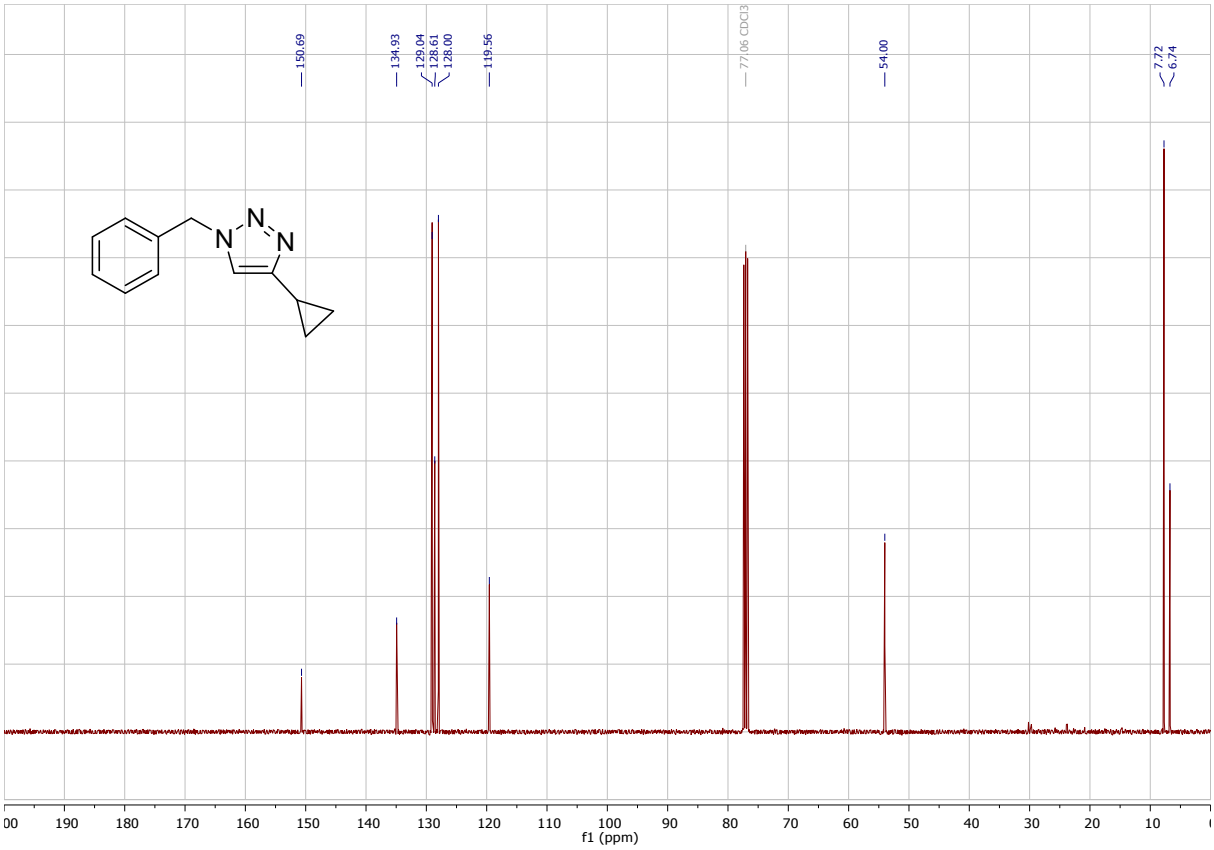


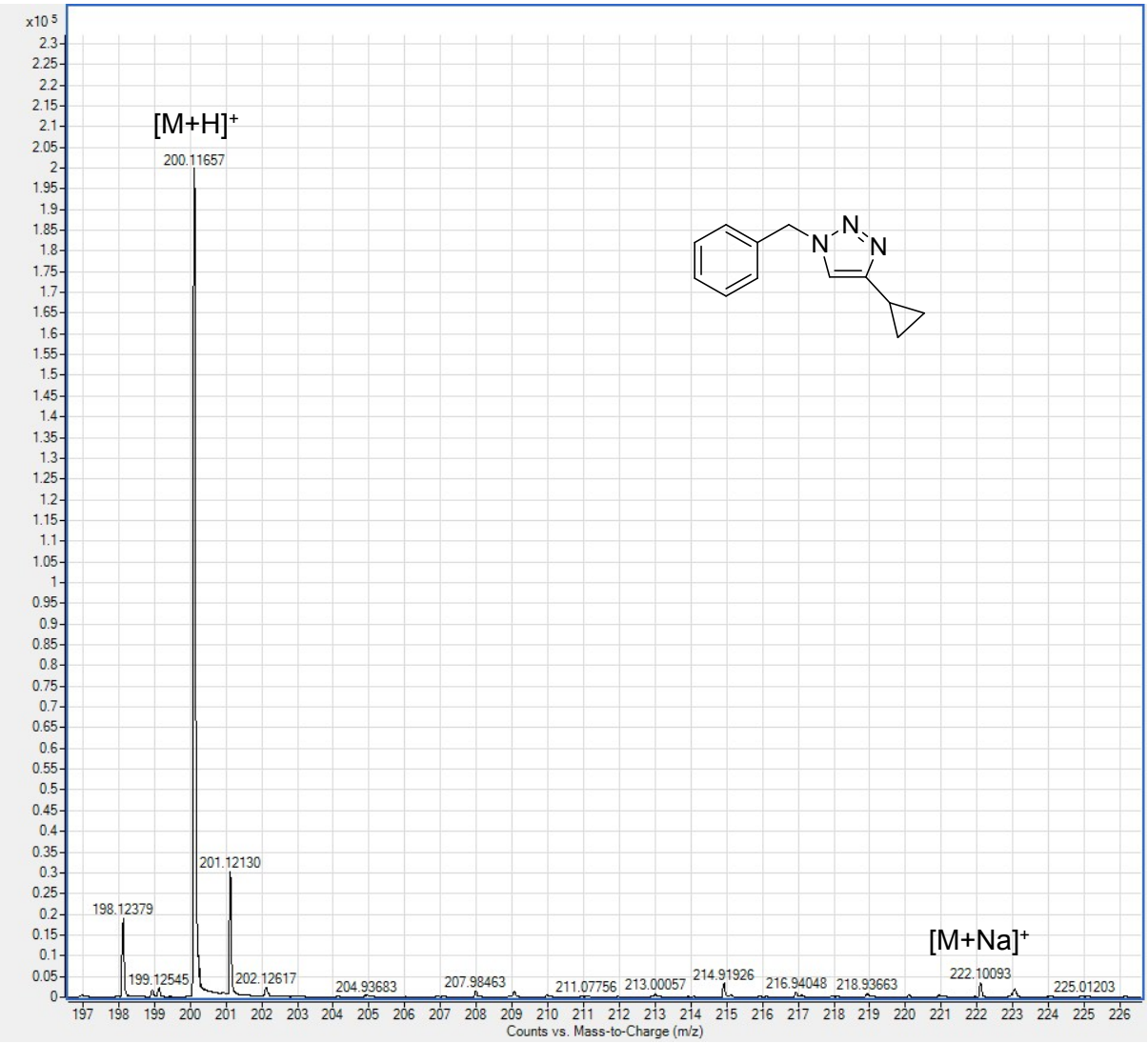



x10 5
[M+H]+
242.07107
243.08164
244.07594
245.07906
247.21807
249.22472
251.16842
253.15093
255.15212
258.10073
261.15803
[M+Na]+
264.05948
265.06413
267.19569
Counts vs. Mass-to-Charge (m/z)

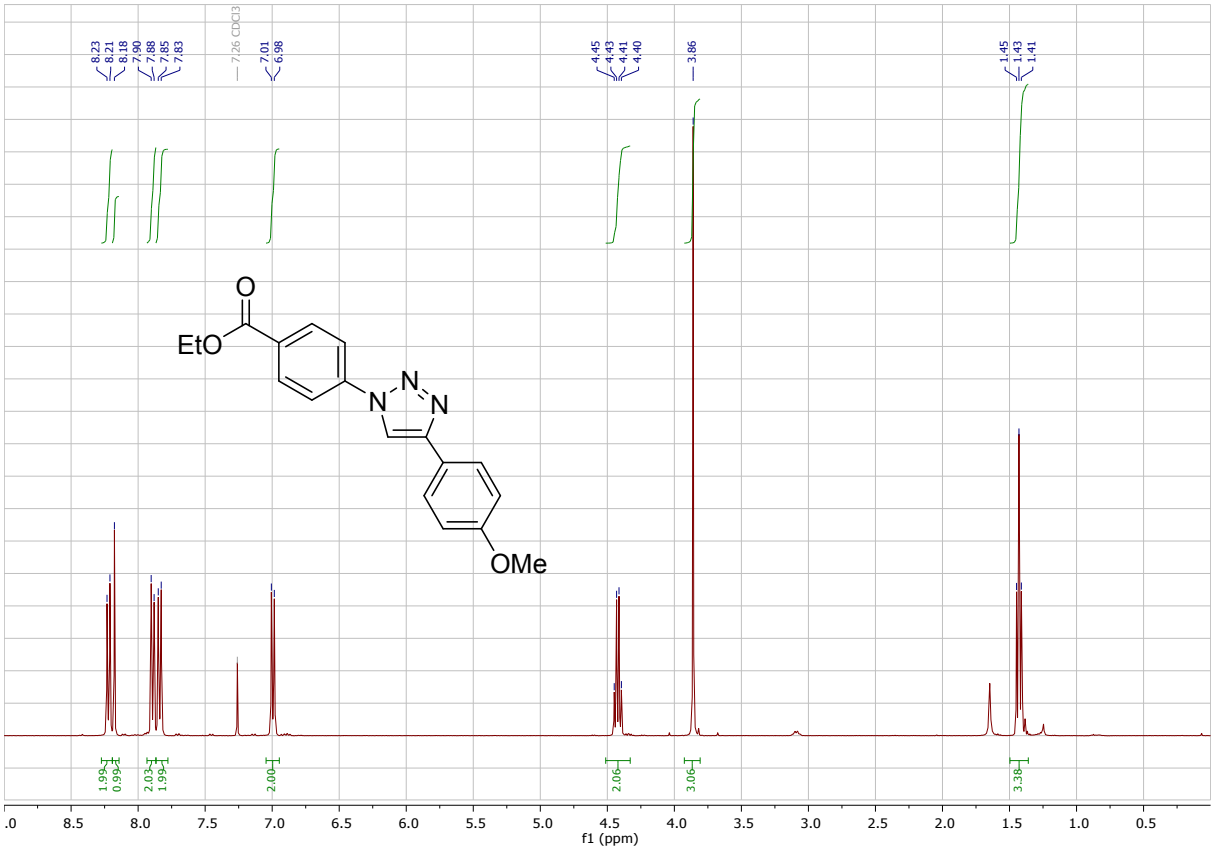
1-Benzyl-4-cyclopropyl-1*H*-1,2,3-triazole (3c)

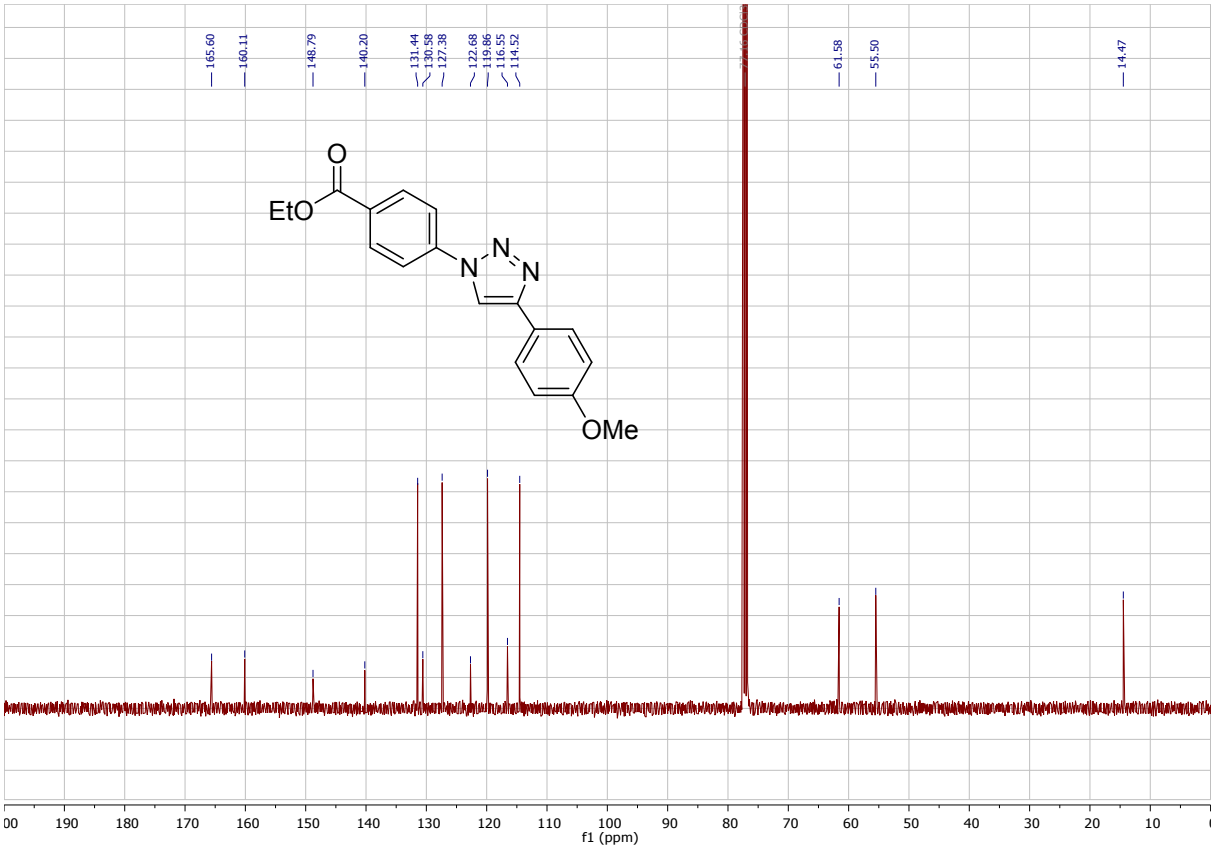


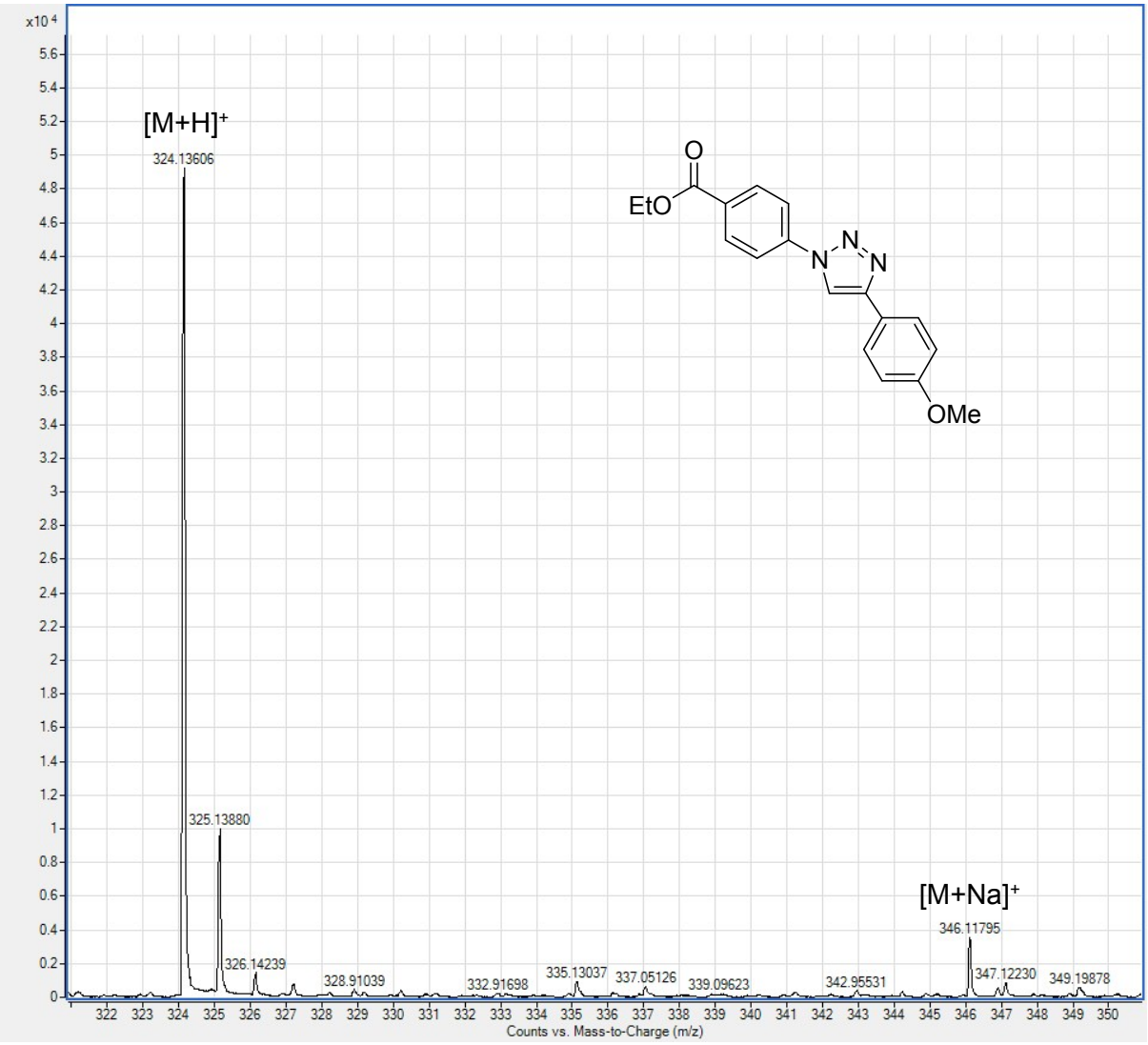



x10 5
[M+H]+
200.11657
201.12130
198.12379
199.12545
202.12617
204.93683
207.98463
211.07756
213.00057
214.91926
216.94048
218.93663
222.10093
225.01203
[M+Na]+
Counts vs. Mass-to-Charge (m/z)

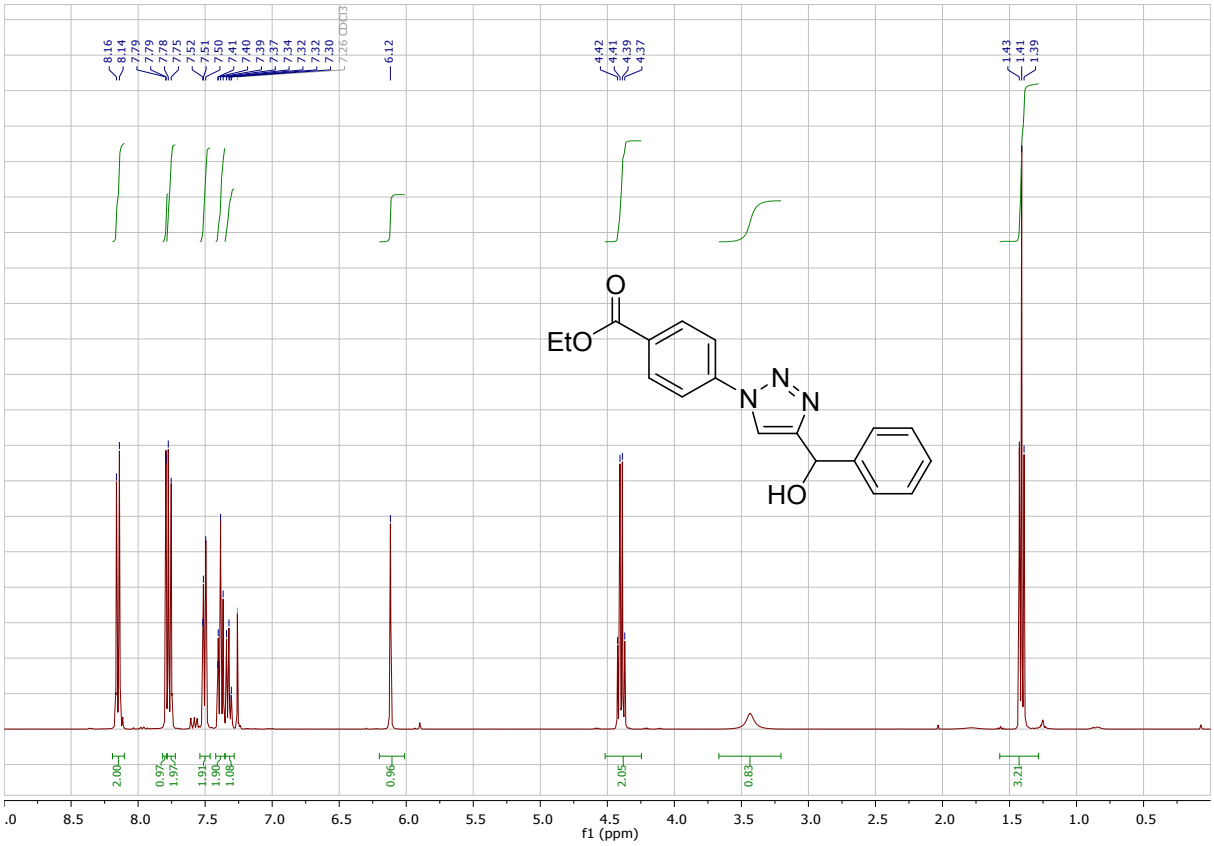
Ethyl 4-(4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl)benzoate (3d)

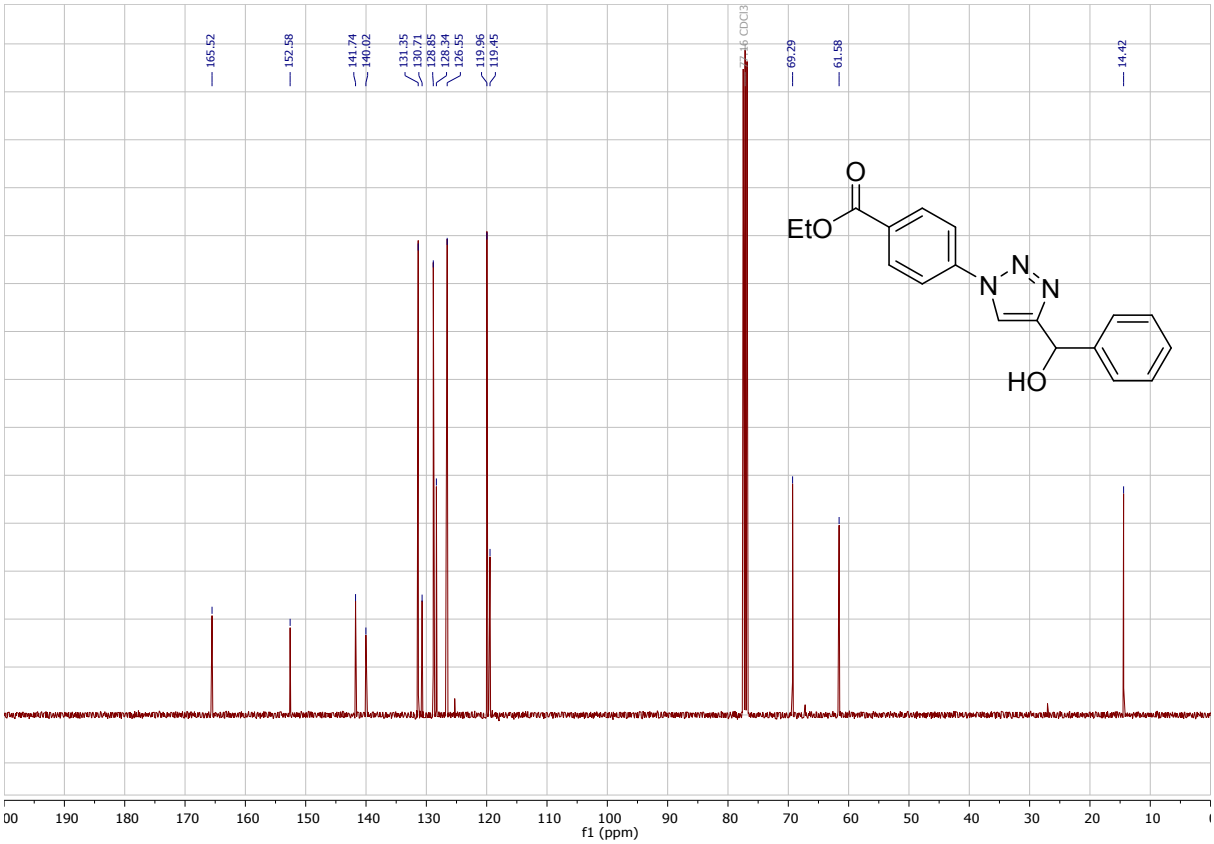


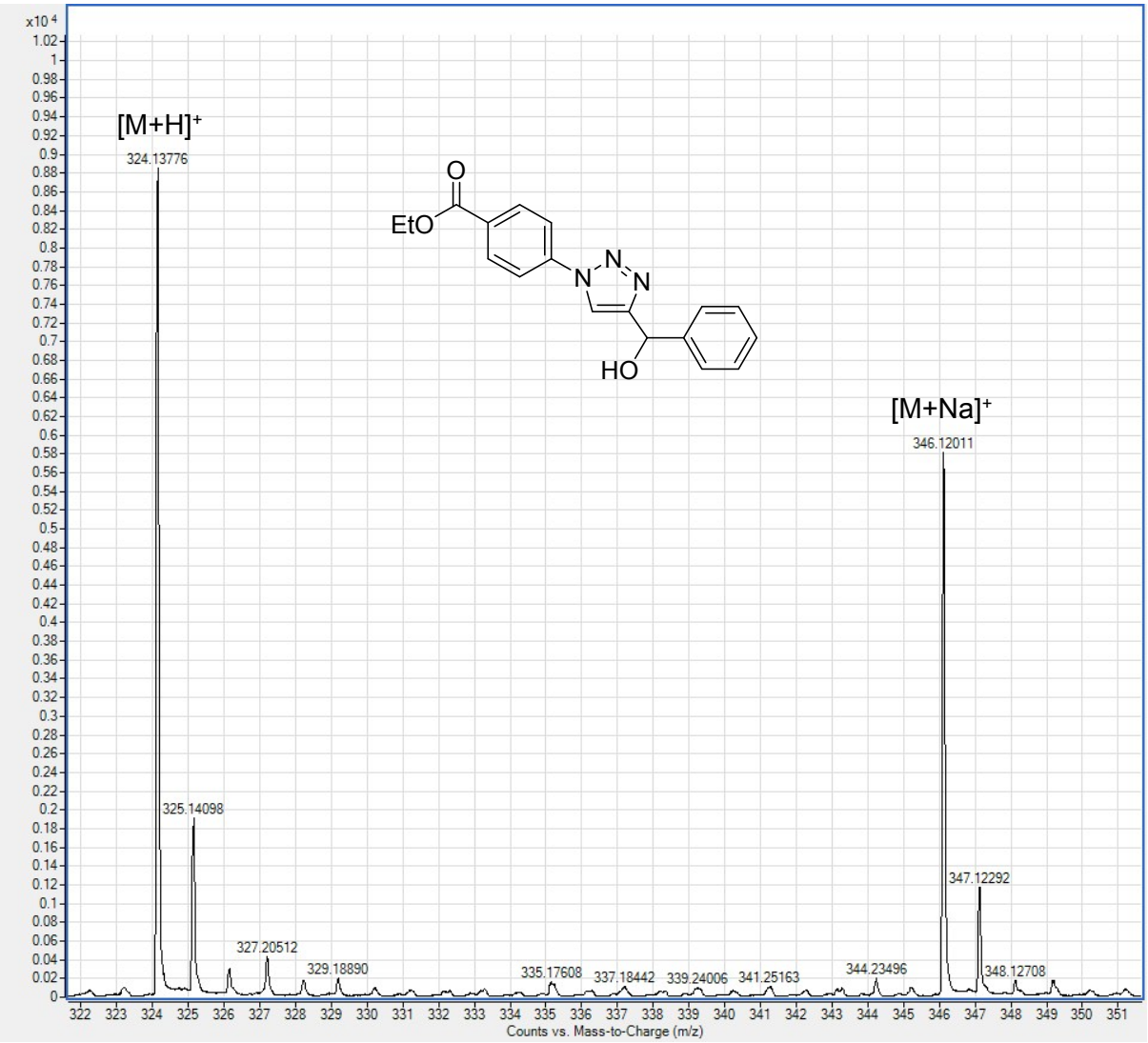



x10 4
[M+H]+
324.13606
325.13880
326.14239
328.91039
332.91698
335.13037
337.05126
339.09623
342.95531
[M+Na]+
346.11795
347.12230
349.19878
EtO
O
N
N
N
OMe
Counts vs. Mass-to-Charge (m/z)

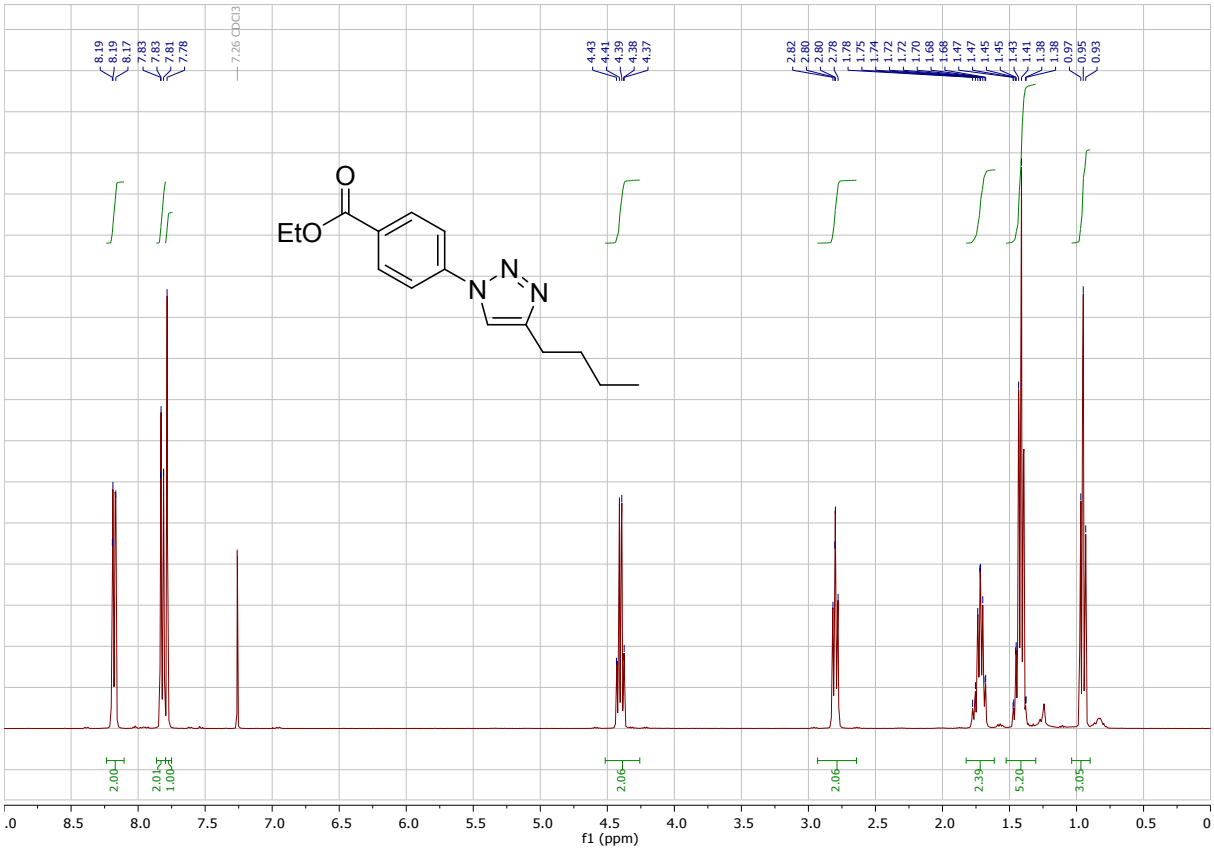
Ethyl 4-(4-(hydroxy(phenyl)methyl)-1*H*-1,2,3-triazol-1-yl)-benzoate (3e)

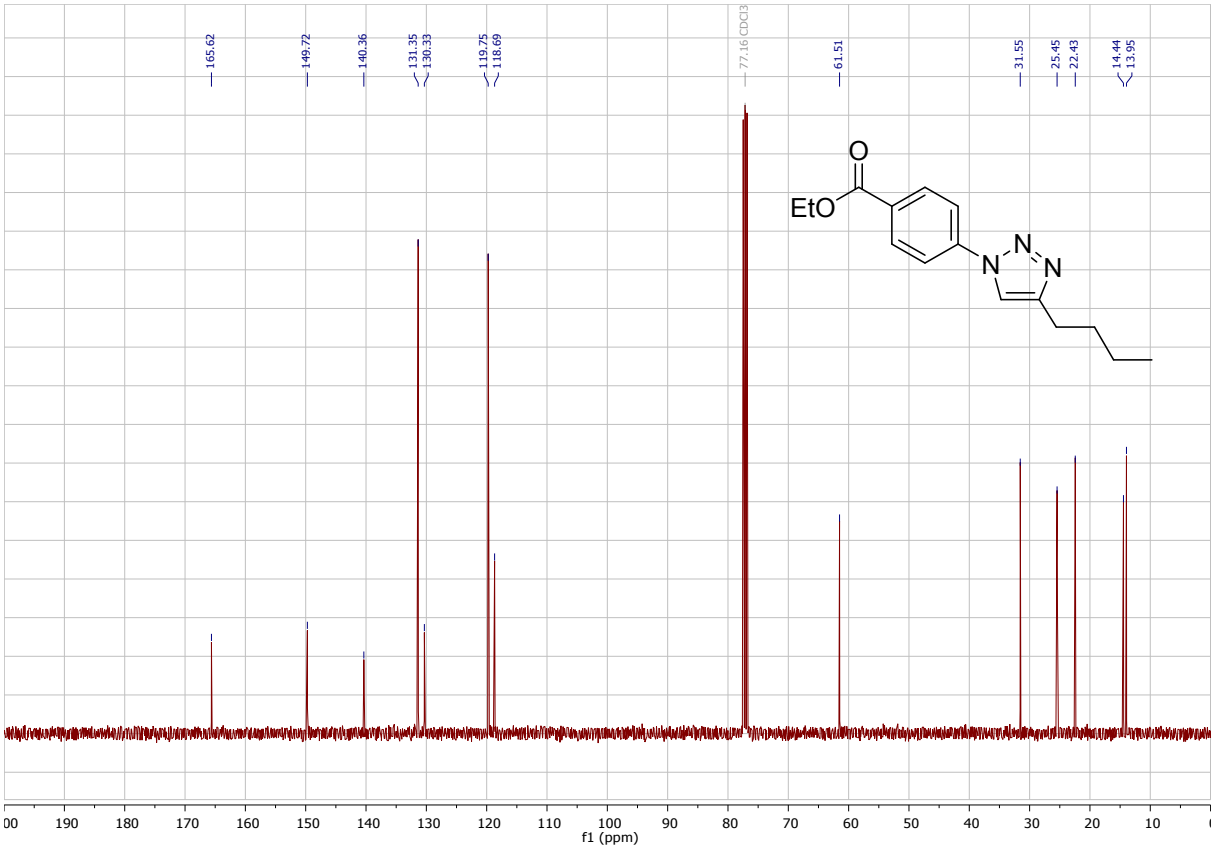


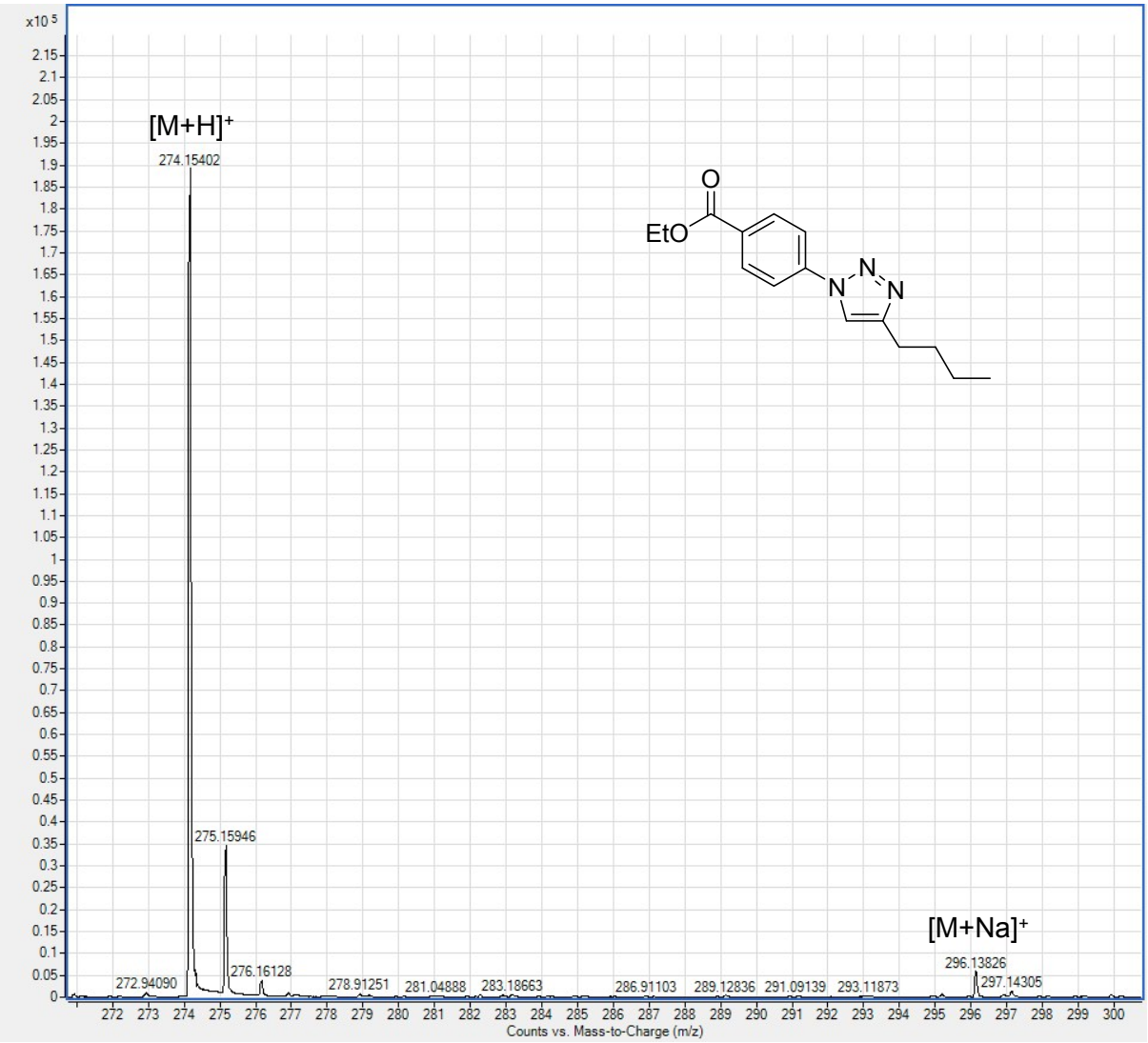



x10 4
[M+H]+
324.13776
[M+Na]+
346.12011
325.14098
327.20512
329.18890
335.17608
337.18442
339.24006
341.25163
344.23496
347.12292
348.12708
Counts vs. Mass-to-Charge (m/z)

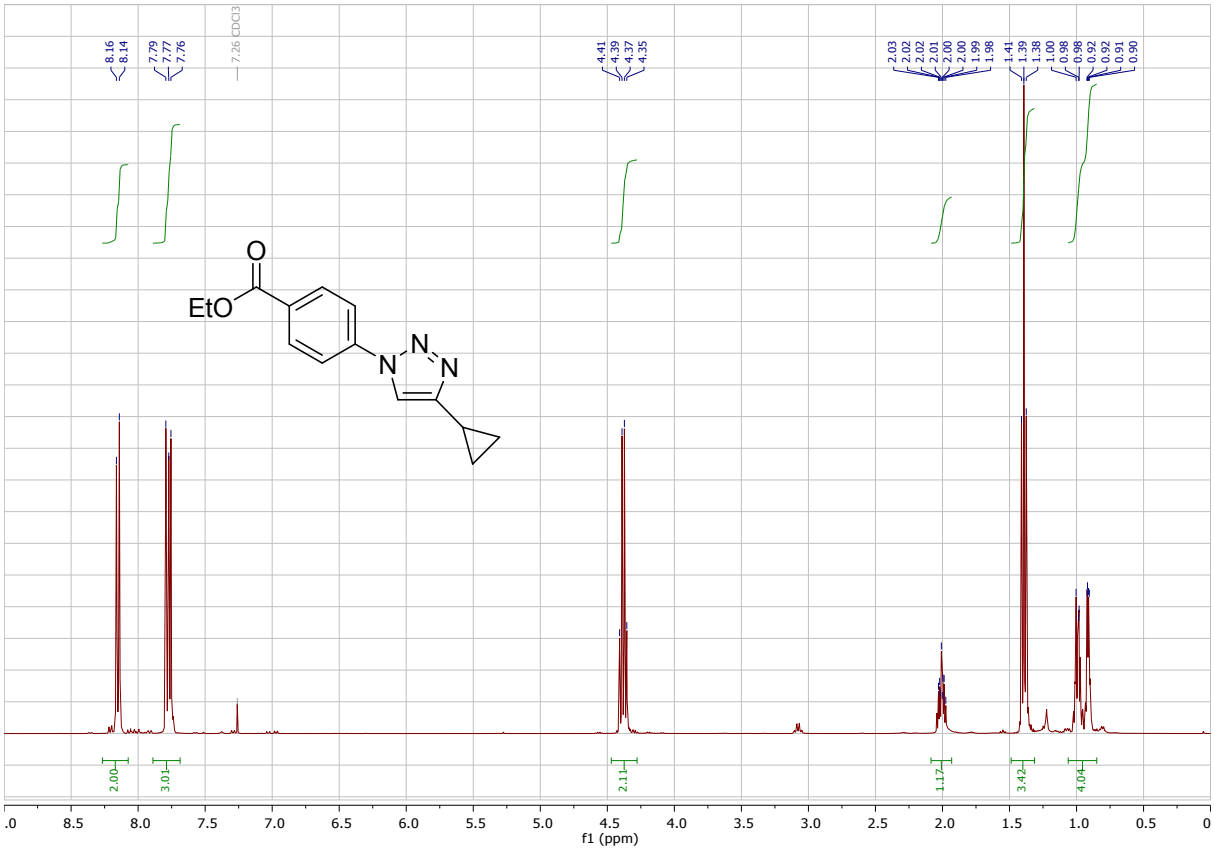
Ethyl 4-(4-butyl-1*H*-1,2,3-triazol-1-yl)benzoate (3f)

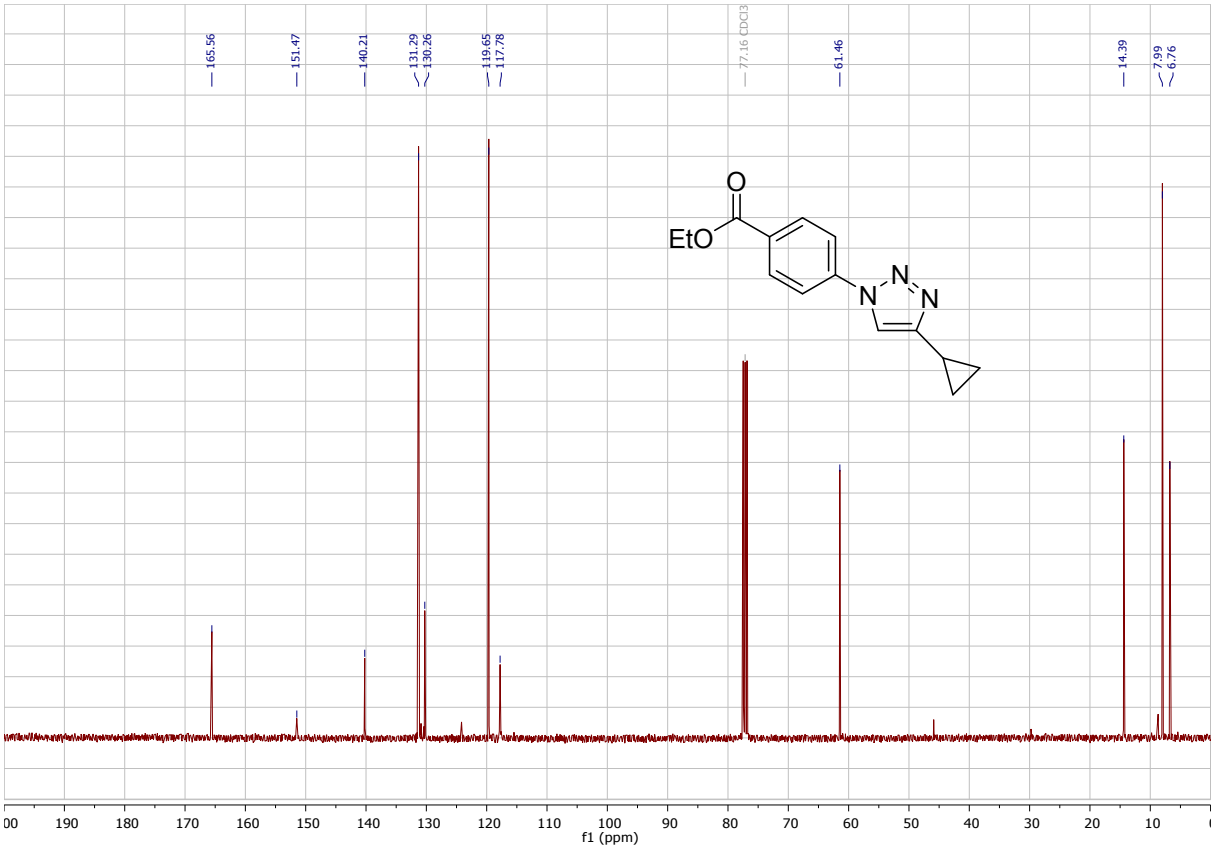


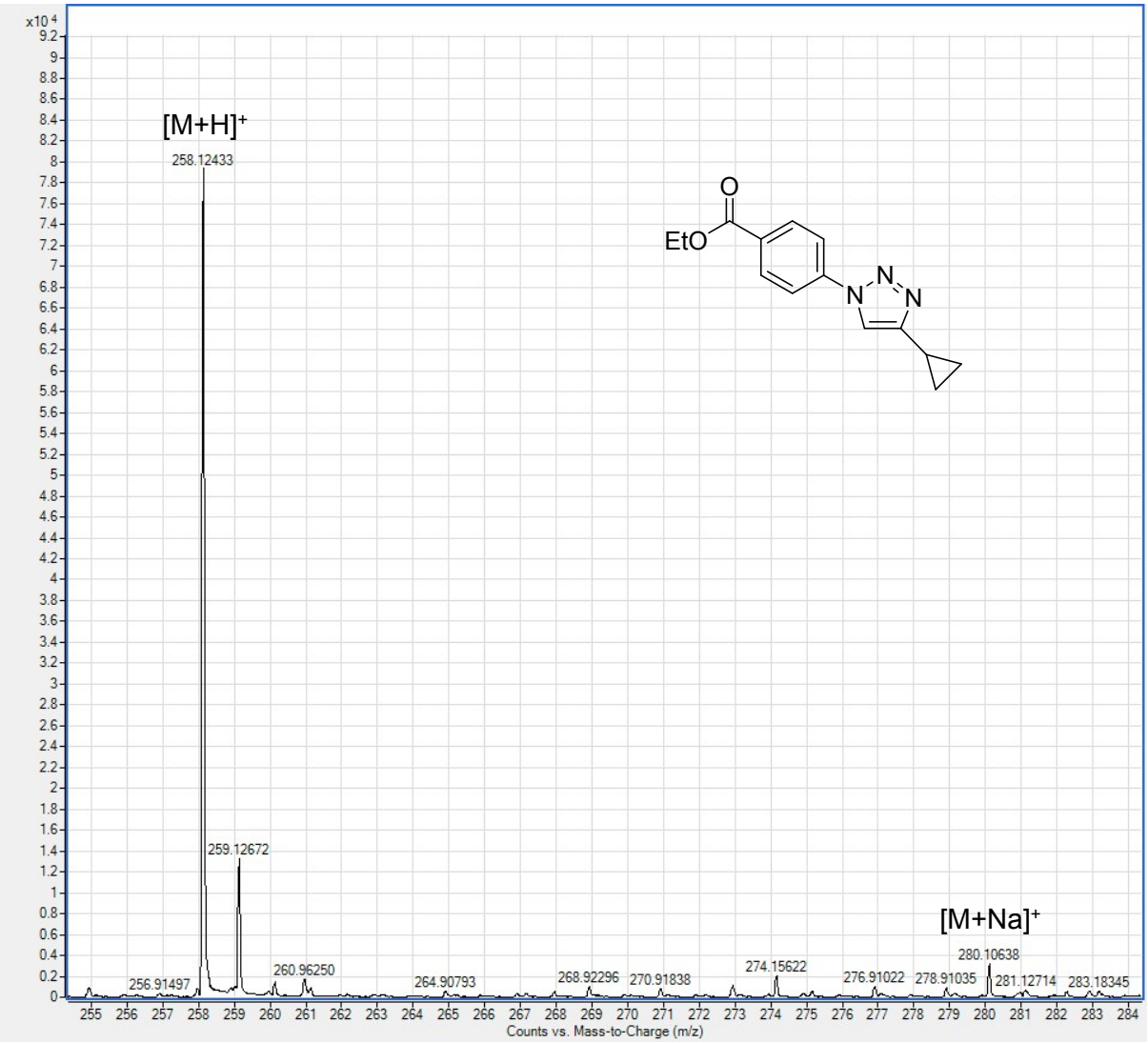



x10 5
[M+H]+
274.15402
275.15946
276.16128
272.94090
278.91251
281.04888
283.18663
286.91103
289.12836
291.09139
293.11873
[M+Na]+
296.13826
297.14305
O
EtO
N
N
N
Counts vs. Mass-to-Charge (m/z)

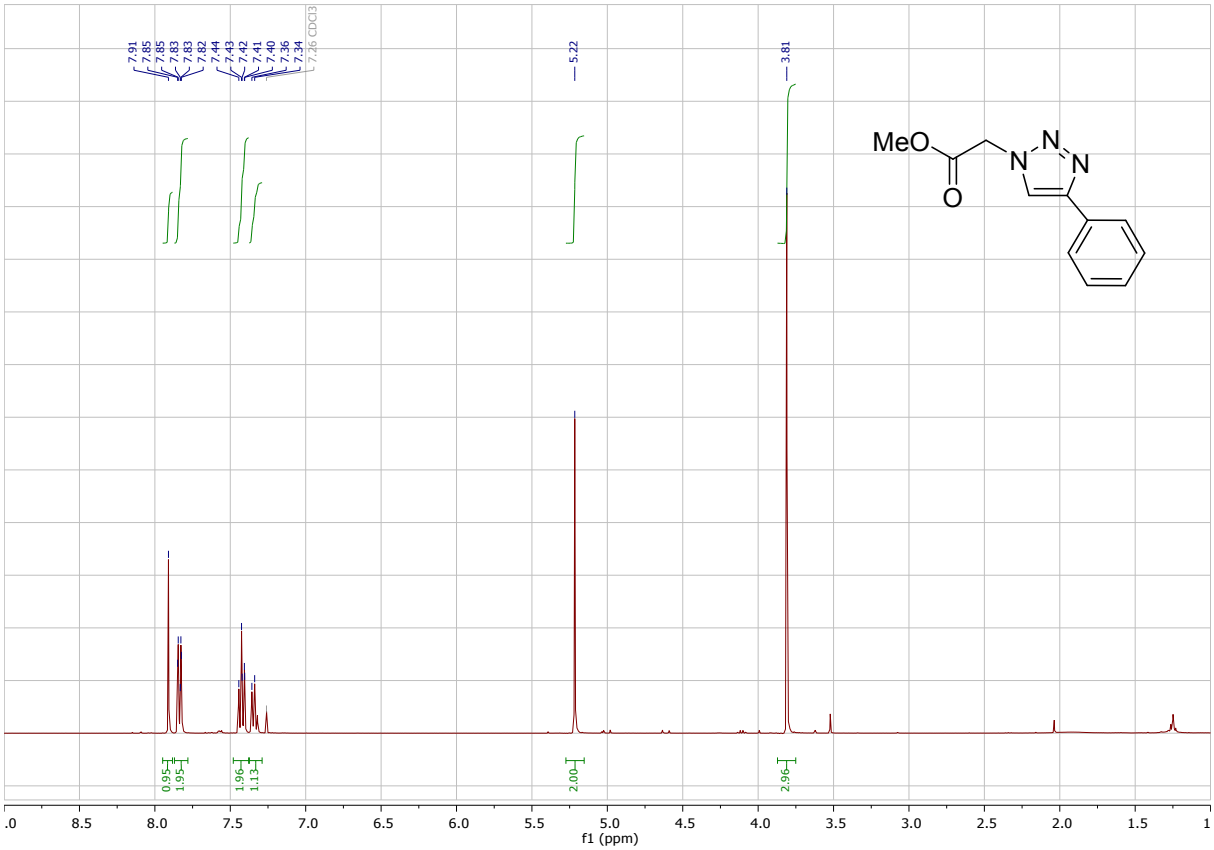
Ethyl 4-(4-cyclopropyl-1*H*-1,2,3-triazol-1-yl)benzoate (3g)

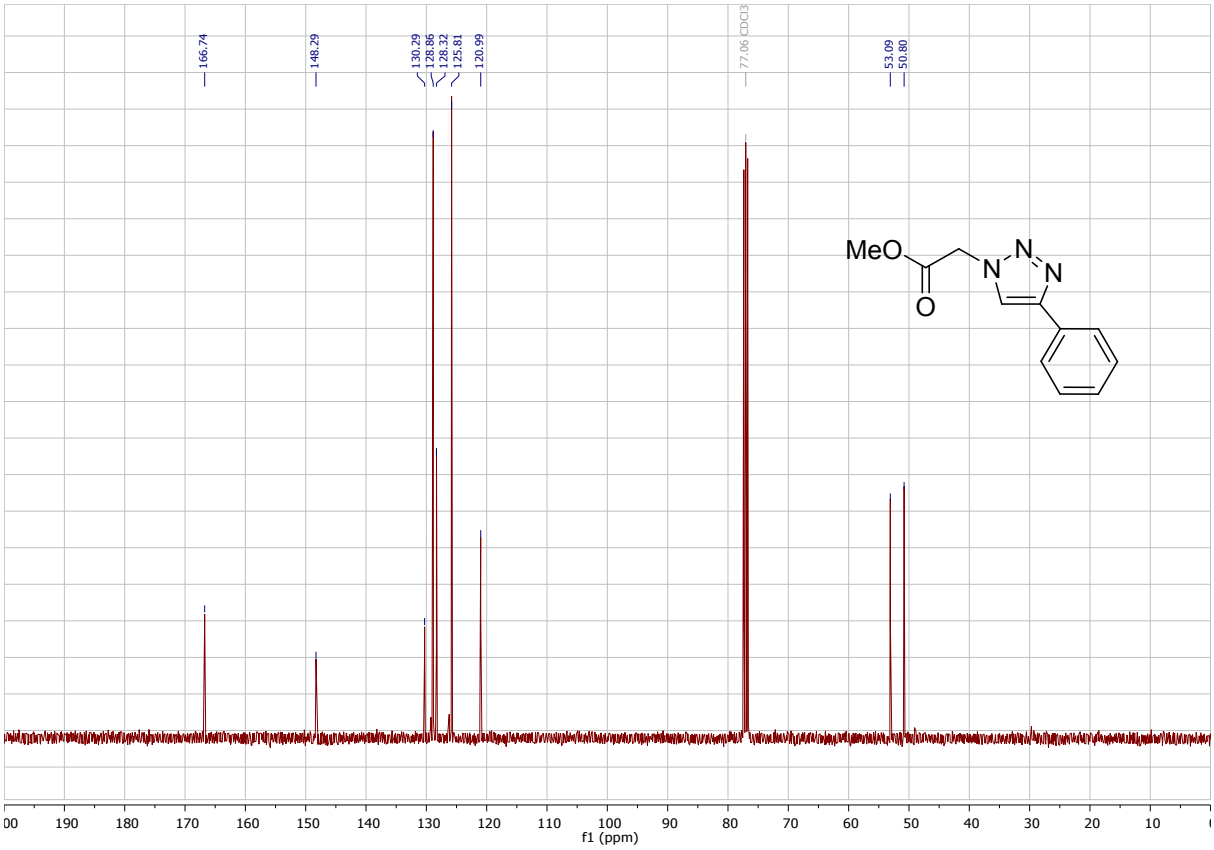


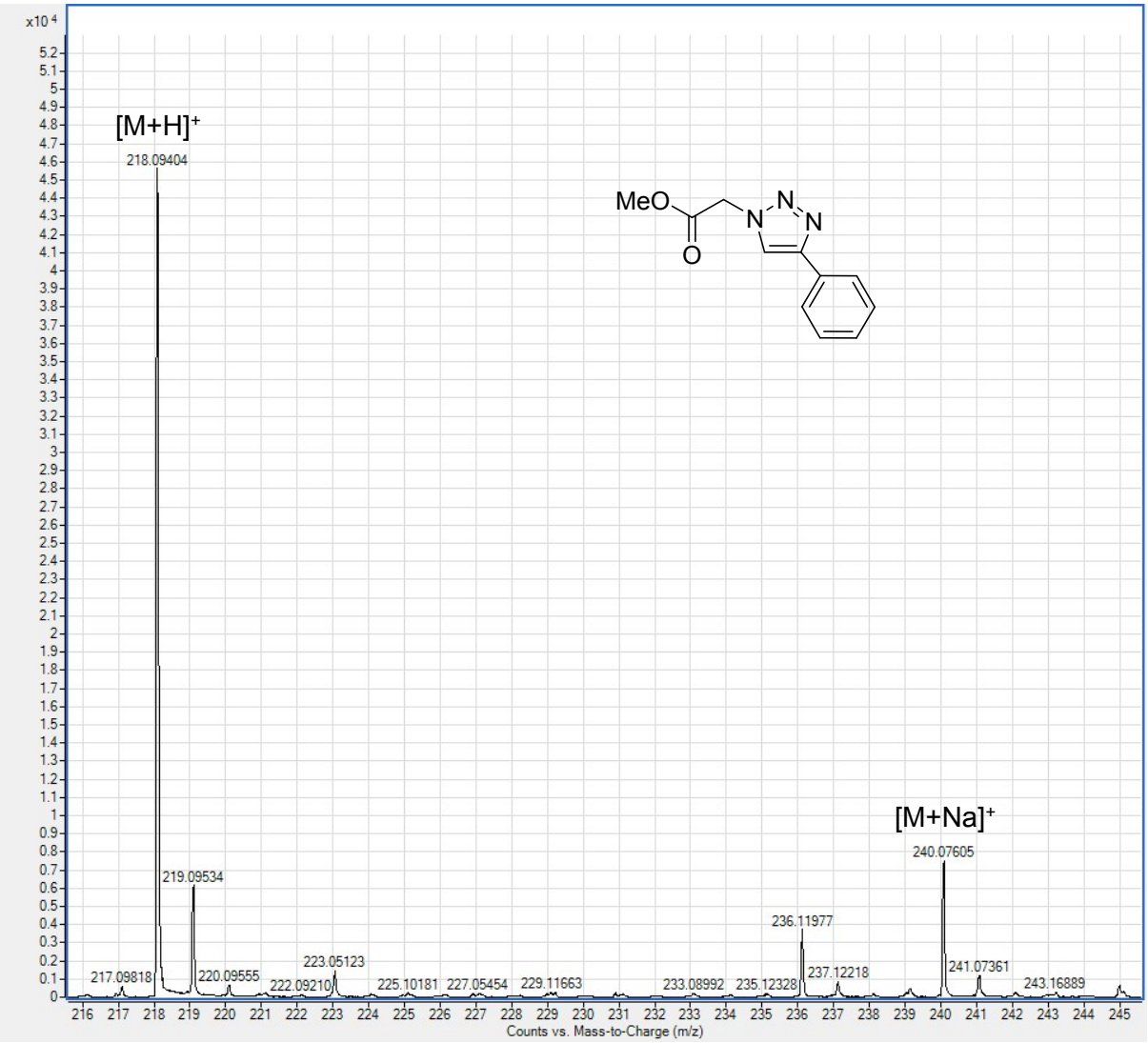



x10 4
[M+H]+
258.12433
259.12672
256.91497
260.96250
264.90793
268.92296
270.91838
274.15622
276.91022
278.91035
280.10638
281.12714
283.18345
[M+Na]+
O
EtO
N
N
N
Counts vs. Mass-to-Charge (m/z)

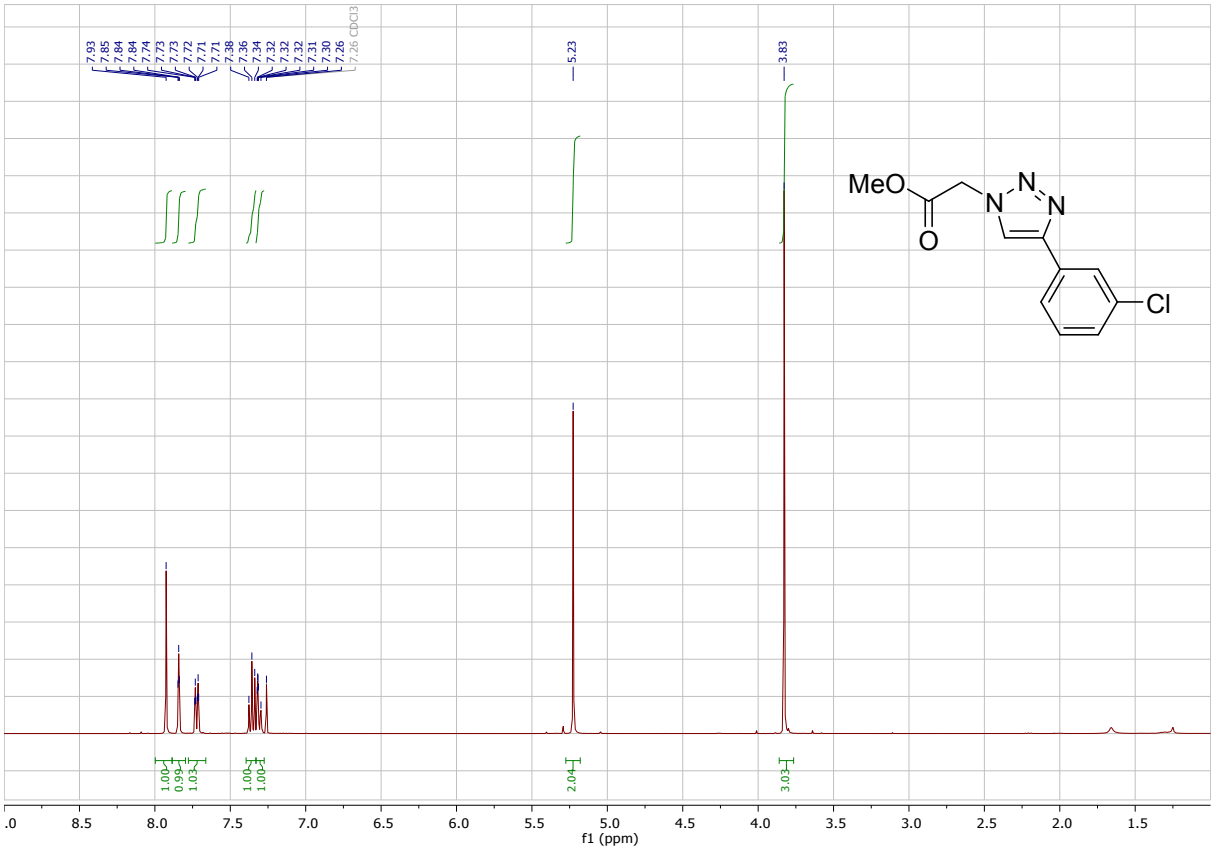
Methyl 2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)acetate (3h)

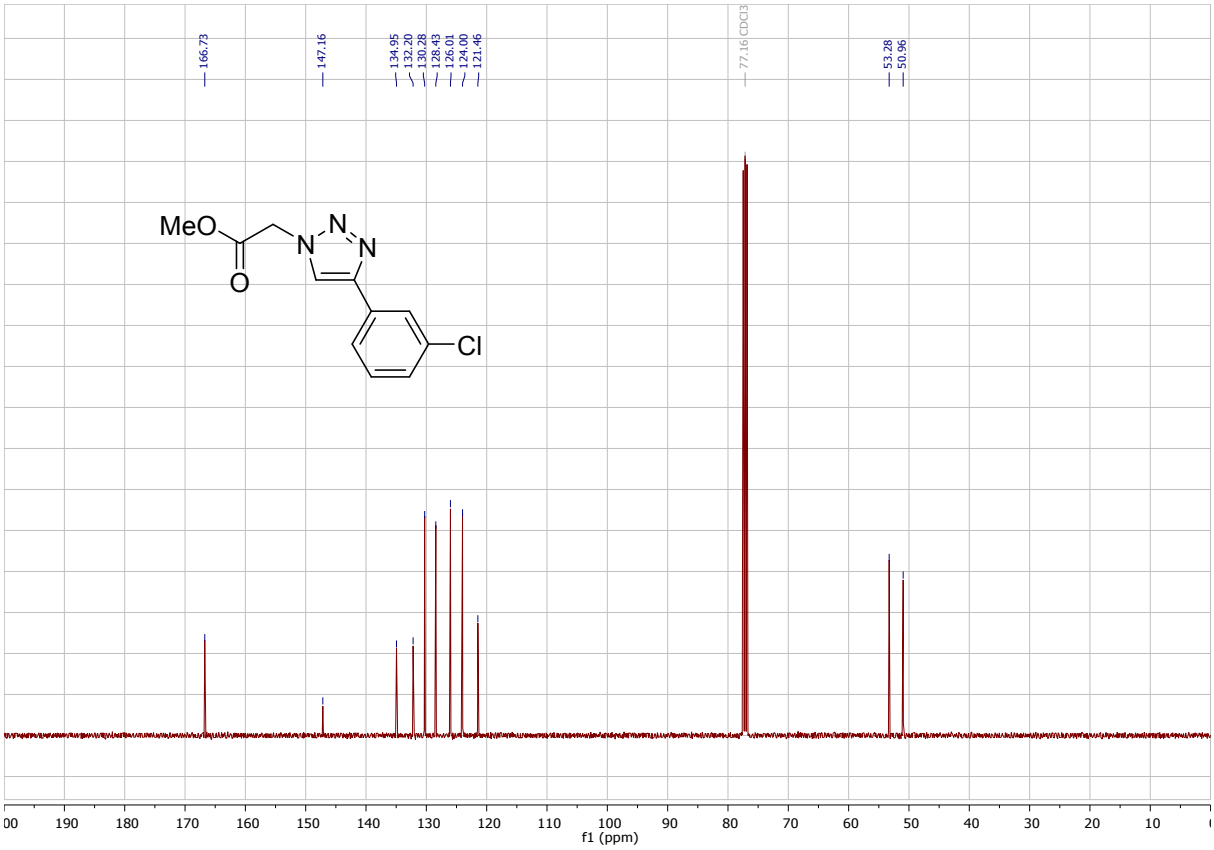


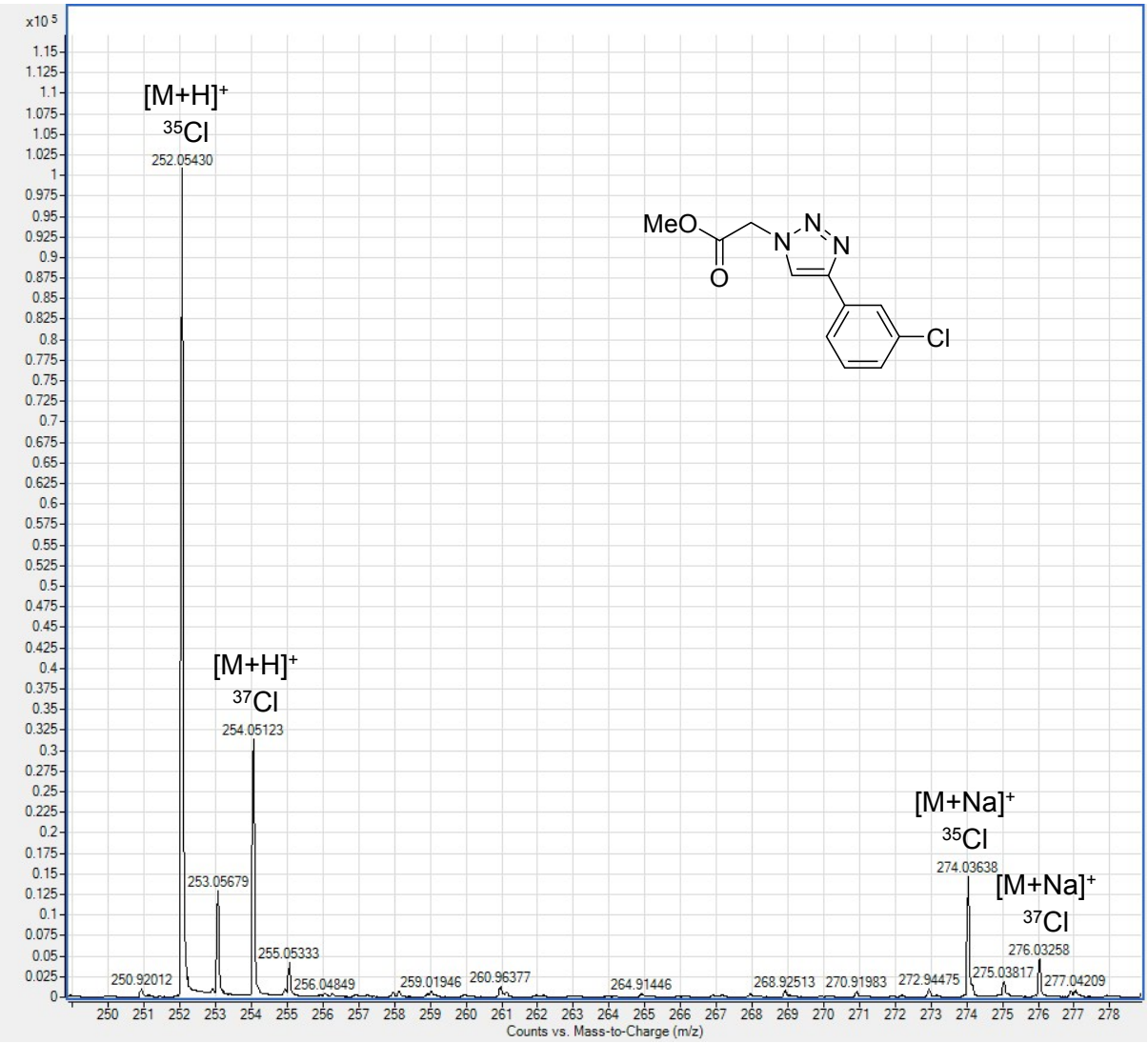



x10 4
[M+H]+
218.09404
MeO
O
N
N
N
[M+Na]+
240.07605
219.09534
236.11977
223.05123
217.09818
220.09555
222.09210
225.10181
227.05454
229.11663
233.08992
235.12328
237.12218
241.07361
243.16889
Counts vs. Mass-to-Charge (m/z)

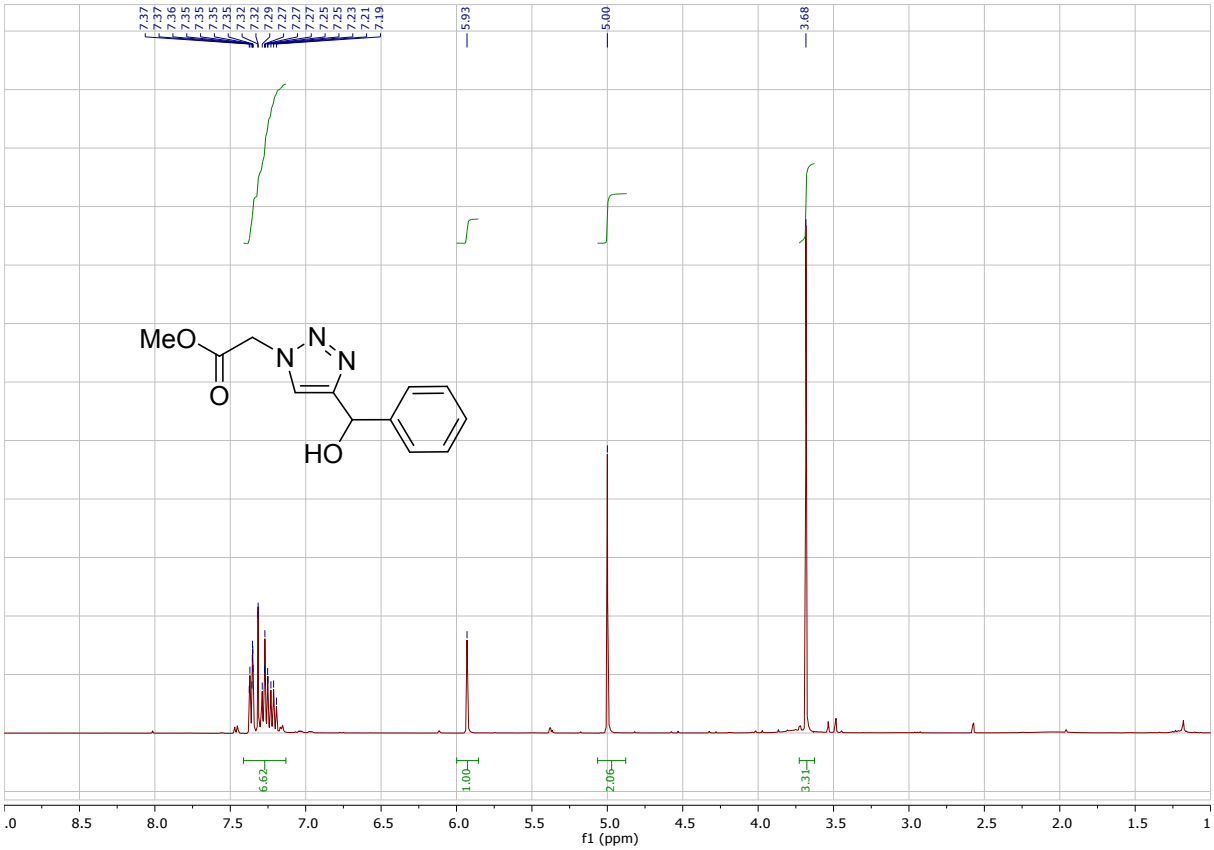
Methyl 2-(4-(3-chlorophenyl)-1*H*-1,2,3-triazol-1-yl)acetate (3i)

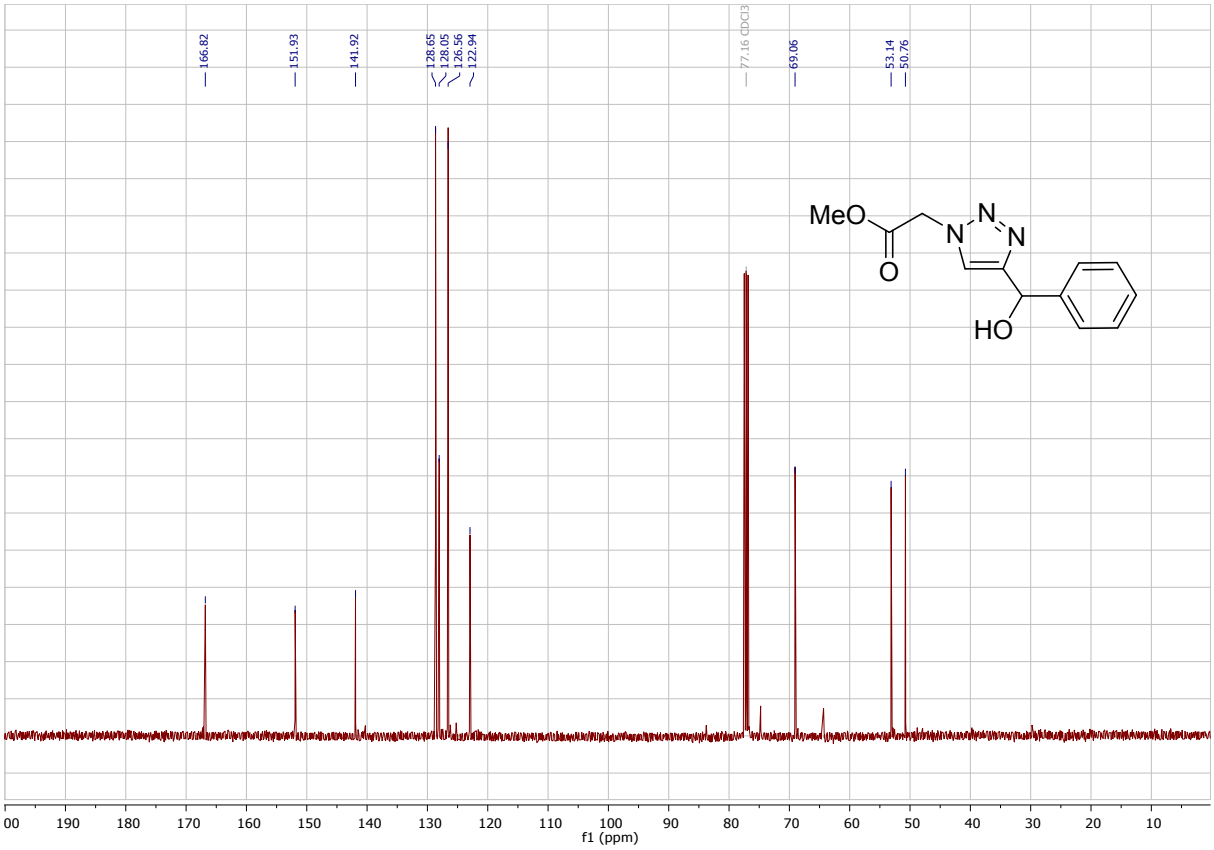


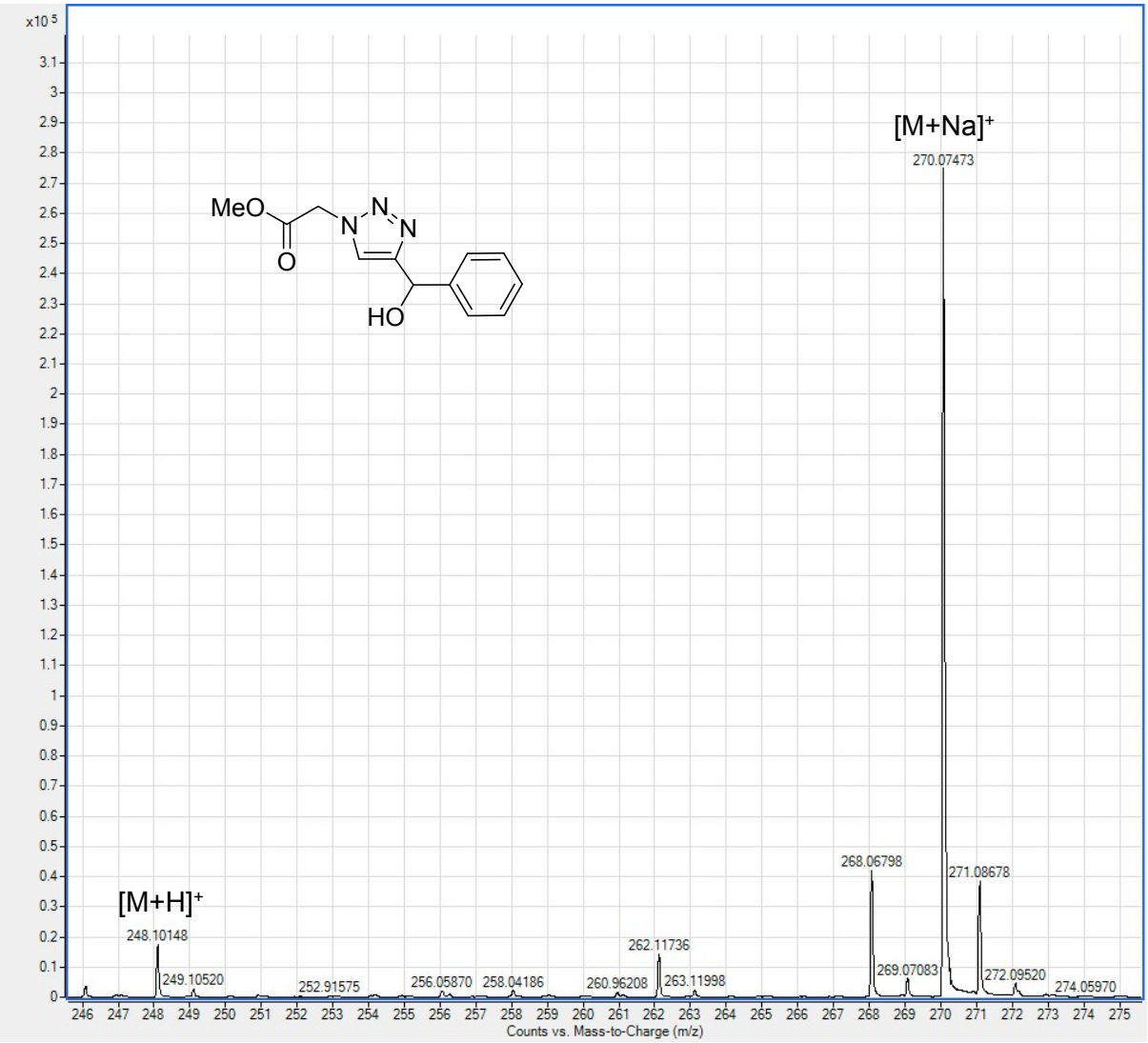



x10 5
[M+H]+
35Cl
252.05430
[M+H]+
37Cl
254.05123
253.05679
255.05333
250.92012
256.04849
259.01946
260.96377
264.91446
268.92513
270.91983
272.94475
[M+Na]+
35Cl
274.03638
[M+Na]+
37Cl
276.03258
275.03817
277.04209
MeO
O
N
N
N
Cl
Counts vs. Mass-to-Charge (m/z)

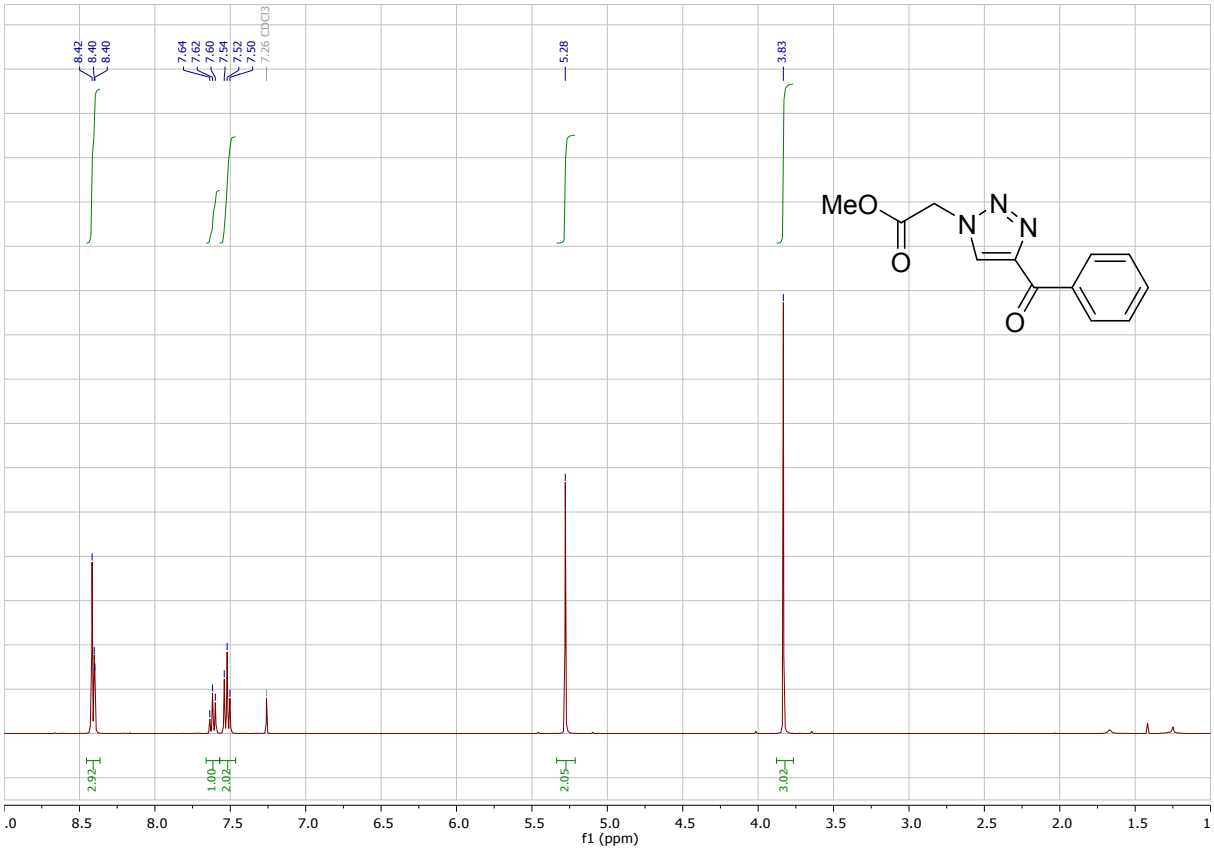
Methyl 2-(4-(hydroxy(phenyl)methyl)-1*H*-1,2,3-triazol-1-yl)-acetate (3j)

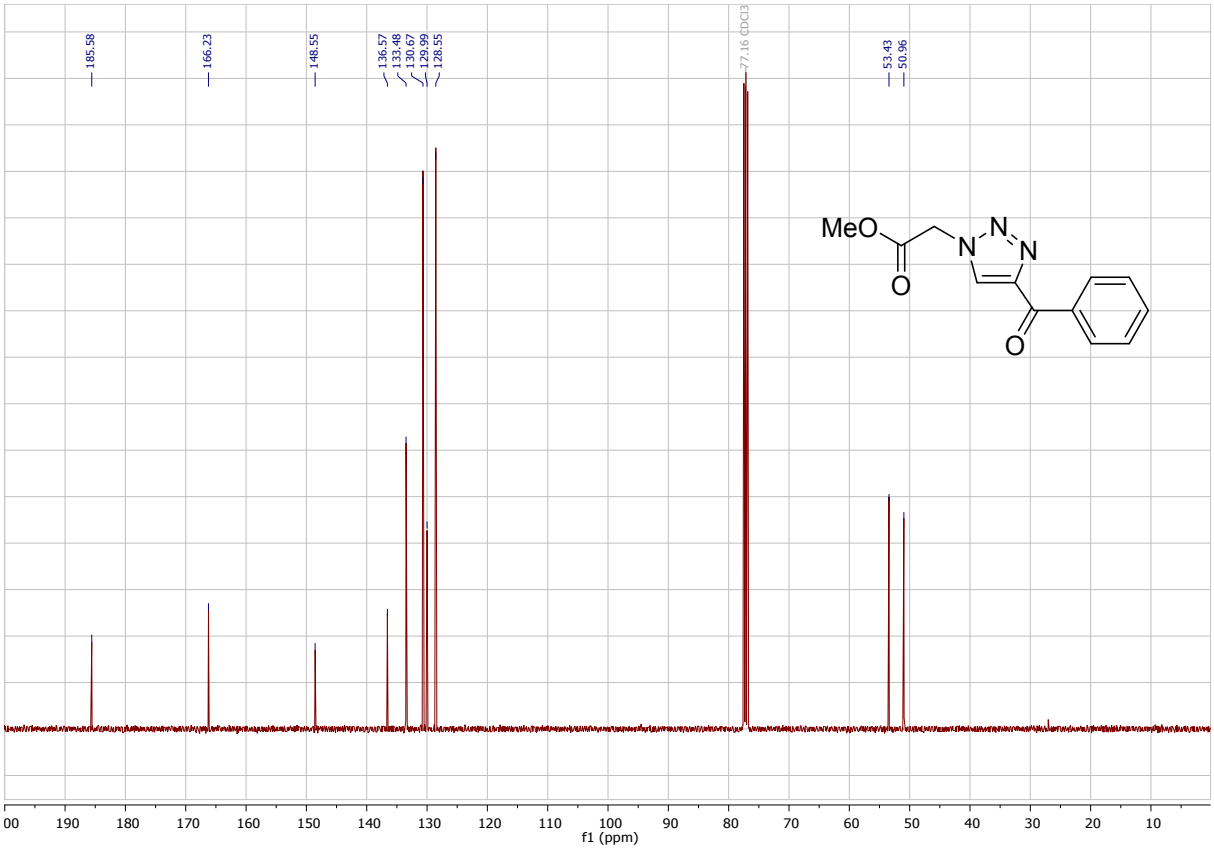


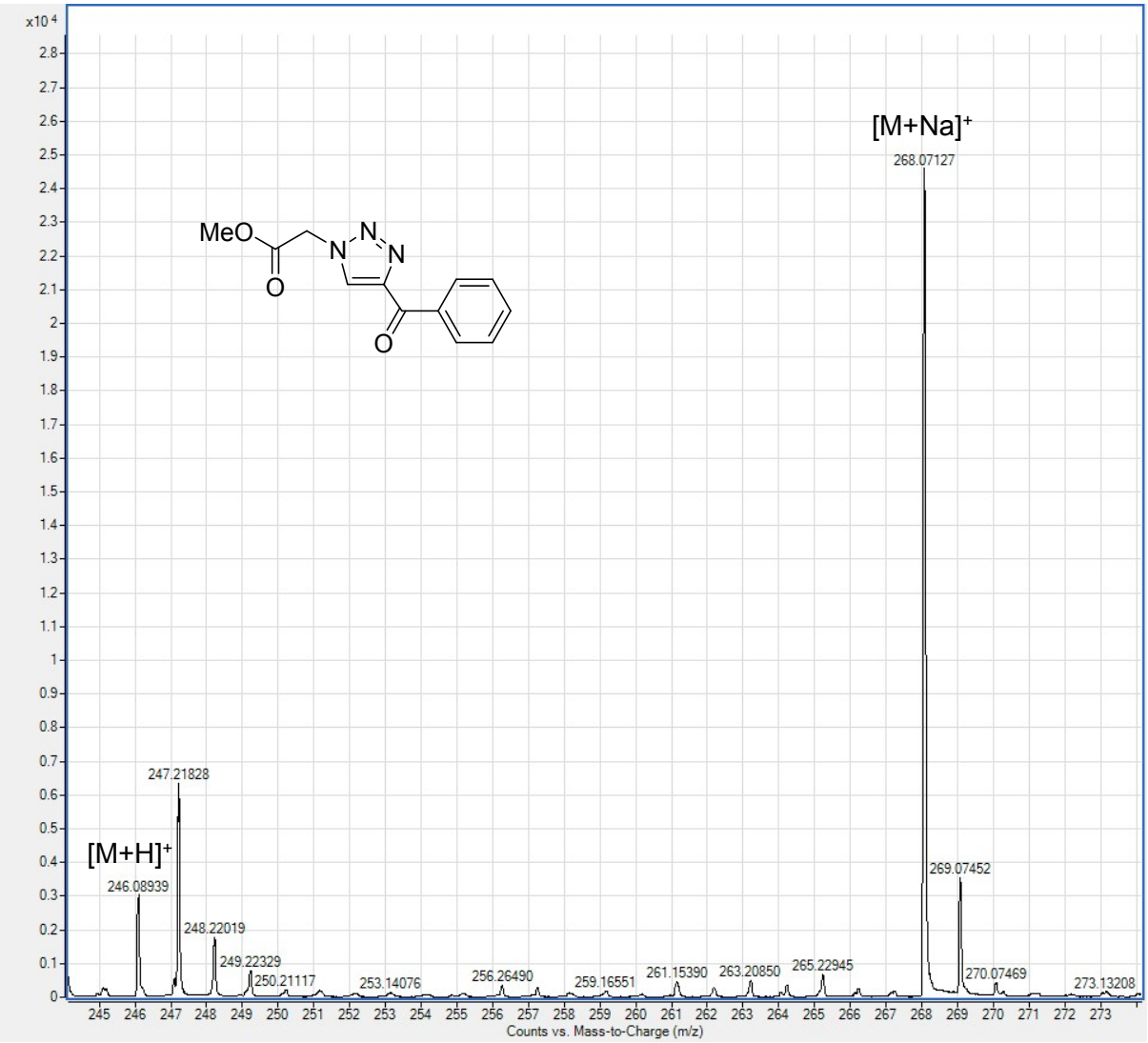



x10 5
[M+Na]+
270.07473
MeO
O
N
N
N
HO
[M+H]+
248.10148
249.10520
252.91575
256.05870
258.04186
260.96208
262.11736
263.11998
268.06798
269.07083
271.08678
272.09520
274.05970
Counts vs. Mass-to-Charge (m/z)

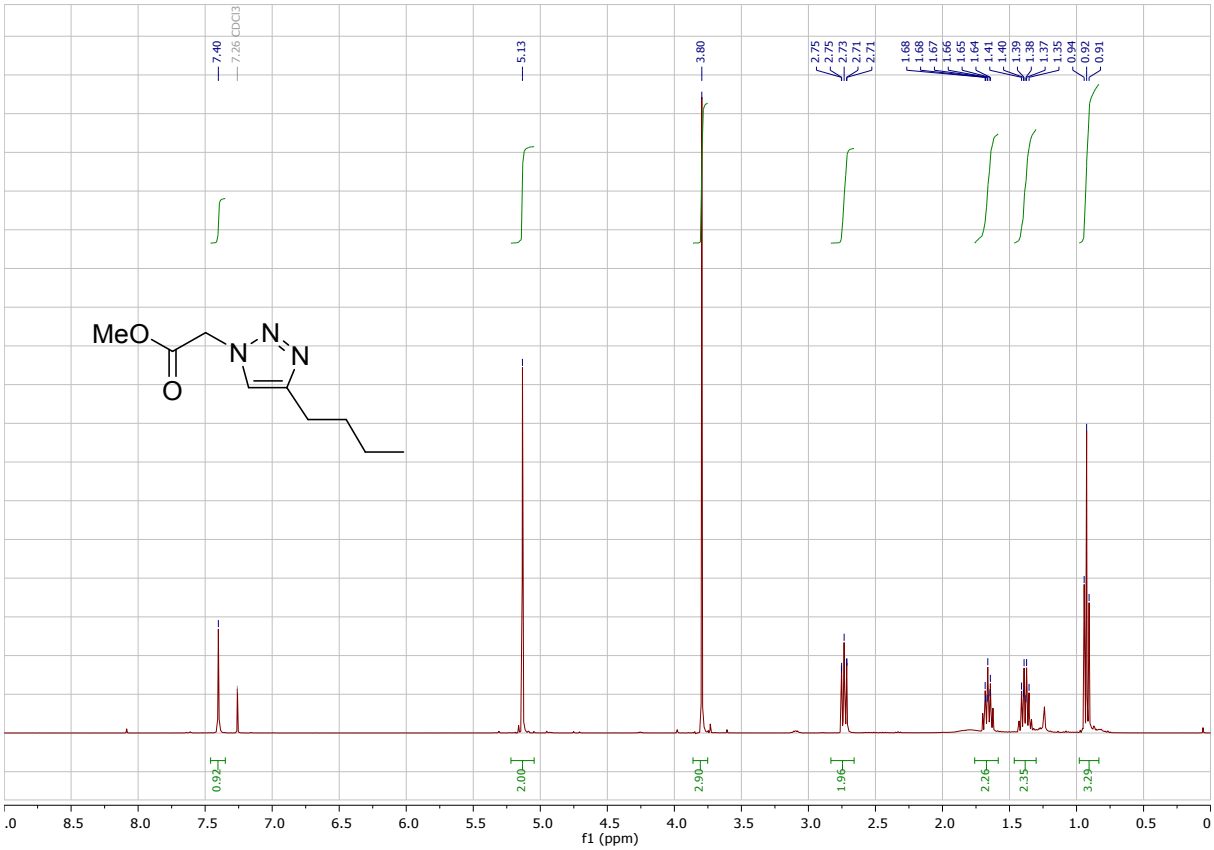
Methyl 2-(4-benzoyl-1*H*-1,2,3-triazol-1-yl)acetate (3k)

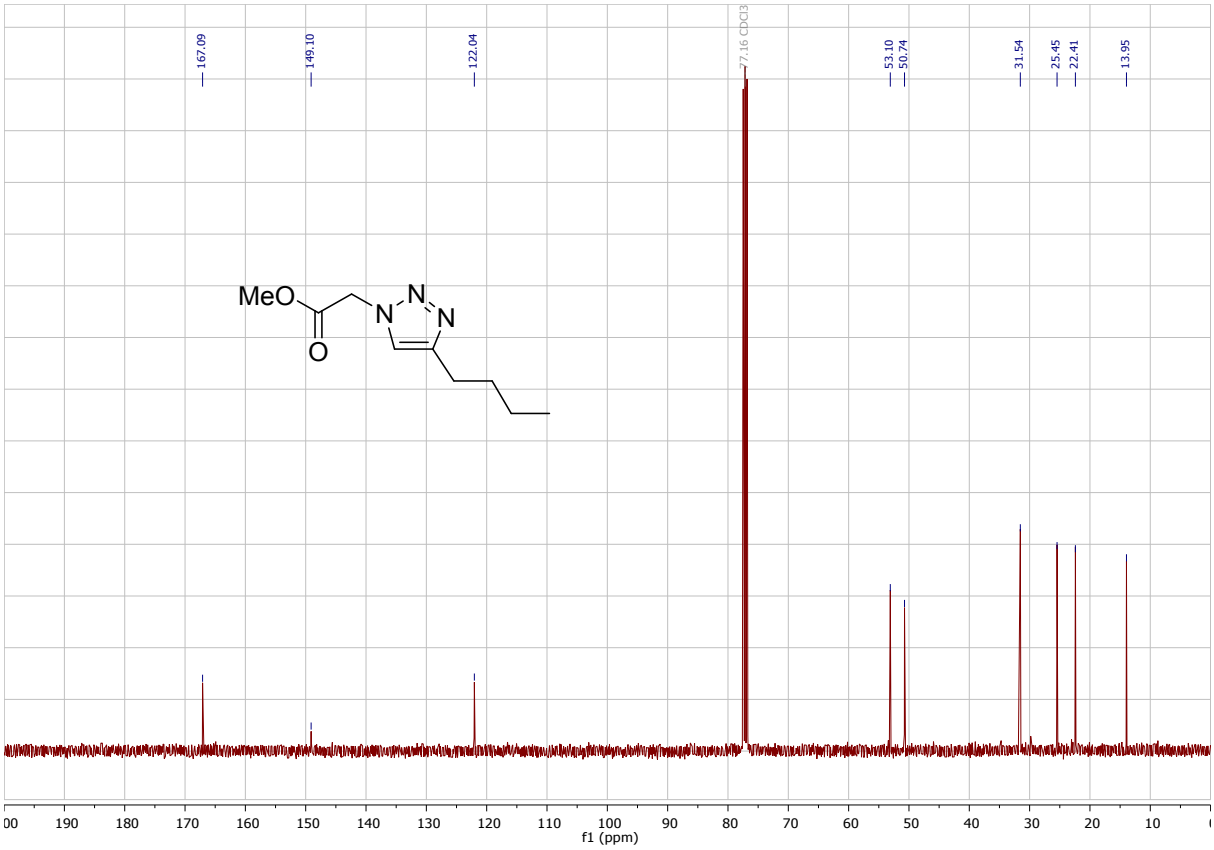


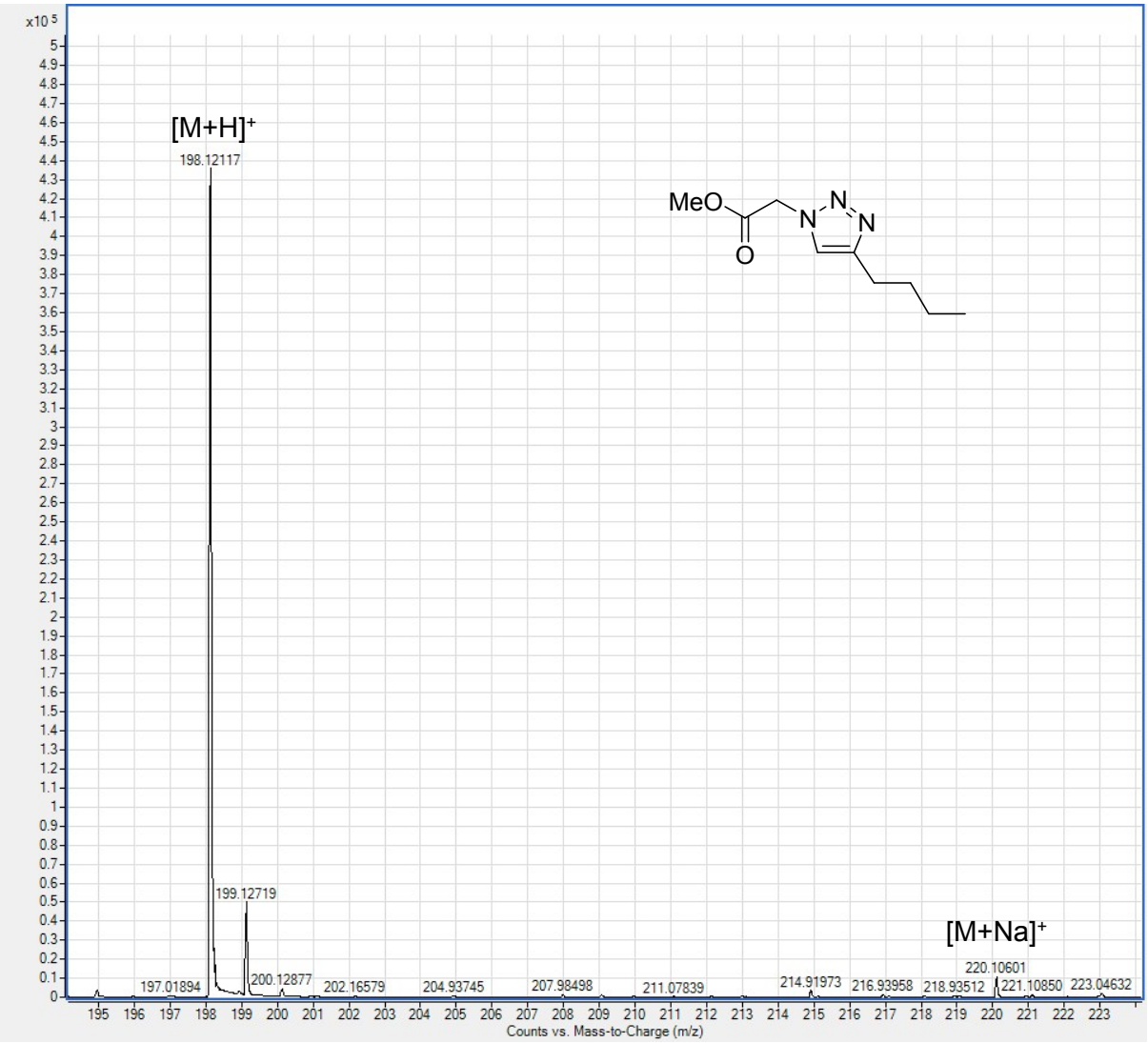



x10 4
[M+Na]+
268.07127
MeO
O
N
N
N
O
[M+H]+
247.21828
246.08939
248.22019
249.22329
250.21117
253.14076
256.26490
259.16551
261.15390
263.20850
265.22945
269.07452
270.07469
273.13208
Counts vs. Mass-to-Charge (m/z)

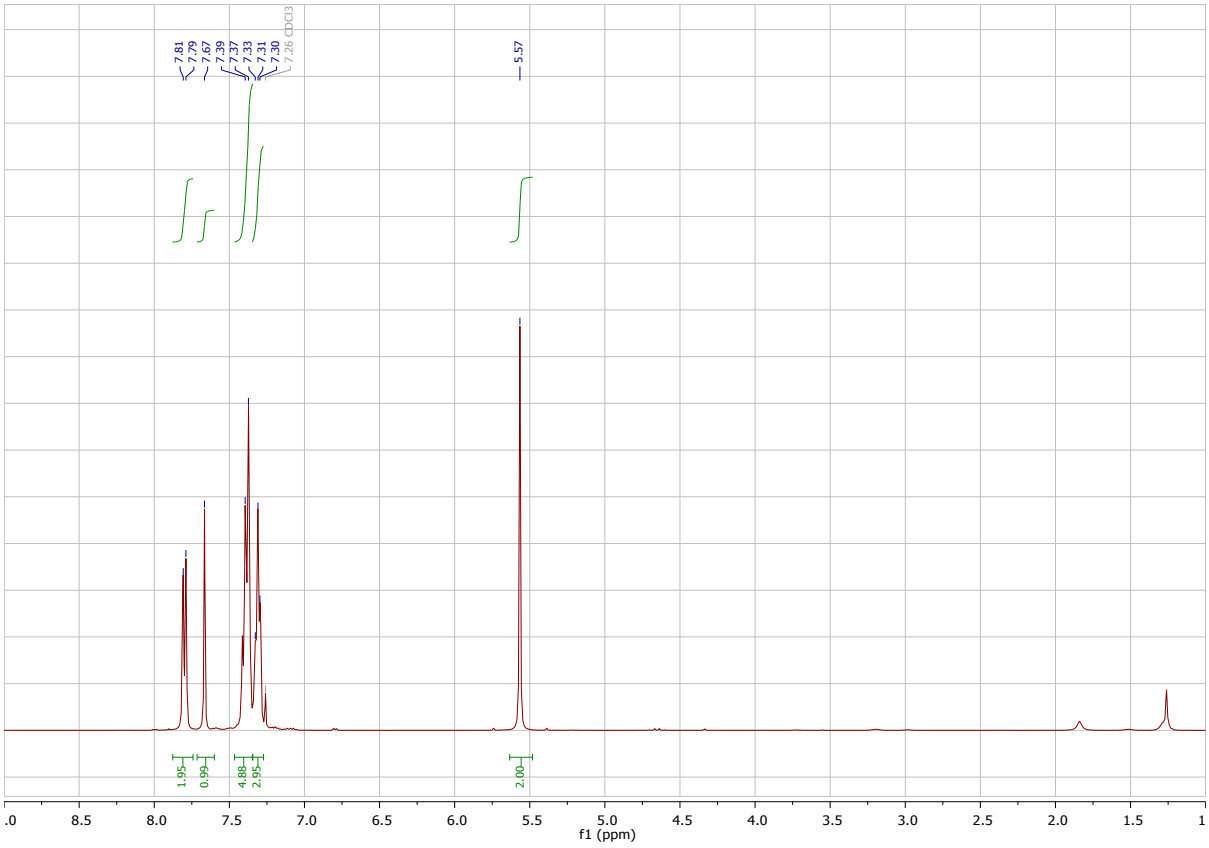
Methyl 2-(4-butyl-1*H*-1,2,3-triazol-1-yl)acetate (3l)

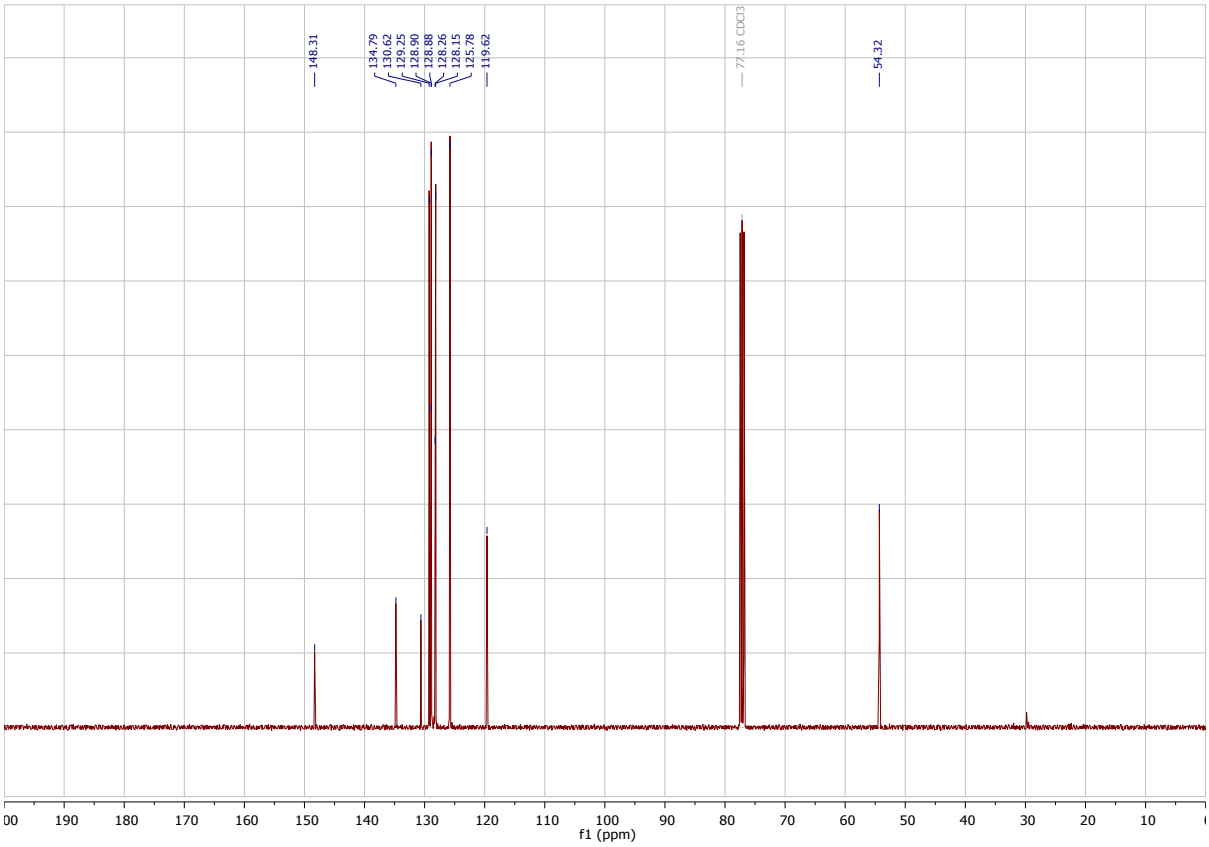





x10 5
[M+H]+
198.12117
199.12719
197.01894
200.12877
202.16579
204.93745
207.98498
211.07839
214.91973
216.93958
218.93512
220.10601
221.10850
223.04632
[M+Na]+
MeO
O
N
N
N
Counts vs. Mass-to-Charge (m/z)

1-Benzyl-4-phenyl-1H-1,2,3-triazole (3a) from gram-scale synthesis





References

[S1] R. S. Cutting, V. S. Coker, J. W. Fellowes, J. R. Lloyd, D. J. Vaughan, Mineralogical and morphological constraints on the reduction of Fe(III) minerals by *Geobacter sulfurreducens*. *Geochim. Cosmochim. Acta* **2009**, *73*, 4004-4022.

[S2] M. C. Biesinger et al., Resolving surface chemical states in XPS analysis of first row transition metals, oxides and hydroxides: Cr, Mn, Fe, Co and Ni. *Appl. Surf. Sci.* **2011**, *257*, 2717-2730.

[S3] N. Joshi et al., Optimising the transport properties and reactivity of microbially-synthesised magnetite for *in situ* remediation. *Sci. Rep.* **2018**, 8, 4246.

[S4] D. R. Lovley, E. J. P. Phillips, Availability of ferric iron for microbial reduction in bottom sediments of the freshwater tidal Potomac river. *Appl. Env. Microbiol.* **1986**, *52*, 751-757.