Analysis of fatty acid metabolism using Click-Chemistry and HPLC-MS

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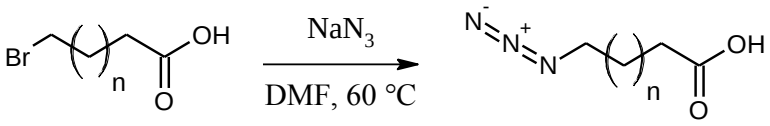
-Supporting Information-

Contents

Experimental section

General procedure for the synthesis of azido fatty acids (2a,b,c,d,e,f,h)

Scheme S1. Synthesis of azido fatty acids.



All azido fatty acids were synthesized in the same way with slightly differing starting amounts. The general procedure of the reaction of the corresponding bromide with 3 equivalents of sodium azide is exemplified for the synthesis of 16-azidohexanoic acid (**1h**):

16-Azidohexadecanoic acid (2h)

To a solution of 16-bromohexadecanoic acid (1.5 g, 4.47 mmol) in 10 mL dry DMF, sodium azide (872 mg, 13.4 mmol, 3 eq.) was added and stirred for 44h at 60 °C. Subsequently the solvent was evaporated and 50 mL of aqueous HCl were added. The raw product was extracted three times with 30 mL of EtOAc, the organic fractions were combined and dried over Na_2SO_4. Purification was conducted by column chromatography on silica, eluting with Hexane to Hexane/EtOAc (1:1), giving the product as a white solid (1.06 g, 3.55 mmol, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.15 -1.43 (m, 22H), 1.49-1.69 (m, 4H), 2.33 (t, J = 7.5 Hz, 2H), 3.23 (t, J = 6.9 Hz); ^{13}C NMR (500 MHz, $CDCl_3$) δ = 24.7, 26.7, 28.1, 29.0, 29.1, 29.2, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 29.6, 34.0, 51.5, 180.0.

12-Azidododecanoic acid (2f)

Yield: 723 mg, 3.0 mmol, 84%. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 1.15-1.41 (m, 14H), 1.49-1.69 (m, 4H), 2.32 (t, J = 7.5 Hz, 2H), 3.23 (t, J = 6.9 Hz); ^{13}C NMR (250 MHz, $CDCl_3$) δ = 24.6, 26.7, 28.8, 29.0, 29.1, 29.2, 29.3, 29.4, 29.4, 34.0, 51.5, 180.2.

11-Azidoundecanoic acid (2e)

Yield: 462 mg, 2.03 mmol, 54%. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 1.19-1.41 (m, 12H), 1.48-1.68 (m, 4H), 2.31 (t, J = 7.5 Hz, 2H), 3.21 (t, J = 6.9 Hz), 11.52 (s, 1H); ^{13}C NMR (250 MHz, $CDCl_3$) δ = 24.6, 26.4, 28.8, 29.0, 29.0, 29.1, 29.2, 29.3, 34.0, 51.4, 180.2.

10-Azidodecanoic acid (2d)

Yield: 608 mg, 2.85 mmol, 72%. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 1.15-1.38 (m, 10H), 1.45-1.64 (m, 4H), 2.28 (t, J = 7.5 Hz, 2H), 3.19 (t, J = 6.9 Hz); ^{13}C NMR (250 MHz, $CDCl_3$) δ = 24.4, 26.5, 28.6, 28.8, 28.9, 28.9, 29.0, 33.9, 51.4, 180.2.

6-Azidohexanoic acid (2c)

Yield: 856 mg, 5.46 mmol, 71%. ^{1}H NMR (250 MHz, $CDCl_3$) δ = 1.32-1.45 (m, 2H), 1.49-1.71 (m, 4H), 3.32 (t, J = 7.3 Hz, 2H), 3.23 (t, J = 6.8 Hz, 2H); ^{13}C NMR (250 MHz, $CDCl_3$) δ = 24.0, 26.0, 28.4, 33.7, 51.0, 179.9.

5-Azidopentanoic acid (2b)

Yield: 332 mg, 3.1 mmol, 37%. ^{1}H NMR (250 MHz, $CDCl_3$) δ =1.51-1.76 (m, 4H), 2.36 (t, J = 7.1 Hz, 2H), 3.26 (t, J = 6.4 Hz, 2H), 11.05 (s, 1H); ^{13}C NMR (250 MHz, $CDCl_3$) δ = 21.7, 28.0, 33.3, 50.9, 179.6.

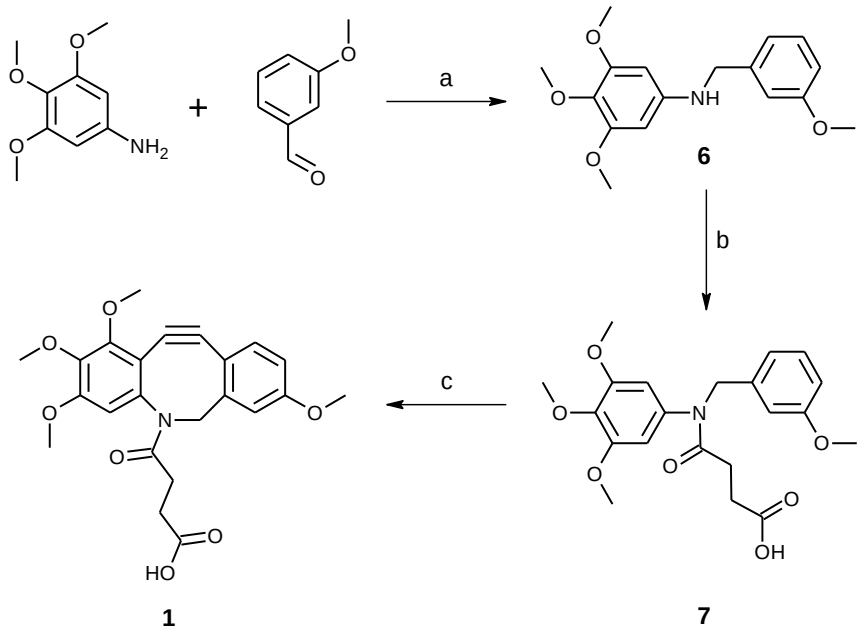
4-Azidobutanoic acid (2a)

Purification gave 34 mg of a mixture of γ-butyrolactone and **2a** with a ^{1}H NMR integral ratio of 31% to 69%. The mixture was used directly for the formation of **3a/4a**. ^{1}H NMR (250 MHz, $CDCl_3$) δ =1.88 (quin, J = 6.9 Hz, 2H), 2.44 (t, J = 7.3 Hz, 2H), 3.34 (t, J = 6.7 Hz, 2H); ^{13}C NMR (250 MHz, $CDCl_3$) δ = 23.9, 30.8, 50.4, 178.3.

γ-Butyrolactone: ^{1}H NMR (250 MHz, $CDCl_3$) δ = 2.15-2.31 (m, 2H), 2.43-2.52 (m, 2H), 4.32 (t, J = 7.0 Hz, 2H); ^{13}C NMR (250 MHz, $CDCl_3$) δ = 22.1, 27.8, 68.6, 178.0.

Synthesis of the TDAC

Scheme S2. Three step synthesis of TDAC.



Three step synthesis of TDAC. (a) 1. MeOH, RT, 5h, 2. $NaBH_4$ (1.7 eq.), 0 °C, 30 min. (b) Succinic anhydride (2 eq.), NEt_3 (1.5 eq.), DMAP (0.1 eq.), DCM, RT, 20h. (c) 1. Tetrachlorocyclopropene (1.01 eq.), $AlCl_3$ (3.8 eq.), DCM, -78 °C, 3h, 2.hv, MeCN, 24h.

3,4,5-Trimethoxy-*N*-(3-methoxybenzyl)aniline (6)

3,4,5-Trimethoxyaniline (3.0 g, 16.4 mmol, 1 eq) and 3-methoxybenzaldehyde (2.0 mL, 16.4 mmol, 1 eq) were stirred for 3.5h in dry Methanol, before the solution was cooled to 0° C and sodium borohydride (930 mg, 24.6 mmol, 1.3 eq) was added carefully. When gas formation ceased after 30 min of stirring at room temperature, the reaction was quenched by addition of 40 mL 1M NaOH and extracted three times with 50 mL of Et_2O. The organic phases were combined, washed with brine, dried over Na_2SO_4 and the solvents evaporated, giving the product as a yellow solid (4.63 g, 15.3 mmol, 93%). ^{1}H NMR (250 MHz, $CDCl_3$) δ =3.73(s, 3H), 3.75 (s, 3H), 3.77 (s, 3H), 4.24 (s, 2H), 5.85 (s, 2H), 6.79 (ddd, J = 8.4 Hz, 2.5 Hz, 0.9 Hz, 1H), 7.24 (t, J = 7.8 Hz, 1H); ^{13}C NMR (250 MHz, $CDCl_3$) δ =48.8, 55.1, 55.8, 60.9, 90.6, 112.6, 113.1, 119.7, 129.6, 130.2 141.0, 144.9, 153.9, 159.9.

4-((3-Methoxybenzyl)(3,4,5-trimethoxyphenyl)amino)-4-oxobutanoic acid (7)

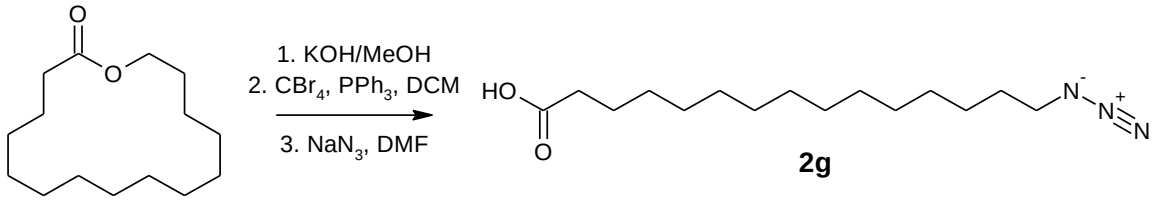
To a solution of **6** (4.49 g, 14.8 mmol) and succinic anhydride (2.96 g, 29.6 mmol, 2 eq) in dry DCM 4-(dimethylamino)-pyridine (180 mg, 1.5 mmol, 0.1 eq) and triethylamine (3.1 mL, 22.2 mmol, 1.5 eq) were added and stirred at room temperature. The solution turned dark after 1h and the reaction was quenched by the addition of 60 mL of 1M NaOH after 24h. After 15 fore minutes of stirring, the solution turned to a brownish color, after which the solution was acidified with 50 mL of 1.8 M HCl and extracted twice with 50 mL $CHCl_3$. The combined organic phases were washed with brine, dried over Na_2SO_4. After evaporation of the solvents the product was purified by column chromatography on silica, eluting with Hexane to Hexane/EtOAc (1:1), giving the product as a brown solid (5.06 g, 12.6 mmol, 85%). ^{1}H-NMR(400 MHz, $CDCl_3$): δ = 2.43 (t, J = 6.4 Hz, 2H), 2.66 (t, J = 6.4 Hz, 2H), 3.68(m, 6H), 3.73 (s, 3H), 3.82 (s, 3H), 4.79 (s, 2H), 6.17 (s, 2H),6.74-6.79 (m, 3H), 7.16 (t, J = 7.9 Hz, 1H); ^{13}C NMR (250 MHz, $CDCl_3$) δ =29.0, 29.5, 53.1, 55.2, 56.1, 60.8, 105.7, 113.2, 114.3, 121.3, 129.3, 137.1, 137.9, 138.9, 153.5, 159.6, 171.7, 177.3.

Oxo(1,2,3,8-tetramethoxy-11,12-didehydrodibenzo[b,f]azocin-5(6H)-yl)butanoic acid (1)

7 (4.91 g, 12.2 mmol) was dissolved in 80 mL of dry DCM and cooled to -80 °C while a portion of aluminium chloride (1.79 g, 13.4 mmol, 1.1 eq) was suspended in a separate flask in dry DCM and tetrachlorocyclopropane (1.49 mL, 12.3 mmol, 1.01 eq) was added. The suspension was diluted with 20 mL of dry DCM and stirred for 10 minutes. Aluminium chloride (4.41 g, 33.1 mmol, 2.7 eq) was added at -80 °C to the solution of **2** before the activated tetrachlorocyclopropane suspension was added by syringe, leading to gas formation. After 3h the reaction mixture was allowed to slowly reach room temperature and stirring was continued for 12h. The reaction was quenched carefully at 0°C by addition of 130 mL of 1M HCl. The clear solution was extracted three times with 50 mL $CHCl_3$ and the combined organic phases washed with brine, dried over Na_2SO_4 and the solvents evaporated. The raw product was purified by column chromatography on silica, eluting with $CHCl_3$ to $CHCl_3$/MeOH (9:1),

giving the cyclopropenone precursor as 1.4 g of a green solid, consisting of a mixture of isomers. The precursor was subsequently dissolved in 31 mL ACN and irradiated with UV light for 24h to give the desired cyclooctyne. The progress of the quantitative photoreaction was monitored by HPLC-MS. ^{1}H-NMR (400 MHz, CD_3CN): δ = 2.27-2.36 (m, 2H), 2.36-2.46 (m, 2H), 3.69 (d, J = 13.9 Hz, 1H), 3.84 (s, 3H), 3.85 (s, 3H),3.87 (s, 3H), 4.07 (s, 3H), 5.00 (d, J = 13.9 Hz, 1H), 6.89 (dd, J = 8.5 Hz, 2.6 Hz, 1H), 7.04 (s, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.22 (d, J = 2.6 Hz, 1H); ^{13}C NMR (400 MHz, CD_3CN) δ = 29.8, 30.1, 56.2, 56.5, 57.1, 61.5, 61.5, 105.4, 109.1, 111,0 113.5, 115.6, 116.2, 119.7, 127.1, 141.9, 148.1, 151.0, 151.2, 154.6, 160.2, 172.9, 174.2. ESI-MS: *m/z* = 426.1 $[M+H]^+$, 448.1 $[M+Na]^+$, 872.8 $[2M+Na]^+$. MALDI-HR-MS: calculated for $C_{23}H_{23}NO_7$ 425.14690, found 425.14674 as $[M]^{+\cdot}$.

Scheme S3. Synthesis of 15-azidopentadecanoic acid by hydrolysis of pentadecanolide, subsequent bromination of the hydroxy fatty acid and final azidation.

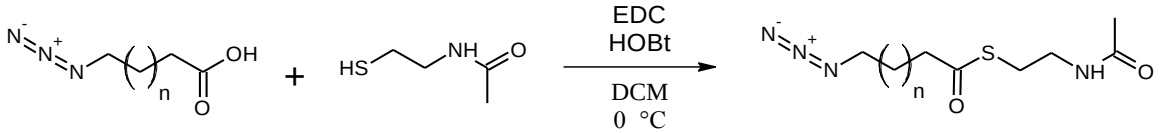


15-Azidopentadecanoic acid (2g)

Pentadecanolide (4.0 g, 16.6 mmol) was dissolved in a mixture of 200 mL MeOH and 20 mL H_2O to which potassium hydroxide (3.7 g, 66.6 mmol, 4 eq) were added. The solution was stirred for 72h, after which it was acidified with 70 mL of 1M HCl and extracted 5 times with 50 mL EtOAc. The organic fractions were combined, dried over Na_2SO_4 and the solvent evaporated, giving 4.14 g of a white solid which consisted almost entirely of the hydrolysis product as confirmed by TLC. A part of the raw product (1.0 g, 3.87 mmol) and triphenylphosphine (1.05 g, 4.0 mmol, 1.05 eq) were then dissolved in 20 mL dry DCM before tetrabromomethane (1.33 g, 4.0 mmol, 1.05 eq) was added. The reaction mixture was stirred at room temperature for 46h before the mixture was diluted with 10 mL H_2O and 20 mL of citric acid solution (20%) were added. The bromination product was extracted three times with 30 mL EtOAc and once with 30 mL of Et_2O. The combined organic phases were dried over

Na_2SO_4 and the solvents evaporated, giving the bromination product as a white solid (977 mg, 3.04 mmol, 1 eq). NMR analysis showed sufficient product purity for direct use in the following substitution step. The bromide was dissolved in 10 mL dry DMF, then sodium azide (593 mg, 9.1 mmol) was added and stirred for 6h at 60 °C. Subsequently the solvent was evaporated and 30 mL of aqueous HCl were added. The raw final product was extracted from the aqueous phase three times with 30 mL of EtOAc, the organic fractions were combined and dried over Na_2SO_4. Purification was conducted by column chromatography on silica, eluting with Hexane to Hexane/EtOAc (1:1), giving the product as a white solid (295 mg, 0.92 mmol, 6%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.17-1.39 (m, 20H), 1.53-1.65 (m, 4H), 2.32 (t, J = 7.6 Hz, 2H), 3.23 (t, J = 7.0 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ = 24.7, 26.7, 28.8, 29.0, 29.1, 29.2, 29.4, 29.5, 29.5, 29.5, 29.6, 29.6, 34.0, 51.5, 180.0.

General procedure for the synthesis of azido fatty acid SNAC esters



n = 2: **2b**; n = 3: **2c**

n = 2: **5a**, n = 3: **5b**

The two azidoacyl-SNACs were synthesized in the same way with equal molar quantities. The general procedure of the reaction of the corresponding AFA with NAC is exemplified for the synthesis of *S*-(5-Azidopentanoyl)-*N*-acetylcysteamine (**5a**):

***S*-(5-Azidopentanoyl)-*N*-acetylcysteamine (5a)**

To a solution of **2b** (200 mg, 1.27 mmol, 1 eq.) in 10 mL of dry DCM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (280 mg, 1.46 mmol, 1.15 eq.) and hydroxybenzotriazole (172 mg, 1.27 mmol, 1 eq.) were added at 0 °C, before N-acetylcysteamine (212 mg, 1.78 mmol, 1.4 eq.) was added by syringe. After stirring for 1h the reaction mixture was allowed to reach room temperature and was stirred for another 3h. The reaction was quenched by addition of 20 mL of 1M HCl and the product was extracted three times with DCM. The combined organic phases were dried over Na_2SO_4, the solvents were evaporated and the raw product was purifiedby

column chromatography on silica, eluting with Hexane to Hexane/EtOAc (1:1), giving **5a** as colorless liquid (102 mg, 0.42 mmol, 42%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.53 (tt, J = 8.2 Hz, 6.9 Hz, 2H), 1.70 (tt, J = 7.8 Hz, 7.0 Hz, 2H), 1.91 (s, 3H), 2.56 (t, J = 7.3 Hz, 2H), 2.97 (t, J = 6.5 Hz, 2H), 3.24 (t, J = 6.7Hz, 2H), 3.36 (q, J = 6.3 Hz, 2H), 6.10 (br, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 22.6, 23.0, 27.9, 28.4, 39.4, 43.2, 50.8, 170.3, 199.1.

_S_-(6-Azidohexanoyl)-_N_-acetylcysteamine (5b)

Yield: 127 mg, 0.49 mmol, 39%. ^{1}H-NMR (400 MHz, $CDCl_3$): δ =1.30-1.38 (m, 2H), 1.53 (quin. J = 7.0, 2H), 1.62 (quin., J = 7.6 Hz, 2H), 1.90 (s, 3H), 2.51 (t, J = 7.4 Hz, 2H), 2.95 (t, J = 6.5 Hz, 2H), 3.20 (t, J = 6.8 Hz, 2H), 3.34 (q, J = 6.4 Hz, 2H), 6.24 (br, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ = 22.9, 24.9, 25.8, 28.3, 28.3, 39.4, 43.6, 51.0, 170.4, 199.4.

Supplementary figures

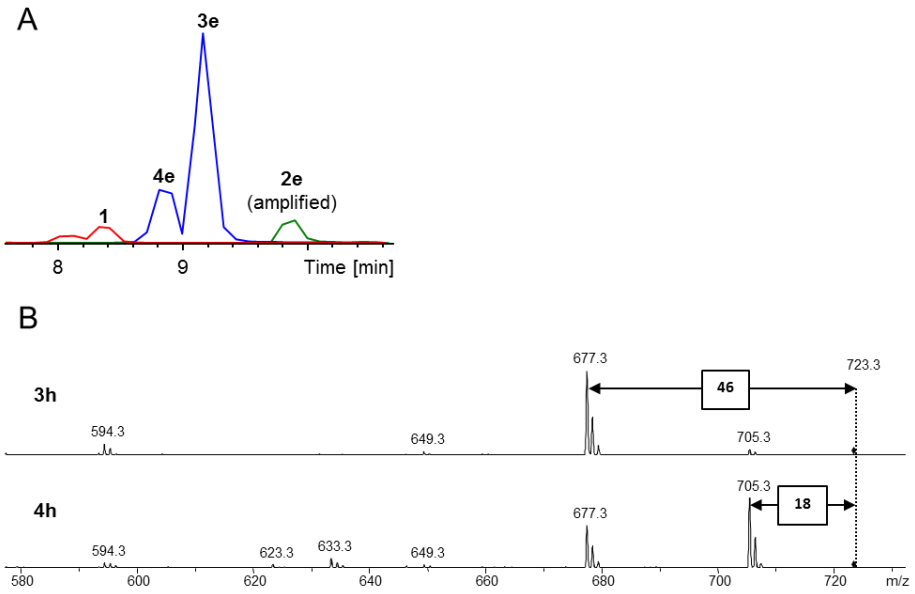


Figure S1. (A) EIC of reactants **1** (as $[M+H]^+$) and **2e** (as $[M-H]^-$, peak amplified 1000-fold for better visibility) in comparison with the EIC of the clicked product (**3e**/**4e**) after the reaction, showing a significant increase in signal strength. (B) MS^2 Fragmentation pattern of click products **3h** and **4h**: *m/z* $[M+H]^+$ = 723.3.

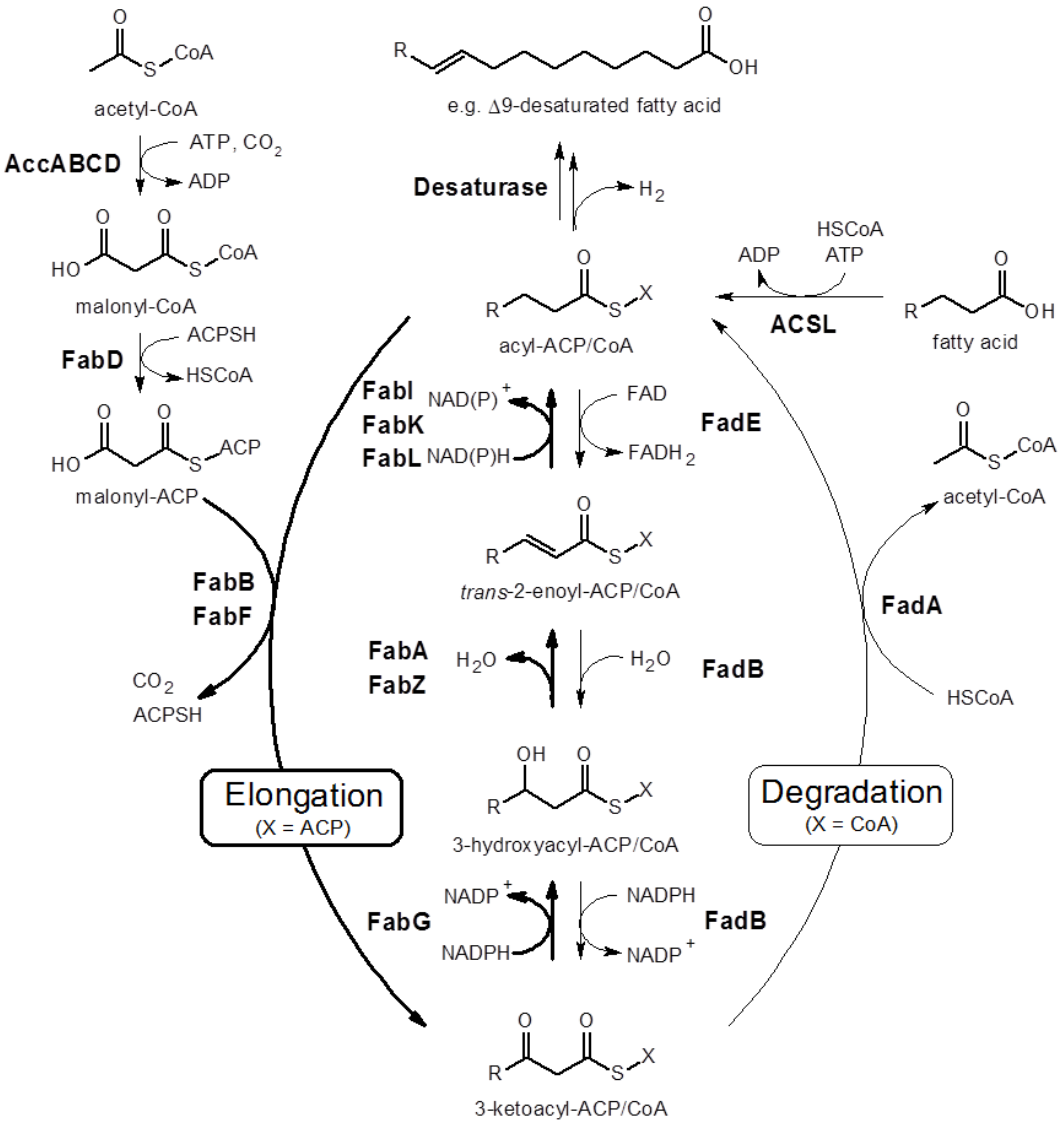
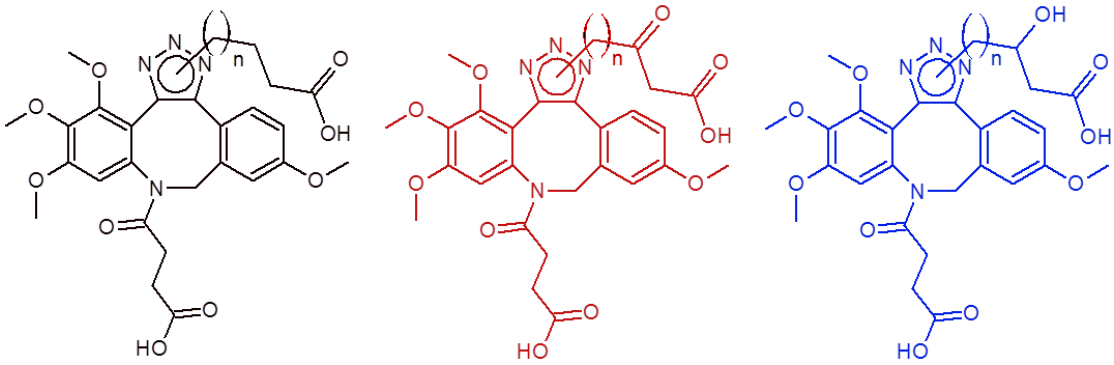


Figure S2. Central steps of fatty acid elongation and degradation in *E. coli*.



3/4d,f,i,l **8a-d** **9a-d**

n			
5	**3/4i**	**8a**	**9a**
7	**3/4d**	**8b**	**9b**
9	**3/4f**	**8c**	**9c**
11	**3/4l**	**8d**	**9d**

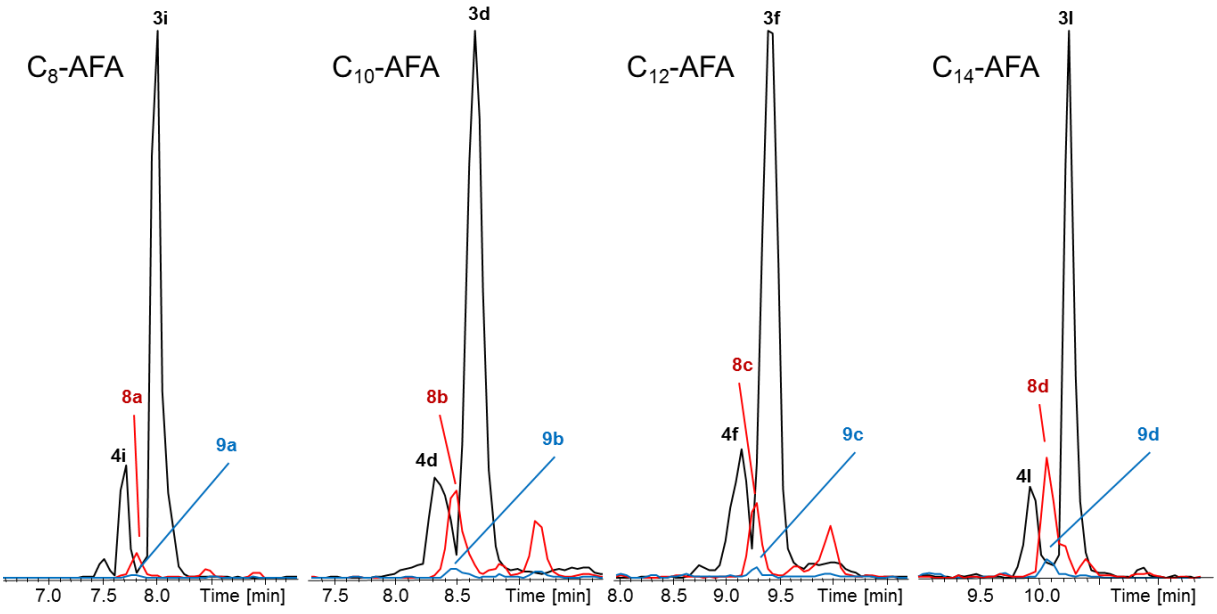


Figure S3. EICs of various clicked degradation products of **2h**. 3-Keto-AFAs (detected as **8a-d**, red) and to a lesser degree 3-hydroxy-AFAs (detected as **9a-d**, blue) can be detected at high concentration of the corresponding AFAs **2i**, **2d**, **2f** and **2l**. Either of the two regioisomers is assumed to attribute to the detected masses.

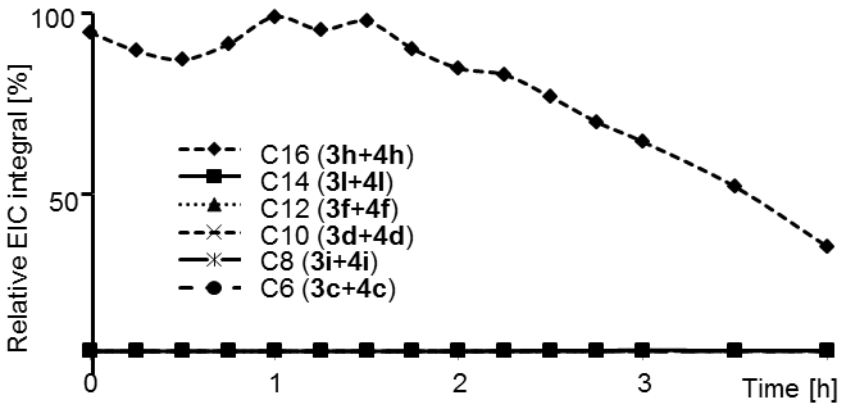


Figure S4. Relative abundance of **2h** degradation products when fed to *fadE*-mutant. All degradation products remain below detectable levels due to inhibition of FA degradation.

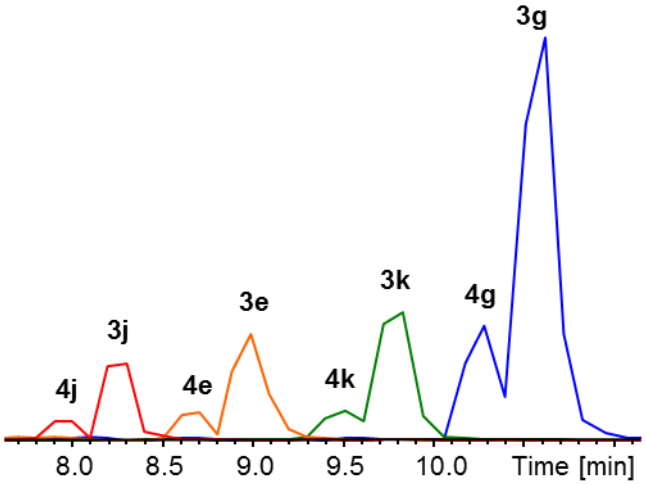
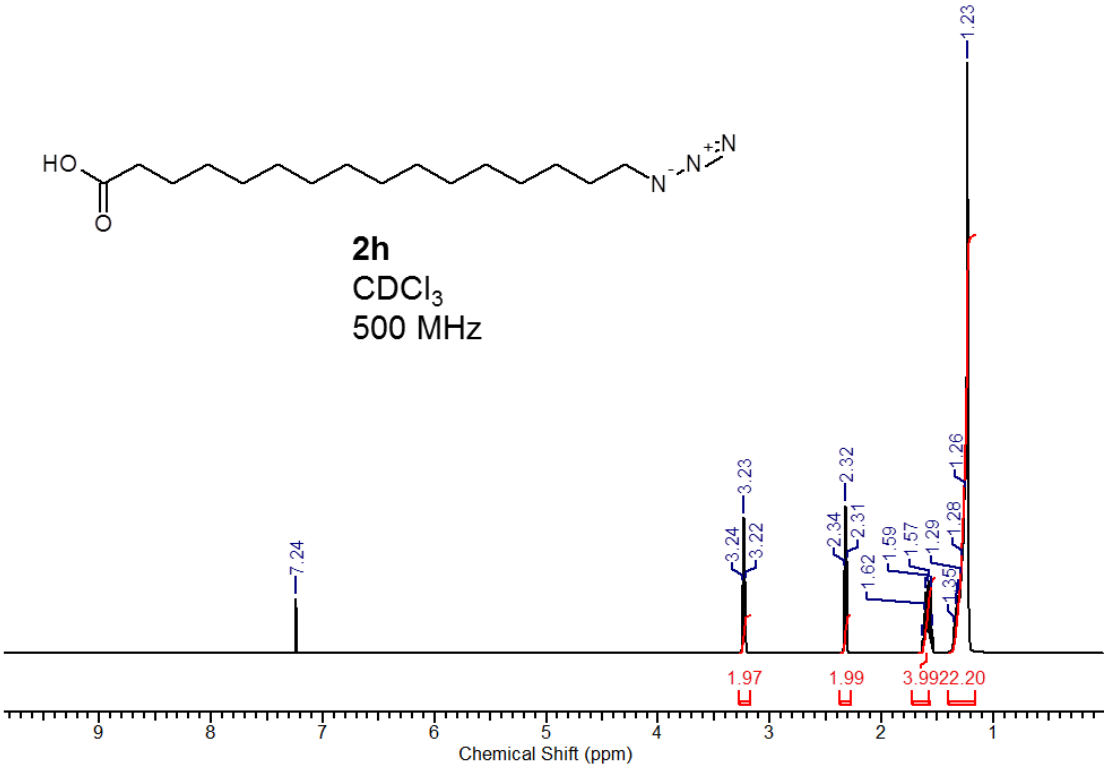
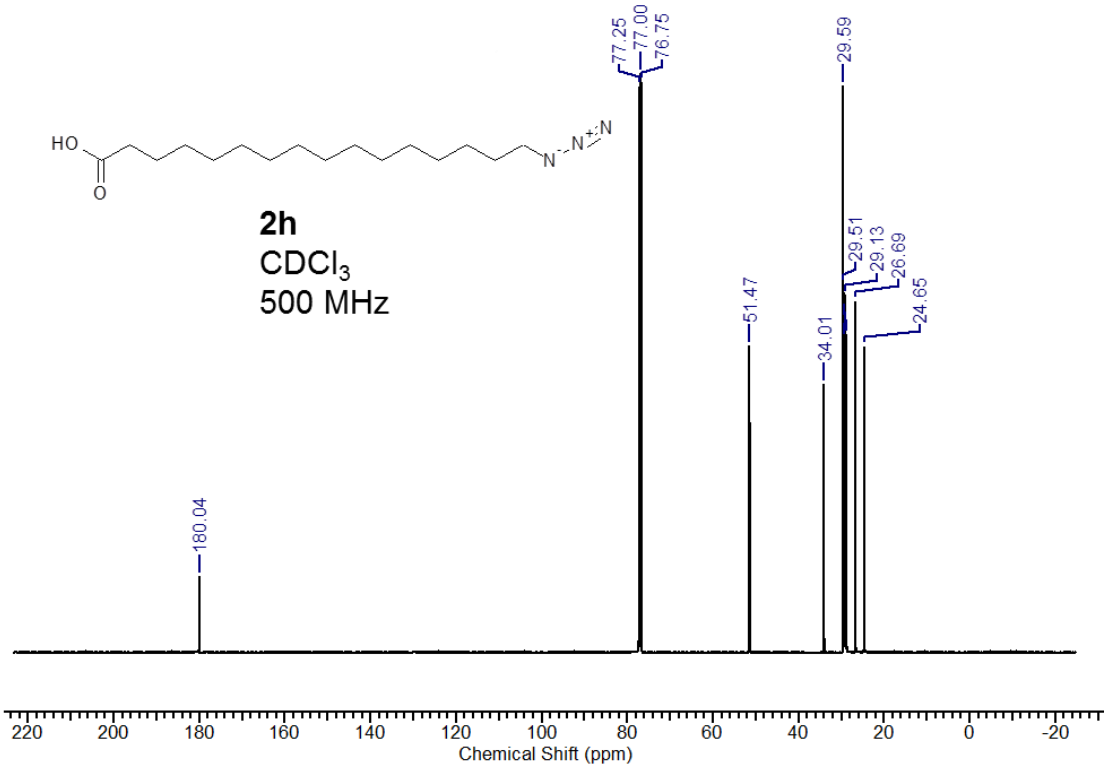


Figure S5. EICs of various clicked degradation products of **2g** (blue) 2h after feeding it to *E. coli* DH10B wildtype, indicating the formation of C_{13}- (green), C_{11}- (orange) and C_9-AFA (red).

NMR Spectra


HO
O
N
N
N
2h
CDCl3
500 MHz
1.23
7.24
3.24
3.23
3.22
2.34
2.32
2.31
1.62
1.59
1.57
1.29
1.28
1.26
1.97
1.99
3.99
22.20
Chemical Shift (ppm)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O
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N
N
2h
CDCl3
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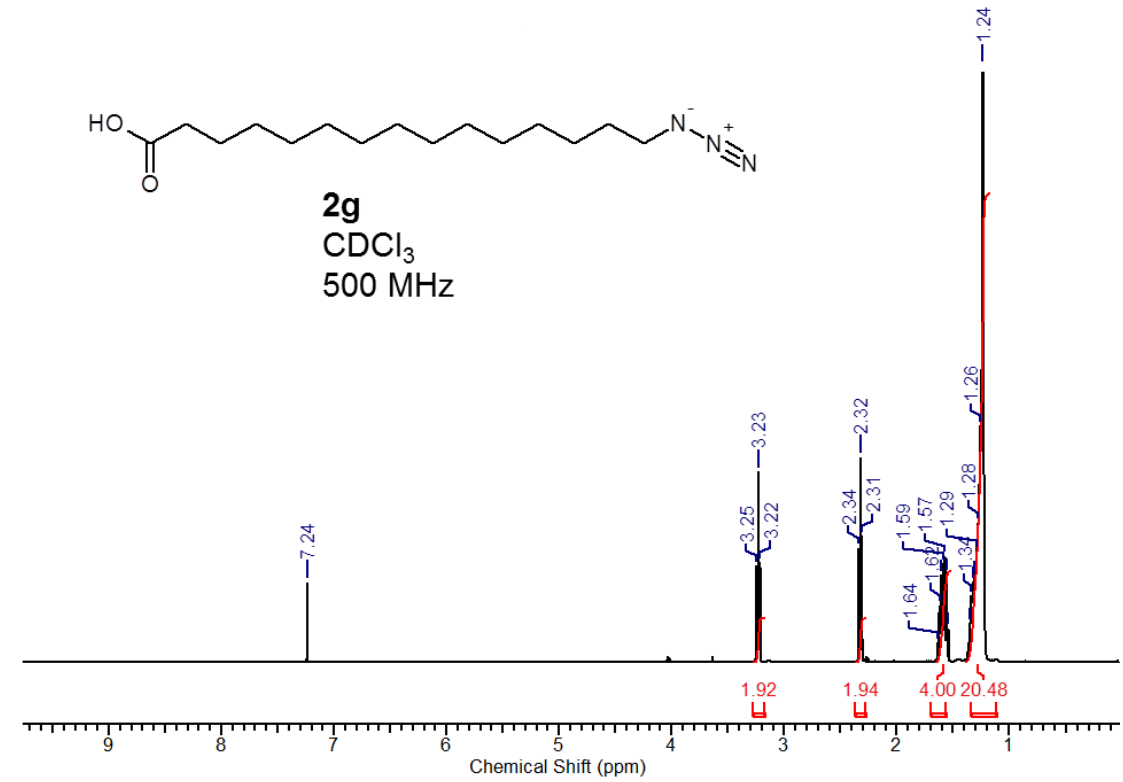
29.13
26.69
24.65
180.04
Chemical Shift (ppm)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O
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N
N
2g
CDCl3
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2.32
1.28
3.25
3.22
2.34
2.31
1.59
1.57
1.29
1.64
1.34
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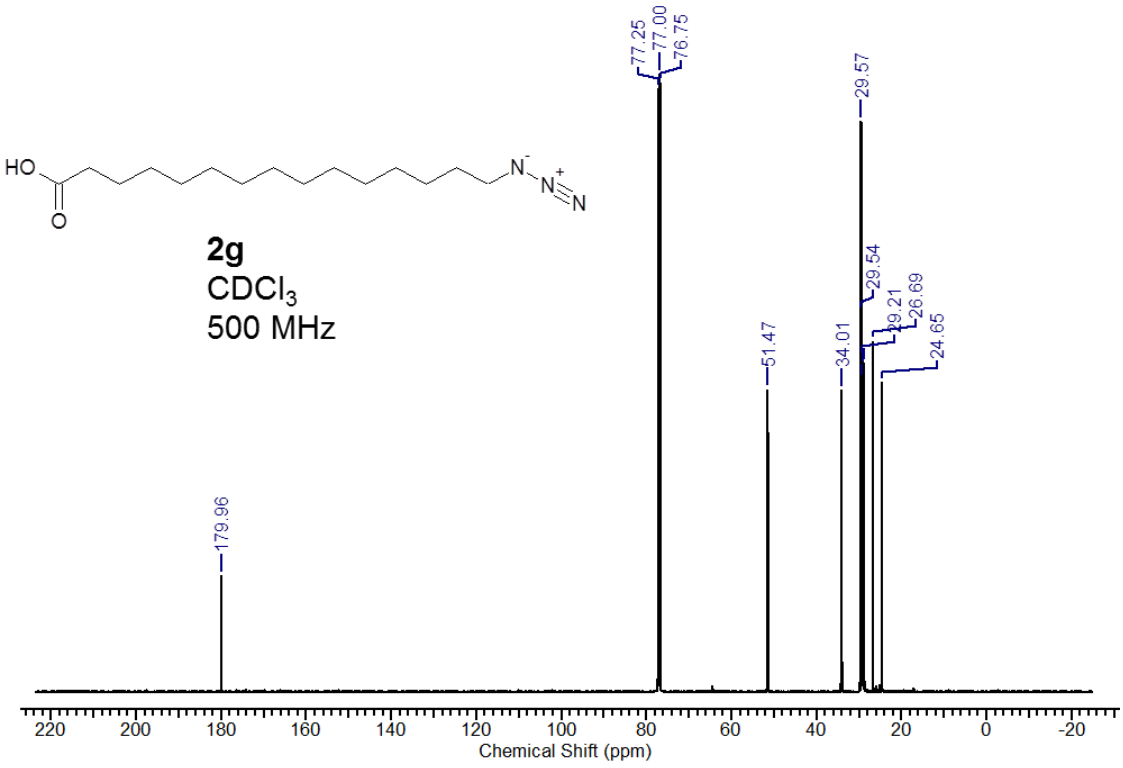
9
8
7
6
5
4
3
2
1
Chemical Shift (ppm)


HO
O
N
N
N
2g
CDCl3
500 MHz
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76.75
29.57
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26.69
24.65
51.47
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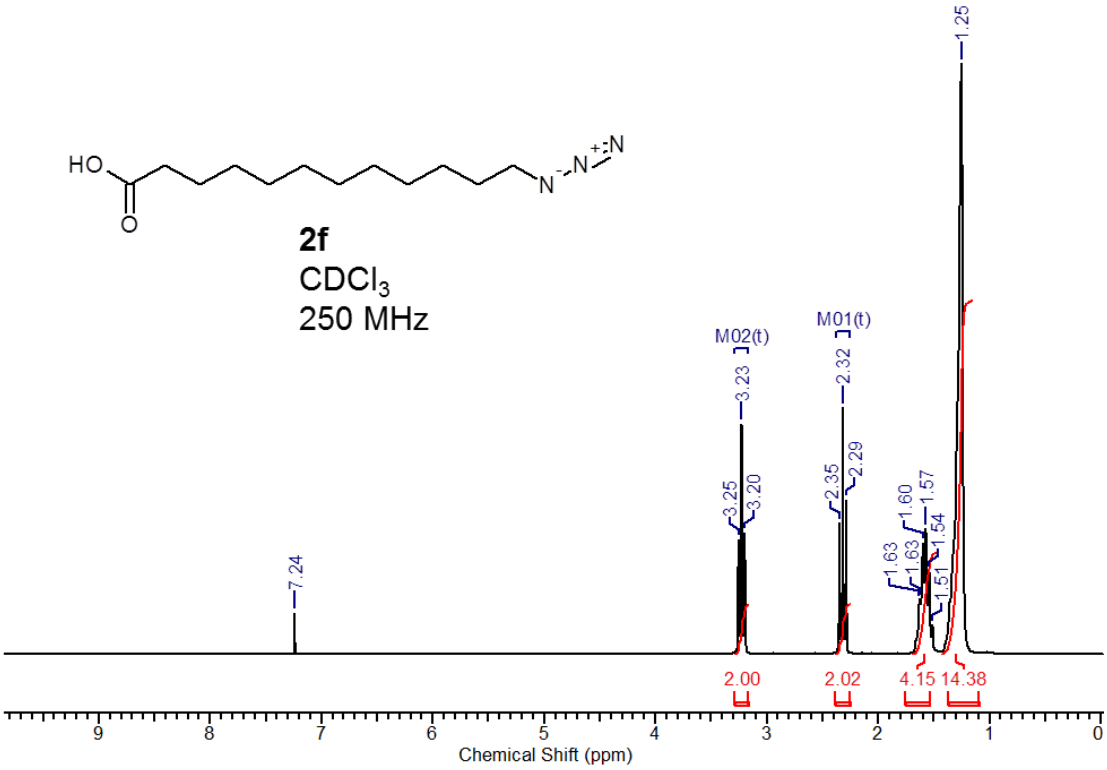
220
200
180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)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O
N
N
N
2f
CDCl3
250 MHz
M02(t)
M01(t)
7.24
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2.32
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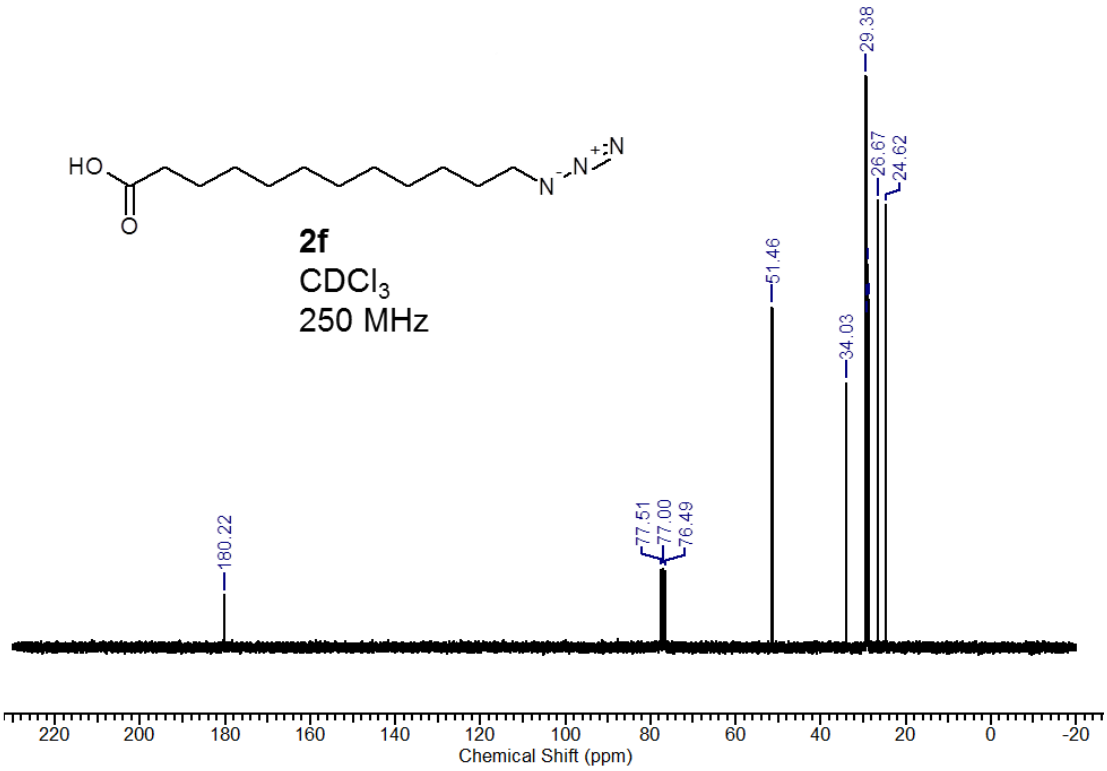
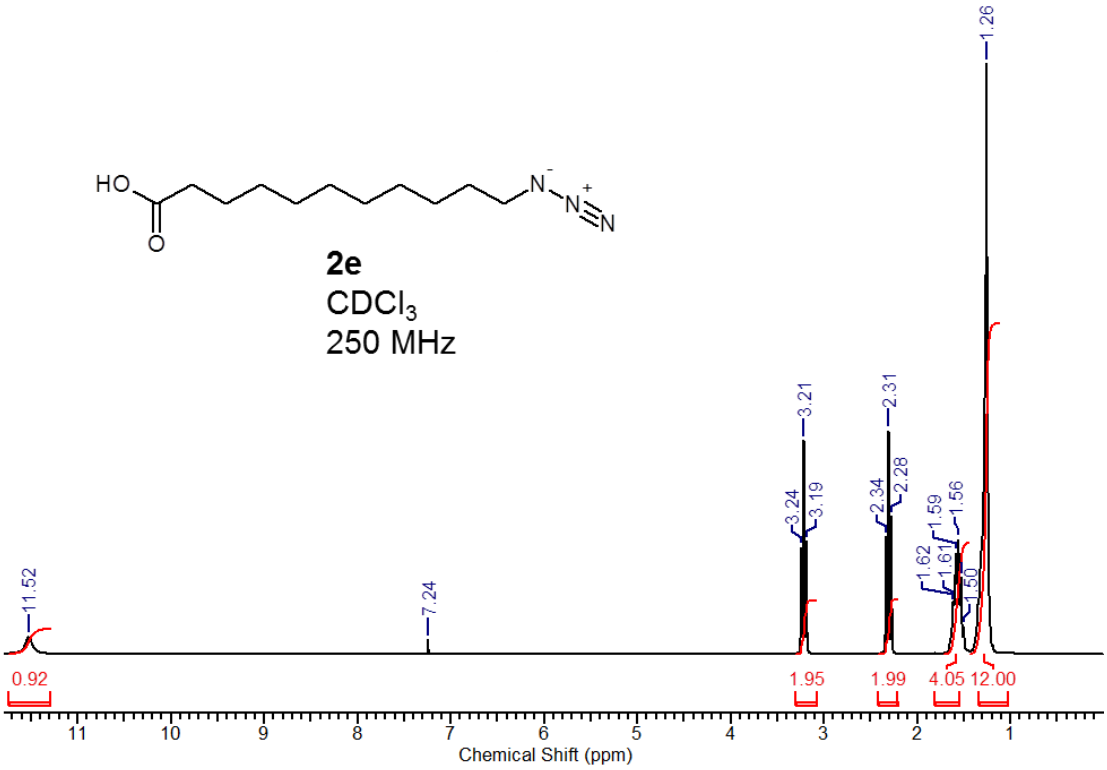
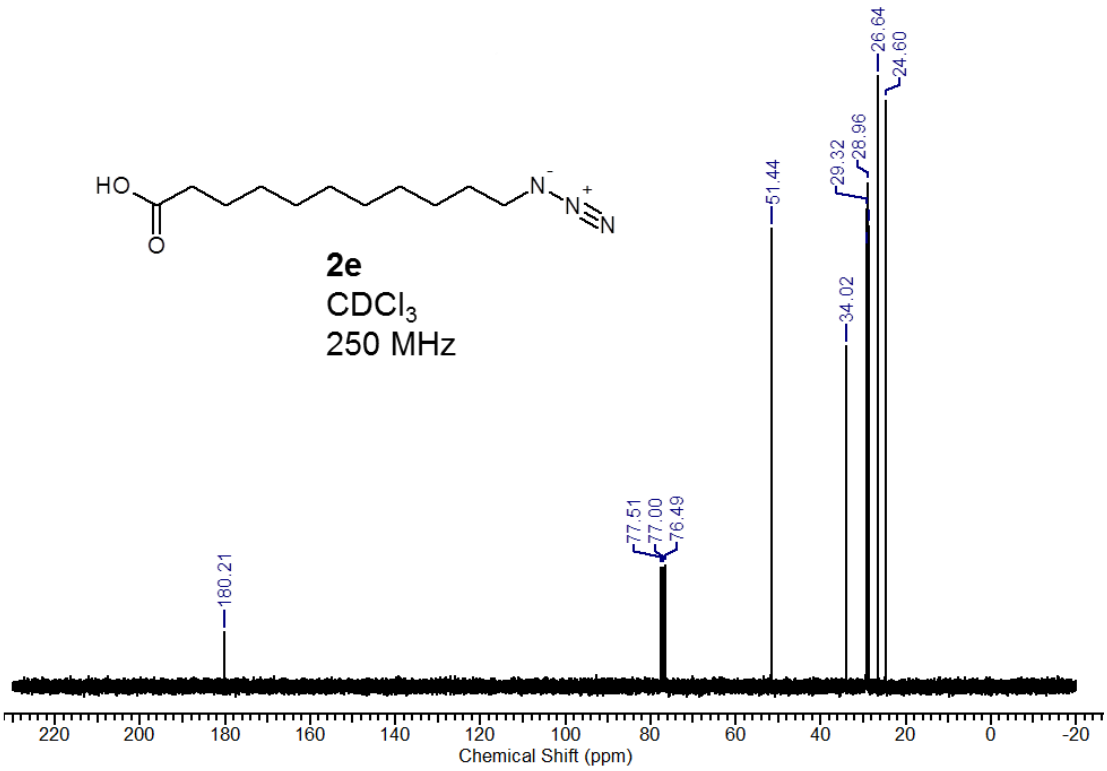
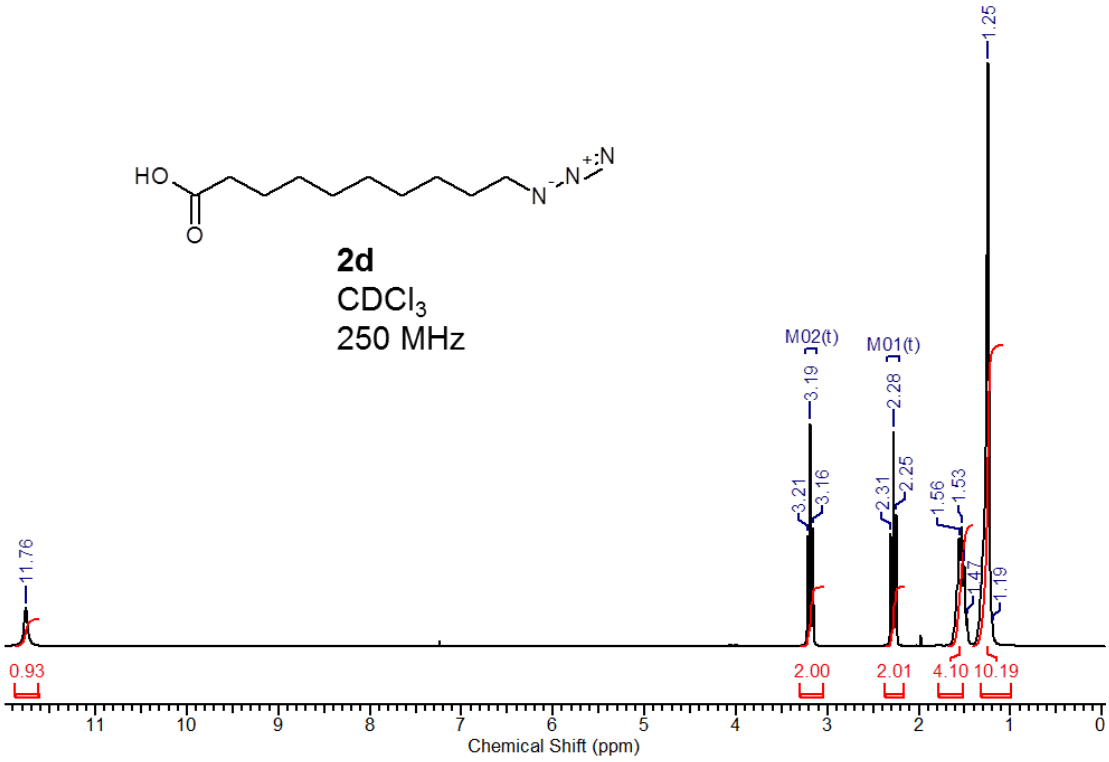
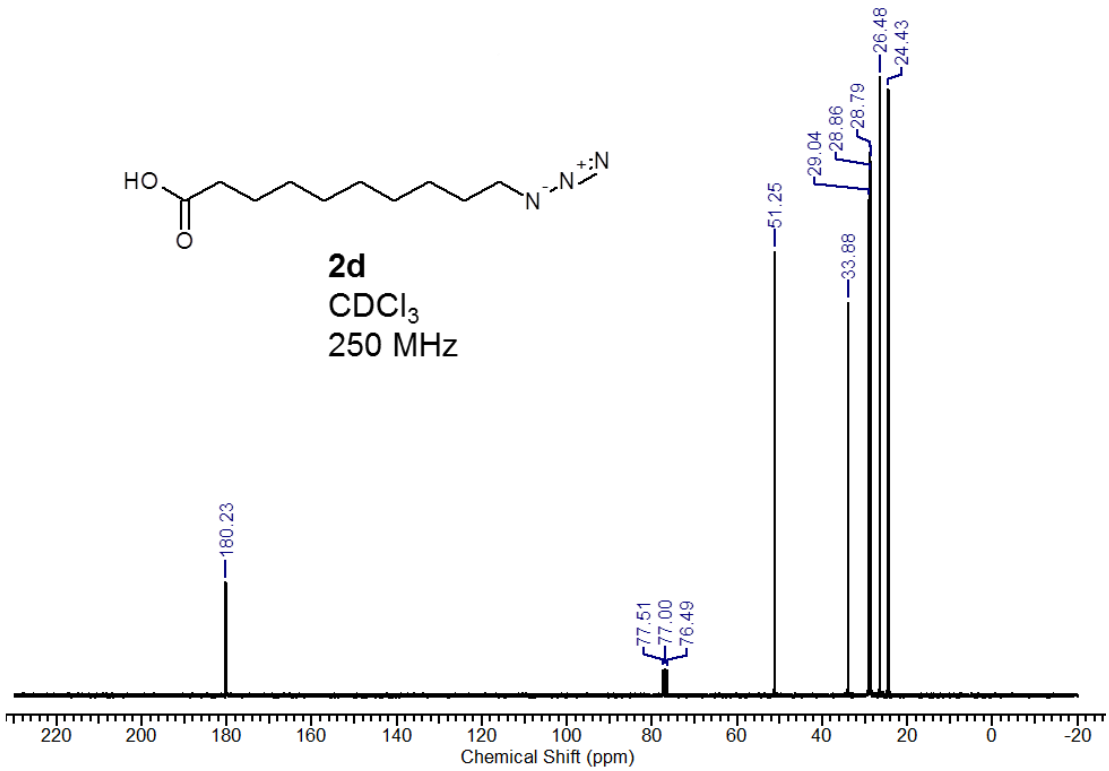
2.00
2.02
4.15
14.38
Chemical Shift (ppm)

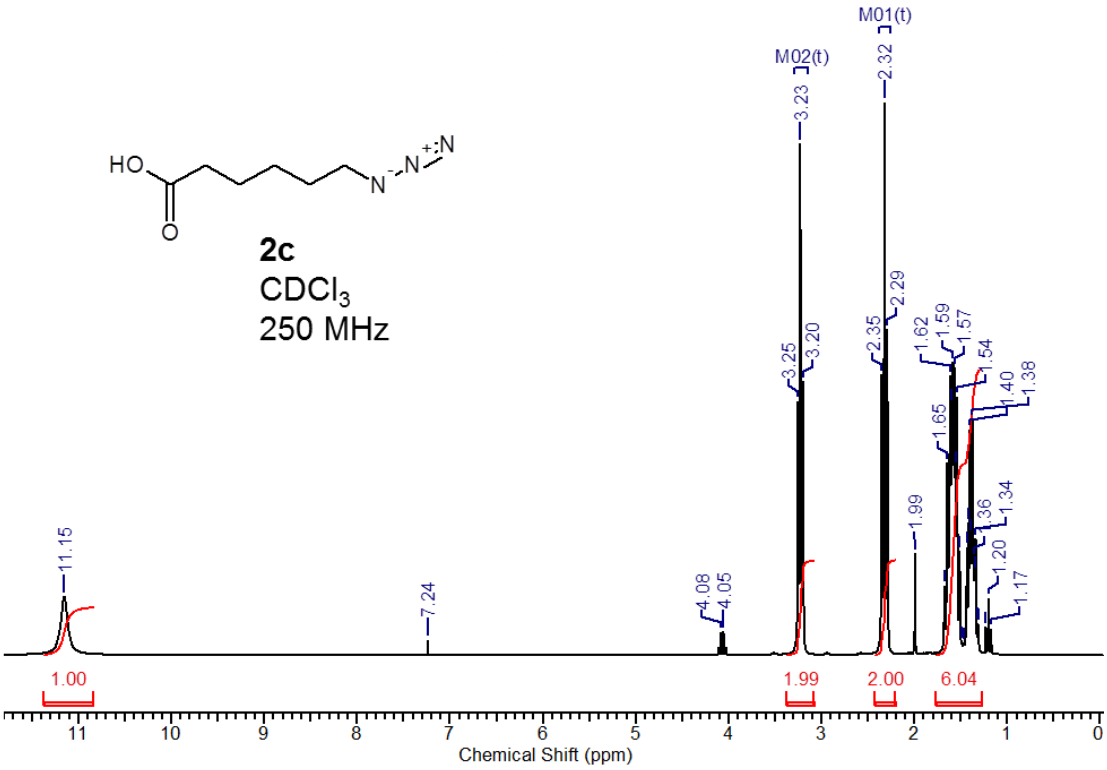

HO
O
N
N
N
2f
CDCl3
250 MHz
180.22
77.51
77.00
76.49
51.46
34.03
29.38
26.67
24.62
Chemical Shift (ppm)

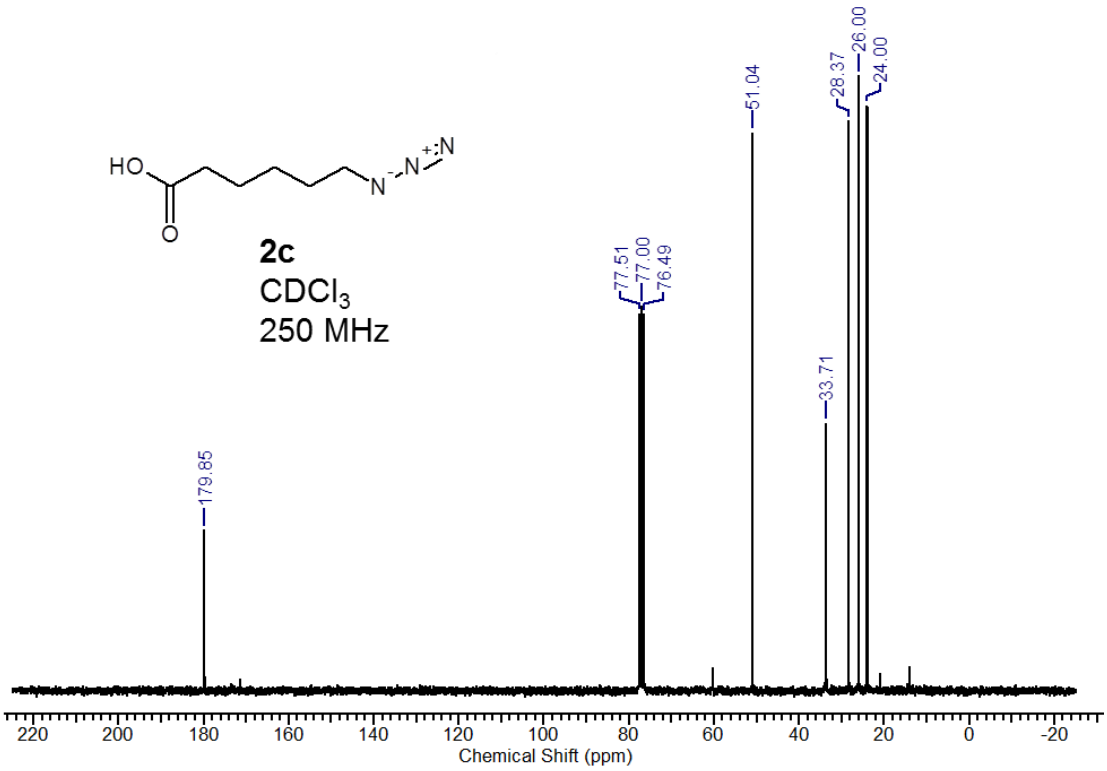

HO
O
N
N
N
2e
CDCl3
250 MHz
11.52
7.24
3.24
3.21
3.19
2.34
2.31
2.28
1.62
1.61
1.59
1.56
1.50
1.26
0.92
1.95
1.99
4.05
12.00
Chemical Shift (ppm)

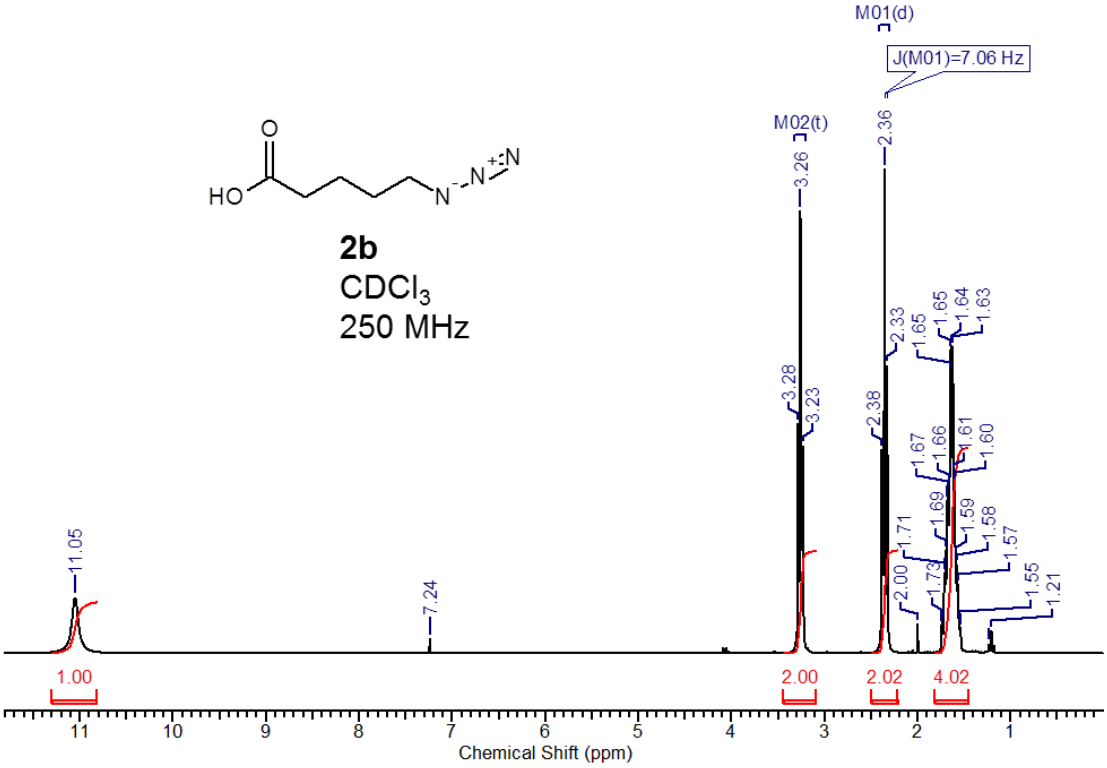

HO
O
N
N
N
2e
CDCl3
250 MHz
180.21
77.51
77.00
76.49
51.44
34.02
29.32
28.96
26.64
24.60
Chemical Shift (ppm)

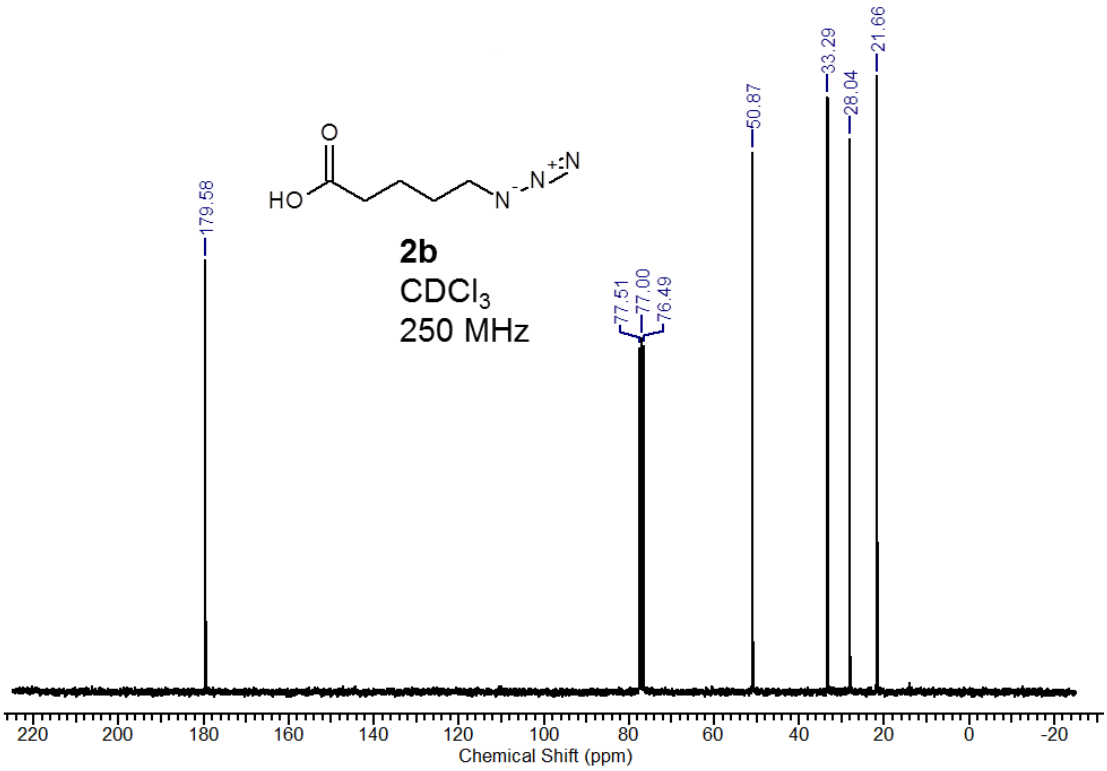

HO
O
N
N
+:N
2d
CDCl3
250 MHz
M02(t)
M01(t)
11.76
3.19
3.21
3.16
2.28
2.31
2.25
1.56
1.53
1.47
1.19
1.25
0.93
2.00
2.01
4.10
10.19
Chemical Shift (ppm)

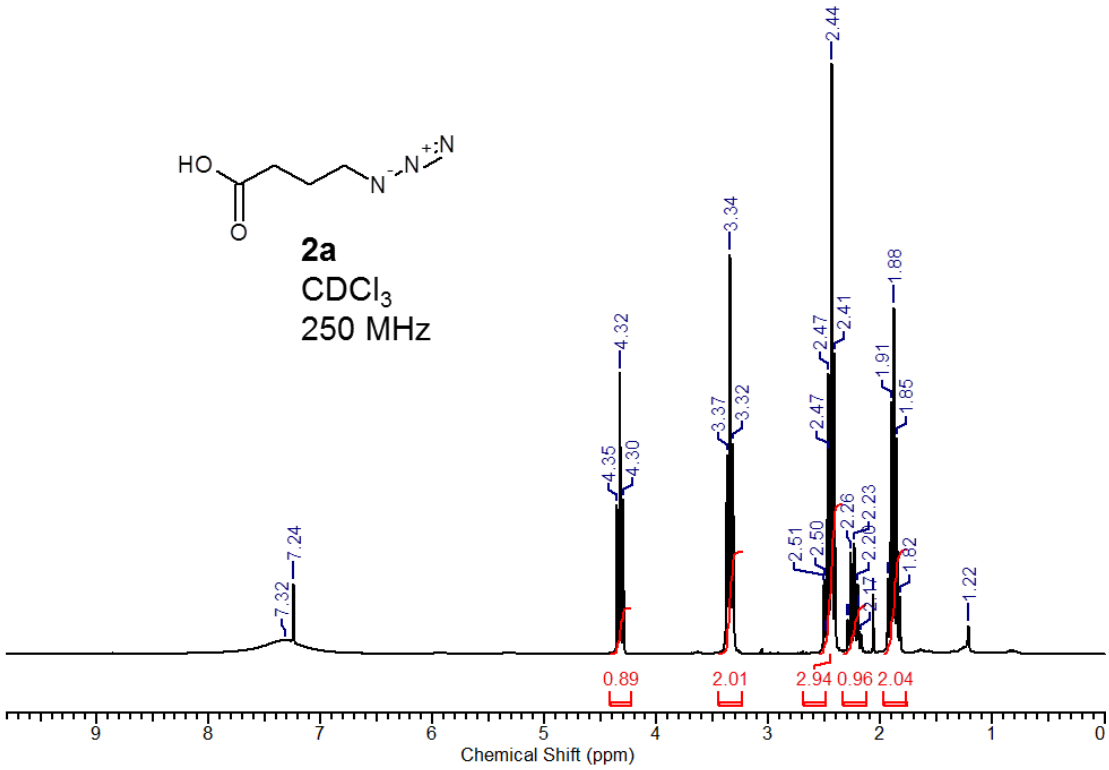

HO
O
N
N
+:N
2d
CDCl3
250 MHz
180.23
77.51
77.00
76.49
51.25
33.88
29.04
28.86
28.79
26.48
24.43
Chemical Shift (ppm)

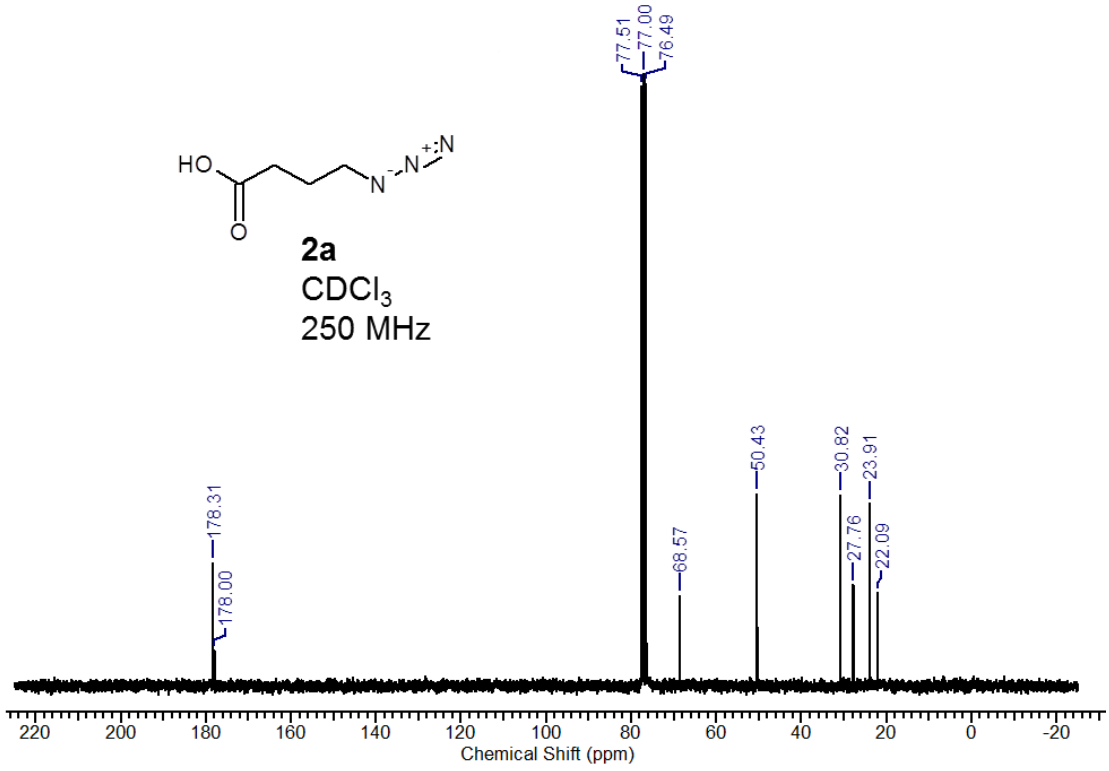

HO
O
N
N
+:N
2c
CDCl3
250 MHz
M01(t)
M02(t)
2.32
3.23
3.25
3.20
2.35
2.29
1.62
1.59
1.57
1.54
1.40
1.38
1.65
1.99
1.36
1.34
1.20
1.17
11.15
7.24
4.08
4.05
1.00
1.99
2.00
6.04
Chemical Shift (ppm)

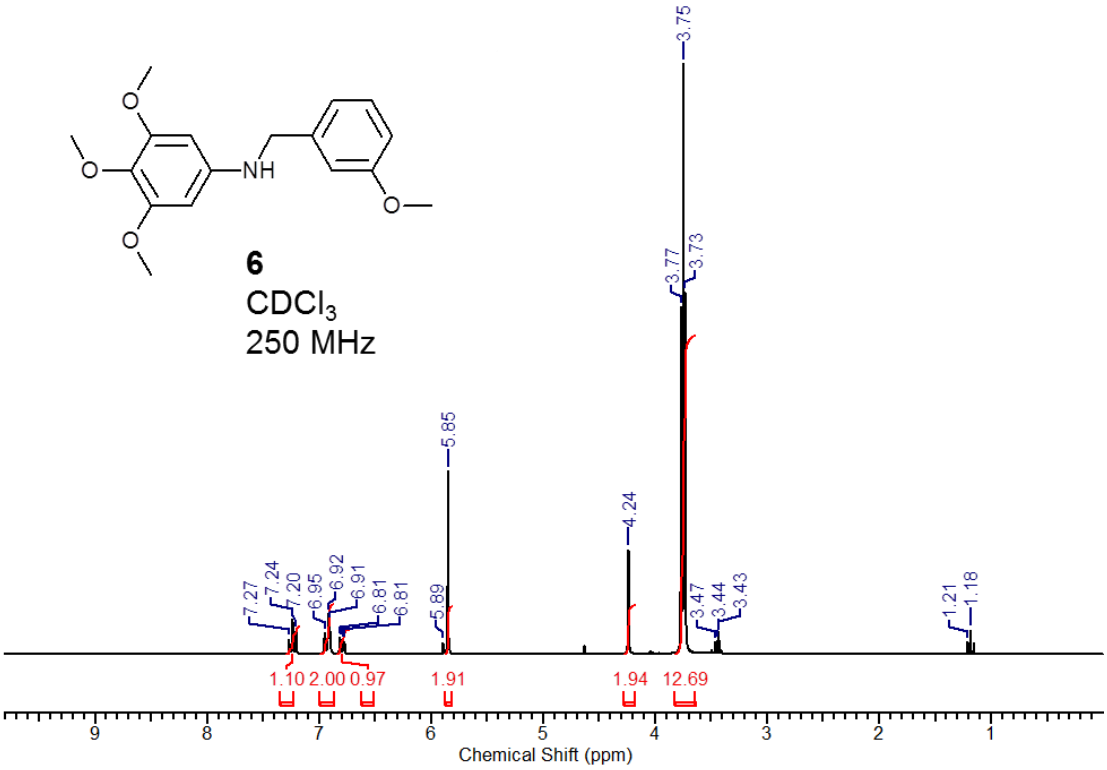

HO
O
N
N
+:N
2c
CDCl3
250 MHz
51.04
28.37
26.00
24.00
77.51
77.00
76.49
33.71
179.85
Chemical Shift (ppm)

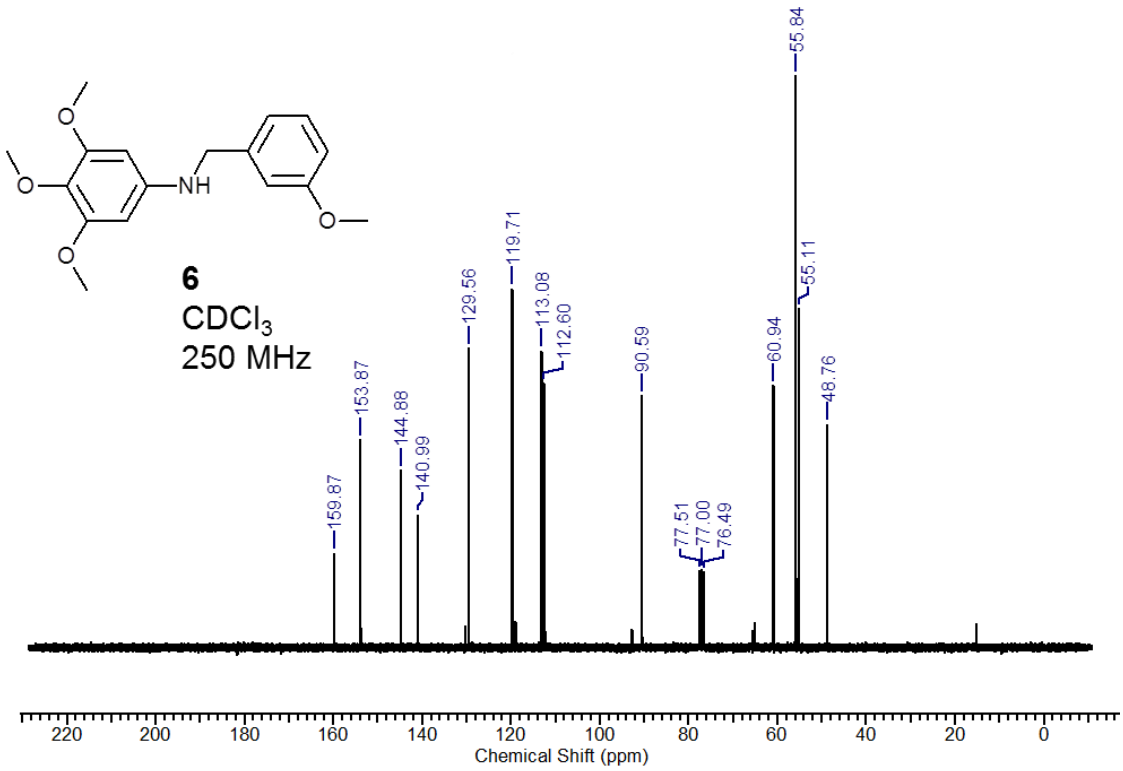

M01(d)
J(M01)=7.06 Hz
M02(t)
2.36
3.26
2.33
1.65
1.64
1.63
3.28
3.23
2.38
1.67
1.66
1.61
1.60
1.69
1.59
1.58
1.57
1.71
2.00
1.73
1.55
1.21
11.05
7.24
1.00
2.00
2.02
4.02
11
10
9
8
7
6
5
4
3
2
1
Chemical Shift (ppm)
HO
O
N
N
2b
CDCl3
250 MHz

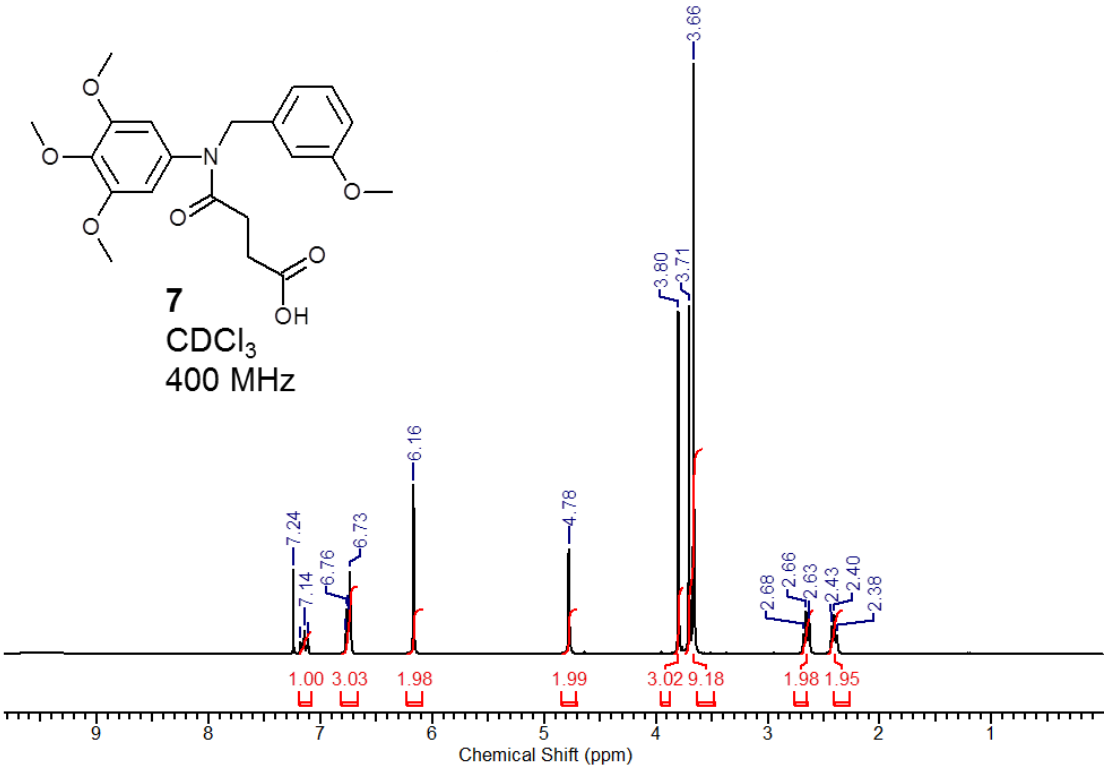

179.58
77.51
77.00
76.49
50.87
33.29
28.04
21.66
220
200
180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)
HO
O
N
N
2b
CDCl3
250 MHz

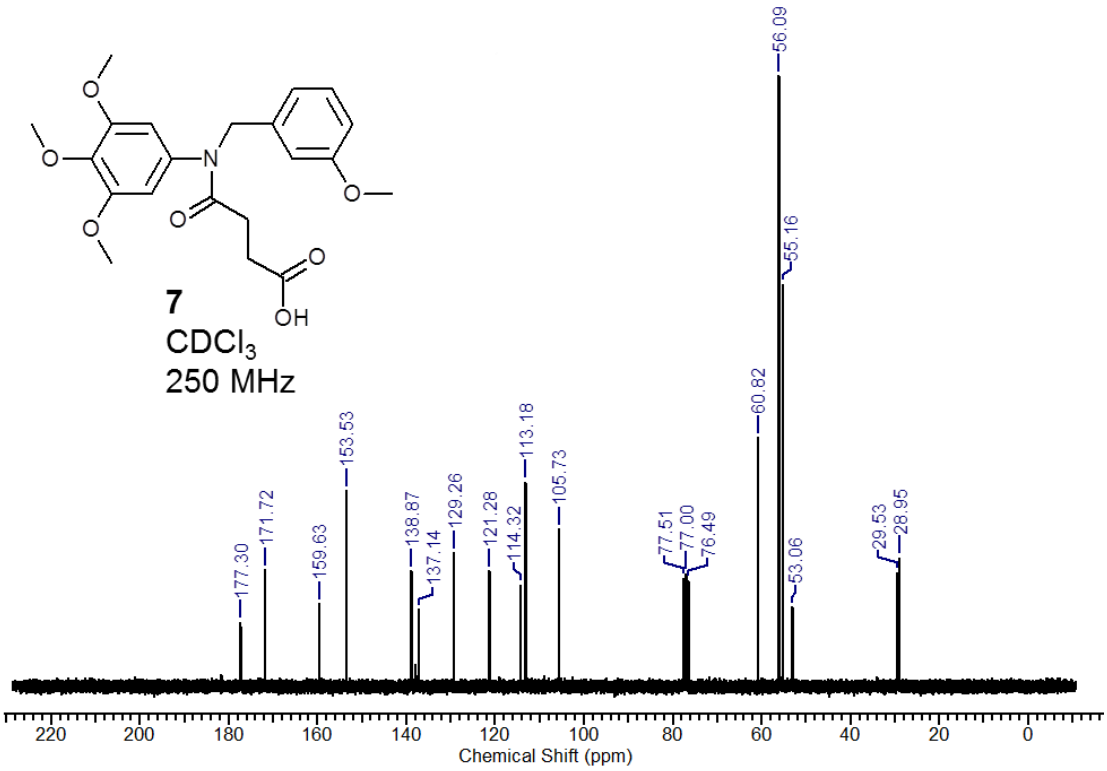

HO
O
N
N
+
N
2a
CDCl3
250 MHz
2.44
3.34
4.32
1.88
2.41
2.47
1.91
3.37
3.32
1.85
2.47
4.35
4.30
2.26
2.23
2.51
2.50
2.20
1.82
7.24
7.32
1.22
0.89
2.01
2.94
0.96
2.04
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

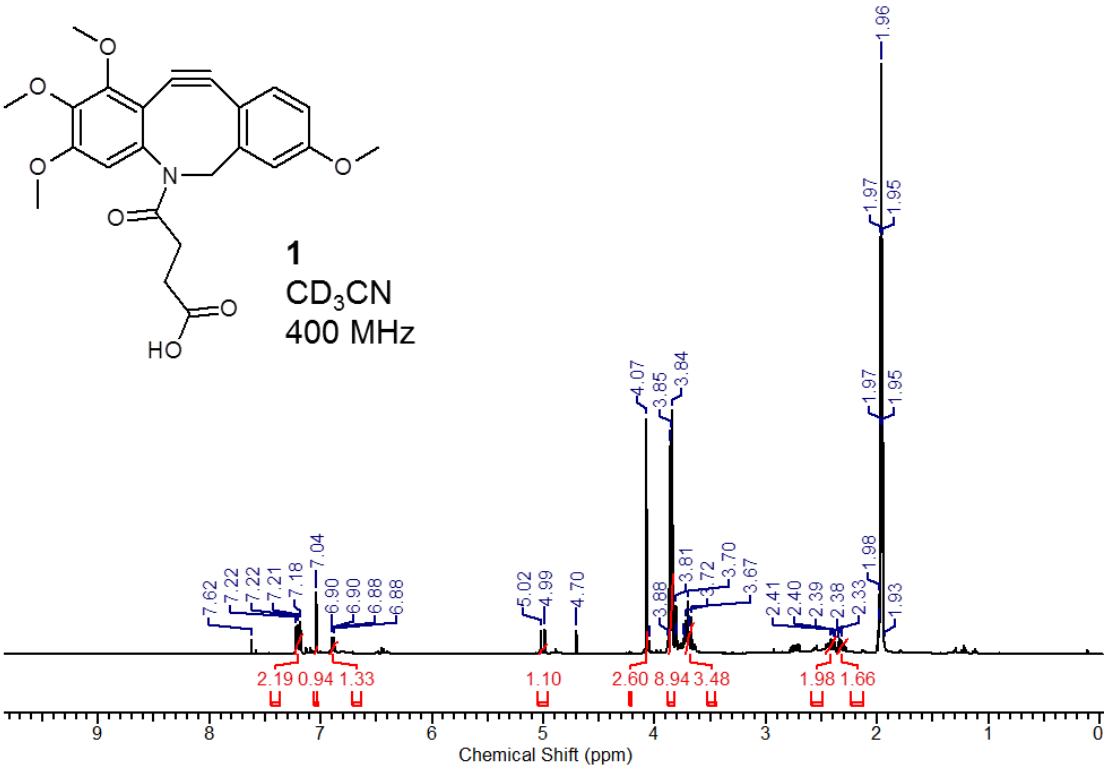

HO
O
N
N
+
N
2a
CDCl3
250 MHz
77.51
77.00
76.49
178.31
178.00
68.57
50.43
30.82
23.91
27.76
22.09
220
200
180
160
140
120
100
80
60
40
20
0
-20
Chemical Shift (ppm)

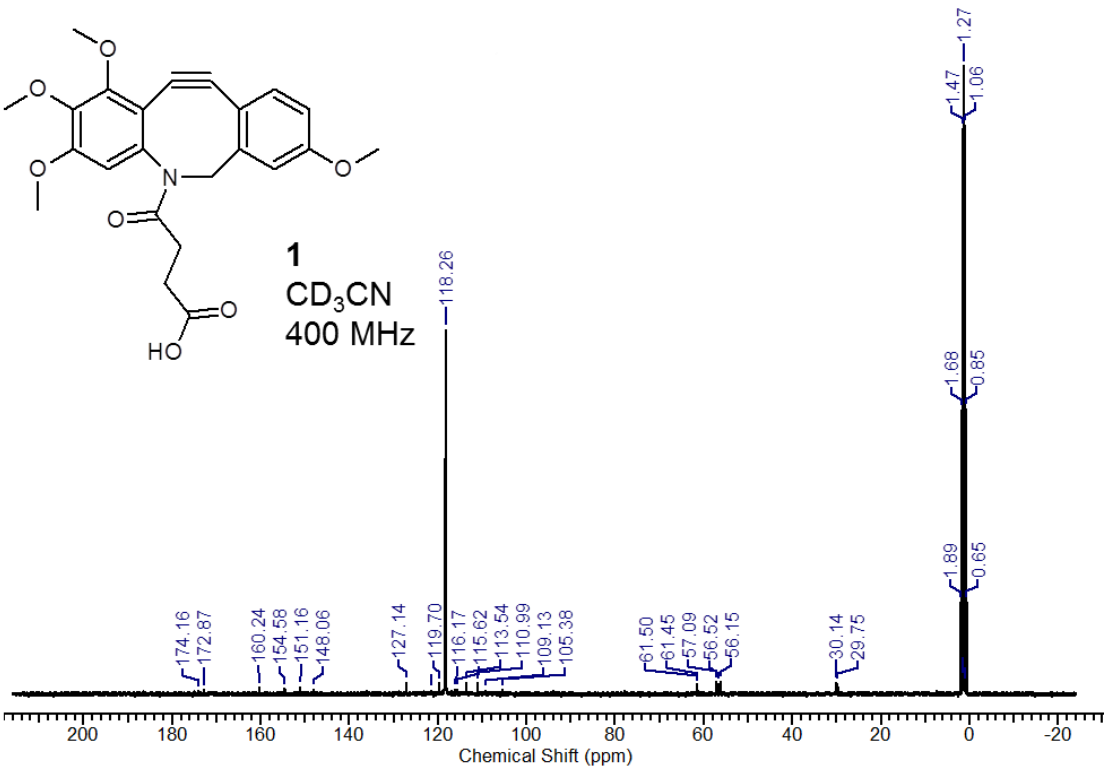

6
CDCl3
250 MHz
3.75
3.77
3.73
5.85
4.24
7.27
7.24
7.20
6.95
6.92
6.91
6.81
6.81
5.89
3.47
3.44
3.43
1.21
1.18
1.10 2.00 0.97
1.91
1.94 12.69
Chemical Shift (ppm)

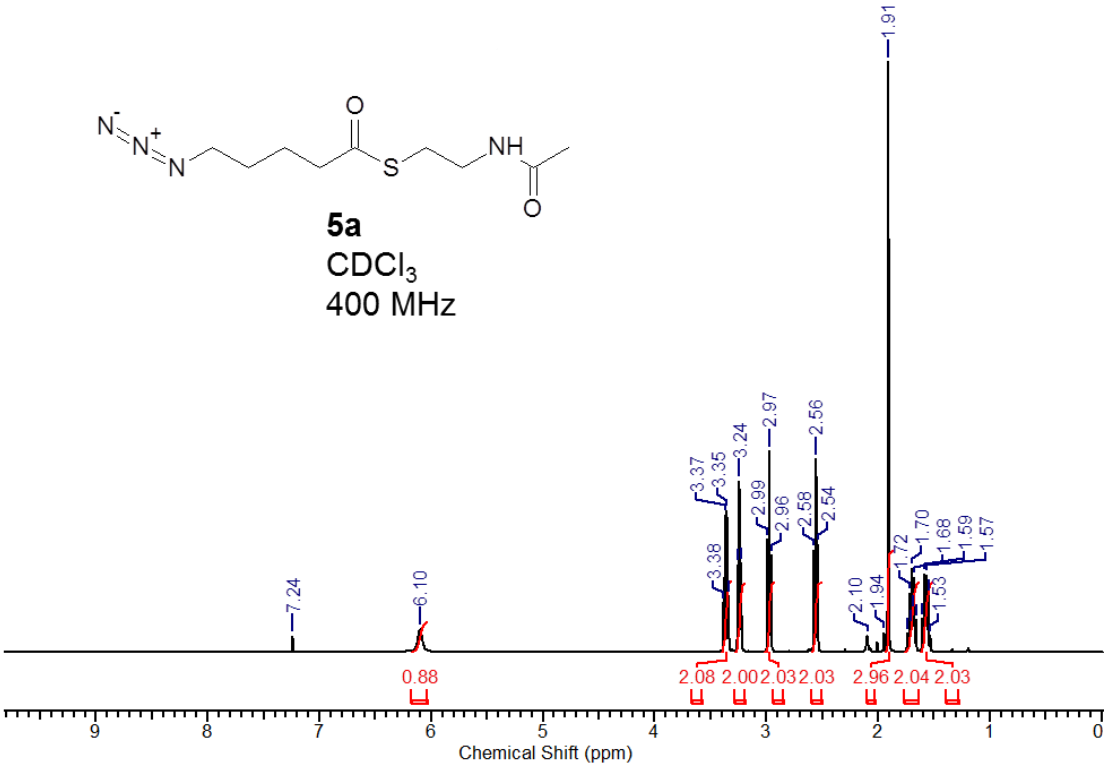

6
CDCl3
250 MHz
55.84
55.11
129.56
119.71
113.08
112.60
153.87
144.88
140.99
159.87
90.59
60.94
48.76
77.51
77.00
76.49
Chemical Shift (ppm)

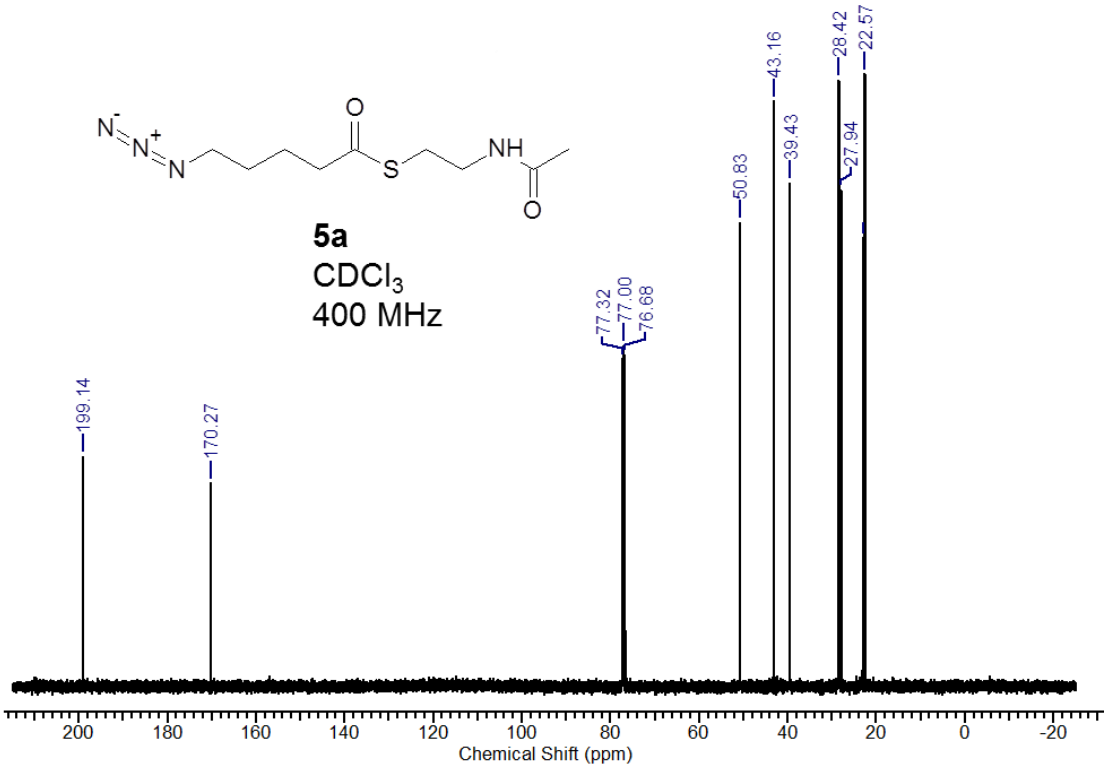

7
CDCl3
400 MHz
3.66
3.80
3.71
6.16
4.78
7.24
6.76
6.73
7.14
2.68
2.66
2.63
2.43
2.40
2.38
1.00 3.03 1.98 1.99 3.02 9.18 1.98 1.95
Chemical Shift (ppm)

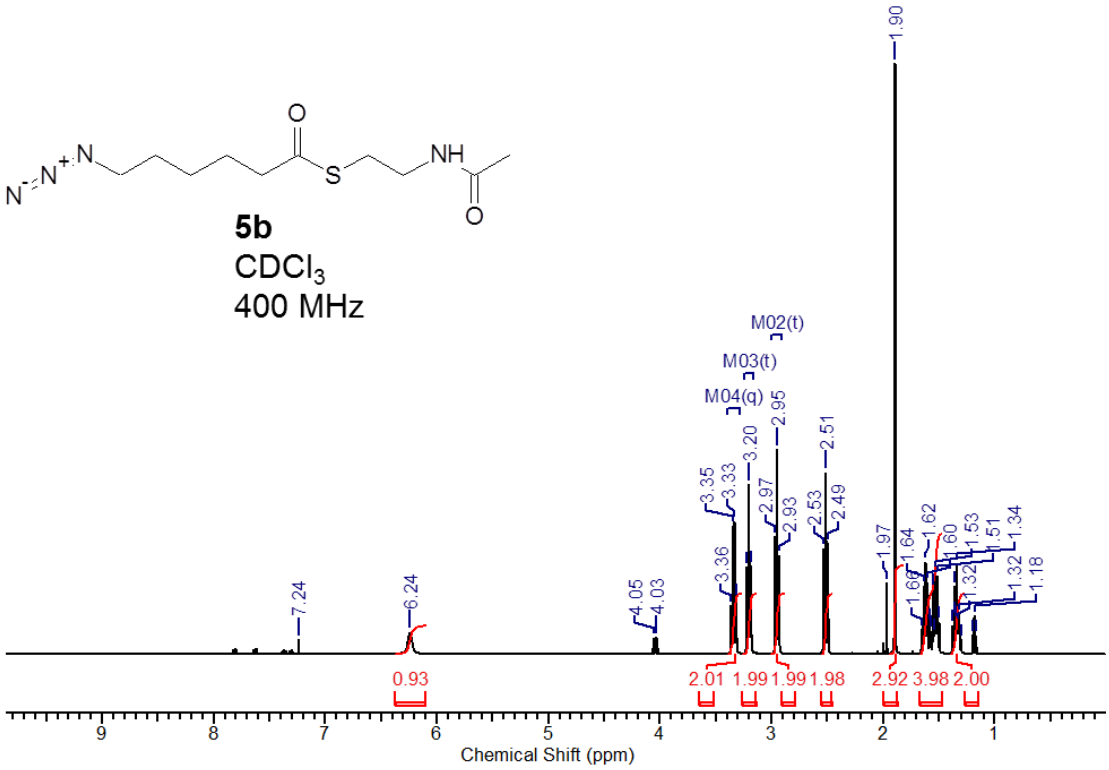

7
CDCl3
250 MHz
56.09
55.16
60.82
153.53
113.18
105.73
177.30
171.72
159.63
138.87
137.14
129.26
121.28
114.32
77.51
77.00
76.49
53.06
29.53
28.95
Chemical Shift (ppm)

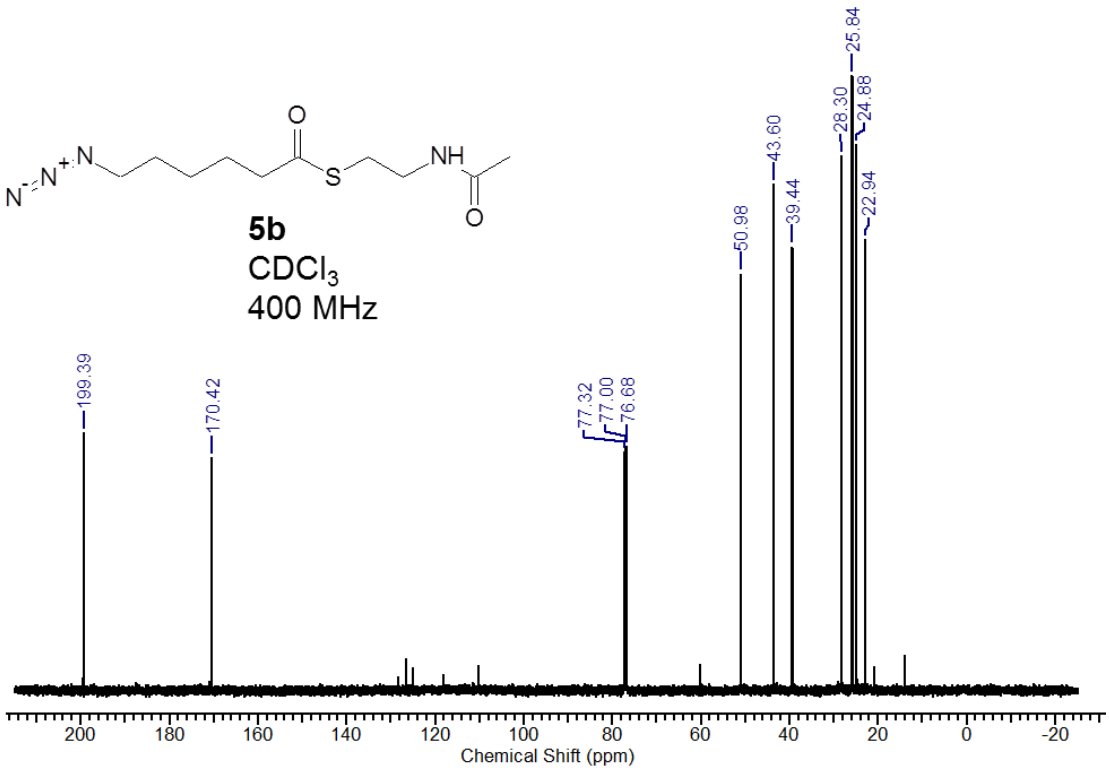

1
CD3CN
400 MHz
Chemical Shift (ppm)


1
CD3CN
400 MHz
Chemical Shift (ppm)


5a
CDCl3
400 MHz
Chemical Shift (ppm)


5a
CDCl3
400 MHz
Chemical Shift (ppm)


5b
CDCl3
400 MHz
M02(t)
M03(t)
M04(q)
1.90
2.95
3.20
2.51
3.35
3.33
2.97
2.93
2.53
2.49
3.36
1.97
1.64
1.62
1.60
1.53
1.51
1.34
1.32
1.18
7.24
6.24
4.05
4.03
0.93
2.01
1.99
1.99
1.98
2.92
3.98
2.00
Chemical Shift (ppm)


5b
CDCl3
400 MHz
199.39
170.42
77.32
77.00
76.68
50.98
43.60
39.44
28.30
25.84
24.88
22.94
Chemical Shift (ppm)