Supporting Information File

Synthesis of 14-membered enediyne-embedded macrocycles

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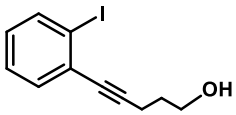
1. SYNTHESIS OF 5-(2-IODOPHENYL)PENT-4-YNAL (1)

Aldehyde **1** was prepared in two reaction steps:

(I) Synthesis of 5-(2-iodophenyl)pent-4-yn-1-ol

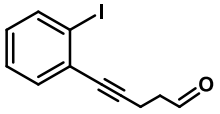


1,2-diiodobenzene (2.29 mmol, 300 µL) and $PdCl_2(PPh_3)_2$ (3 mol%, 0.069 mmol, 48 mg) were dissolved in triethylamine (3 mL) under argon. After 15 minutes was add CuI (3 mol%, 0.069 mmol, 13 mg) and after 5 minutes pent-4-yn-1-ol (2 eq, 4.58 mmol, 425 µL). The reaction mixture was stirred for 24h at room temperature and quenched with saturated NH_4Cl. The product was extracted with ethyl-acetate and organic layer was washed with brine and water, dried over Na_2SO_4 and concentrated under reduced pressure. The product was obtained by flash column chromatography (petroleum ether/ethyl acetate (v/v = 2/1)).

Yield 44 % (580 mg); brown oil; R_f = 0.31 (petroleum ether/ethyl acetate = 2:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.81 (d, J = 8.0 Hz, 1H), 7.44 – 7.35 (m, 1H), 7.30 – 7.22 (m, 1H), 6.98 – 6.93 (m, 1H), 3.89 (t, J = 6.1 Hz, 2H), 2.61 (t, J = 6.9 Hz, 2H), 1.95 – 1.87 (m, 2H), 1.66 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.7, 132.6, 130.3, 129.0, 127.9, 101.1, 93.8, 85.6, 61.9, 31.2, 16.2.

(II) Synthesis of 5-(2-iodophenyl)pent-4-ynal (**1**)



5-(2-iodophenyl)pent-4-yn-1-ol (0.24 mmol, 69 mg) was dissolved in dichlormethane and DMP (2 eq, 0.48 mmol, 204 mg) was add. The reaction mixture was stirred for 2h at room temperature and quenched with aqueous mixture of $Na_2S_2O3/NaHCO_3$. The product was extracted with dichlormethane and organic layer was washed with brine and water, dried over Na_2SO_4 and concentrated under reduced pressure. The product was obtained by flash column chromatography (petroleum ether/ethyl acetate (v/v = 2/1))

Yield 88 % (60 mg); yellow oil; R_f = 0.60 (petroleum ether/ethyl acetate = 2:1).

^{1}H NMR (600 MHz, $CDCl_3$): δ = 9.90 (s, 1H), 7.82 (d, J = 7.9 Hz, 1H), 7.39 (dd, J = 7.7 Hz, J = 1.5 Hz, 1H), 7.28 – 7.25 (m, 1H), 6.98 – 6.95 (m, 1H), 2.84 – 2.78 (m, 4H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 200.5, 138.8, 132.6, 129.7, 129.2, 127.9, 101.2, 92.1, 83.9, 42.5, 13.1.

2. MACROCYCLIZATION REACTION OPTIMIZATION

Table 1. Optimization of reaction conditions for cyclization of **2a**[a]

Condensation regent (eq.)	Base (eq.)	t/°C	Solvent (c/M)	Yield/%[b]
BOP/HOBt (3)	DIPEA (6)	RT	DCM (0.01)	12
BOP/HOBt (3)	TEA (5)	RT	DCM (0.01)	12
BOP/HOBt (3)	DIPEA (6)	40	DMF (0.01)	7
PyBOP (3)	DIPEA (6)	RT	DCM (0.01)	68
PyBOP (3)	DIPEA (6)	40	DMF (0.01)	18

[a] Reactions were carried out on a 0.15 mmol scale; [b] Yields refer to the isolated product.

3. HPLC ANALYSIS OF MACROCYCLIZATION REACTION

To elucidate the formation of macrocyclic compound **4g**, rather than expected product **3g** during the macrocyclization of acyclic enediyne **2g**, we followed the reaction by HPLC.

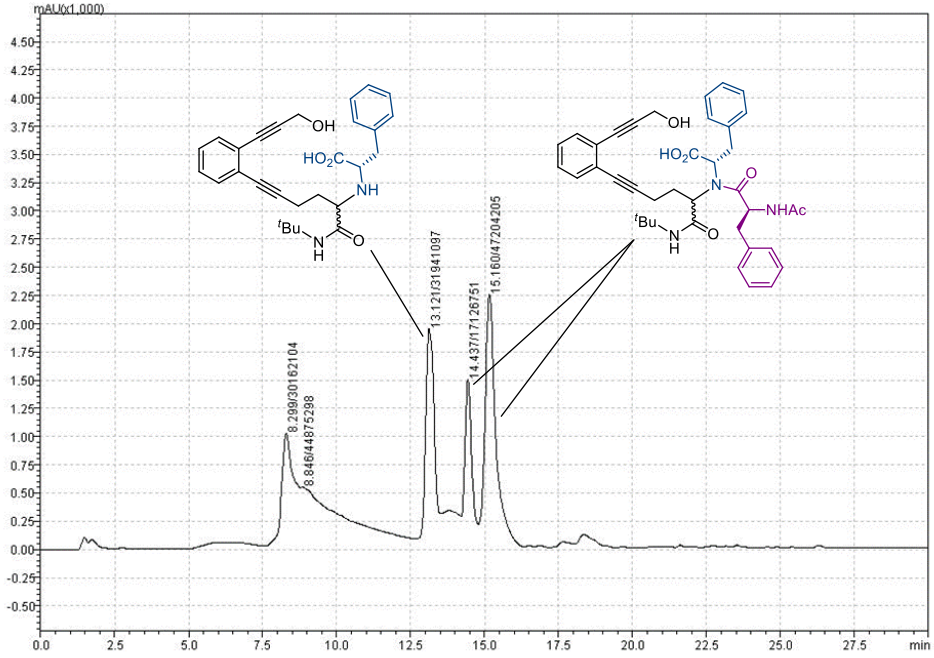


Fig SI-1. HPLC spectrum of deprotected **2g** before macrocyclization reaction. Chromatogram indicate presence of acyclic enediyne with hydrolyzed tertiary amide bond along with non-hydrolyzed ones.

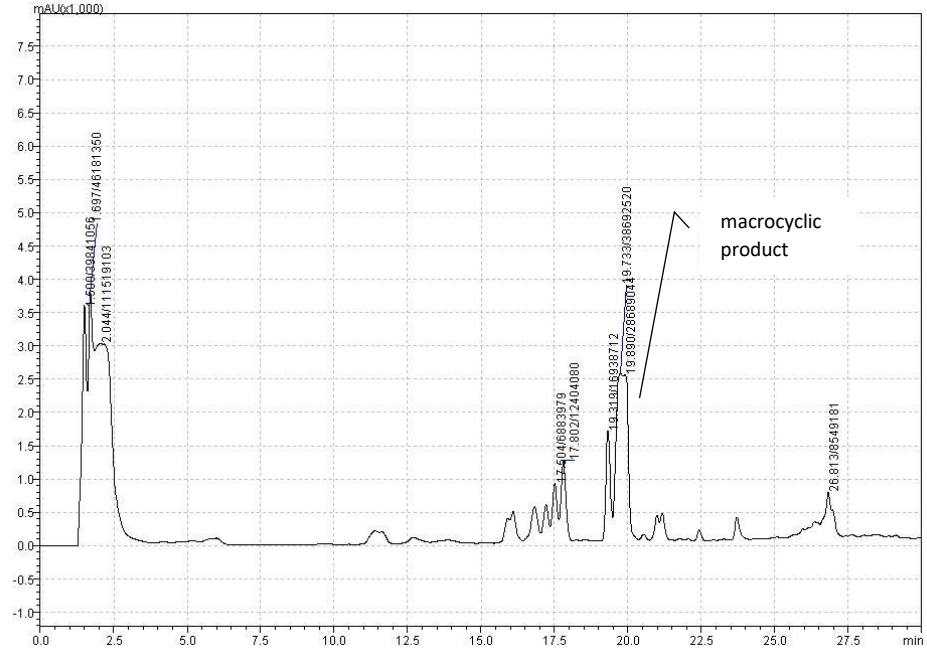


Fig SI-2. HPLC spectrum of deprotected **2g** during macrocyclization reaction (after 20h). Chromatogram show disappearance of **2g** and predominant formation of single macrocyclic product.

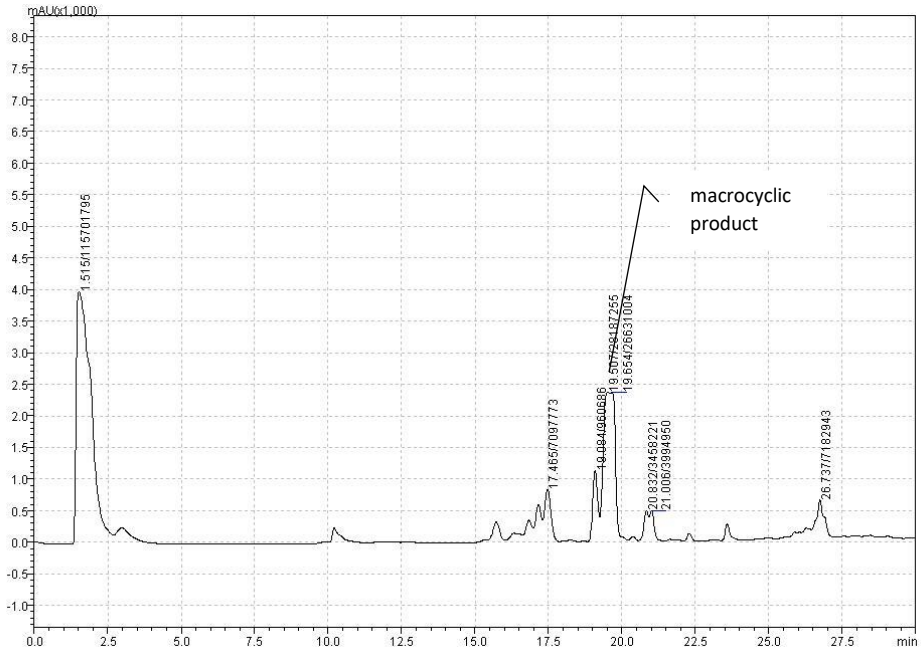


Fig. SI-3. HPLC spectrum of deprotected **2g** during macrocyclization reaction (after 40h).

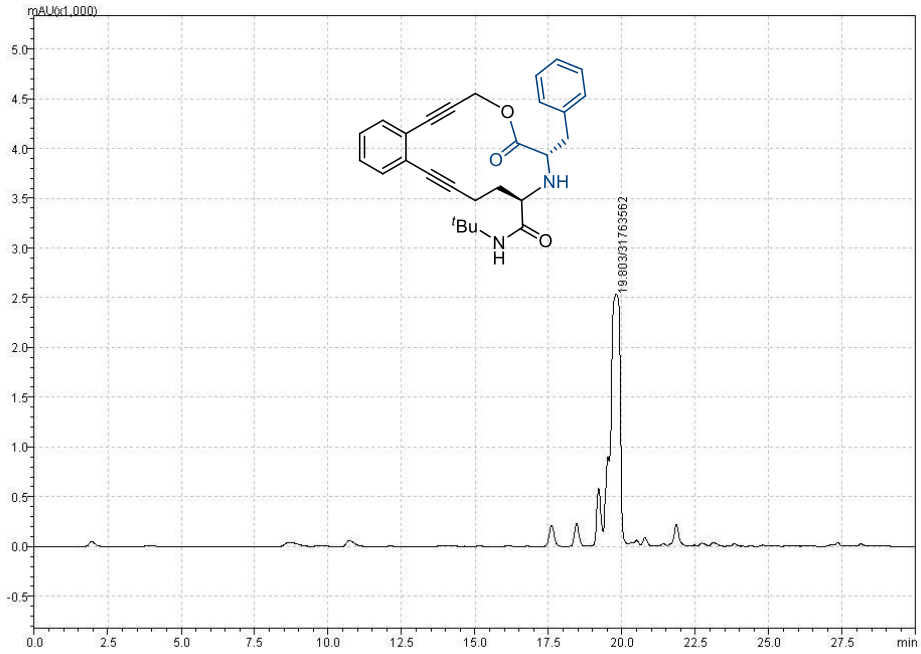


Fig. SI-4. HPLC spectrum of isolated **4g**[1]

Then we performed analysis on the formation of **3a**, where to diastereoisomers of expected macrocycle **3a** have been isolated.

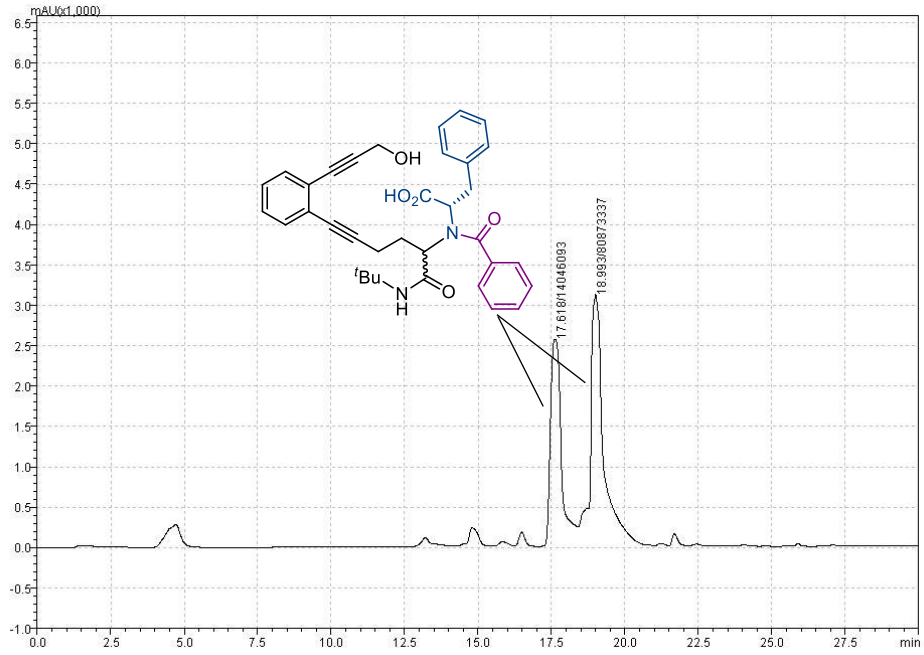


Fig SI-5. HPLC spectrum of deprotected **2a** before macrocyclization reaction.

Chromatogram shows presence of two diastereoisomers of deprotected **2a**.

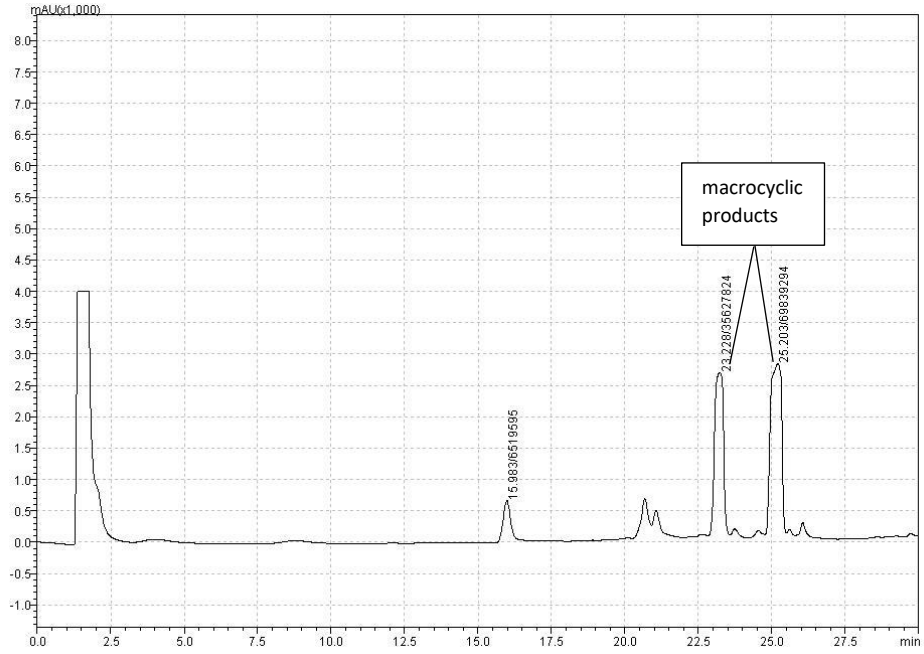


Fig. SI-6. HPLC spectrum of deprotected **2a** during macrocyclization reaction (after 20h).

Chromatogram show disappearance of deprotected **2a** and formation of two diastereoisomers of macrocycle **3a**.

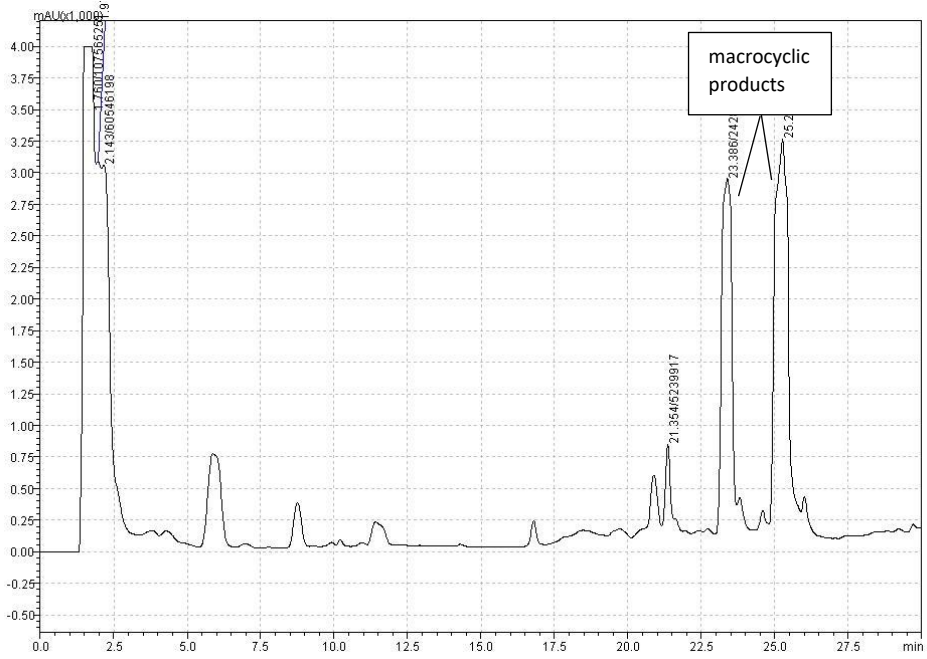


Fig. SI-7. HPLC spectrum of deprotected **2a** during macrocyclization reaction (after 40h).

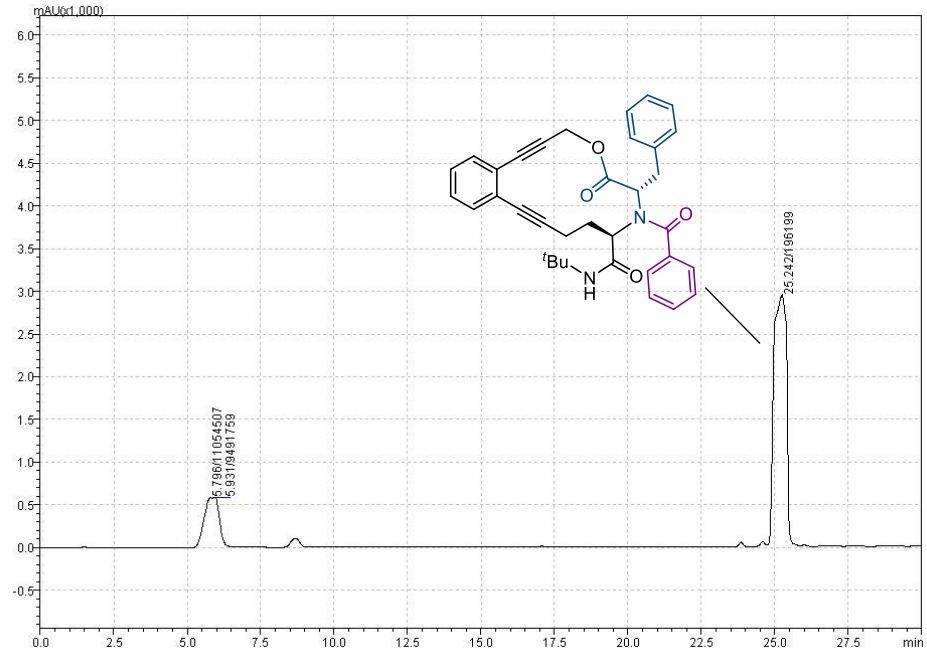


Fig. SI-8. HPLC spectrum of isolated **3a**[1]

HPLC analysis was performed on Zorbax RF XDB-C18 column (3,5 µm, 4,6 x 75 mm). Solvents for the analysis were 0.1% acetic acid in water (solvent A) and methanol (solvent B). The gradient was applied as follows: 0-5 min, 50 % B/50 % A, 5-25 min, 70 % B/30 % A, 25-27 min, 100% B; 27-27,1 min, 50 % B/50 A; 27,1-30 min, 50 % B/50 % A. The flow rate was 0.5 mL/ min. UV detection was performed at 254 nm and 280 nm.

4. EVALUATION OF ANTIPROLIFERATIVE EFFECT OF SELECTED MACROCYCLIC COMPOUNDS *IN VITRO*

This study has been conducted in the Laboratory of Experimental Therapy, Ruđer Bošković Institute.The purpose of this study was to investigate the effects of several compounds on proliferation of different human cell lines. The experiments were carried out on 2 human cell lines: HCT116 (colon carcinoma) and HEK293T (embryonic kidney). The following macrocyclic compounds have been tested.

Compound	Stock Solution/ Solvent	Additional information
3e[1]	4×10^{-2} M/DMSO	Precipitated in medium at max. tested concentration.
4g[1]	4×10^{-2} M/DMSO	Precipitated in medium at max. tested concentration.
3h[1]	4×10^{-2} M/DMSO	Precipitated in medium at max. tested concentration.
3i	4×10^{-2} M/DMSO	Precipitated in medium at max. tested concentration.
3m[1]	4×10^{-2} M/DMSO	Precipitated in medium at max. tested concentration.

Cell culturing

HCT116 and HEK 293T cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 2 mM L^{-1}glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin in a humidified atmosphere with 5% CO_2 at 37˚C.

Proliferation assays[1]

The panel cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 1.5×10^4 cells/ml, depending on the doubling times of specific cell line. Test agents were then added in five 10-fold dilutions (10^{-8} to 10^{-4} M) and incubated for a further 72 hours. Working dilutions were freshly prepared on the day of testing.

After 72 hours of incubation the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenase activity in viable cells. The MTT Cell Proliferation Assay is a colorimetric assay system, which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. For this purpose the substance treated medium was discarded and MTT was added to each well at a concentration of 20 µg / 40 µl. After four hours of

incubation the precipitates were dissolved in 160 µl of dymethyl-sulphoxide (DMSO). The absorbance (OD, optical density) was measured on a microplate reader at 570 nm. The absorbance is directly proportional to the cell viability. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

If (mean OD_{test} – mean OD_{tzero}) ≥ 0 then

$$PG = 100 \times (\text{mean } OD_{test} - \text{mean } OD_{tzero}) / (\text{mean } OD_{ctrl} - \text{mean}OD_{tzero}).$$

If (mean OD_{test} – mean OD_{tzero}) < 0 then:

$$PG = 100 \times (\text{mean } OD_{test} - \text{mean } OD_{tzero}) / OD_{tzero}.$$

Where:

Mean OD_{tzero} = the average of optical density measurements before exposure of cells to the test compound.

Mean OD_{test} = the average of optical density measurements after the desired period of time.

Mean OD_{ctrl} = the average of optical density measurements after the desired period of time with no exposure of cells to the test compound.

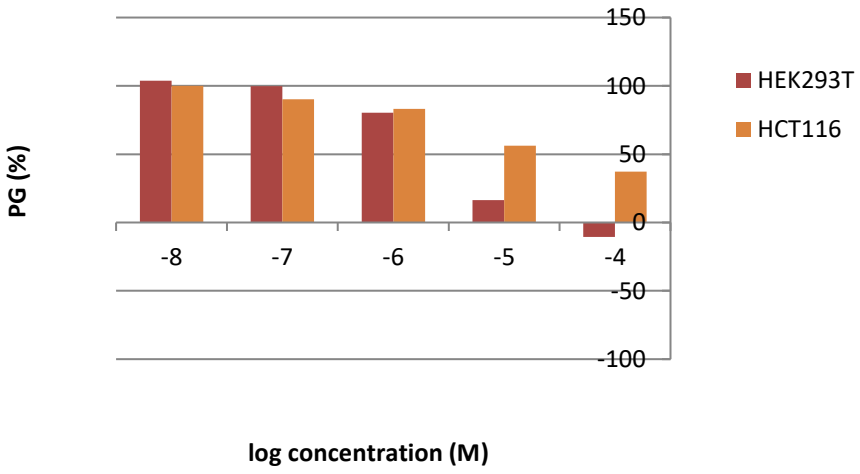
Each test point was performed in quadruplicate in three individual experiments. The results were expressed as GI_{50}, a concentration necessary for 50% of inhibition. Each result is a mean value from at least two separate experiments.

GI_{50} calculations

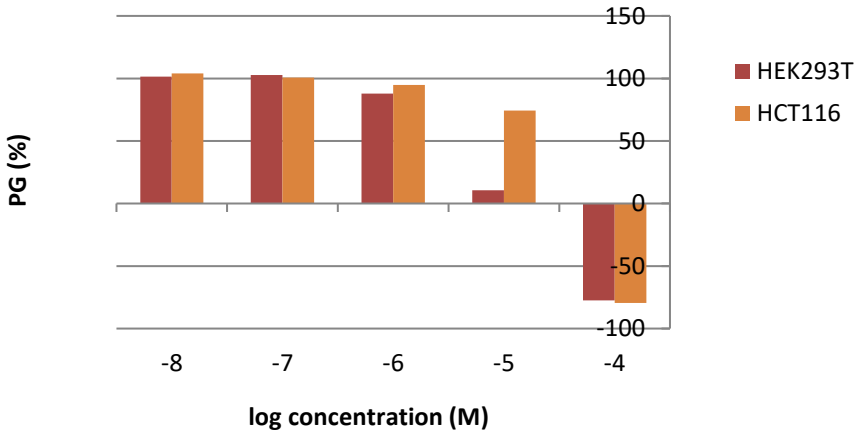
The GI_{50} measures the growth inhibitory power of the test agent and represents the concentration that causes 50% growth inhibition. The GI_{50} values for each compound are calculated from dose-response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the respective reference value (e.g. 50 for GI_{50}). Therefore, a "real" value for any of the response parameters is obtained only if at least one of the tested drug concentrations falls above, and likewise at least one falls below the respective reference value. If however, for a given cell line all of the tested concentrations produce PGs exceeding the respective reference level of effect (e.g. PG value of 50), then the highest tested concentration is assigned as the default value. In the screening data report, that default value is preceded by a ">" sign.

3) Results

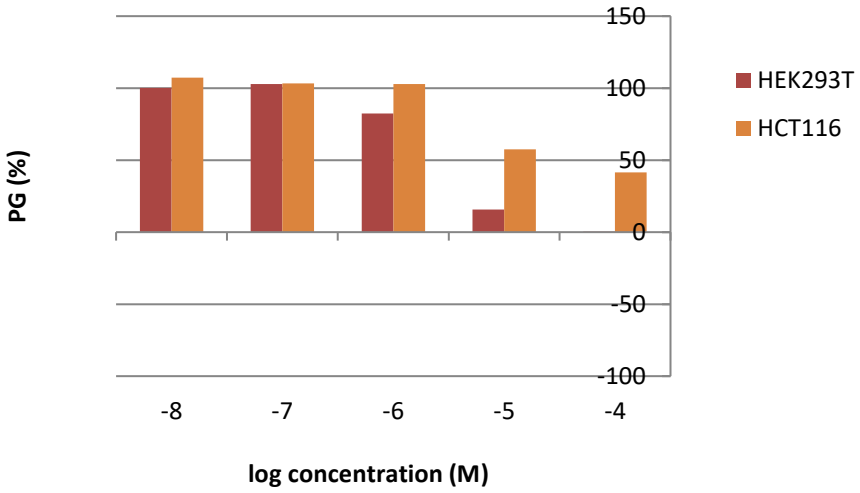
3e[1]



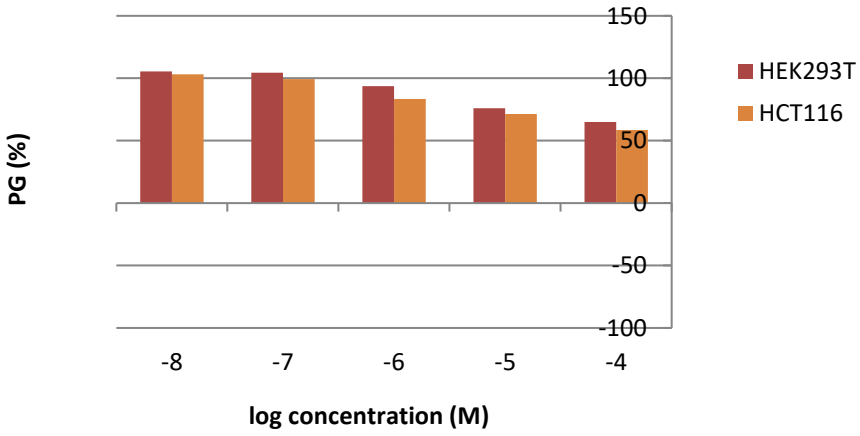
4g[1]



3h[1]


150
100
50
0
-50
-100
PG (%)
-8
-7
-6
-5
-4
log concentration (M)
HEK293T
HCT116

3i


150
100
50
0
-50
-100
PG (%)
-8
-7
-6
-5
-4
log concentration (M)
HEK293T
HCT116

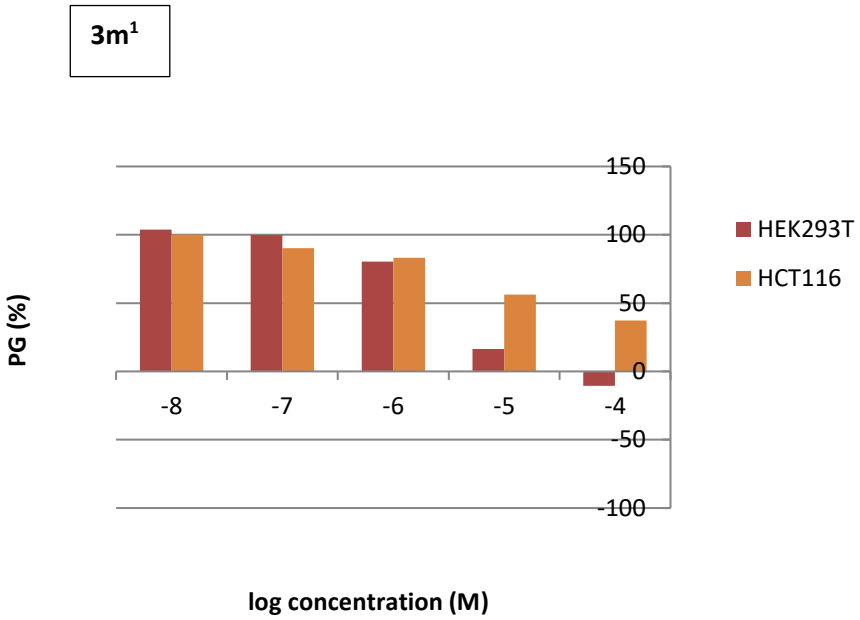


Figure SI-9. Dose-response profiles for selected macrocycles tested *in vitro* on HCT116 and HEK293T cell lines.

5. STABILITY OF COMPOUND 3m[1] IN PHOSPHATE BUFFER

Compound **3m[1]** (5 mg) was dissolved in DMSO (150 µL) and was added phosphate buffer (2.85 mL, pH 7.2). The buffer was made from solution A (720 µL), solution B (280 µL) and mQ water (9 mL). Solution A was made from Na_2HPO_4 anhydrous (1.4196 g) was dissolved in mQ water (10 mL) and solution B from $Na_2H_2PO_4$ x H_2O (1.3795 g) was dissolved in mQ water (10 mL). The reaction mixture was heated in drying chamber for 24 h at 37 °C, and for additional 24 h at 65 °C. We observed precipitation of **3m[1]** with time. The reaction mixture (40 µL) was dissolved in methanol and checked by HPLC.

nm.

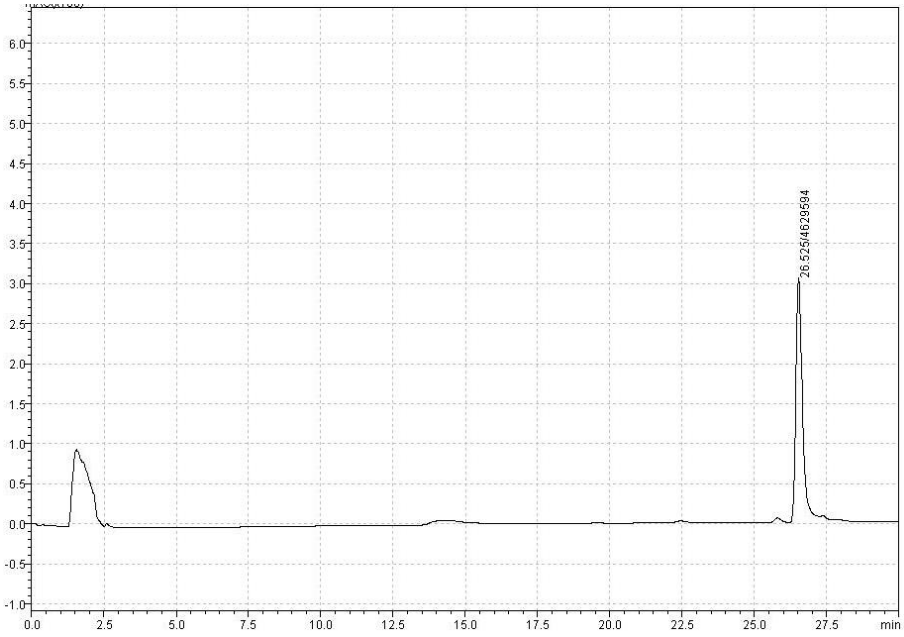


Fig. SI-10. HPLC spectrum of **3m[1]** dissolved in buffer before heating.

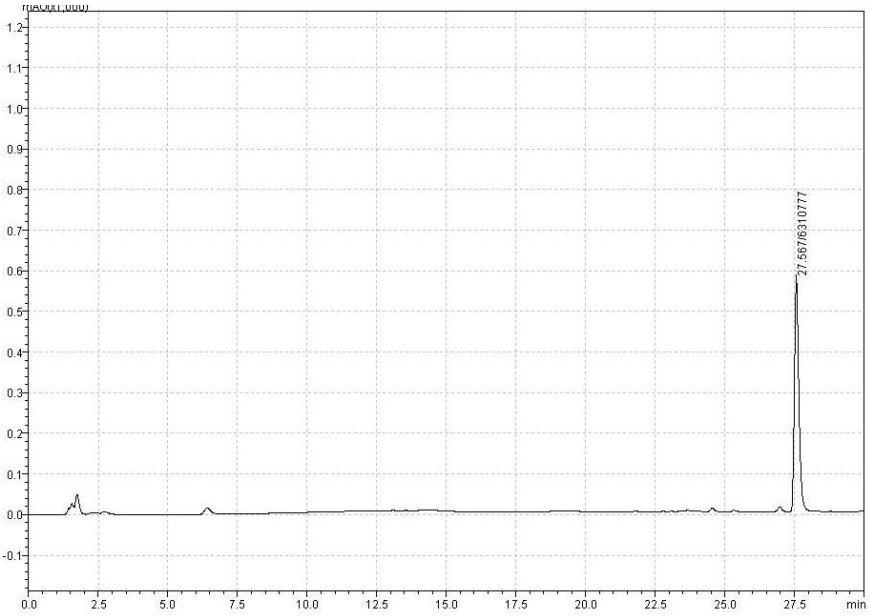
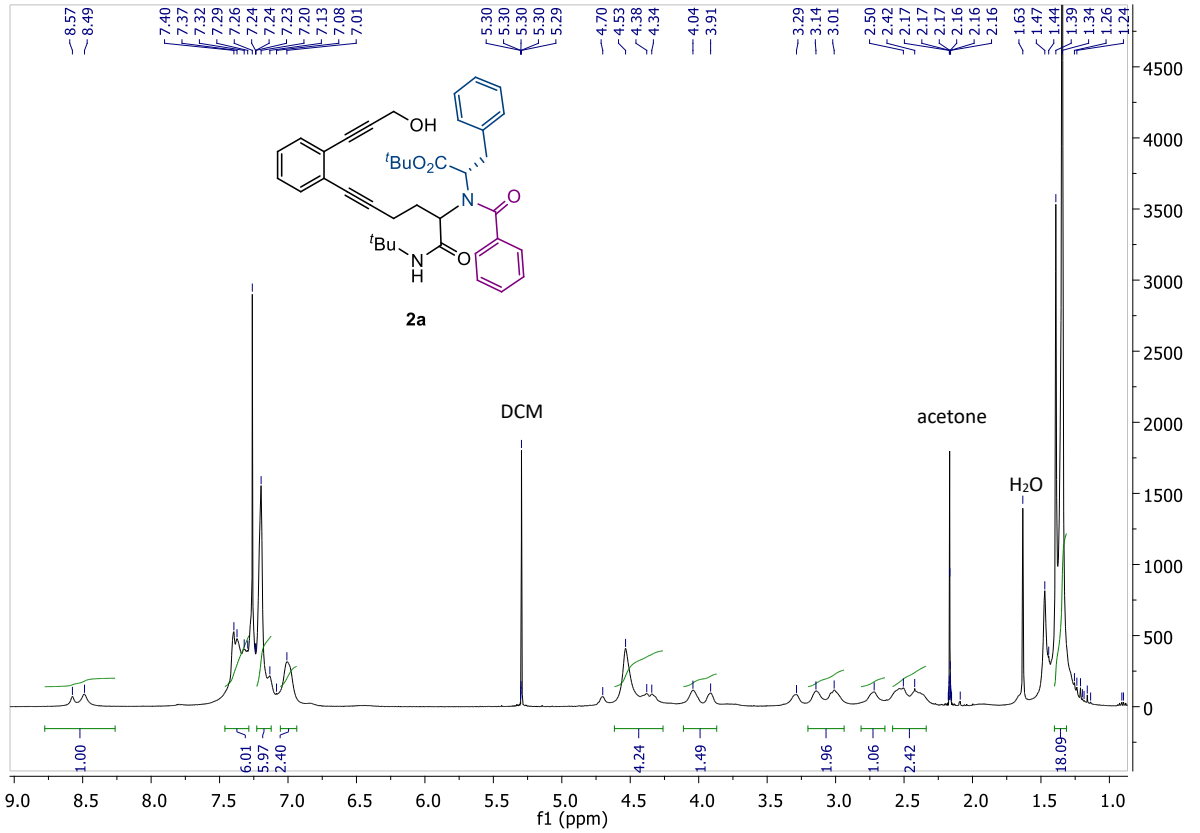


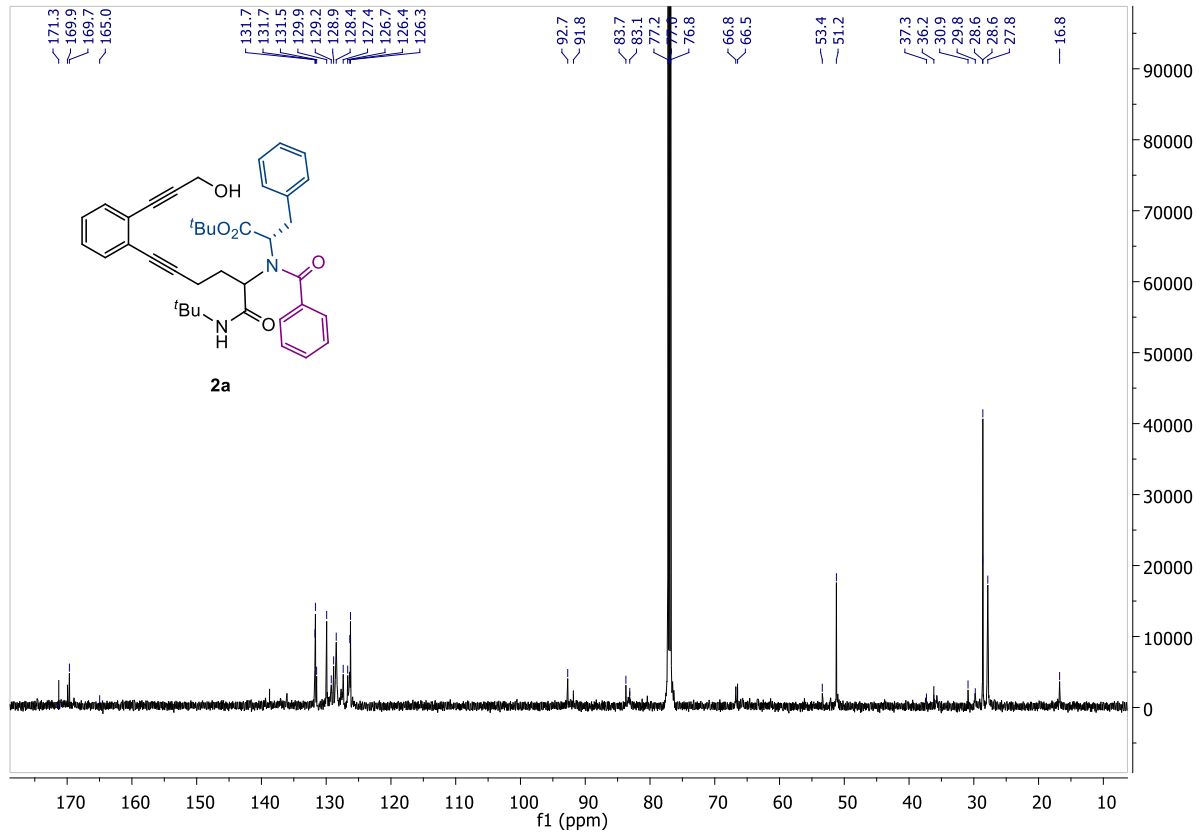
Fig SI-11. HPLC spectrum of **3m**[1] after 24h at 37 °C, and 24 hat 65 °C.

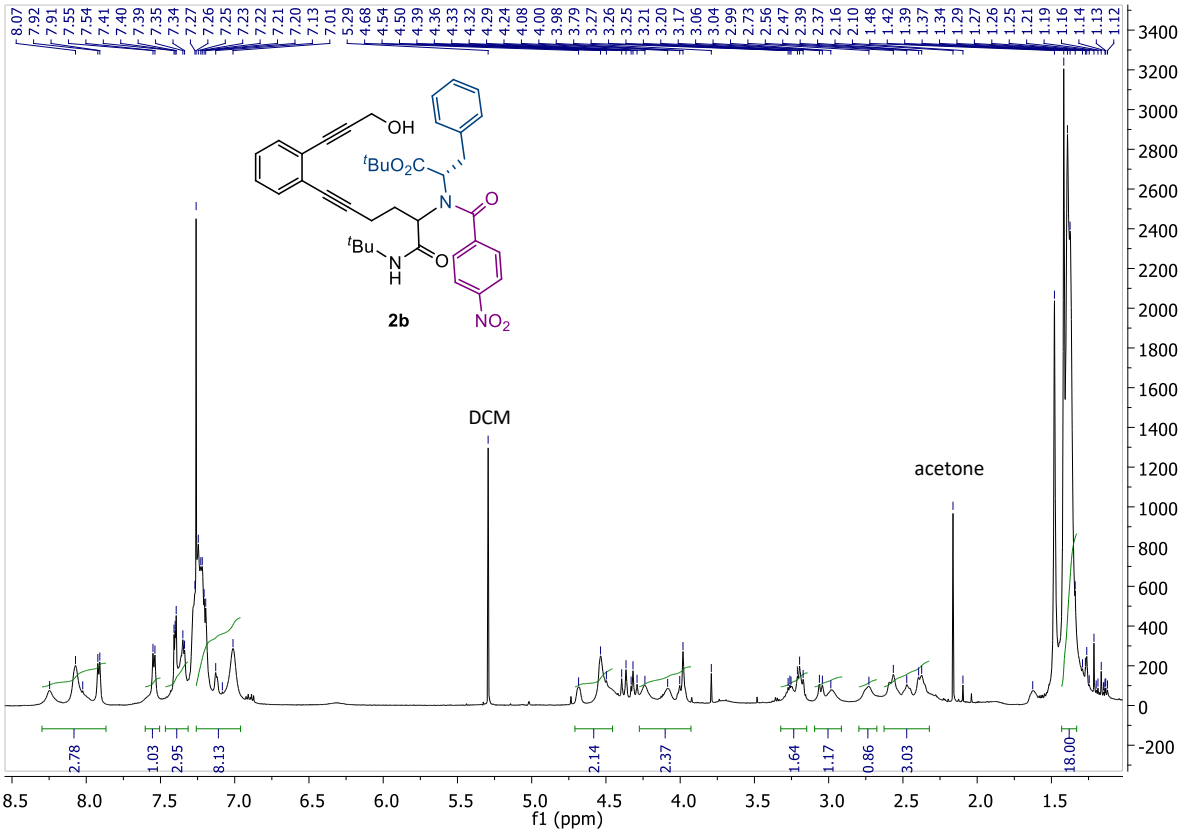
Analysis was performed on Zorbax RF XDB-C18 column (3,5 µm, 4,6 · 75 mm). Solvents for the analysis were 0.1% acetic acid in water (solvent A) and methanol (solvent B). The gradient was applied as follows: 0-5 min, 50 % B/50 % A, 5-25 min, 70 % B/30 % A, 25-27 min, 100% B; 27-27,1 min, 50 % B/50 A; 27.1-30 min, 50 % B/50 % A. The flow rate was 0.5 mL/ min. UV detection was performed at 254 nm and 280 nm.

6. NMR SPECTRA OF ACYCLIC ENEDIYNES 2

All acyclic enediynes **2** are isolated as mixtures of diastereoisomers. Presence of two diastereoisomers, as well as rotamers (tertiary amide bond), cause proton signal broadening.



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N
O
^{t}Bu
N
H
O
2b
NO$_2$
DCM
acetone
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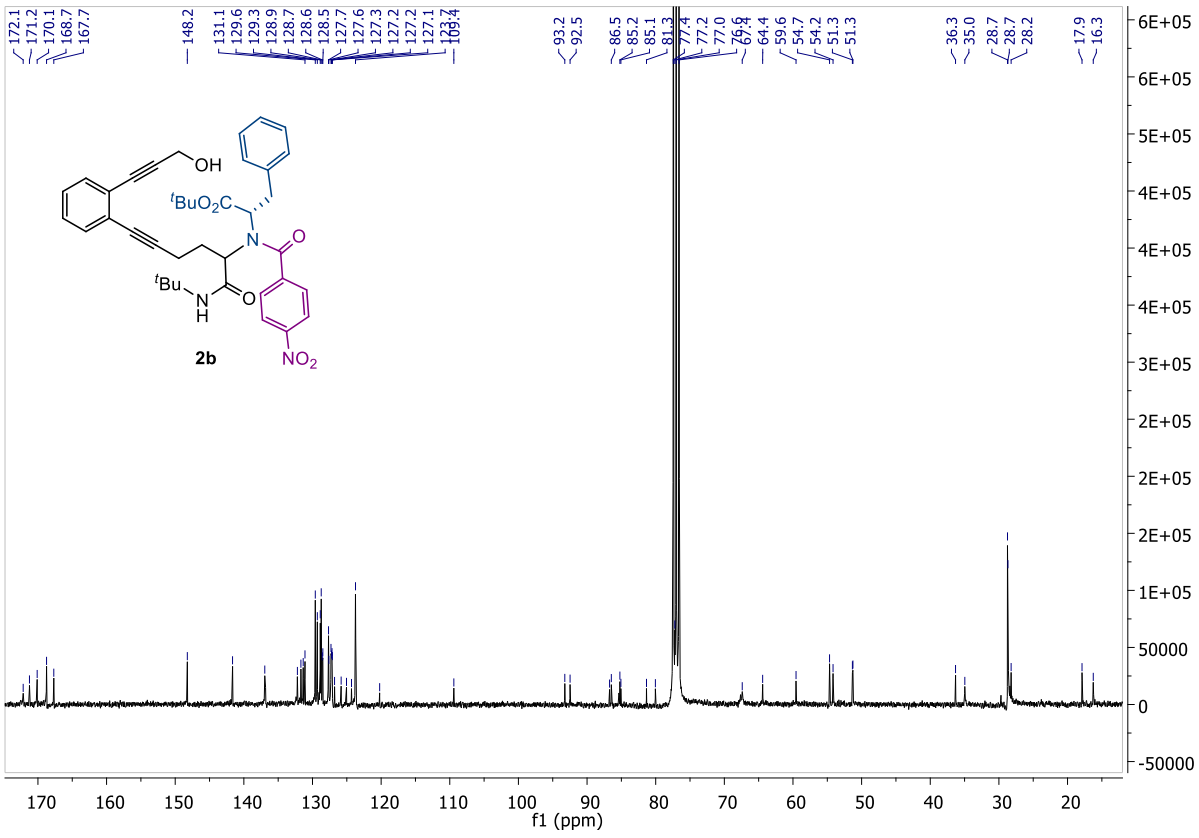
3.03
18.00
f1 (ppm)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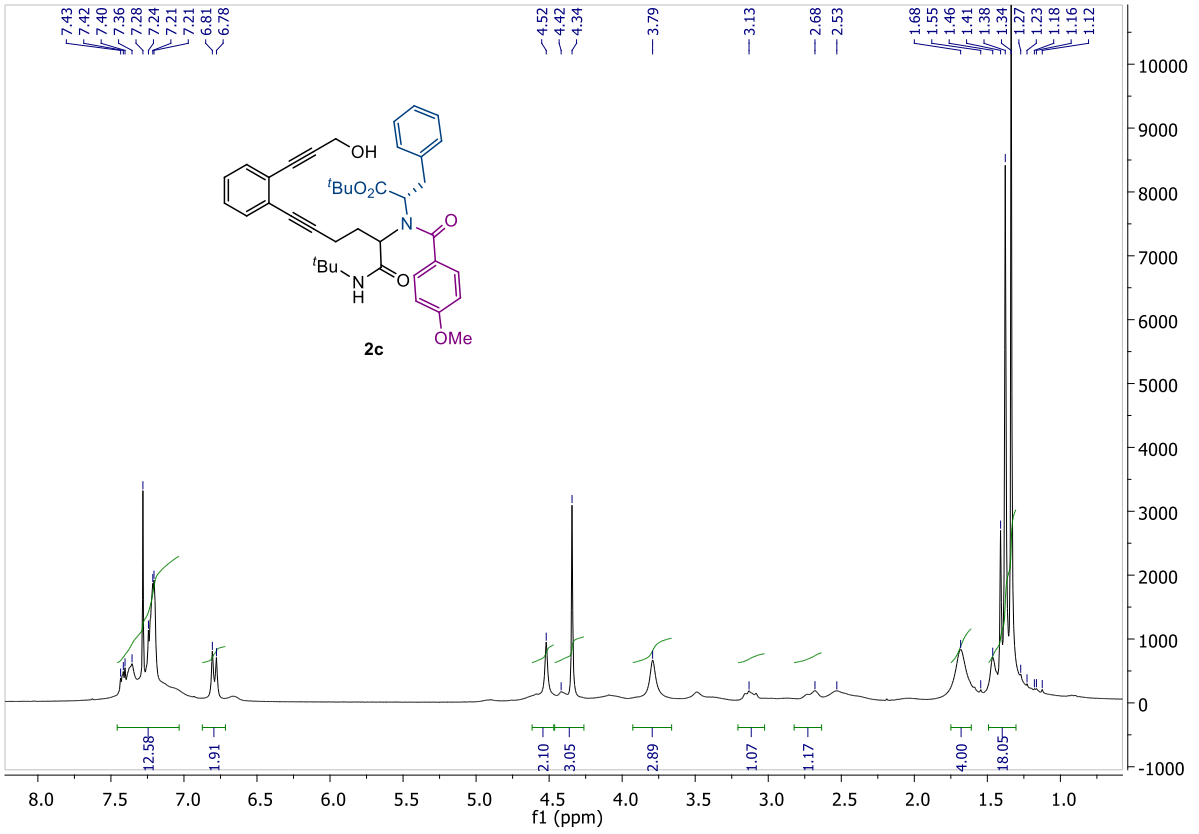
N
O
^{t}Bu
N
H
O
2b
NO$_2$
f1 (ppm)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6.78
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4.34
3.79
3.13
2.68
2.53
1.68
1.55
1.46
1.41
1.38
1.34
1.27
1.23
1.18
1.16
1.12
OH
tBuO2C
N
O
tBu
N
H
O
OMe
2c
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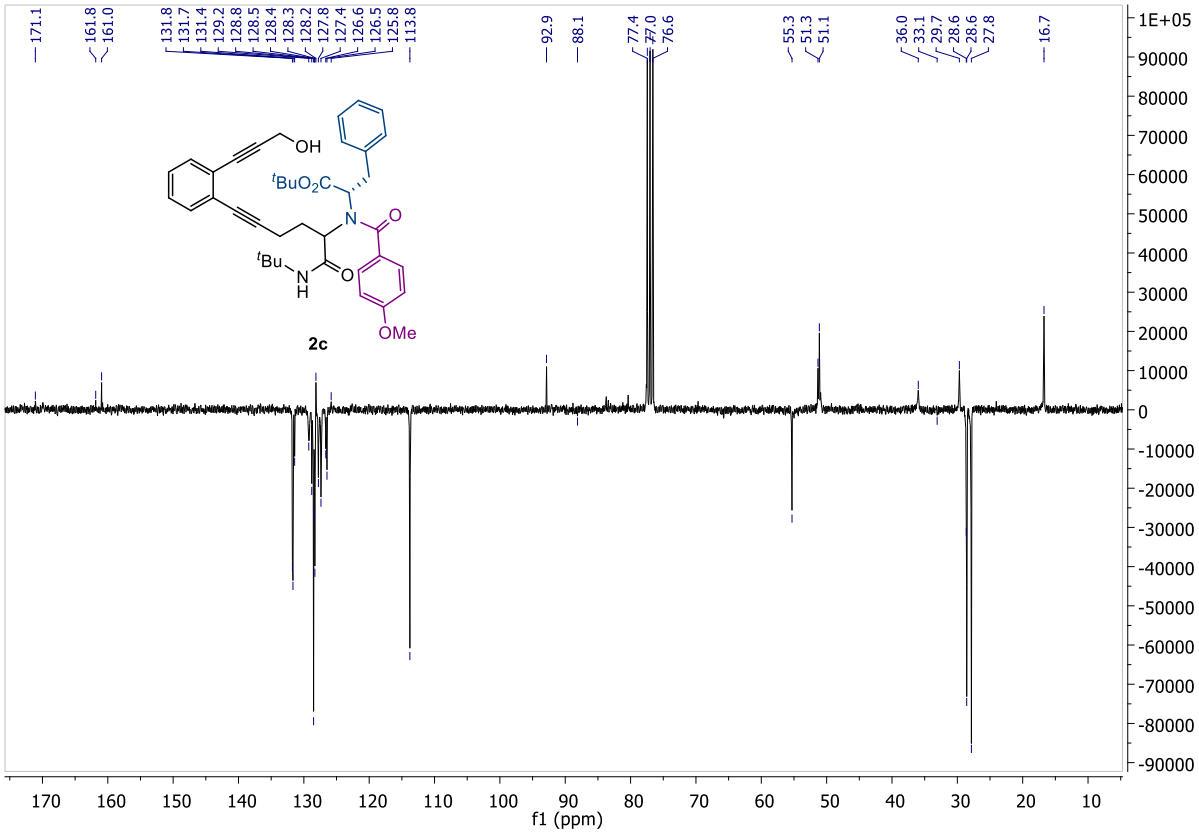
1.07
1.17
4.00
18.05
f1 (ppm)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161.0
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127.8
127.4
126.6
126.5
125.8
113.8
92.9
88.1
77.4
77.0
76.6
55.3
51.3
51.1
36.0
33.1
29.7
28.6
28.6
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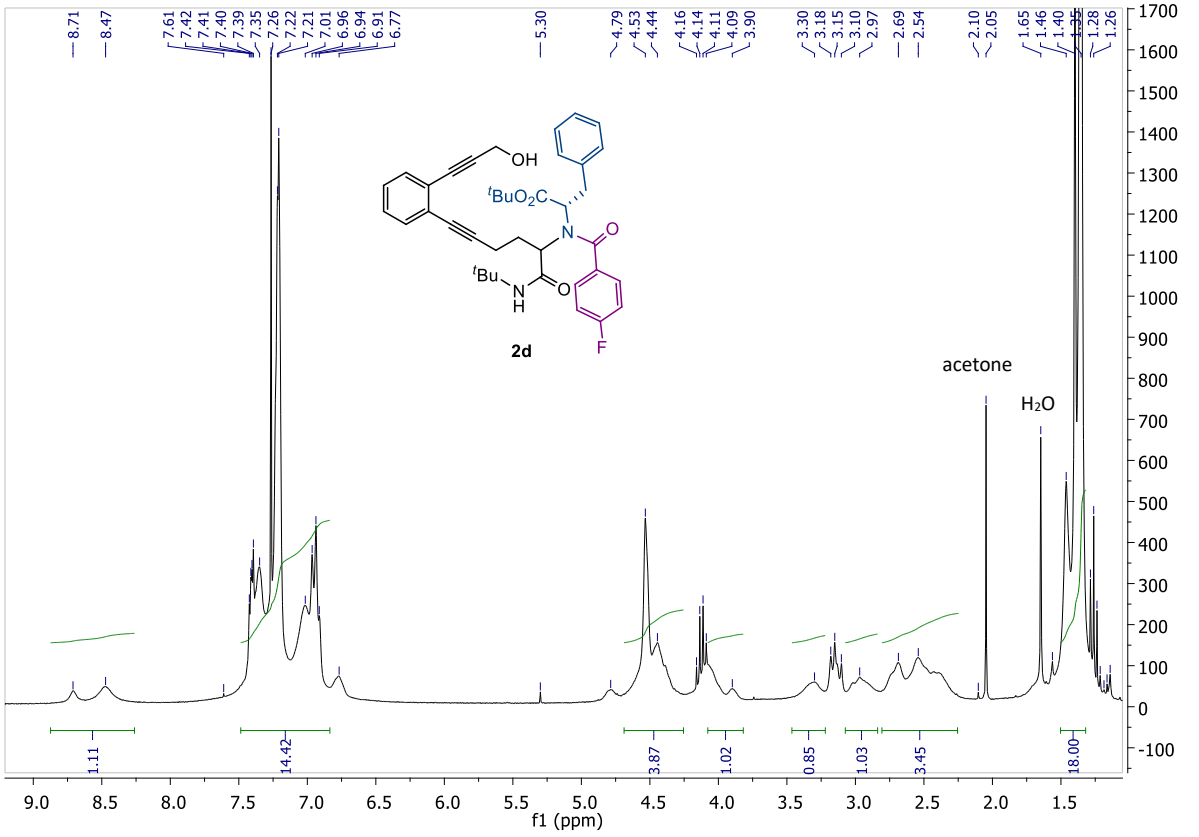
16.7
OH
tBuO2C
N
O
tBu
N
H
O
OMe
2c
f1 (ppm)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7.35
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7.22
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6.96
6.94
6.91
6.77
5.30
4.79
4.53
4.44
4.16
4.14
4.11
4.09
3.90
3.30
3.18
3.15
3.10
2.97
2.69
2.54
2.10
2.05
1.65
1.46
1.40
1.35
1.28
1.26
OH
tBuO2C
N
O
tBu
N
H
O
F
2d
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H2O
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1.02
0.85
1.03
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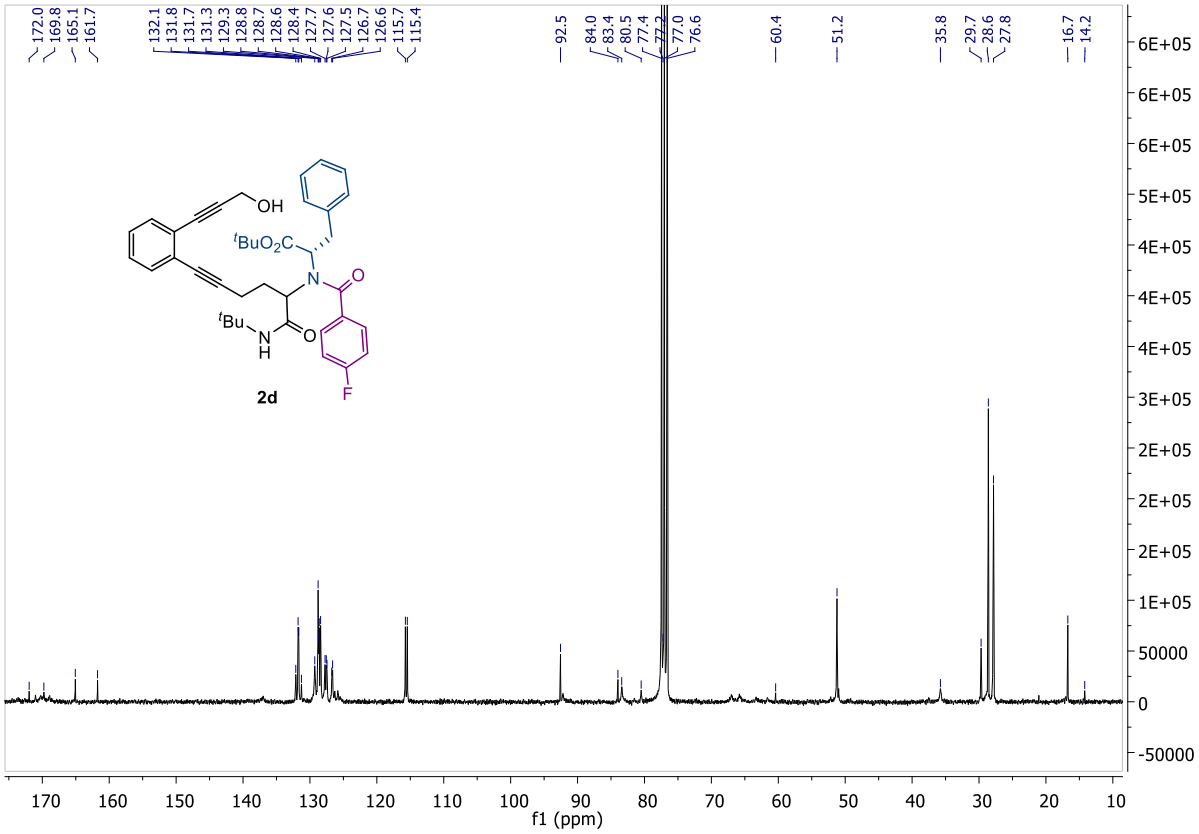
f1 (ppm)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132.1
131.8
131.7
131.3
129.3
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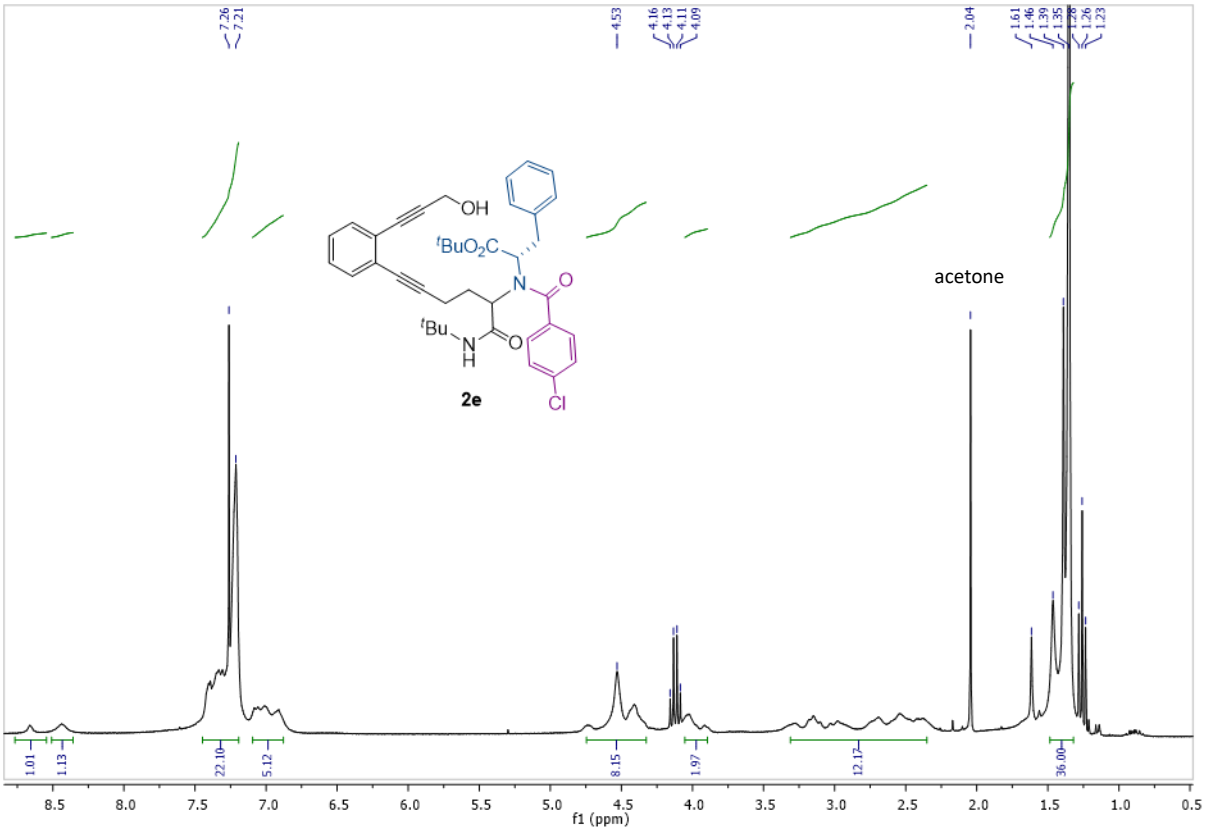
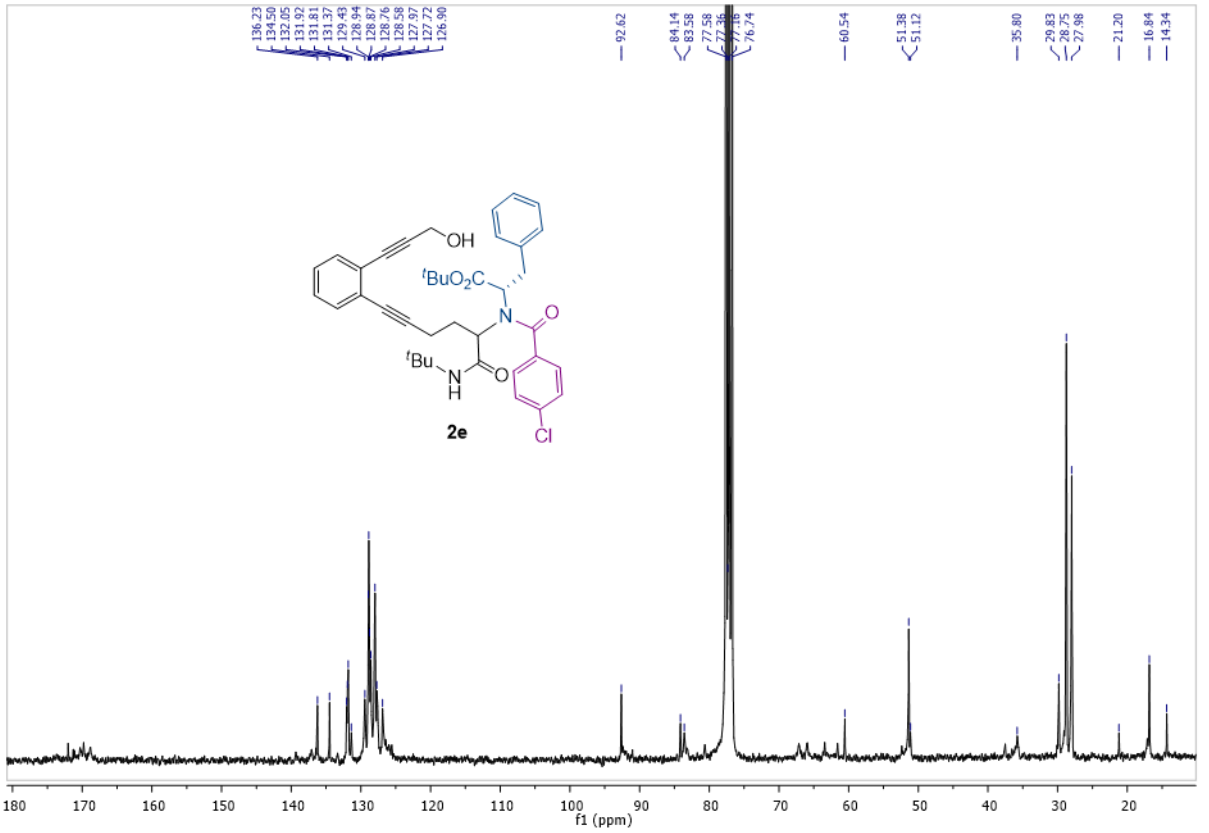
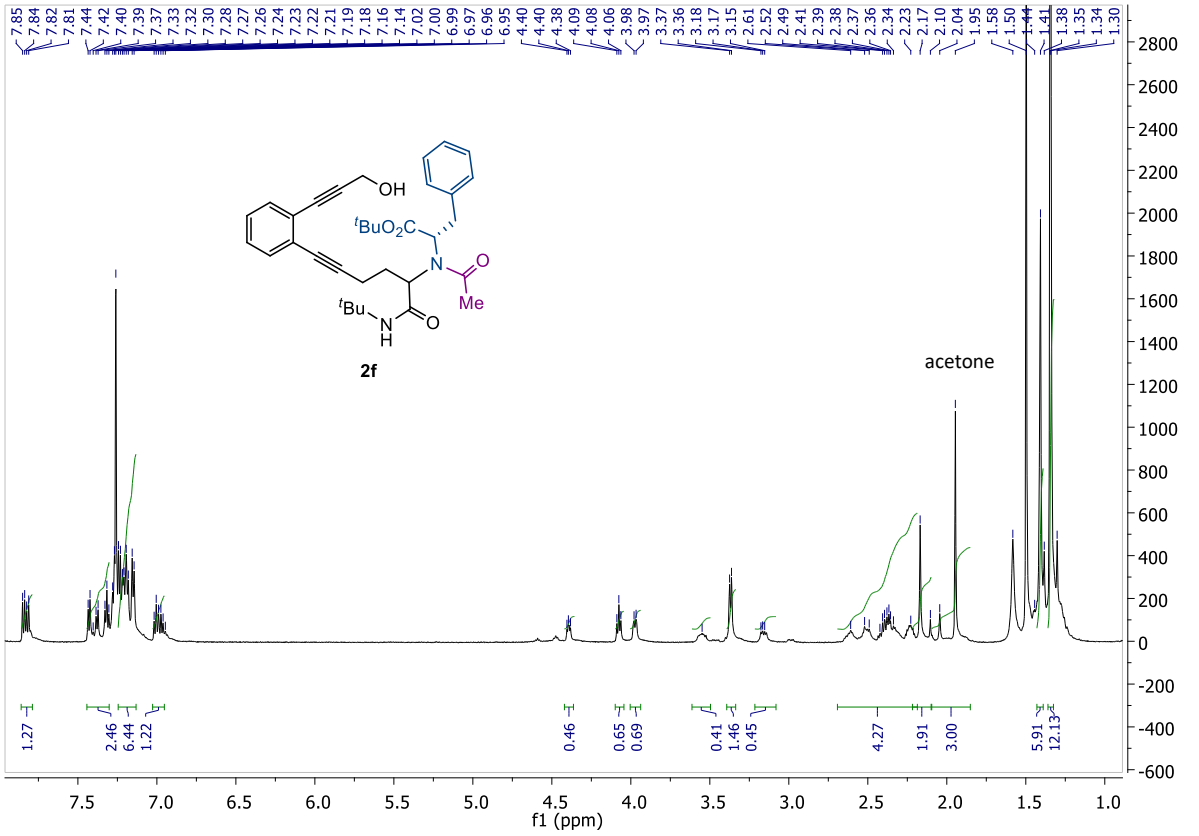
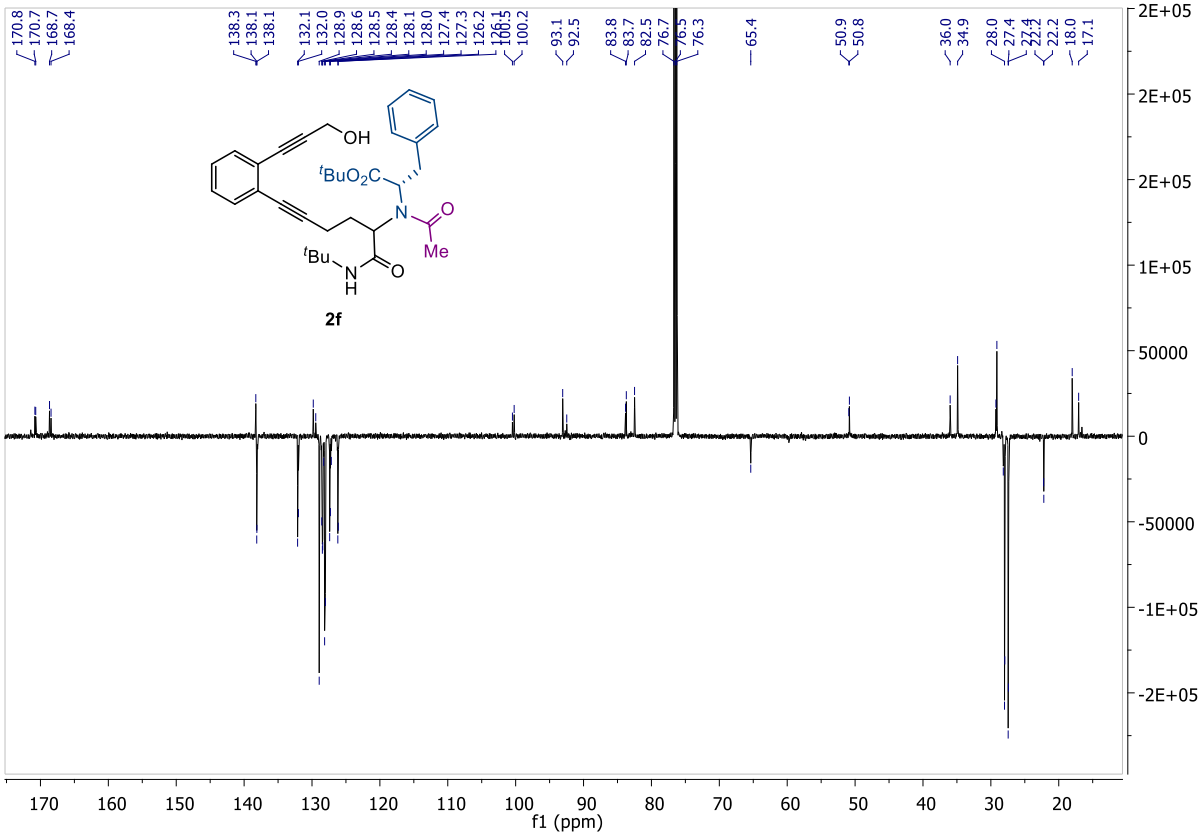
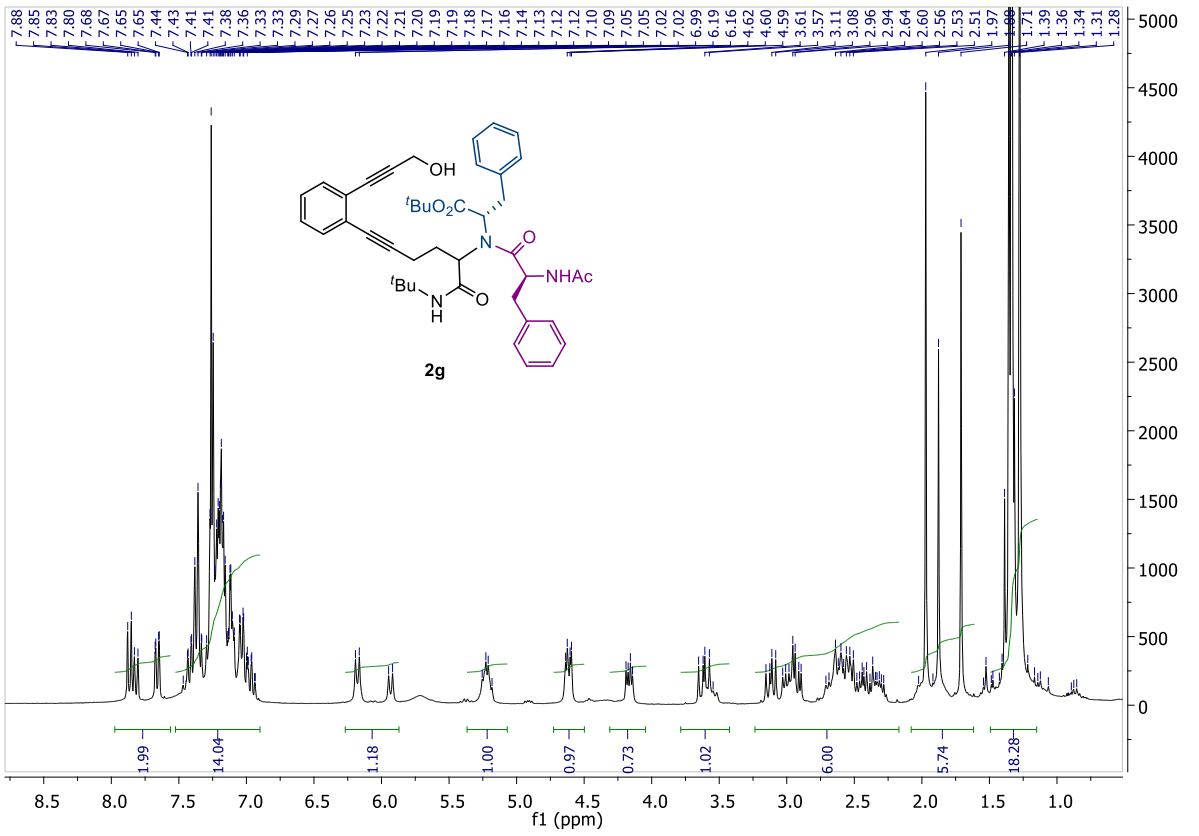
128.4
127.7
127.6
127.5
126.7
126.6
115.7
115.4
92.5
84.0
83.4
80.5
77.4
77.2
77.0
76.6
60.4
51.2
35.8
29.7
28.6
27.8
16.7
14.2
OH
tBuO2C
N
O
tBu
N
H
O
F
2d
f1 (ppm)

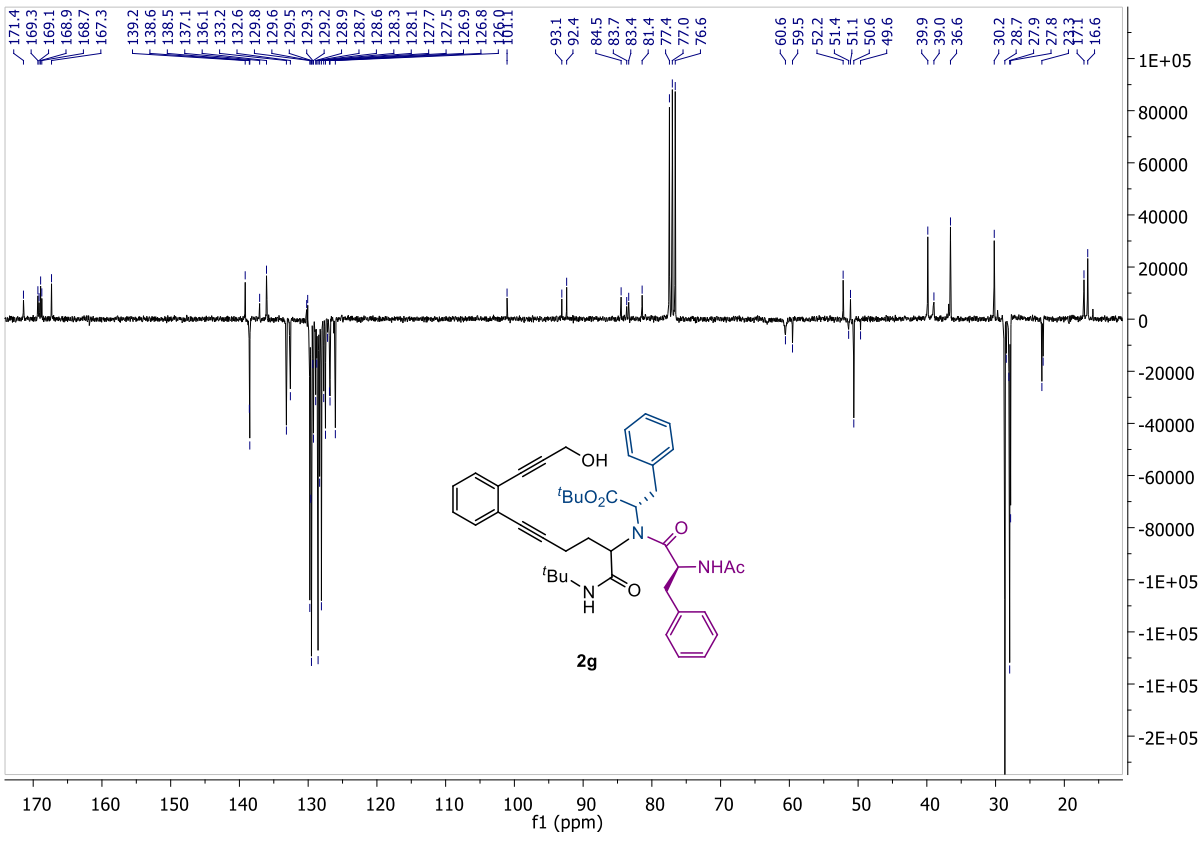

acetone
2e
f1 (ppm)

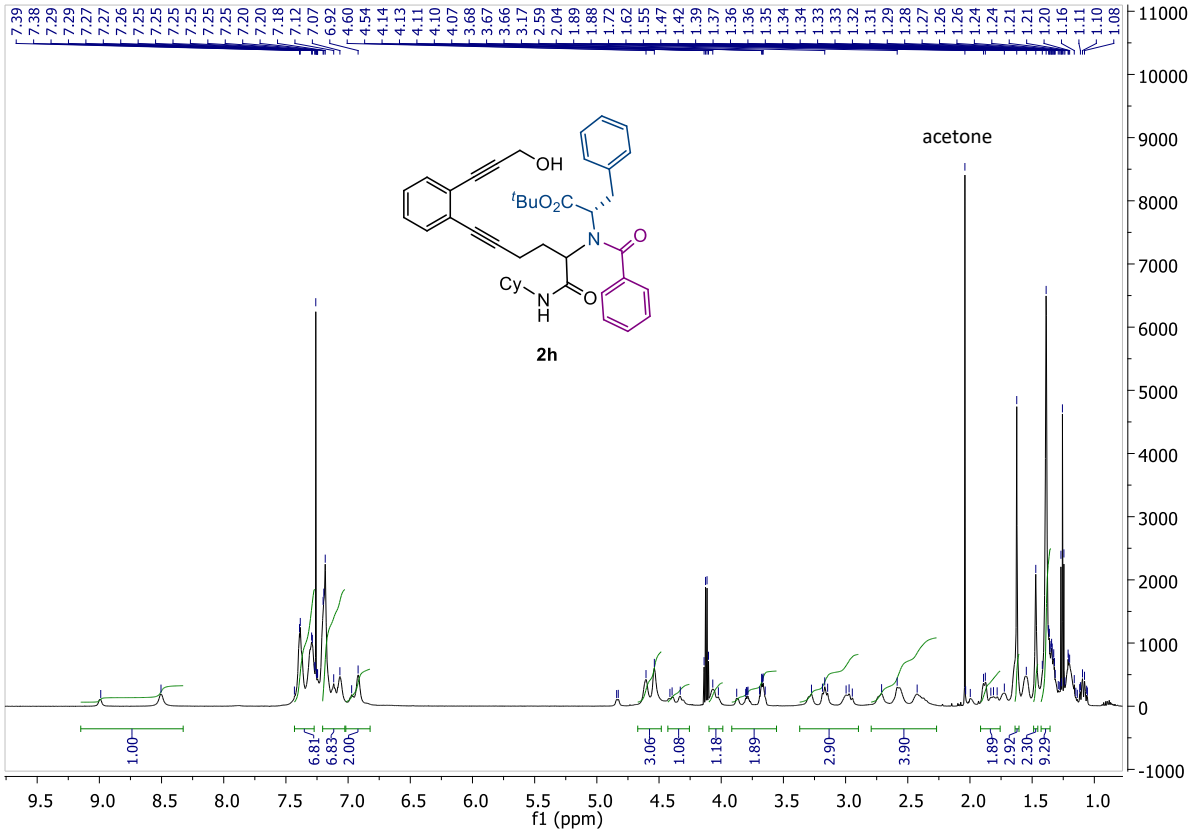

2e
f1 (ppm)

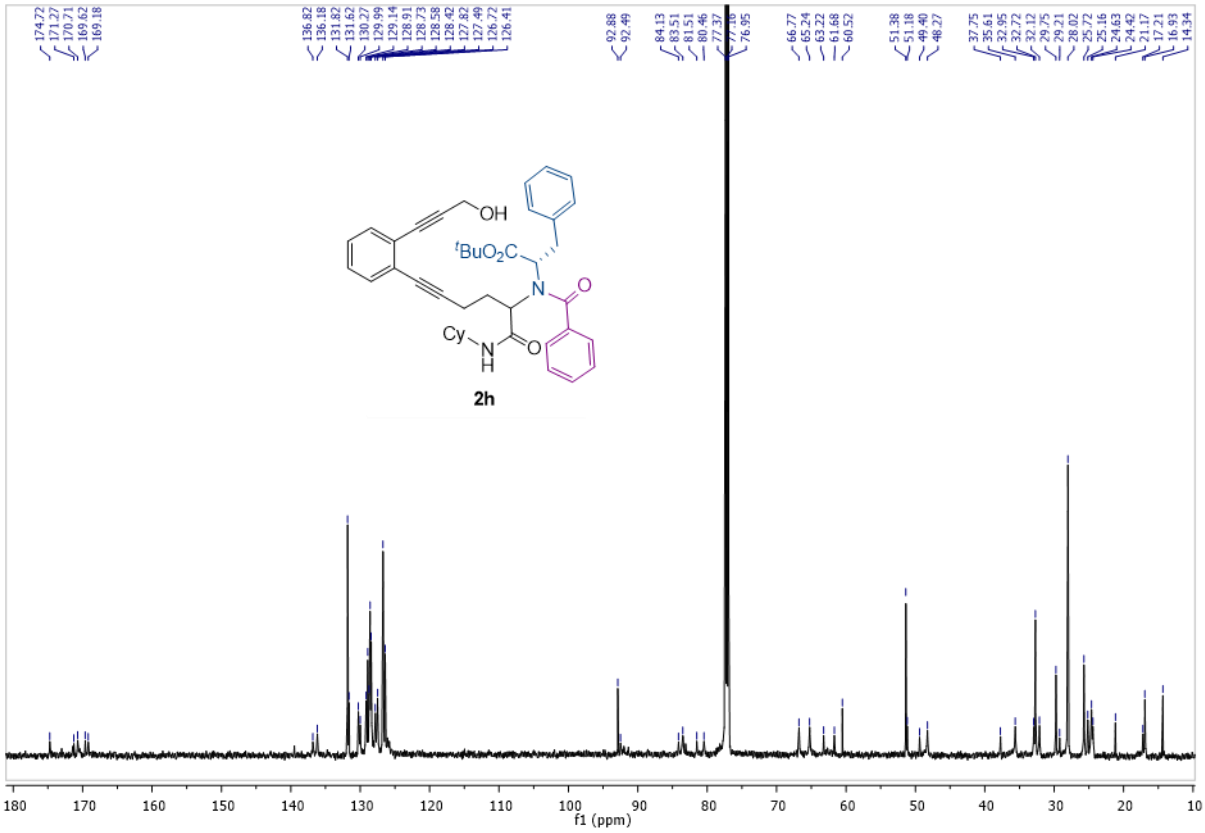

OH
tBuO2C
N
O
Me
tBu
N
H
O
2f
acetone
f1 (ppm)

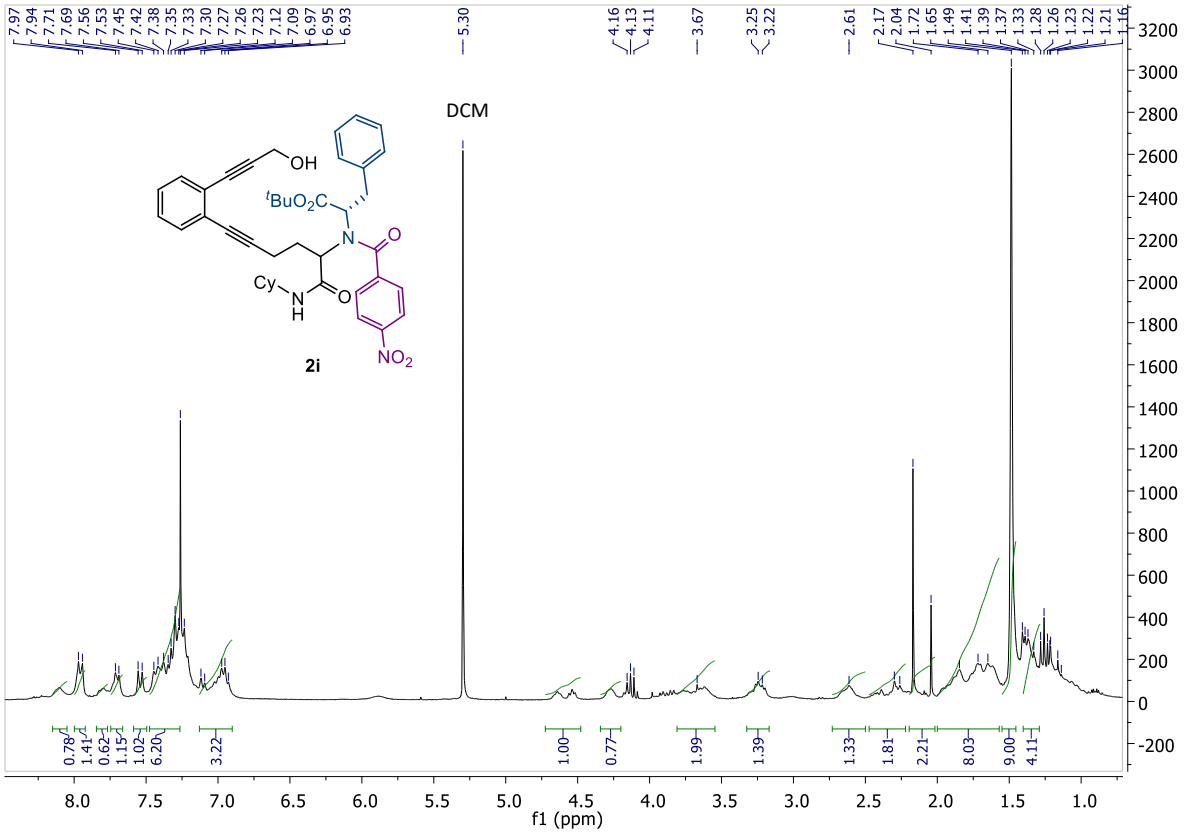

OH
tBuO2C
N
O
Me
tBu
N
H
O
2f
f1 (ppm)

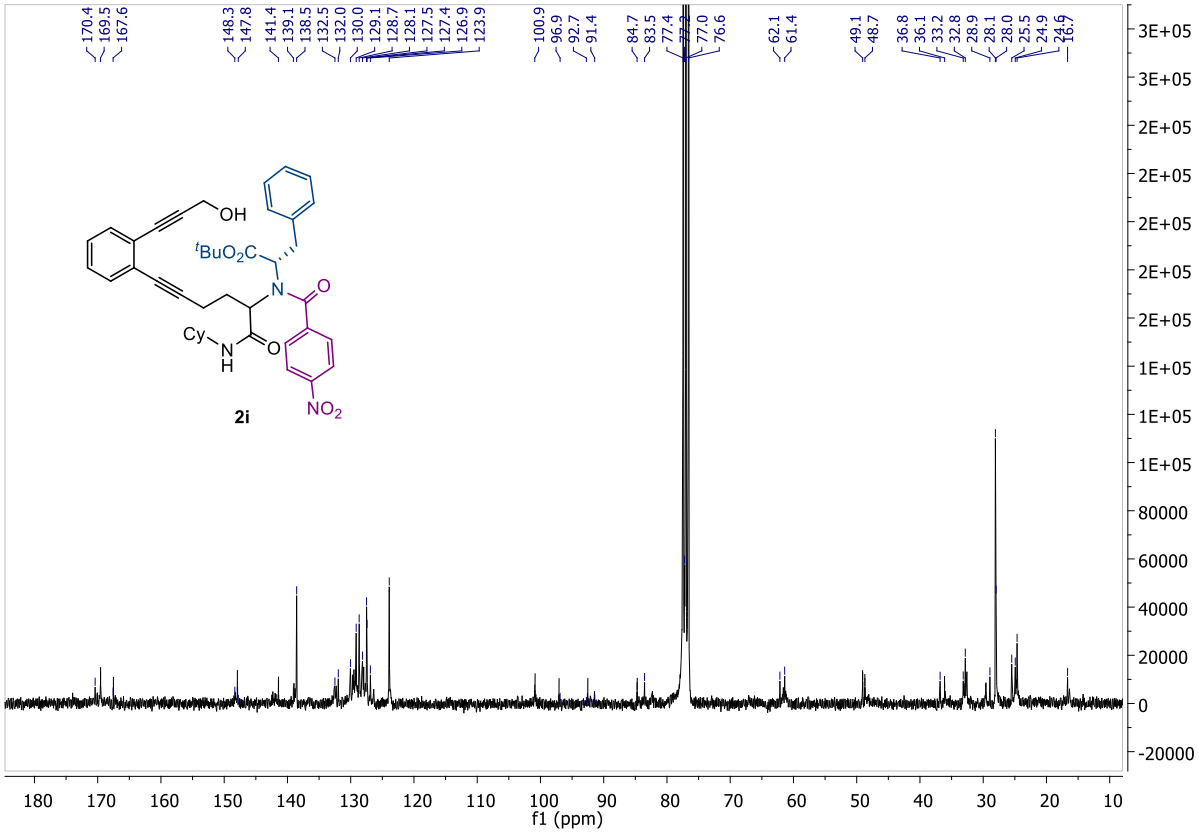

OH
tBuO2C
N
O
NHAc
tBu
N
H
O
2g
f1 (ppm)

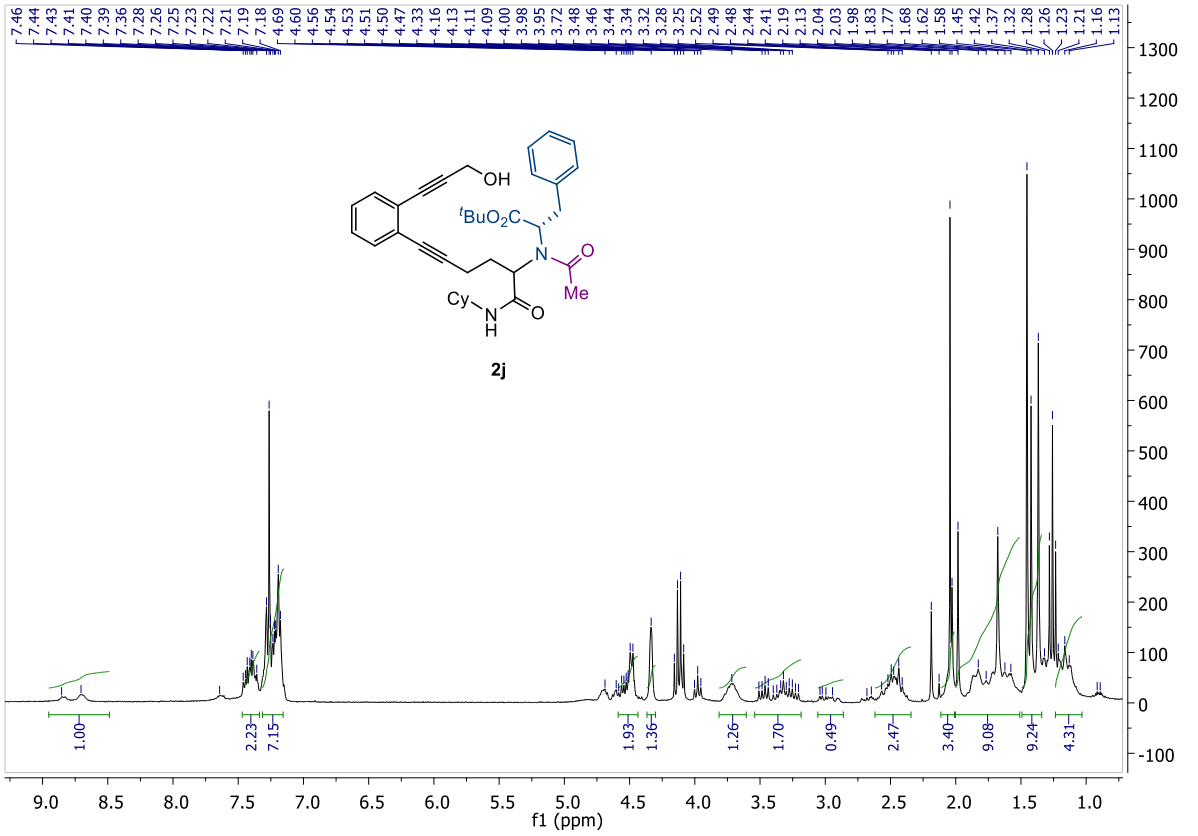

OH
tBuO2C
N
O
NHAc
tBu
N
H
O
2g
f1 (ppm)

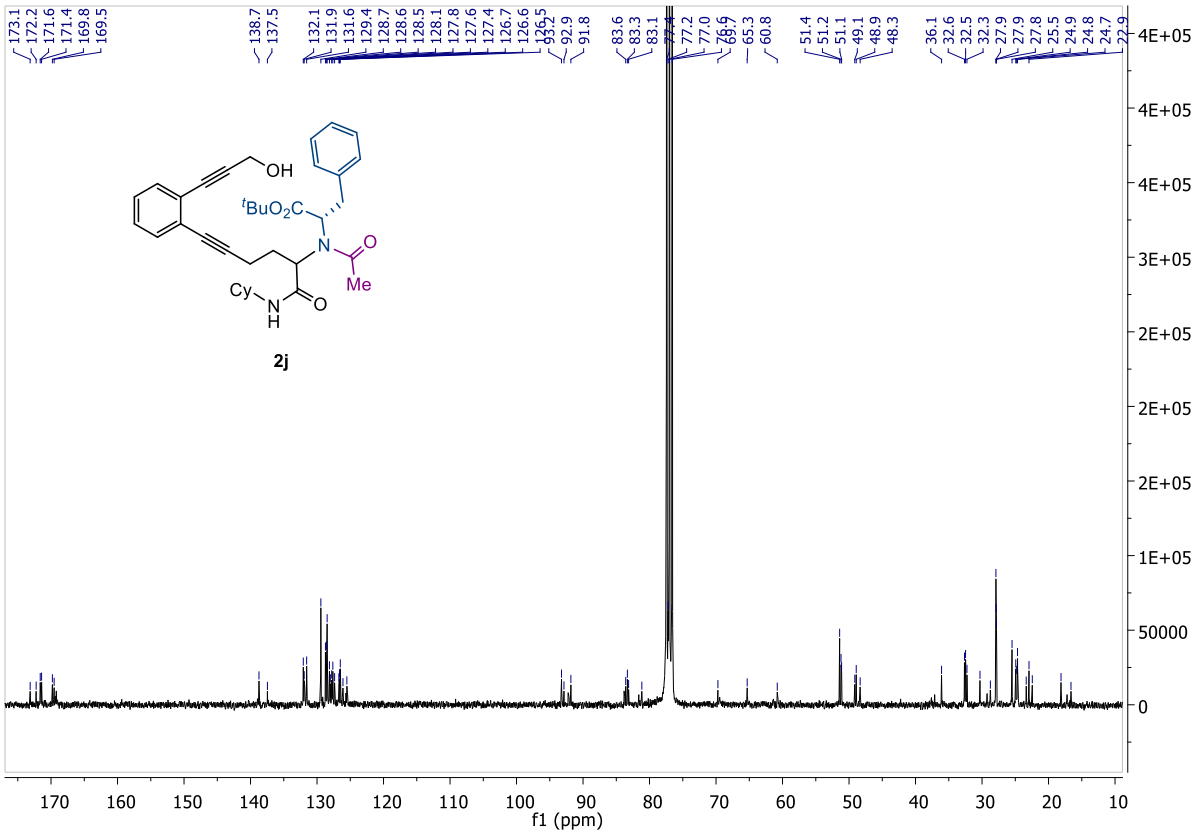

acetone
OH
tBuO2C
N
O
Cy
N
H
O
2h
f1 (ppm)

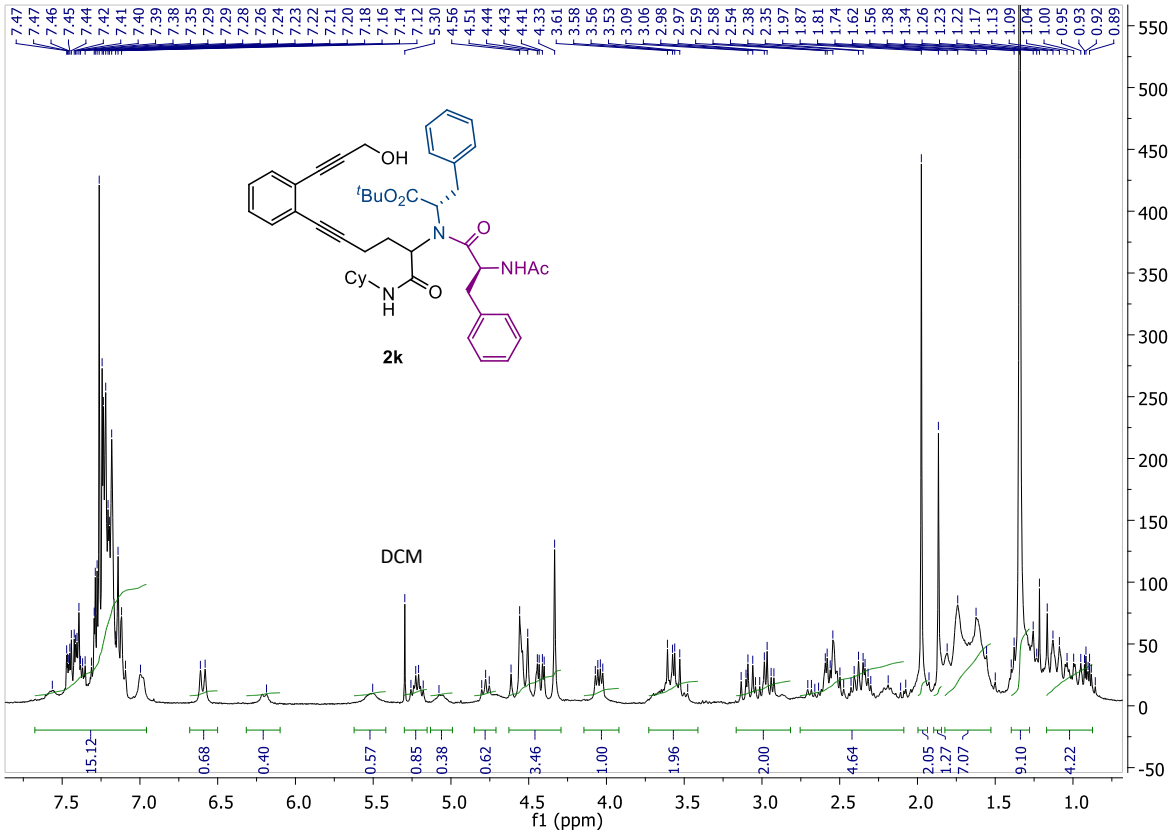

OH
tBuO2C
N
O
Cy
N
H
O
2h
f1 (ppm)

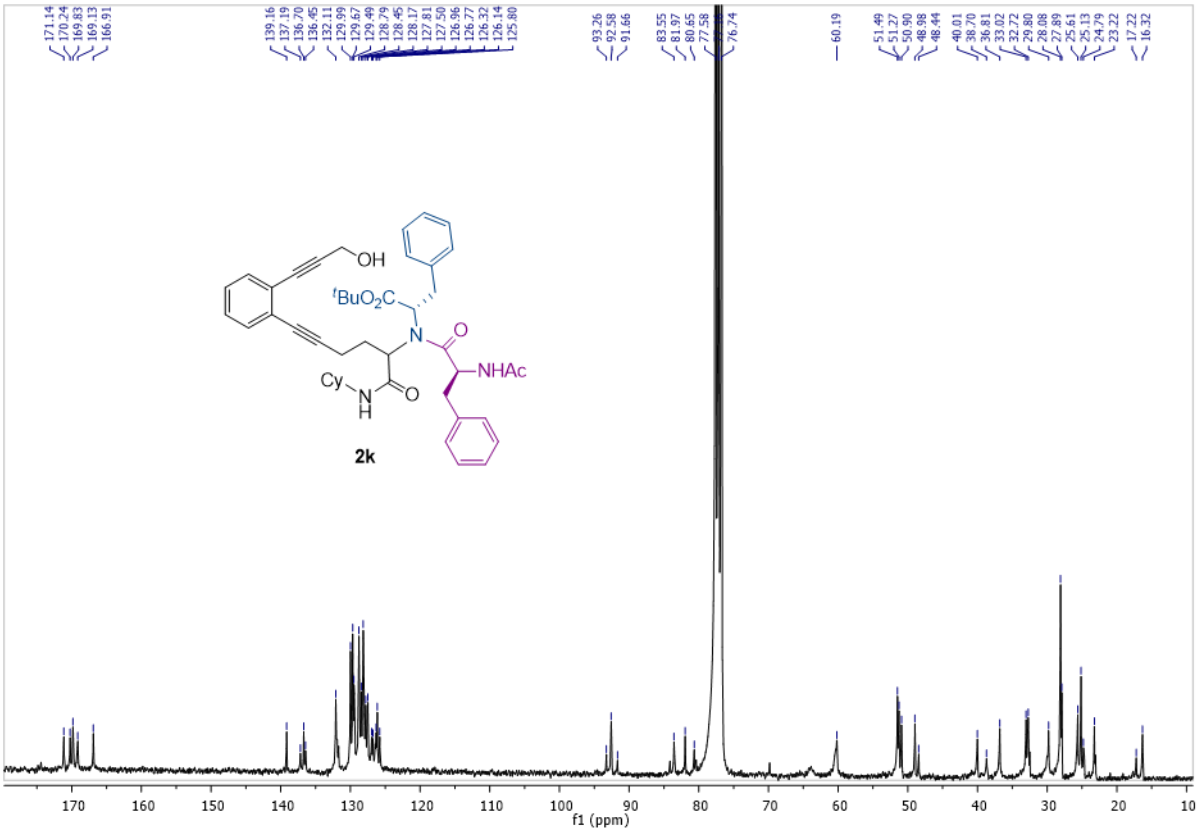

DCM
OH
tBuO2C
N
O
Cy
N
H
O
NO2
2i
f1 (ppm)

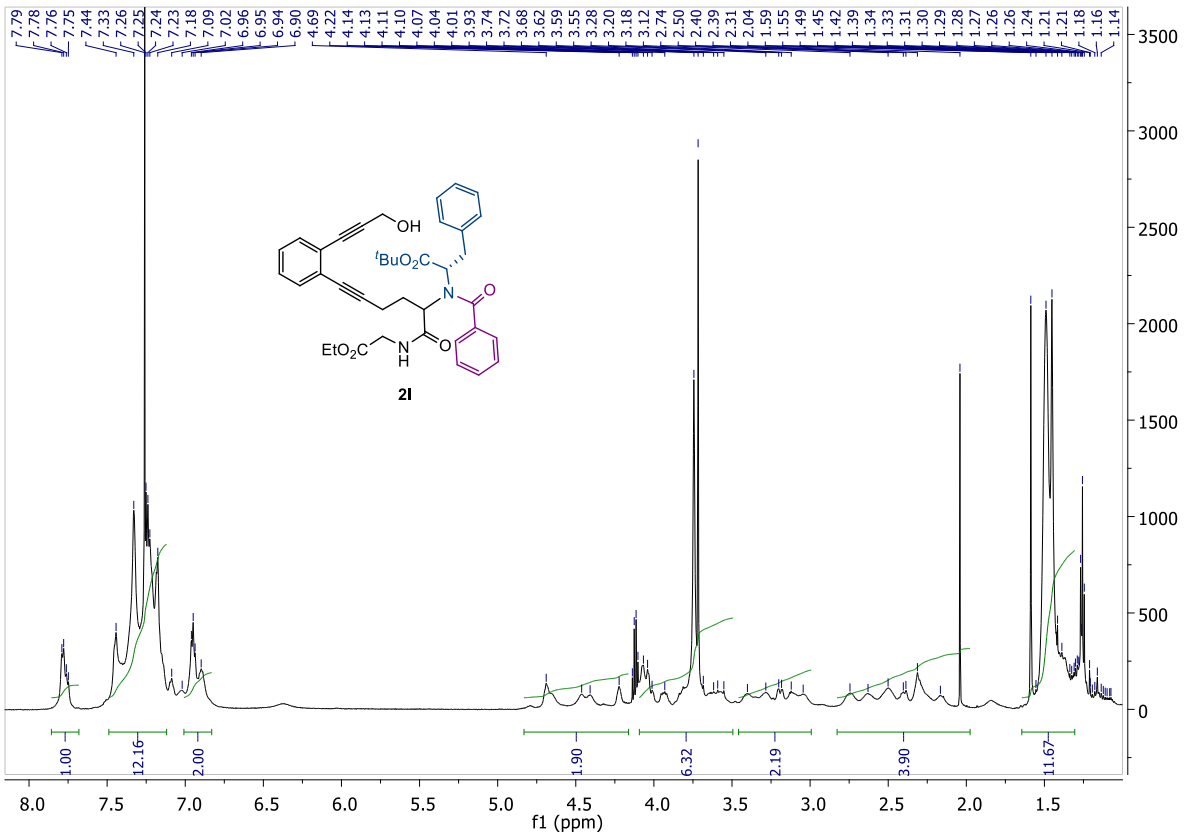

OH
tBuO2C
N
O
Cy
N
H
O
NO2
2i
f1 (ppm)

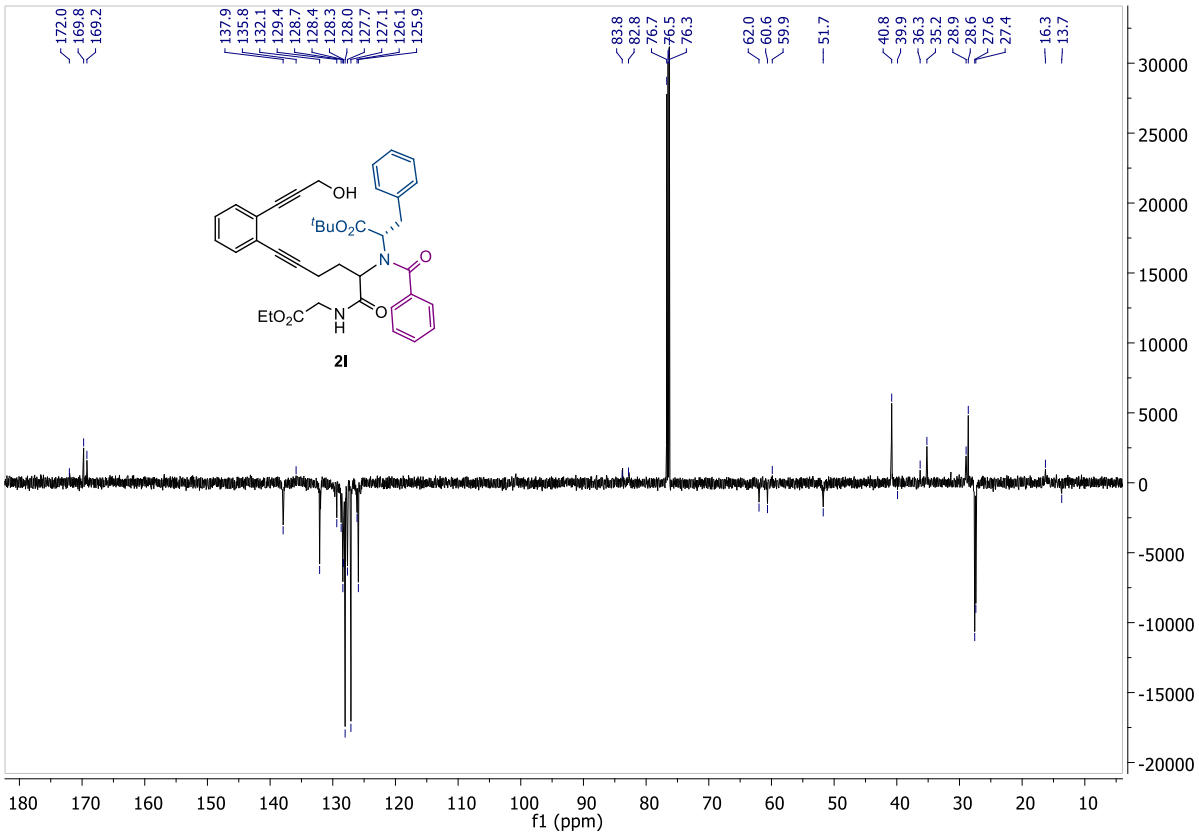

OH
tBuO2C
N
O
Me
Cy
N
H
O
2j
f1 (ppm)

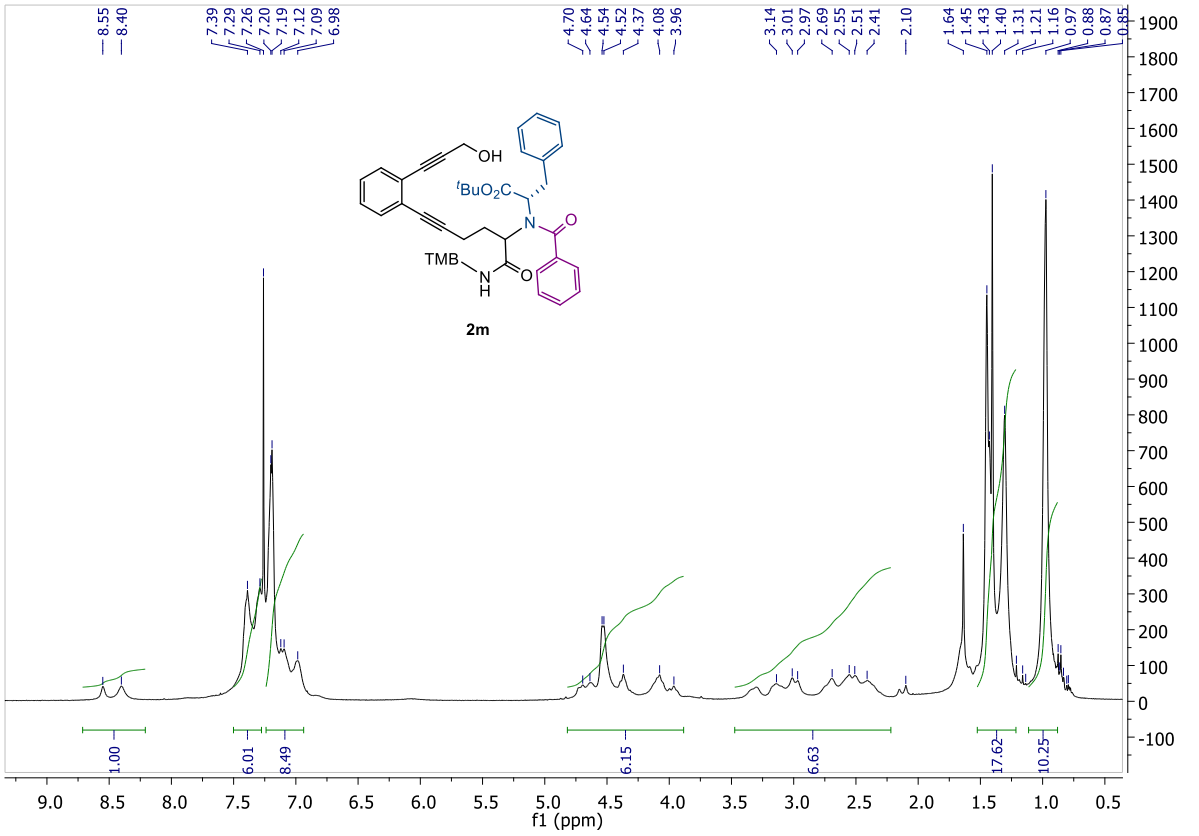

OH
tBuO2C
N
O
Me
Cy
N
H
O
2j
f1 (ppm)

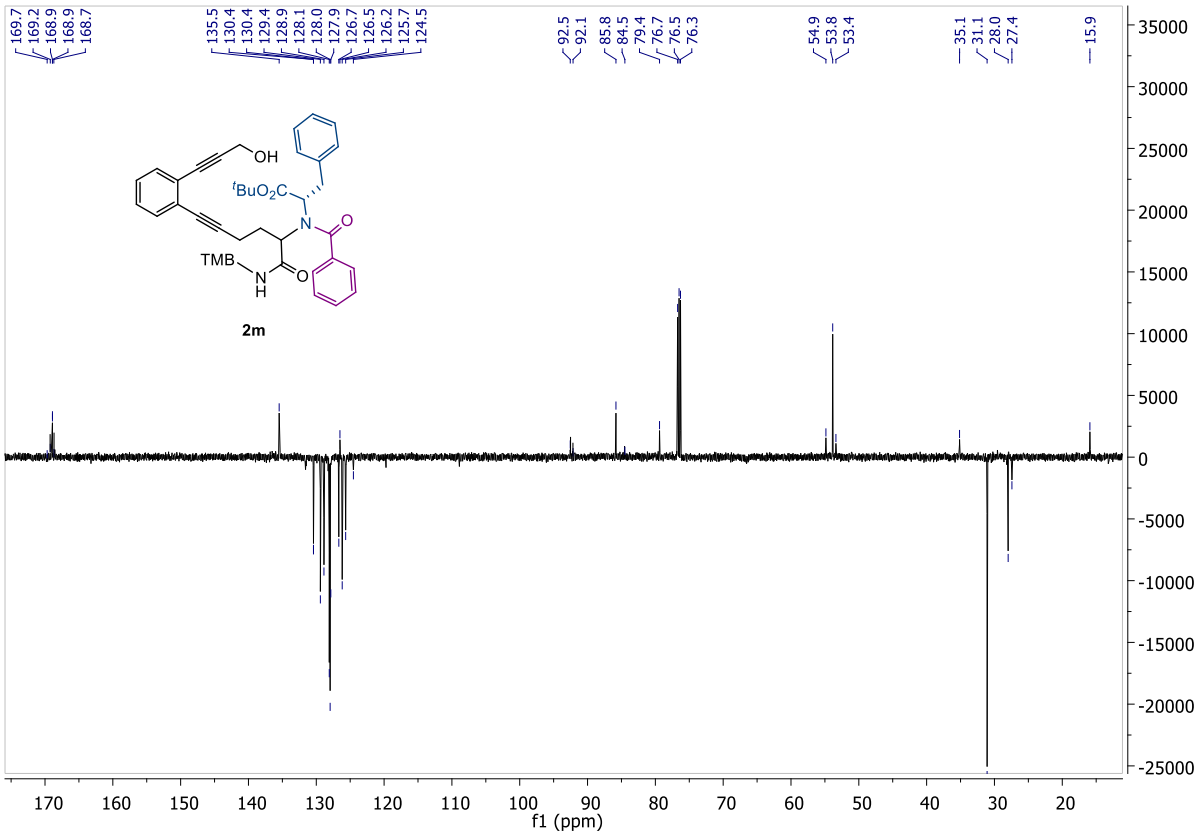

OH
tBuO2C
N
O
NHAc
Cy
N
H
O
2k
DCM
f1 (ppm)

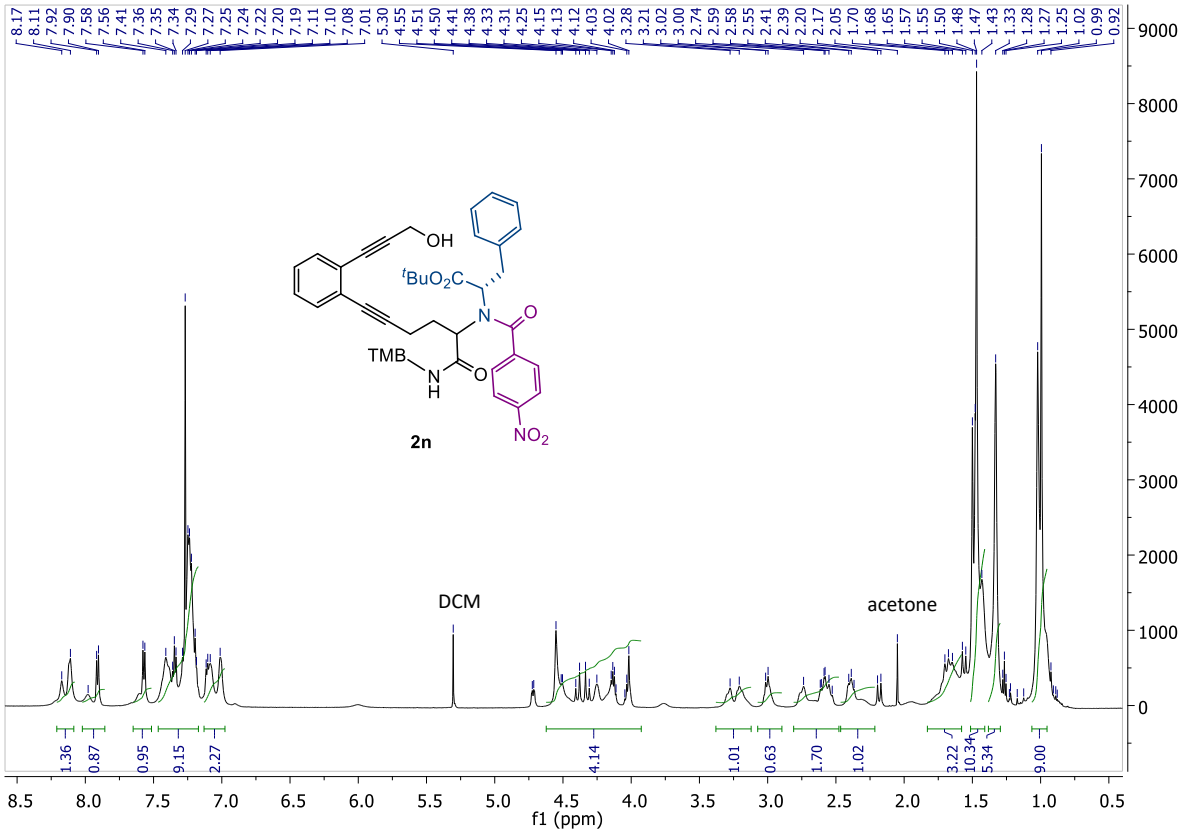

OH
tBuO2C
N
O
NHAc
Cy
N
H
O
2k
f1 (ppm)

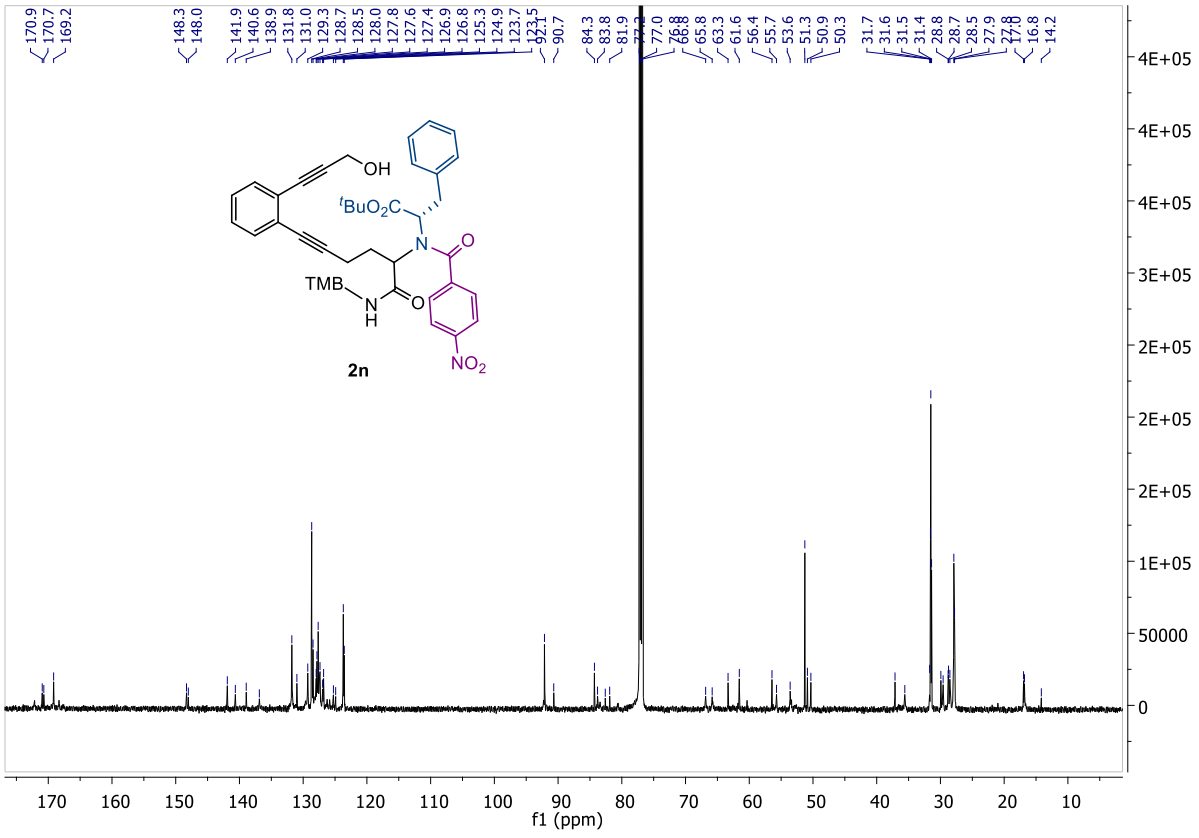

OH
tBuO2C
N
O
EtO2C
N
H
O
2l
7.79 7.78 7.76 7.75 7.44 7.33 7.26 7.25 7.24 7.23 7.18 7.09 7.02 6.96 6.95 6.94 6.90 4.69 4.22 4.14 4.13 4.11 4.10 4.07 4.04 4.01 3.93 3.74 3.72 3.68 3.62 3.59 3.55 3.28 3.20 3.18 3.12 2.74 2.50 2.40 2.39 2.31 2.04 1.59 1.55 1.49 1.45 1.42 1.39 1.34 1.33 1.31 1.30 1.29 1.28 1.27 1.26 1.26 1.24 1.21 1.21 1.18 1.16 1.14
1.00 12.16 2.00 1.90 6.32 2.19 3.90 11.67
f1 (ppm)

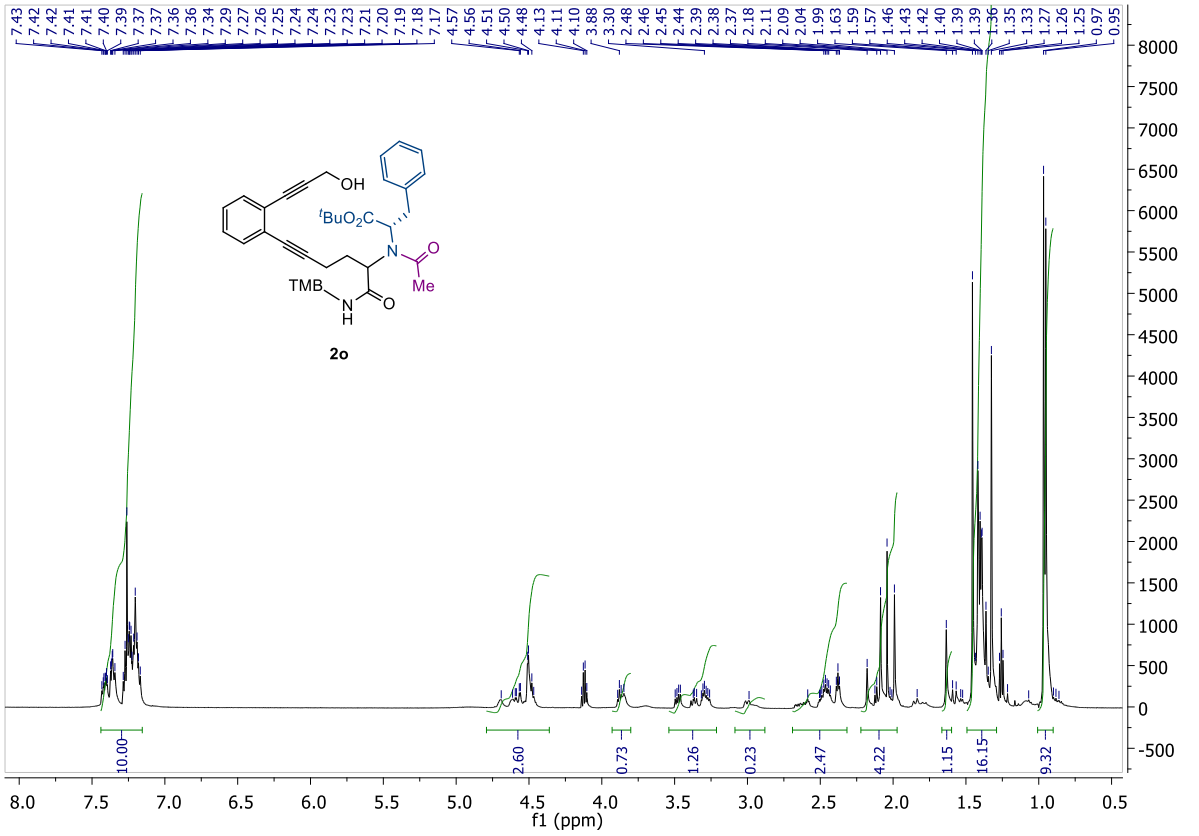

OH
tBuO2C
N
O
EtO2C
N
H
O
2l
172.0 169.8 169.2 137.9 135.8 132.1 129.4 128.7 128.4 128.3 128.0 127.7 127.1 126.1 125.9 83.8 82.8 76.7 76.5 76.3 62.0 60.6 59.9 51.7 40.8 39.9 36.3 35.2 28.9 28.6 27.6 27.4 16.3 13.7
f1 (ppm)

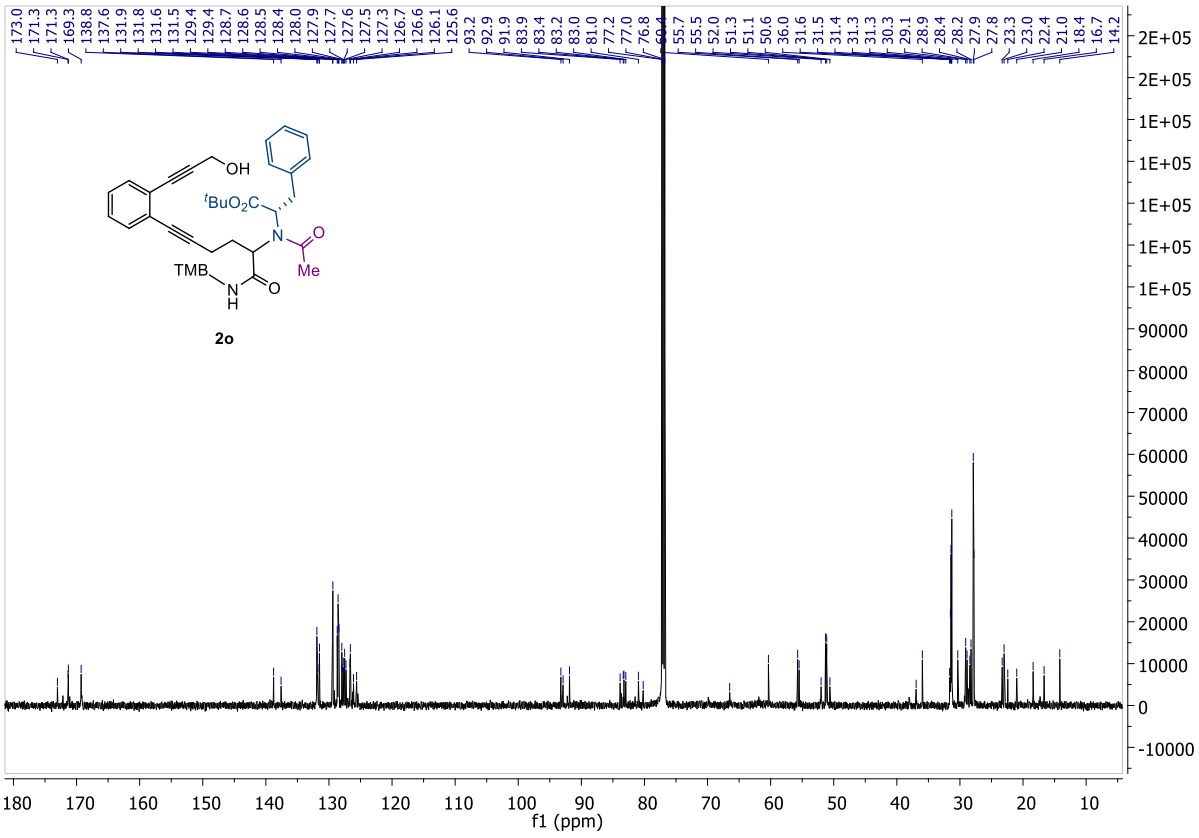

8.55
8.40
7.39
7.29
7.26
7.20
7.19
7.12
7.09
6.98
4.70
4.64
4.54
4.52
4.37
4.08
3.96
3.14
3.01
2.97
2.69
2.55
2.51
2.41
2.10
1.64
1.45
1.43
1.40
1.31
1.21
1.16
0.97
0.88
0.87
0.85
OH
tBuO2C
N
O
TMB
N
H
O
2m
1.00
6.01
8.49
6.15
6.63
17.62
10.25
f1 (ppm)

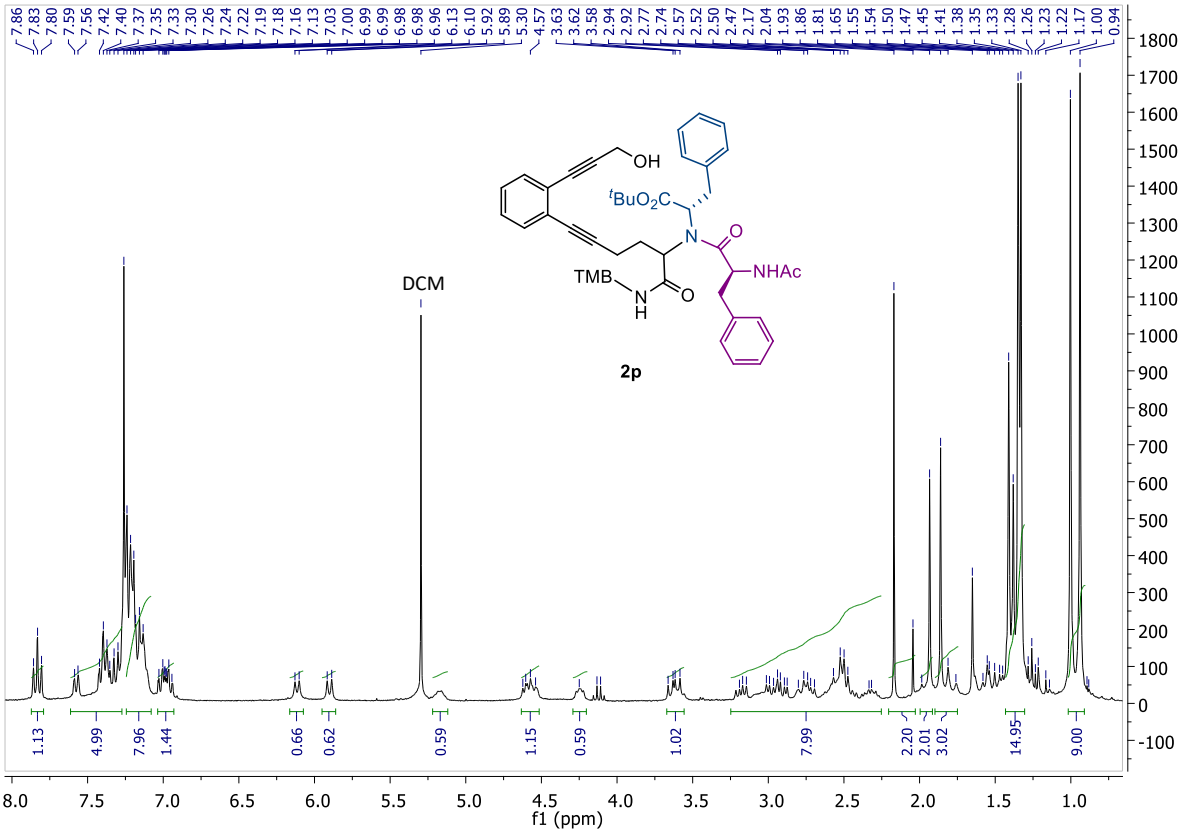

169.7
169.2
168.9
168.9
168.7
135.5
130.4
130.4
129.4
128.9
128.1
128.0
127.9
126.7
126.5
126.2
125.7
124.5
92.5
92.1
85.8
84.5
79.4
76.7
76.5
76.3
54.9
53.8
53.4
35.1
31.1
28.0
27.4
15.9
OH
tBuO2C
N
O
TMB
N
H
O
2m
f1 (ppm)

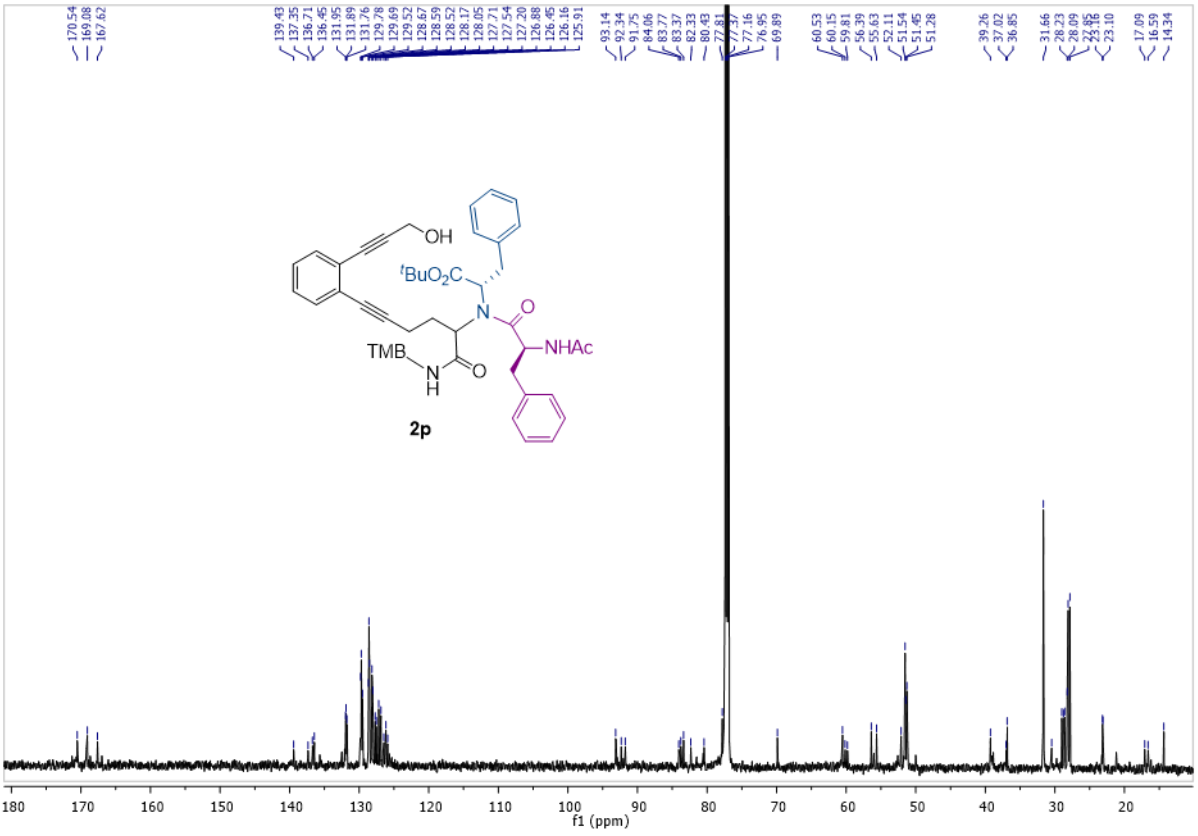

OH
tBuO2C
N
O
TMB
N
H
O
NO2
2n
DCM
acetone
f1 (ppm)

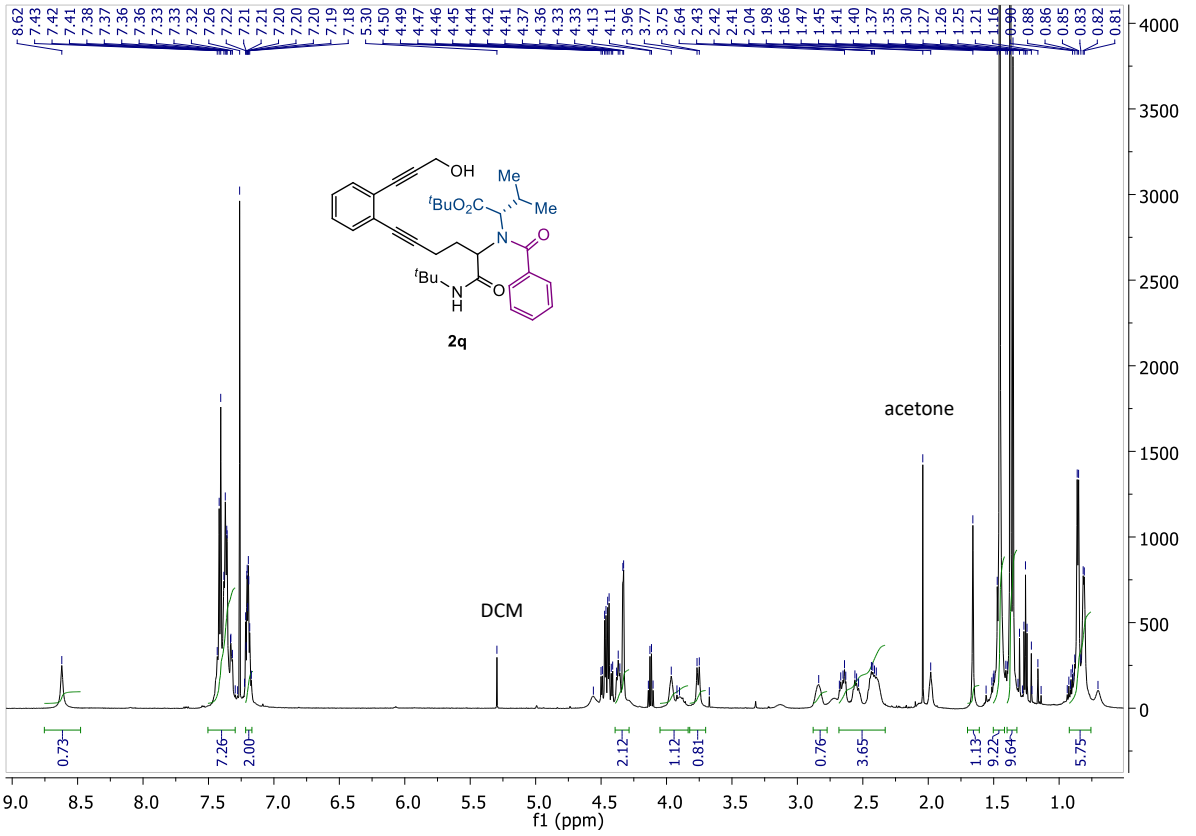

OH
tBuO2C
N
O
TMB
N
H
O
NO2
2n
f1 (ppm)

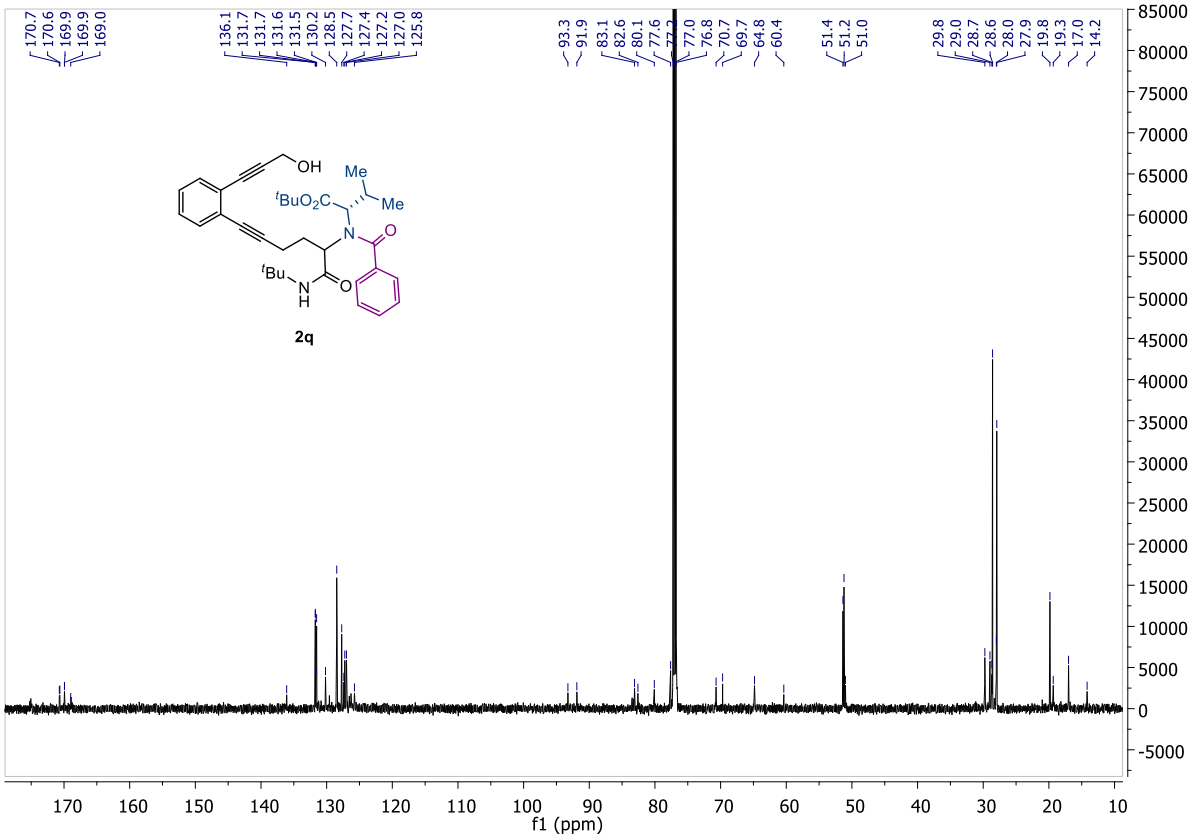

OH
tBuO2C
N
O
Me
TMB
N
H
O
2o
f1 (ppm)

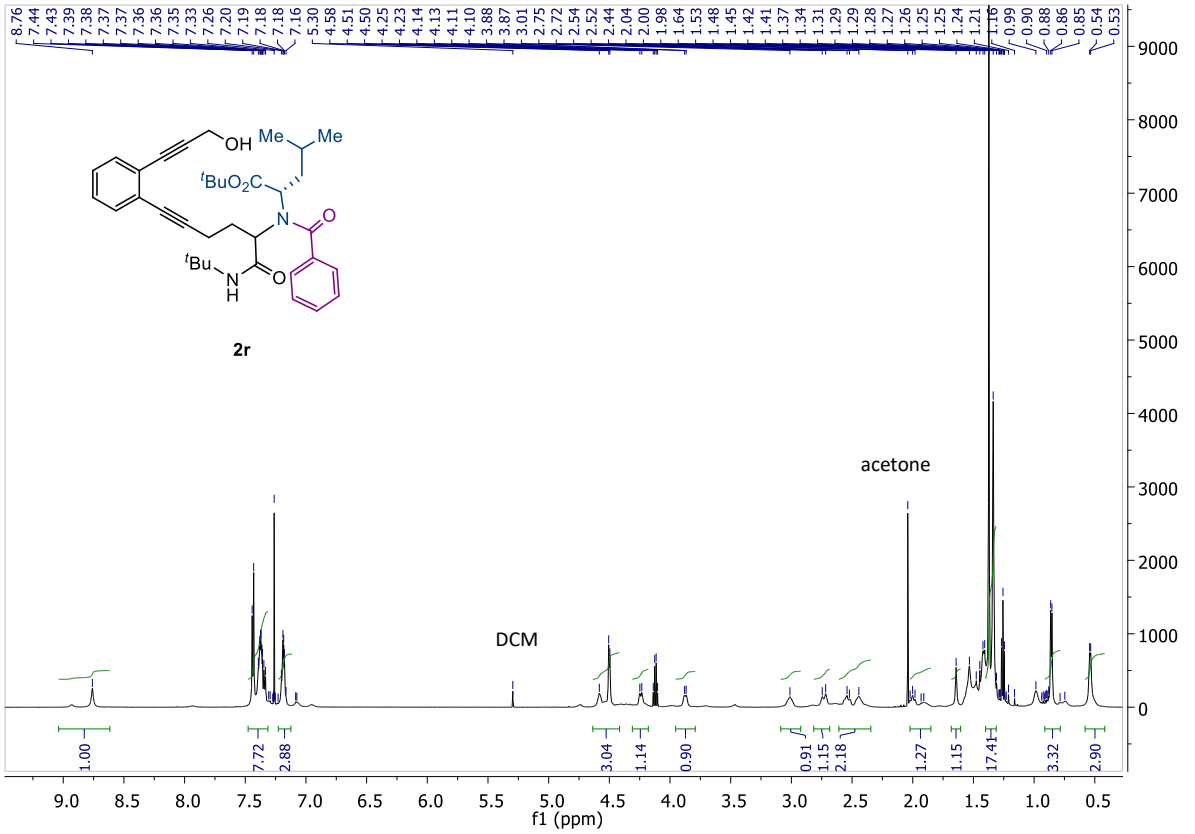

OH
tBuO2C
N
O
Me
TMB
N
H
O
2o
f1 (ppm)

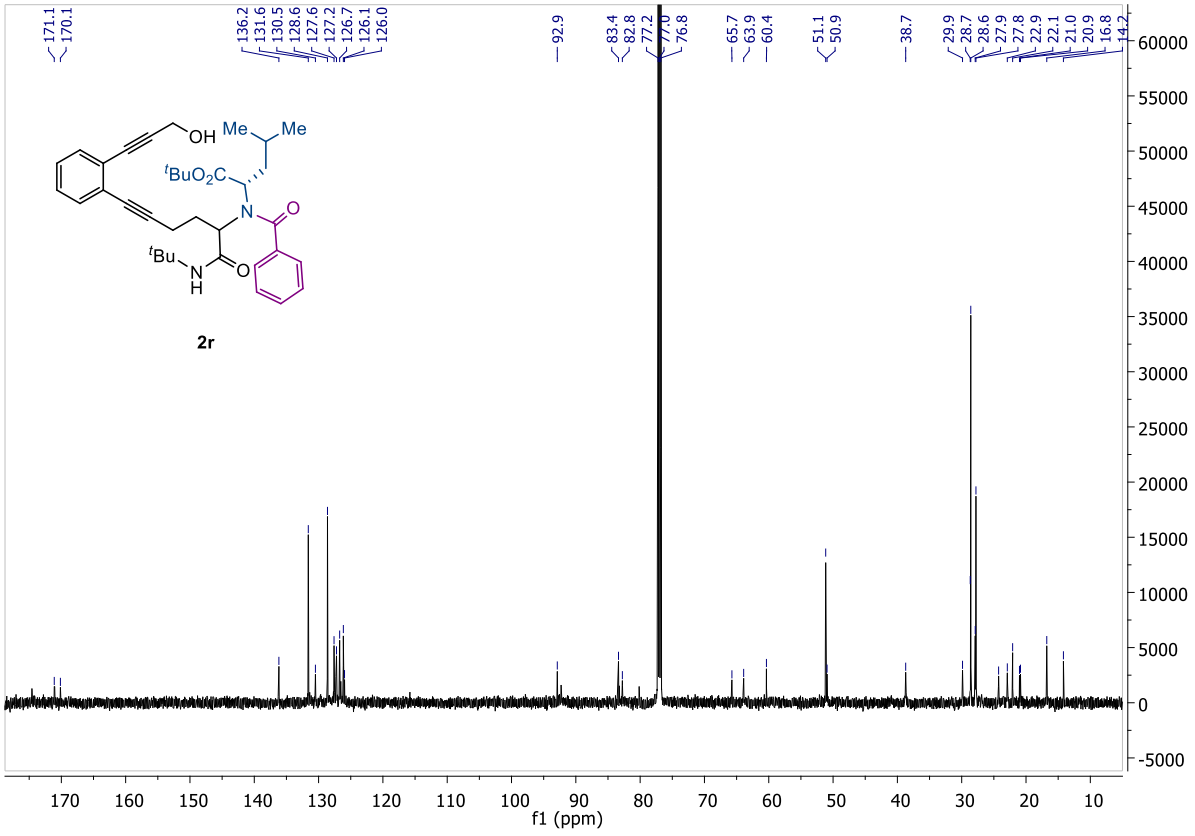

DCM
OH
^{t}BuO$_2$C
N
O
NHAc
TMB
N
H
O
2p
f1 (ppm)


OH
^{t}BuO$_2$C
N
O
NHAc
TMB
N
H
O
2p
f1 (ppm)

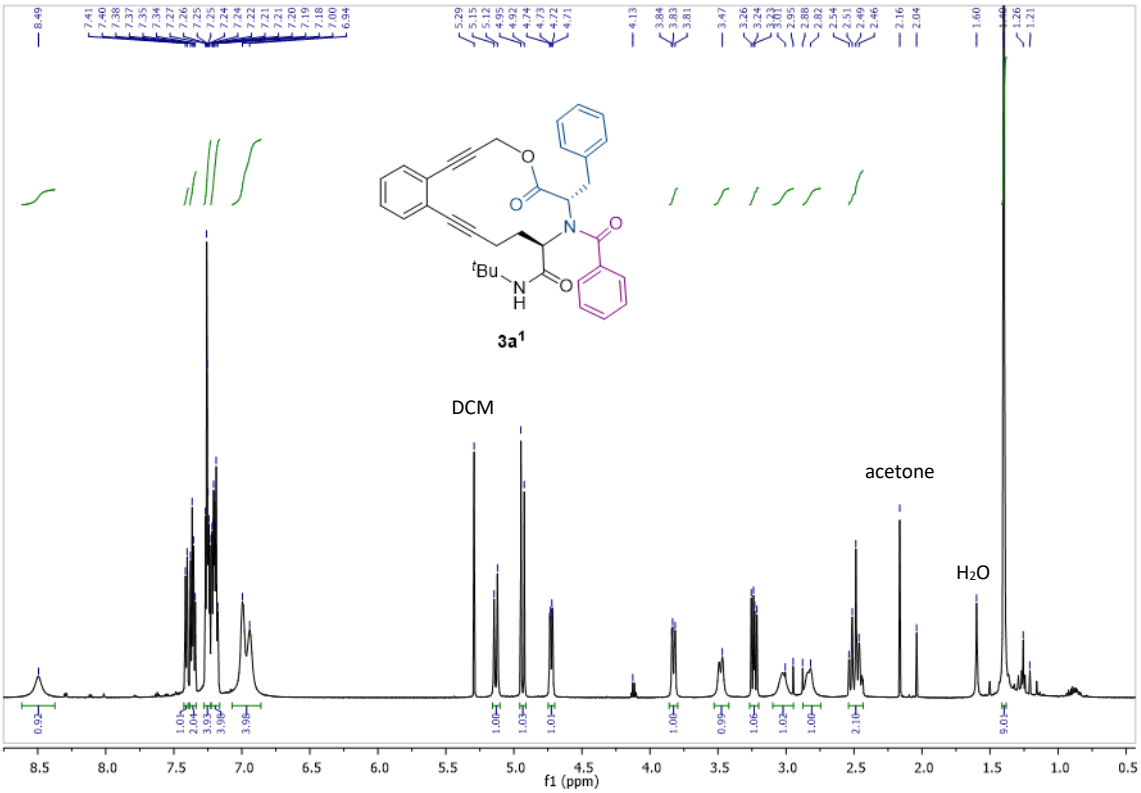

OH
Me
tBuO2C
Me
N
O
tBu
N
H
O
2q
acetone
DCM
f1 (ppm)

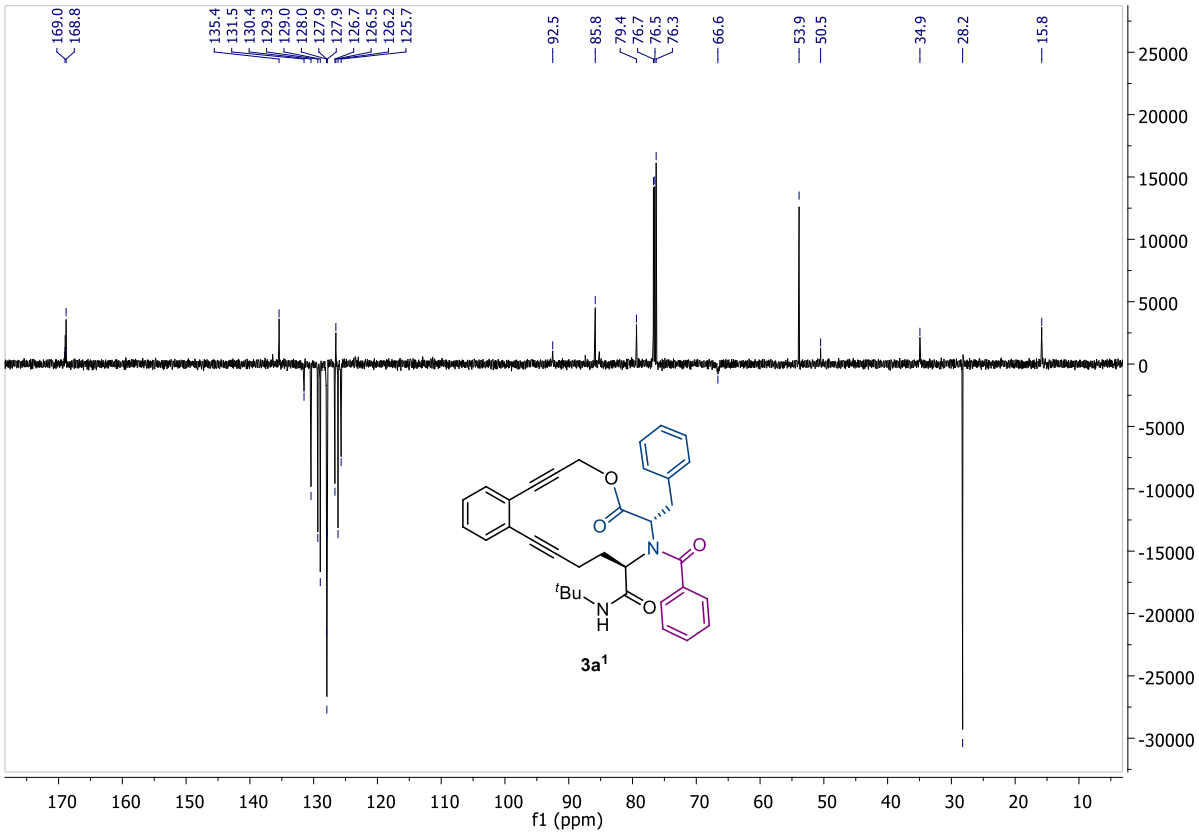

OH
Me
tBuO2C
Me
N
O
tBu
N
H
O
2q
f1 (ppm)

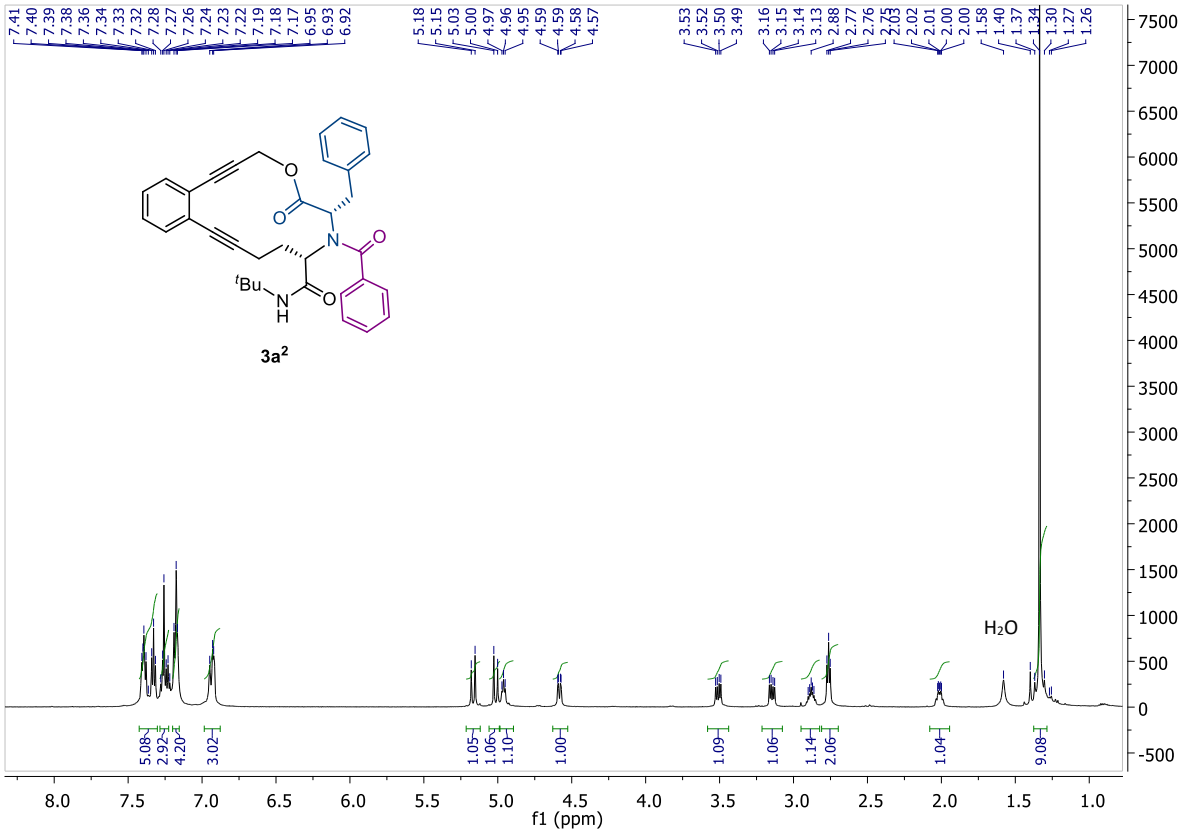

2r
acetone
DCM
f1 (ppm)

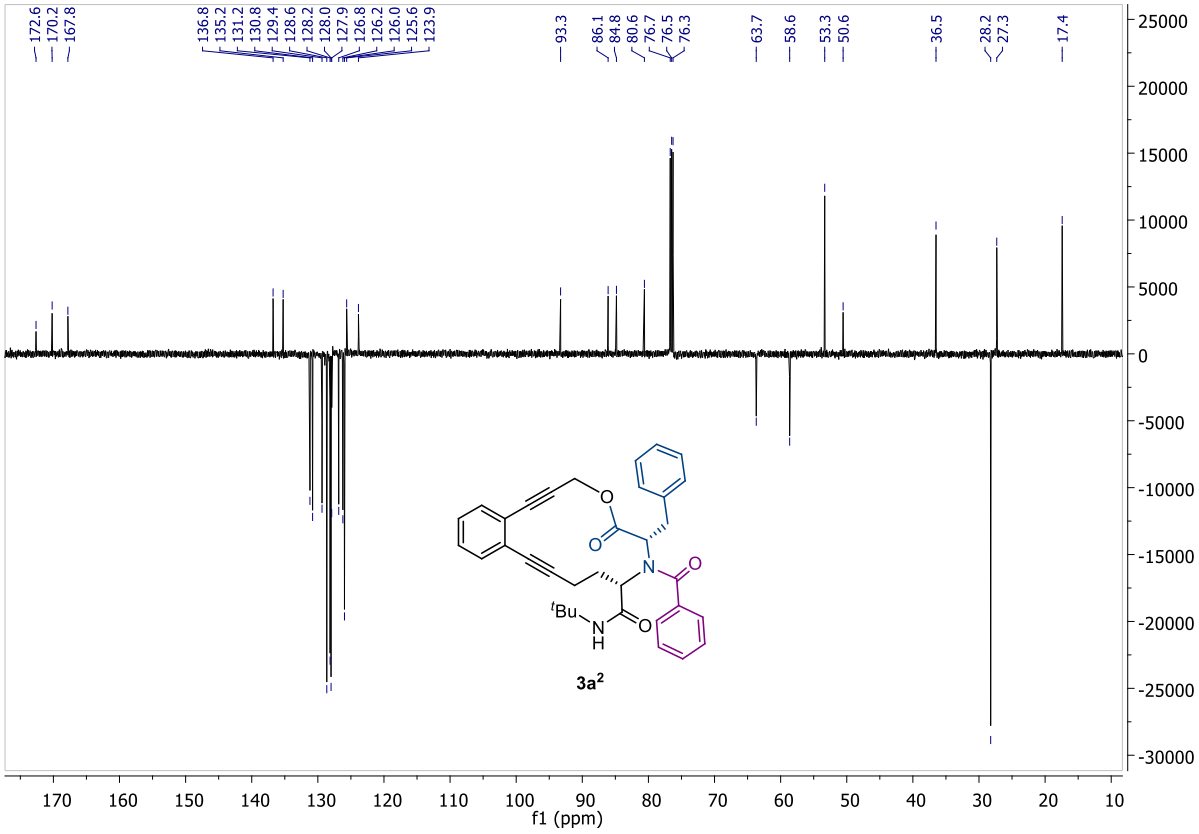

2r
f1 (ppm)

7. NMR SPECTRA OF MACROCYCLIC COMPOUNDS 3 AND 4

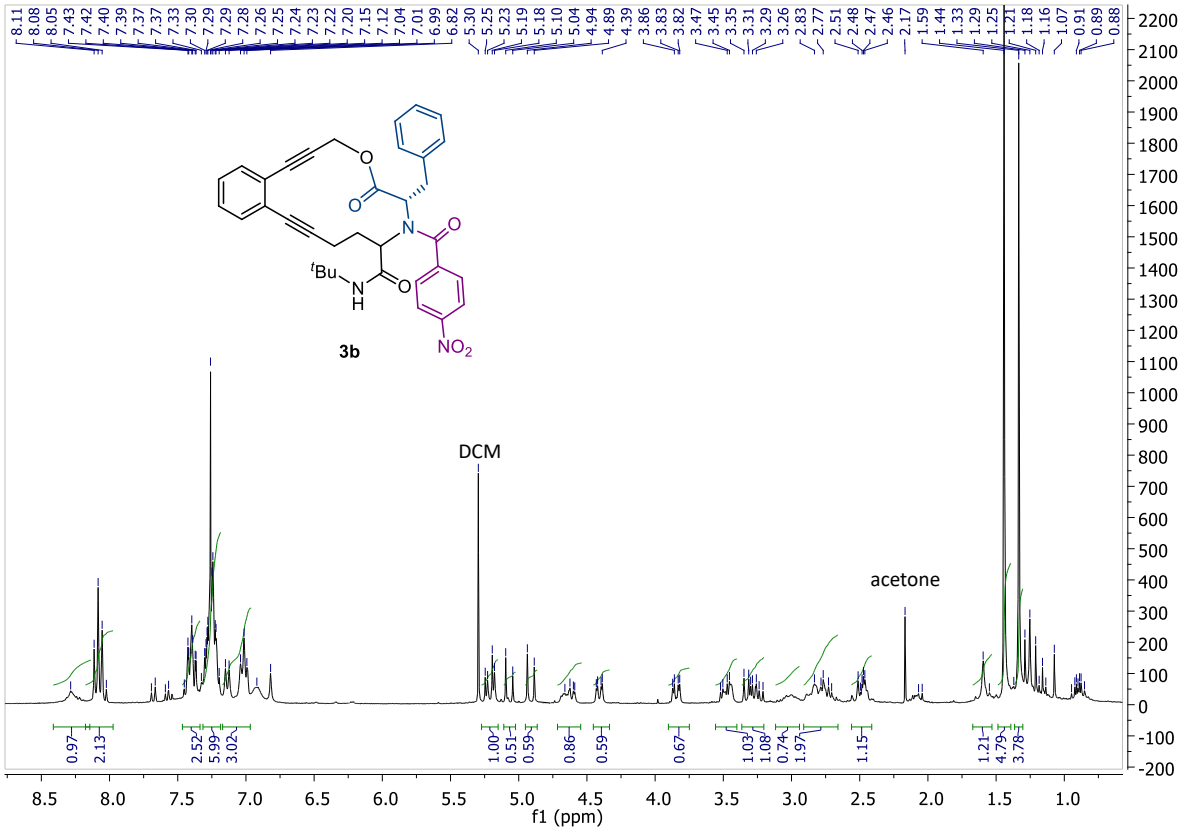


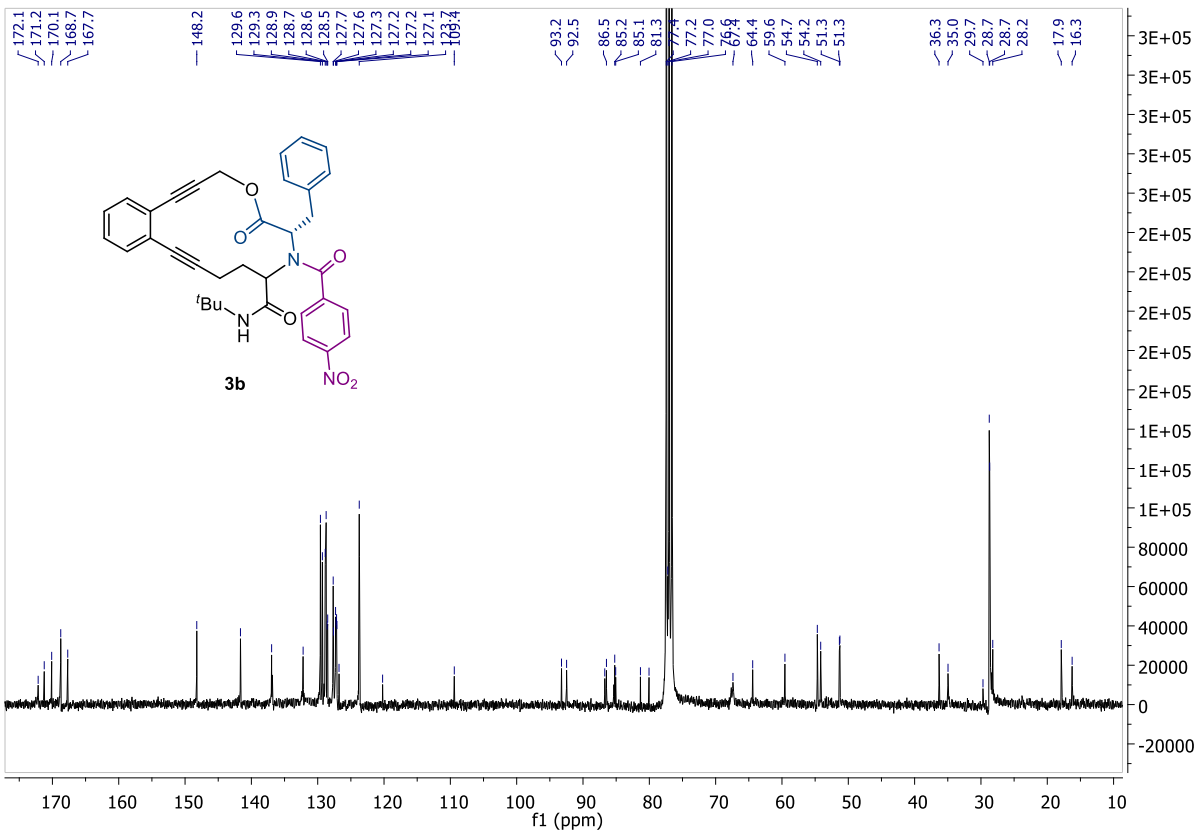



7.41 7.40 7.39 7.38 7.36 7.34 7.33 7.32 7.28 7.27 7.26 7.24 7.23 7.22 7.19 7.18 7.17 6.95 6.93 6.92
5.18 5.15 5.03 5.00 4.97 4.96 4.95 4.59 4.59 4.58 4.57
3.53 3.52 3.50 3.49 3.16 3.15 3.14 3.13 2.88 2.77 2.76 2.03 2.02 2.01 2.00 2.00 1.58 1.40 1.37 1.34 1.30 1.27 1.26
H2O
5.08 2.92 4.20 3.02 1.05 1.06 1.10 1.00 1.09 1.06 1.14 2.06 1.04 9.08
f1 (ppm)
3a2

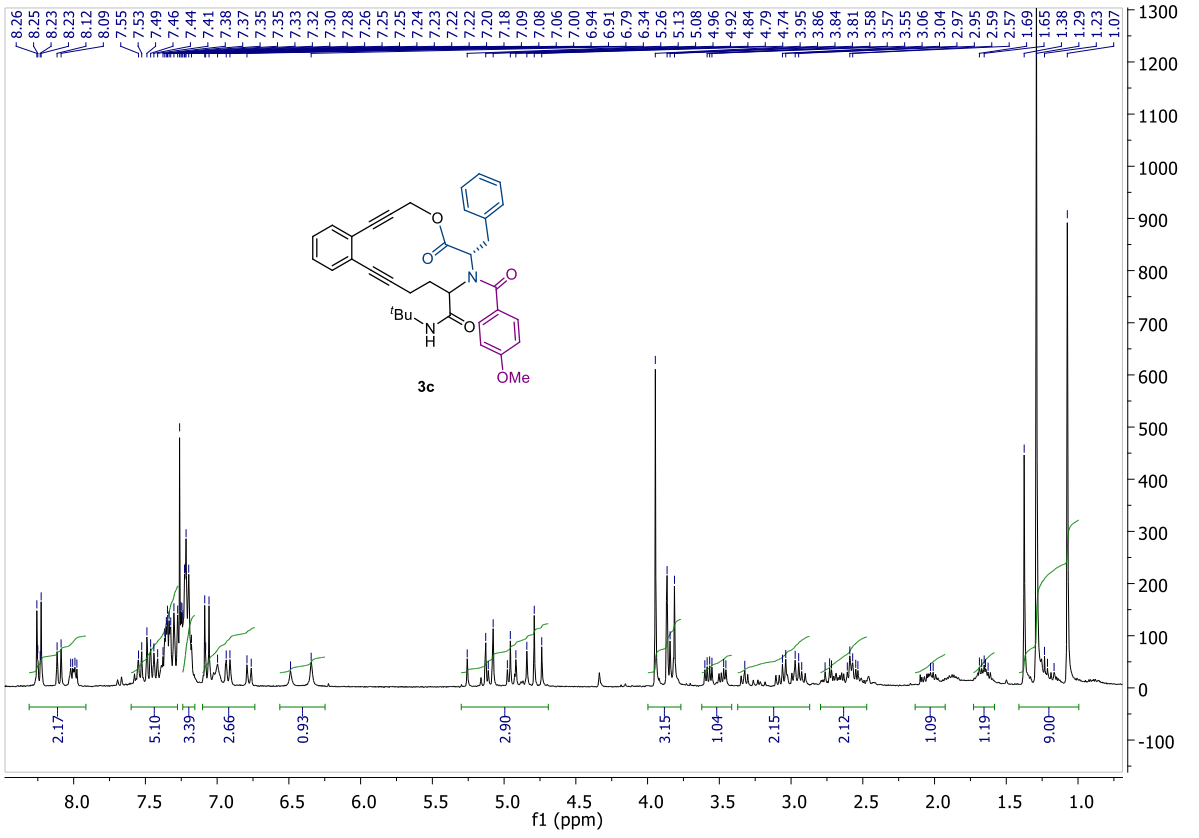

172.6 170.2 167.8
136.8 135.2 131.2 130.8 129.4 128.6 128.2 128.0 127.9 126.8 126.2 126.0 125.6 123.9
93.3 86.1 84.8 80.6 76.7 76.5 76.3
63.7 58.6 53.3 50.6 36.5 28.2 27.3 17.4
f1 (ppm)
3a2

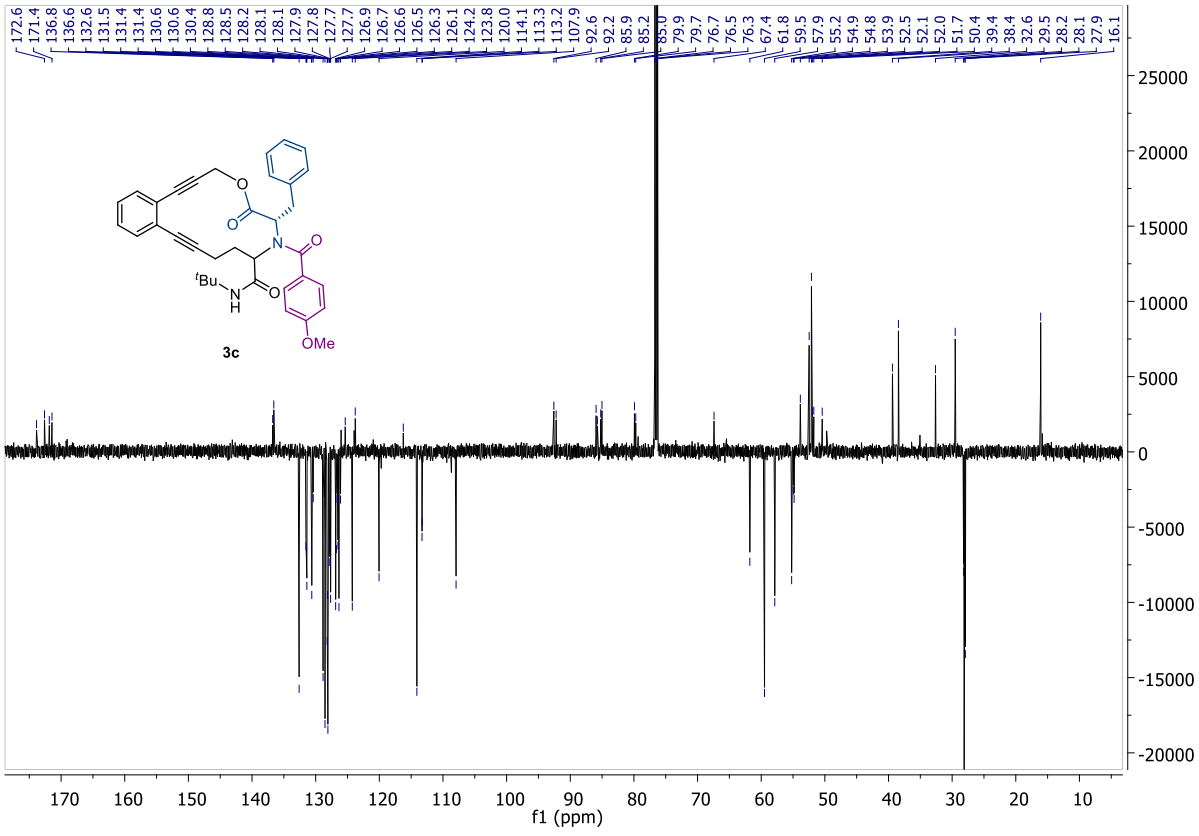
Macrocycle **3b** is isolated as a mixture of diastereoisomers

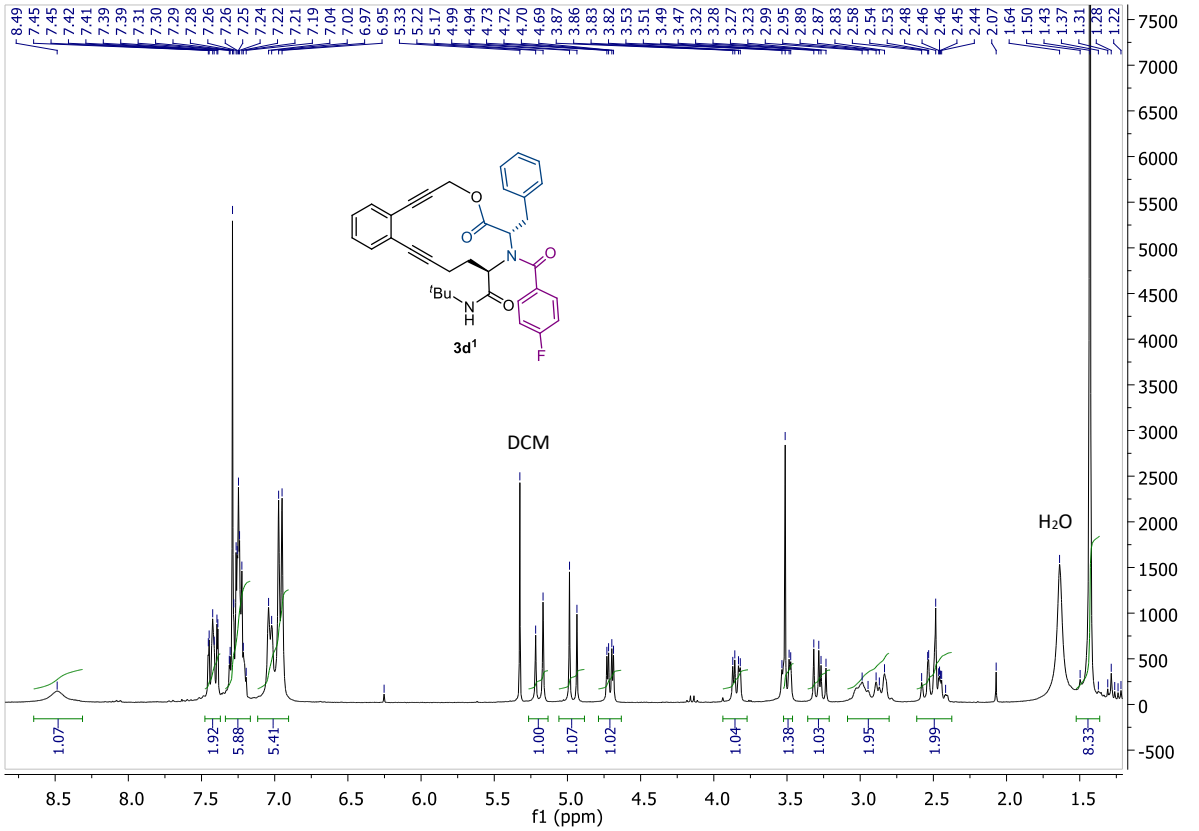


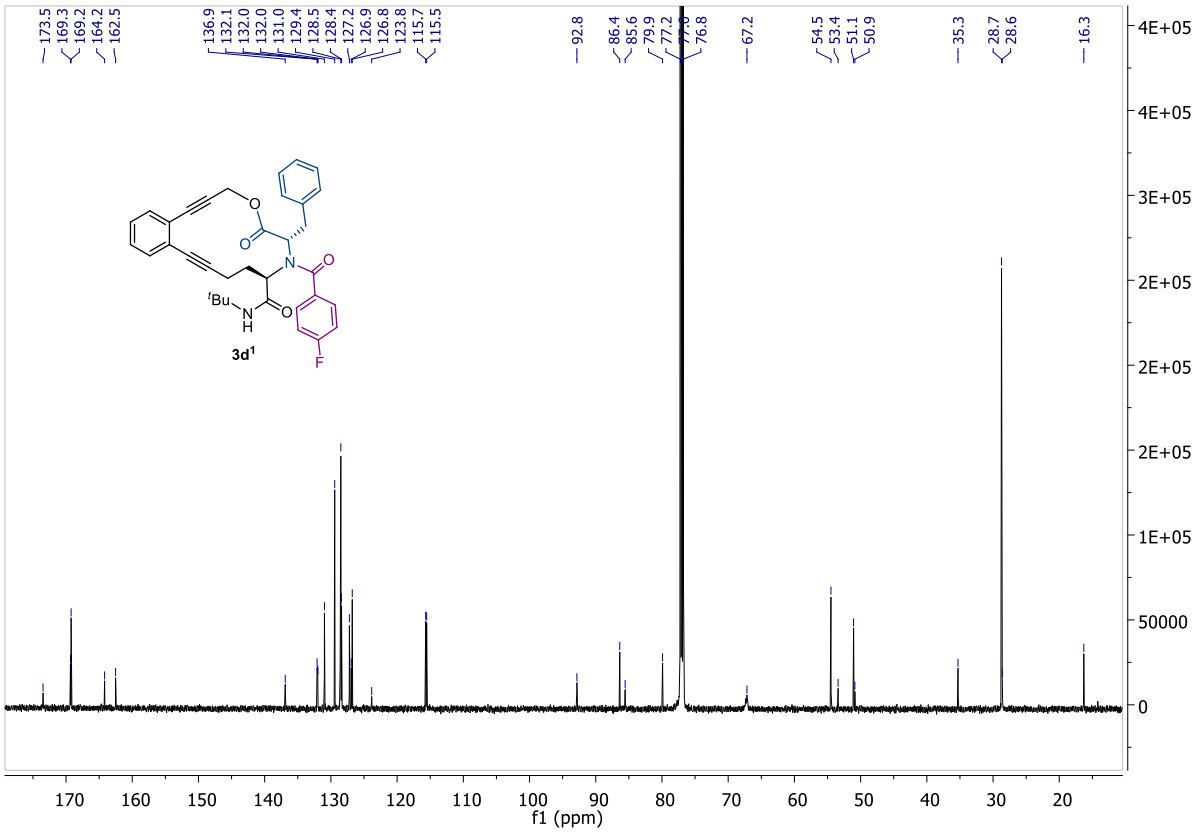


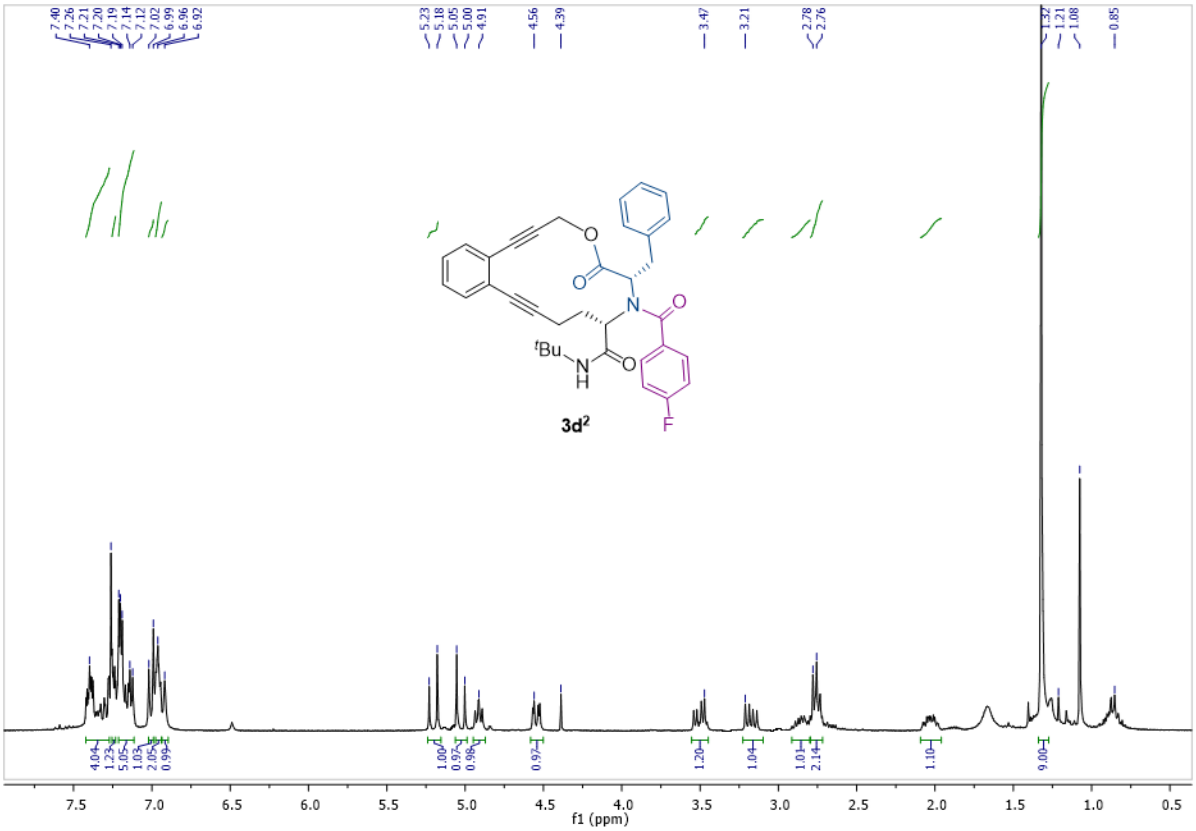
Macrocycle **3c** is isolated as a mixture of diastereoisomers

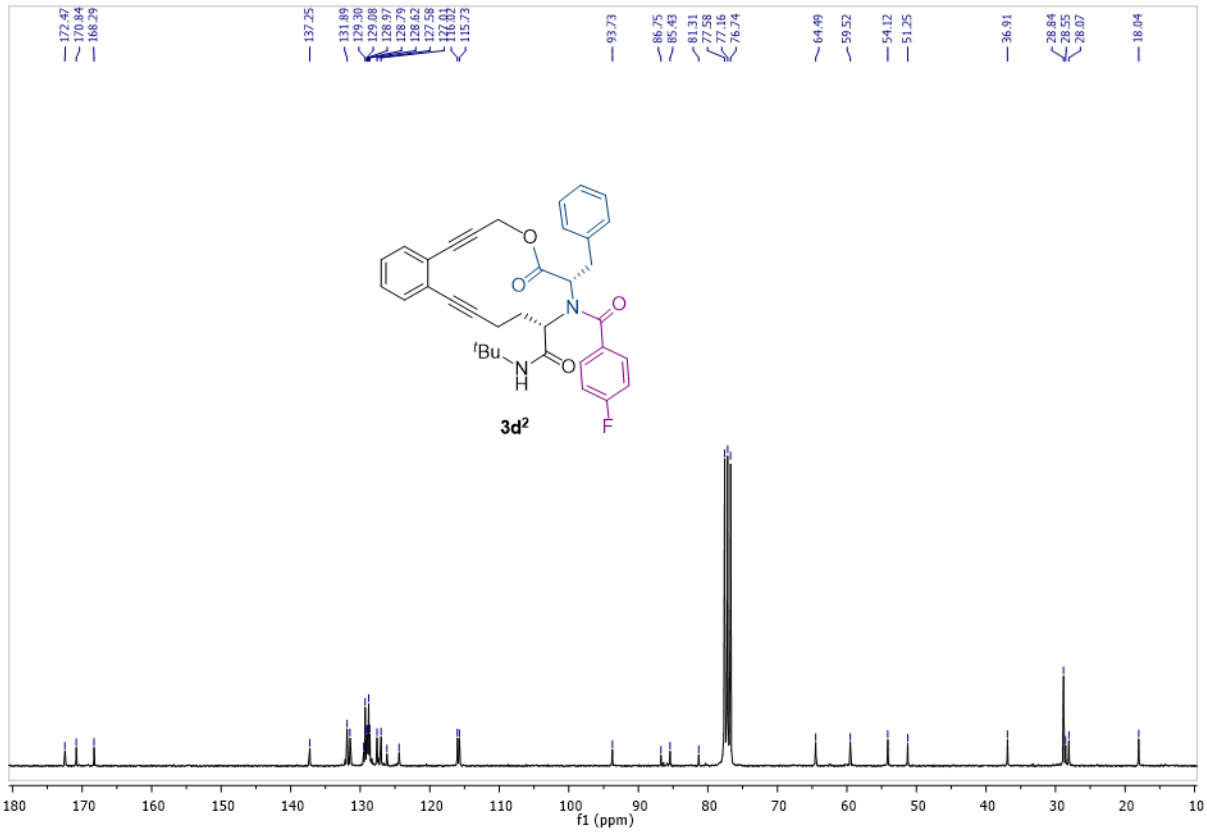


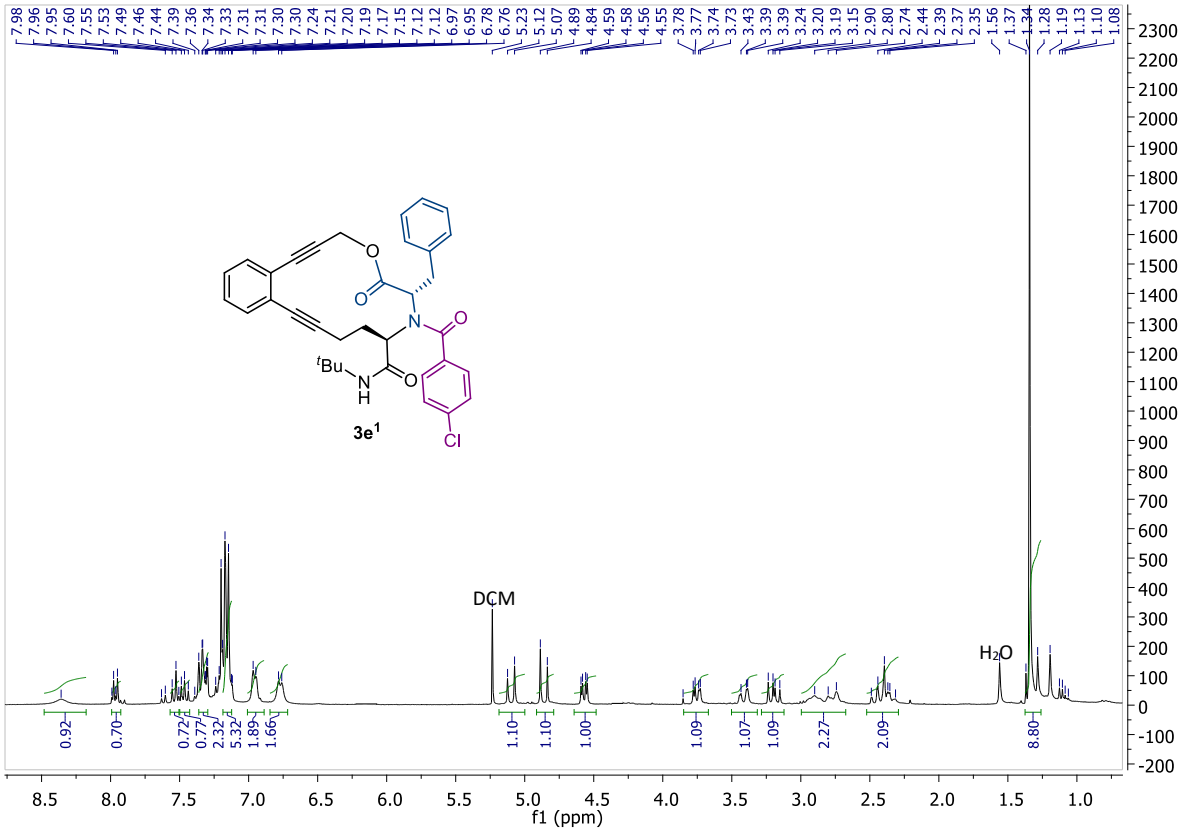


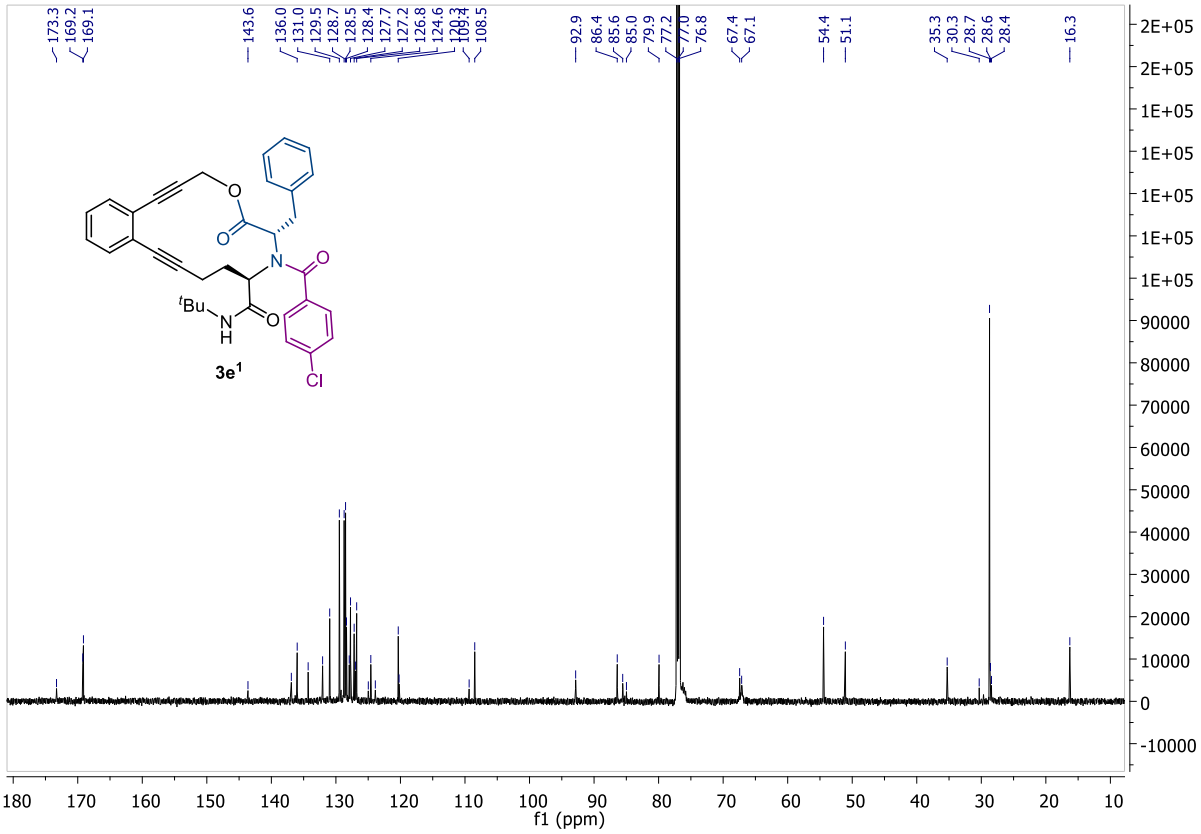

DCM
H2O
3d1
f1 (ppm)

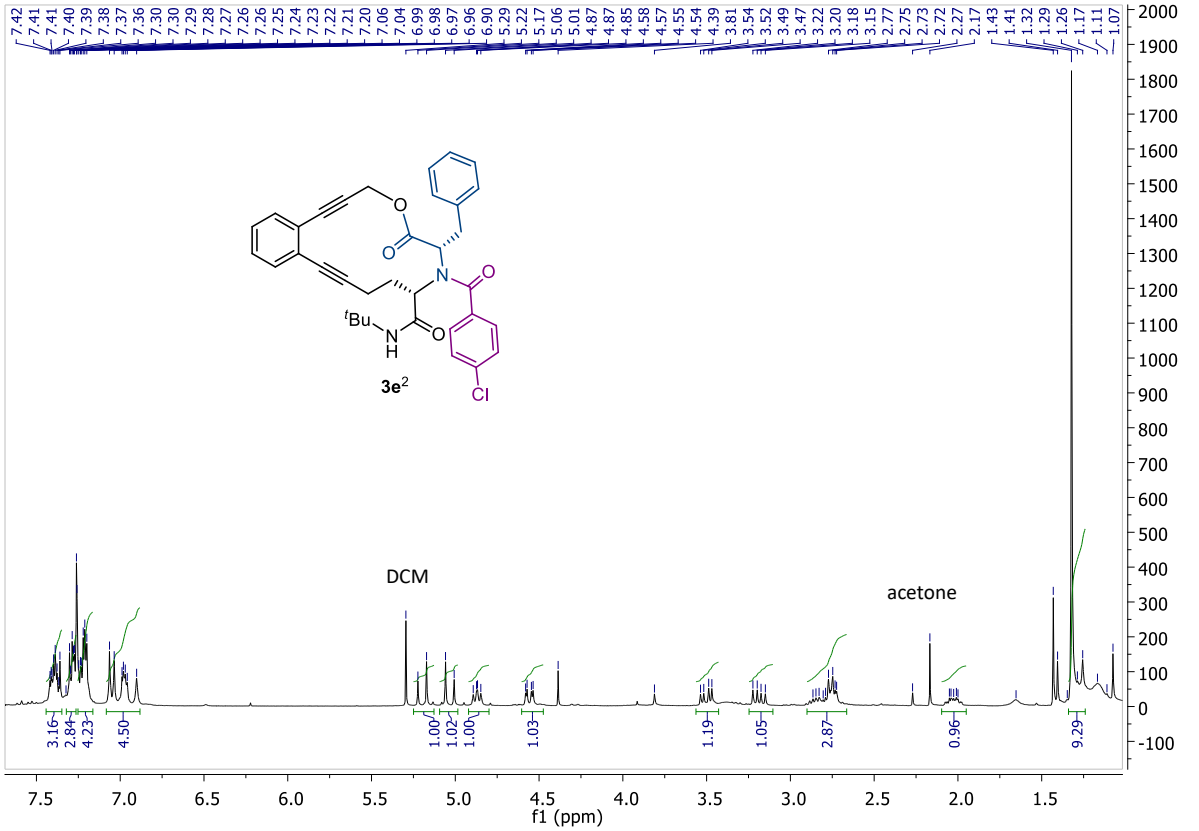

3d1
f1 (ppm)

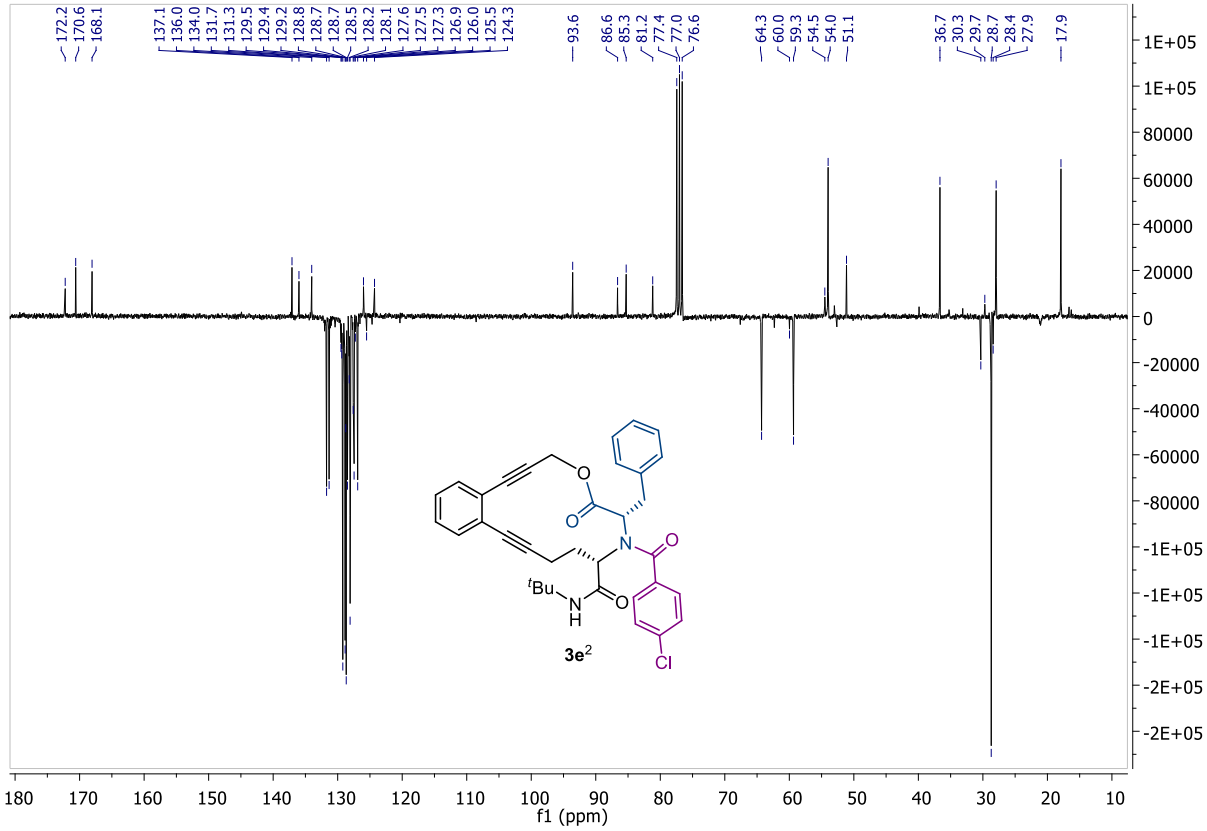

7.40
7.26
7.21
7.20
7.19
7.14
7.12
7.02
6.99
6.96
6.92
5.23
5.18
5.05
5.00
4.91
4.56
4.39
3.47
3.21
2.78
2.76
1.32
1.21
1.08
0.85
4.04
1.23
5.05
1.03
2.05
0.99
1.00
0.97
0.98
0.97
1.20
1.04
1.01
2.14
1.10
9.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)
3d2

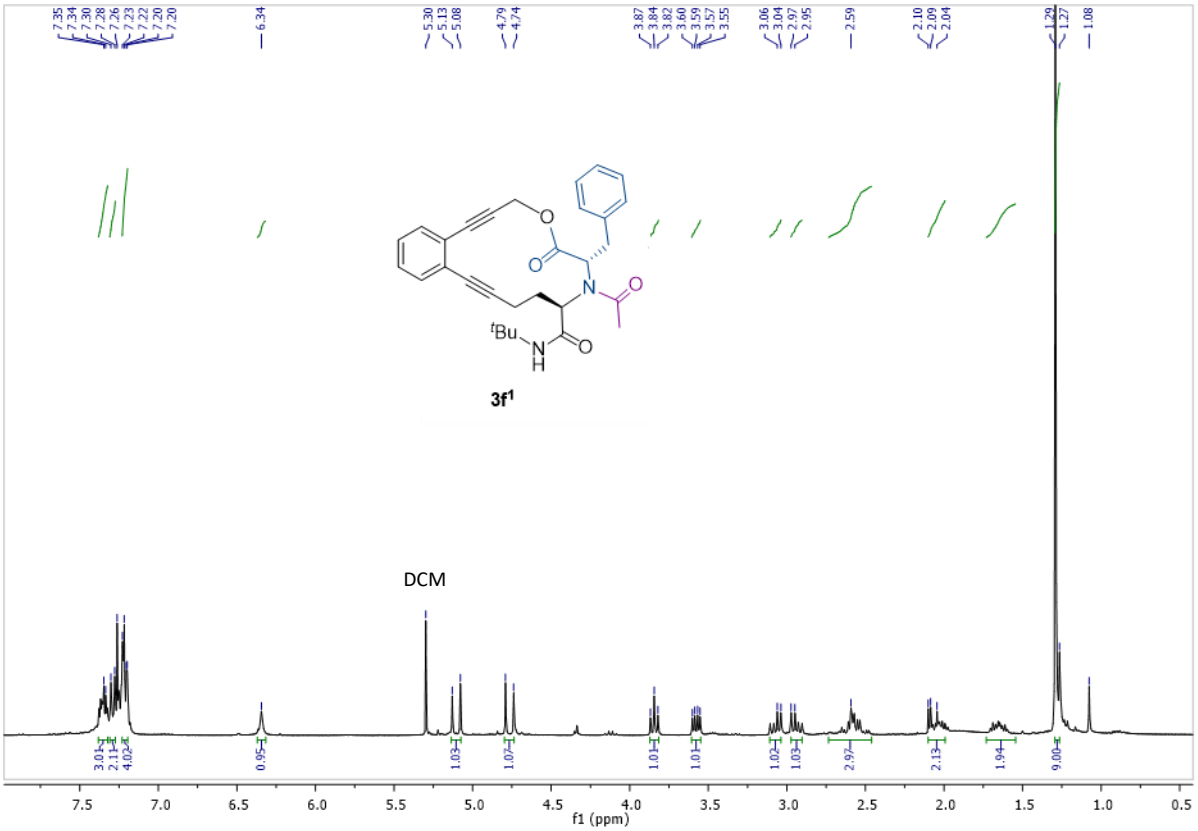

172.47
170.84
168.29
137.25
131.89
129.30
129.08
128.97
128.79
128.62
127.58
116.01
115.73
93.73
86.75
85.43
81.31
77.58
77.16
76.74
64.49
59.52
54.12
51.25
36.91
28.84
28.55
28.07
18.04
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)
3d2


7.98 7.96 7.95 7.60 7.55 7.53 7.49 7.46 7.44 7.39 7.36 7.34 7.33 7.31 7.31 7.30 7.30 7.24 7.21 7.20 7.19 7.17 7.15 7.12 7.12 6.97 6.95 6.78 6.76 5.23 5.12 5.07 4.89 4.84 4.59 4.58 4.56 4.55 3.78 3.77 3.74 3.73 3.43 3.39 3.39 3.24 3.20 3.19 3.15 2.90 2.80 2.74 2.44 2.39 2.37 2.35 1.56 1.37 1.34 1.28 1.19 1.13 1.10 1.08
DCM
H2O
0.92 0.70 0.72 0.77 2.32 5.32 1.89 1.66 1.10 1.10 1.00 1.09 1.07 1.09 2.27 2.09 8.80
f1 (ppm)
3e1

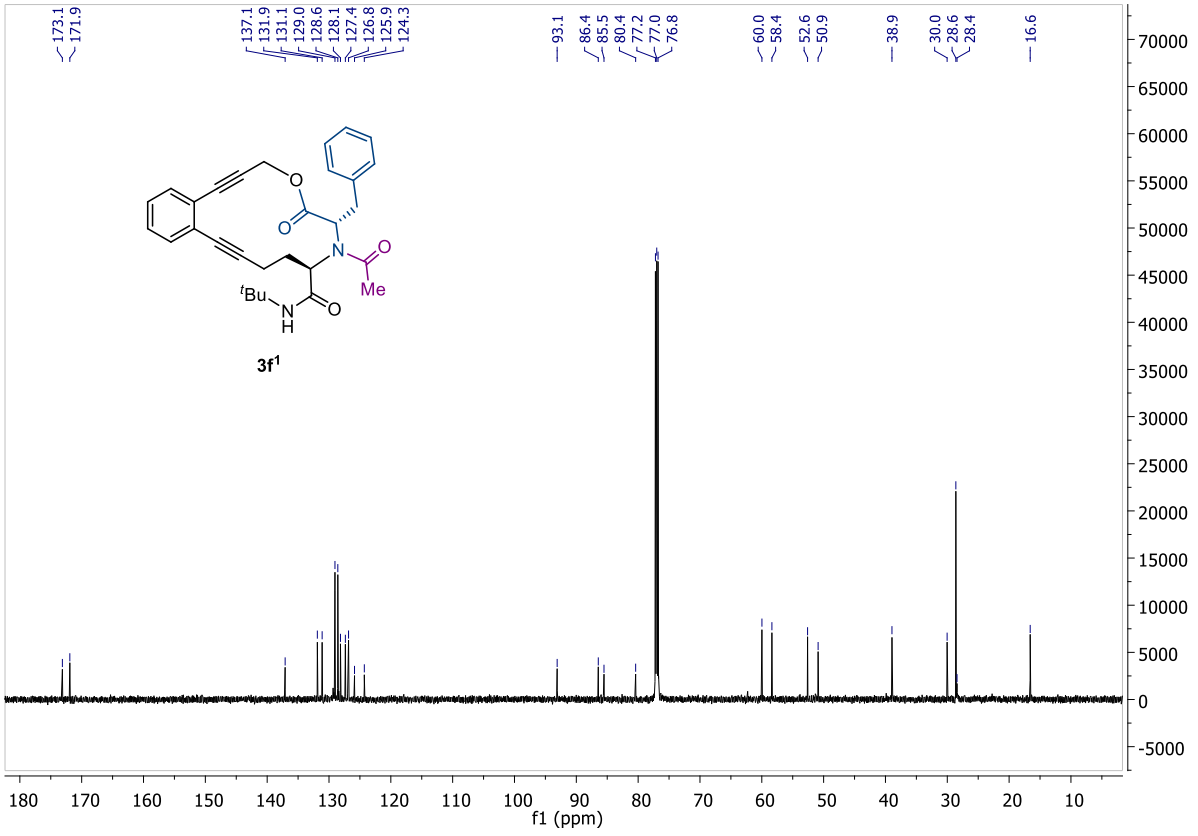

173.3 169.2 169.1 143.6 136.0 131.0 129.5 128.7 128.5 128.4 127.7 127.2 126.8 124.6 108.5 92.9 86.4 85.6 85.0 79.9 77.2 77.0 76.8 67.4 67.1 54.4 51.1 35.3 30.3 28.7 28.6 28.4 16.3
f1 (ppm)
3e1


DCM
acetone
3e2
f1 (ppm)

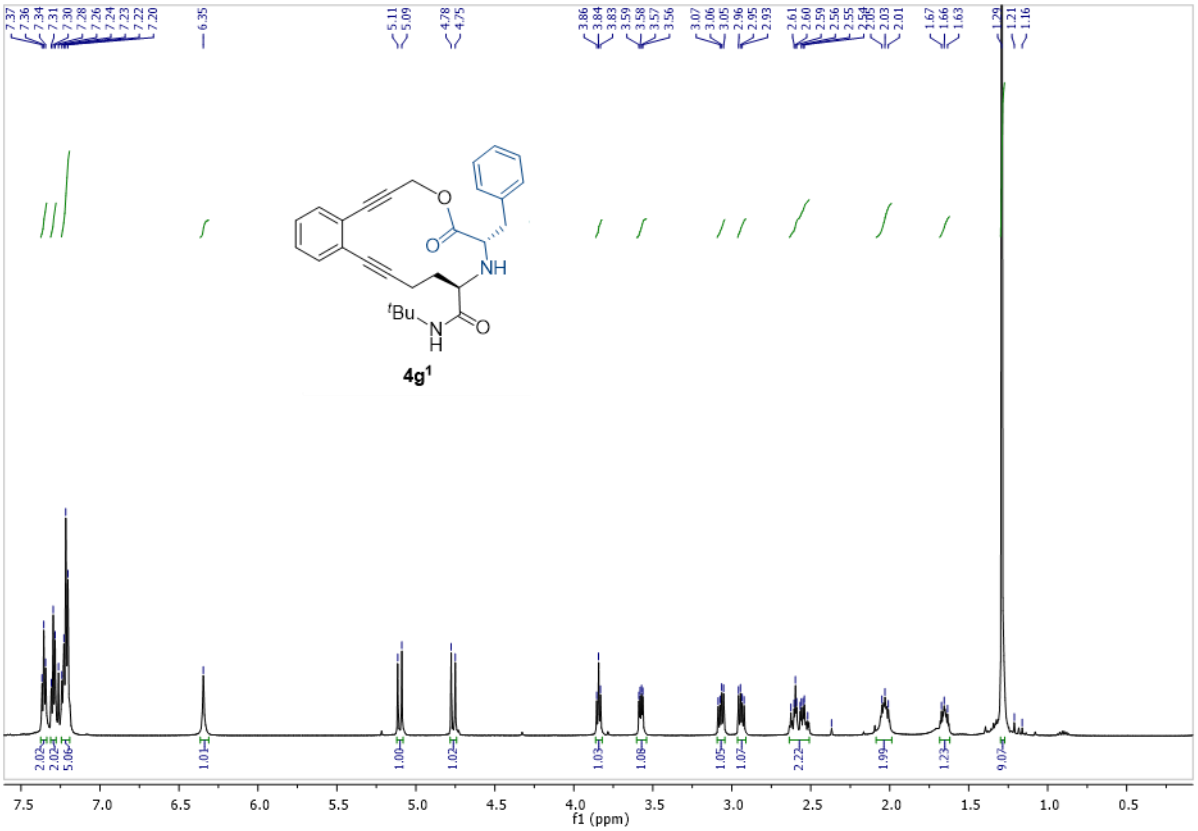

3e2
f1 (ppm)



3f[1]



3f[1]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7.36
7.34
7.31
7.30
7.28
7.26
7.24
7.23
7.22
7.20
6.35
5.11
5.09
4.78
4.75
3.86
3.84
3.83
3.59
3.58
3.57
3.56
3.07
3.06
3.05
2.96
2.95
2.93
2.61
2.60
2.59
2.56
2.55
2.05
2.03
2.01
1.67
1.66
1.63
1.29
1.21
1.16
O
O
NH
tBu
N
H
O
4g1
2.02
2.02
5.06
1.01
1.00
1.02
1.03
1.08
1.05
1.07
2.22
1.99
1.23
9.07
7.5
7.0
6.5
6.0
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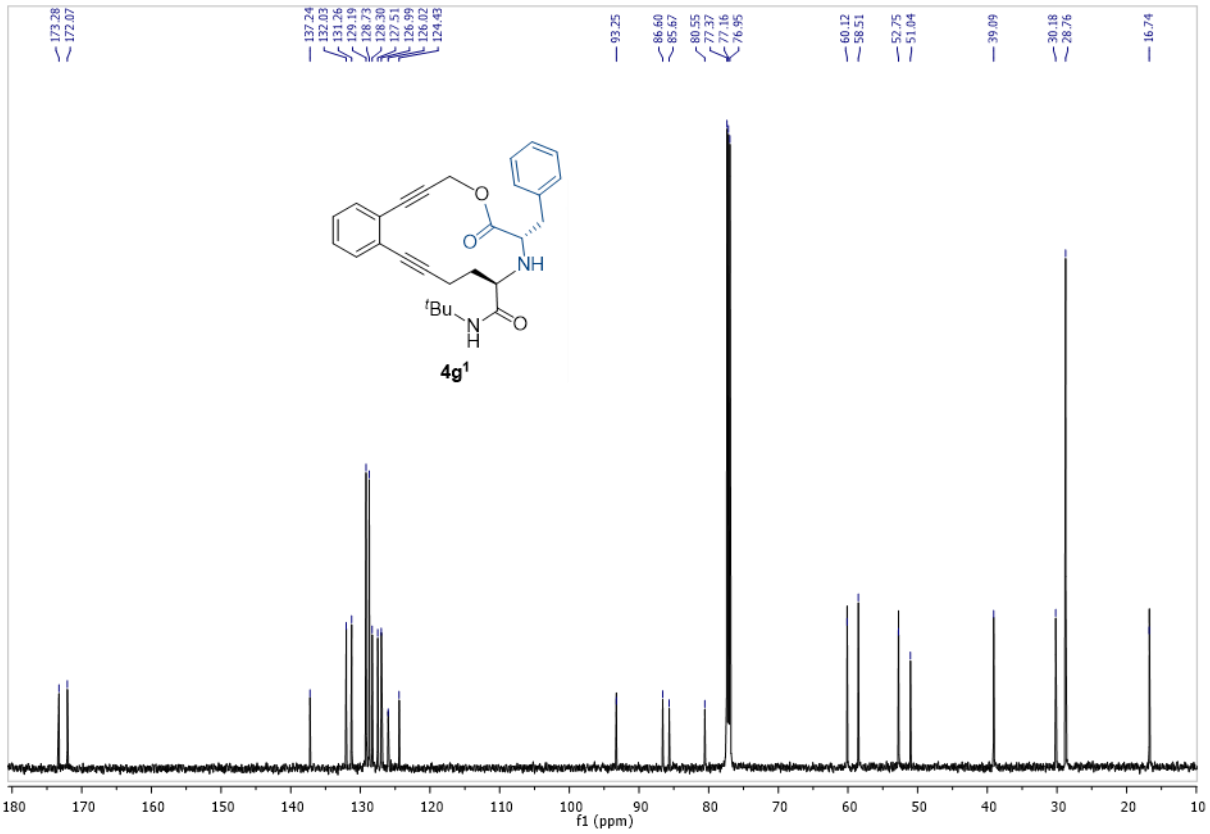
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)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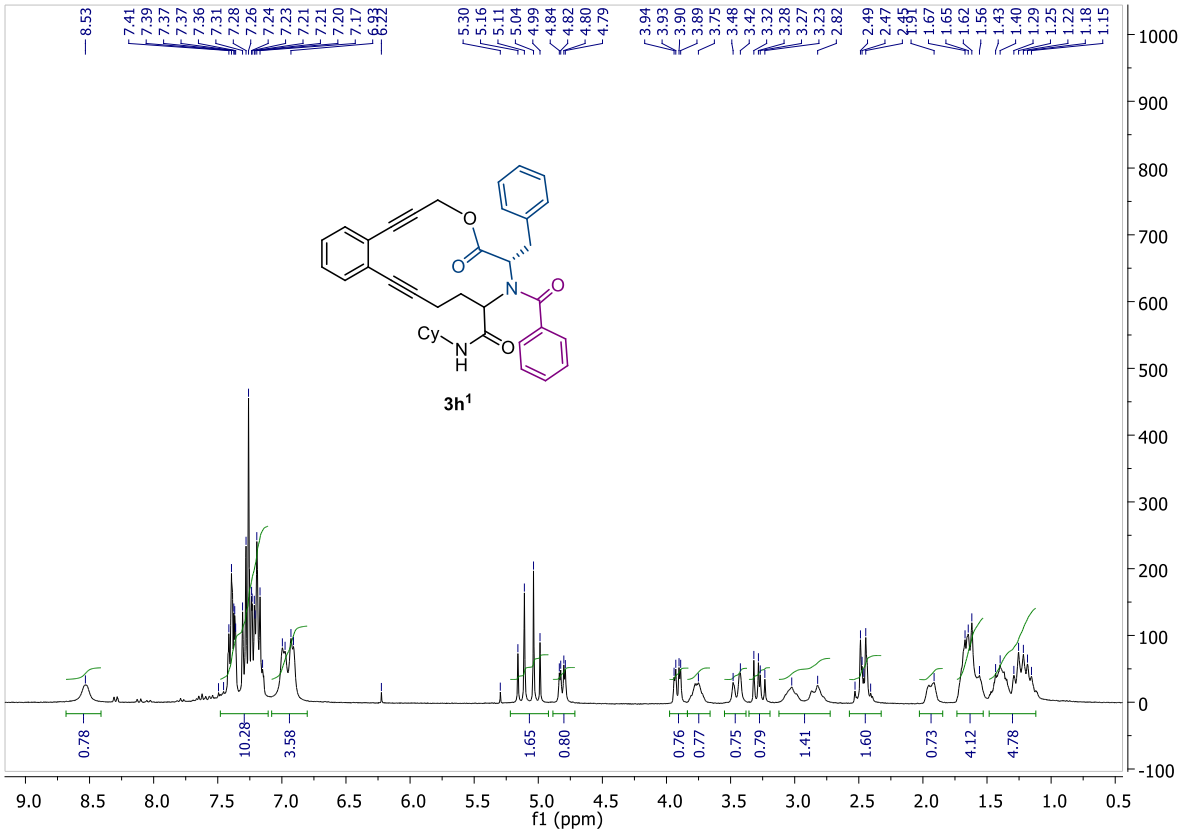
172.07
137.24
132.03
131.26
129.19
128.73
128.30
127.51
126.99
126.02
124.43
93.25
86.60
85.67
80.55
77.37
77.16
76.95
60.12
58.51
52.75
51.04
39.09
30.18
28.76
16.74
O
O
NH
tBu
N
H
O
4g1
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)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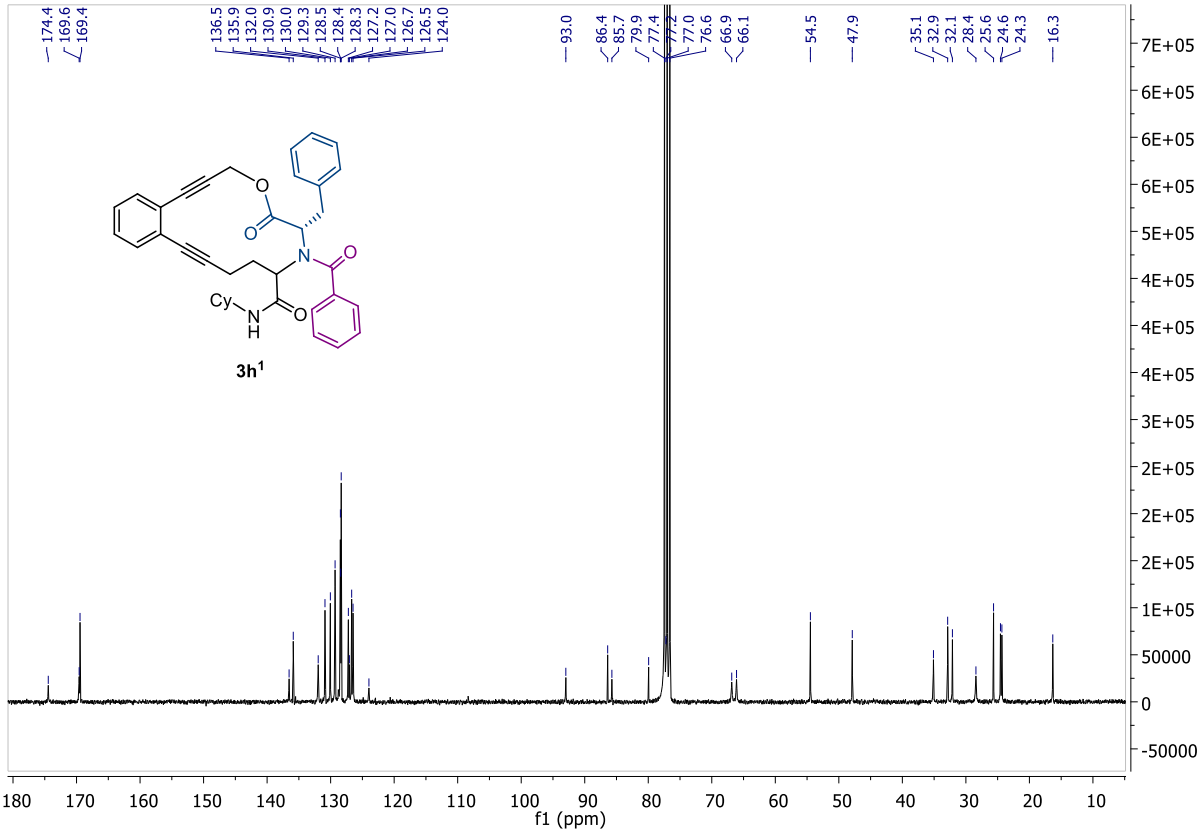
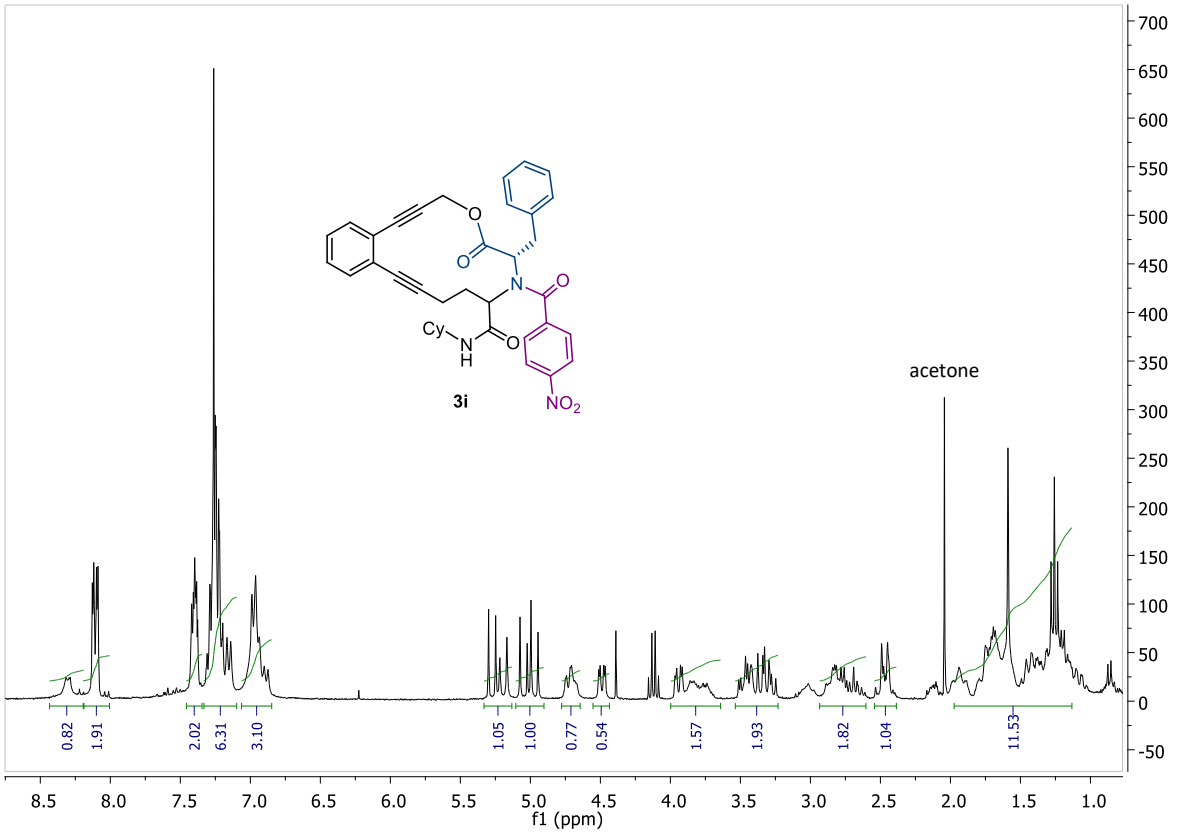
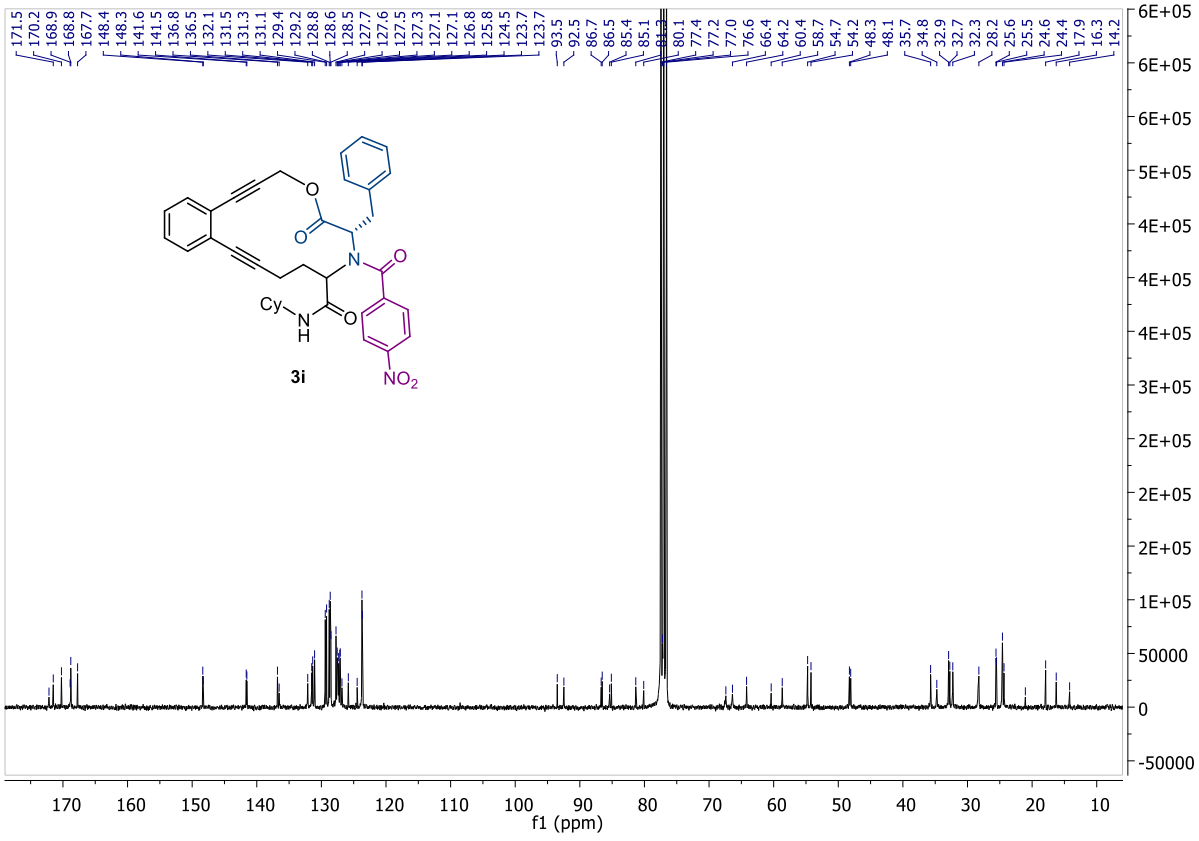
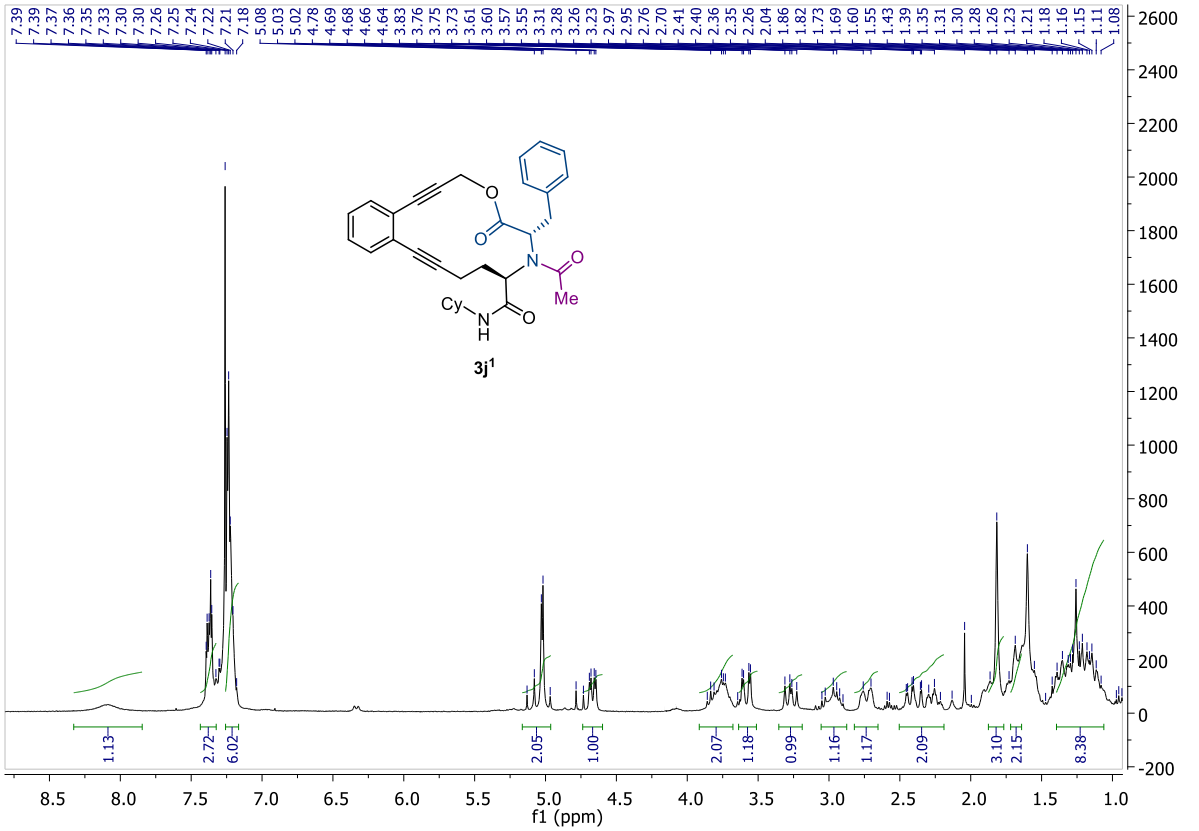
7.41
7.39
7.37
7.37
7.36
7.31
7.28
7.26
7.24
7.23
7.21
7.21
7.20
7.17
6.93
6.92
5.30
5.16
5.11
5.04
4.99
4.84
4.82
4.80
4.79
3.94
3.93
3.90
3.89
3.75
3.48
3.42
3.32
3.28
3.27
3.23
2.82
2.49
2.47
2.45
1.91
1.67
1.65
1.62
1.56
1.43
1.40
1.29
1.25
1.22
1.18
1.15
3h1
0.78
10.28
3.58
1.65
0.80
0.76
0.77
0.75
0.79
1.41
1.60
0.73
4.12
4.78
f1 (ppm)

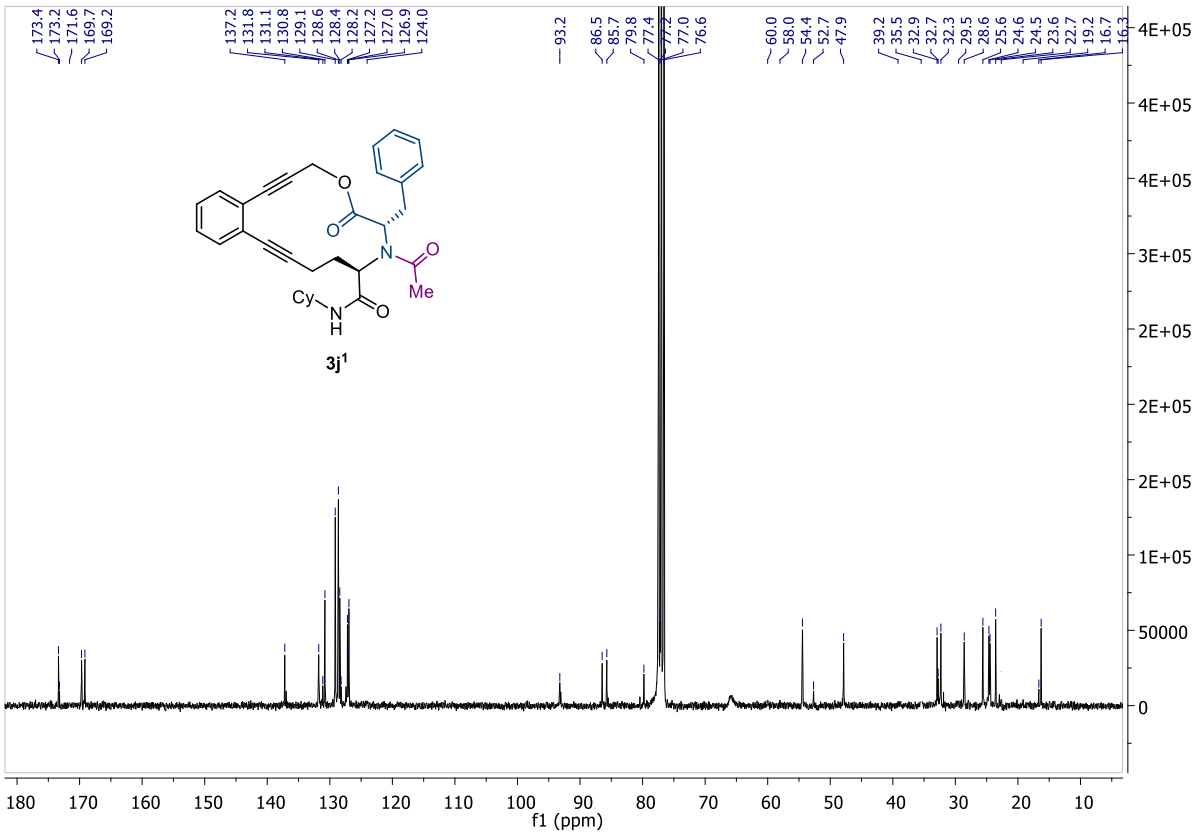

174.4
169.6
169.4
136.5
135.9
132.0
130.9
130.0
129.3
128.5
128.4
128.3
127.2
127.0
126.7
126.5
124.0
93.0
86.4
85.7
79.9
77.4
77.2
77.0
76.6
66.9
66.1
54.5
47.9
35.1
32.9
32.1
28.4
25.6
24.6
24.3
16.3
3h1
f1 (ppm)

Macrocycle **3i** is isolated as a mixture of diastereoisomers






7.39 7.39 7.37 7.36 7.35 7.33 7.30 7.30 7.26 7.25 7.24 7.22 7.21 7.18 5.08 5.03 5.02 4.78 4.69 4.68 4.66 4.64 3.83 3.76 3.75 3.73 3.61 3.60 3.57 3.55 3.31 3.28 3.26 3.23 2.97 2.95 2.76 2.70 2.41 2.40 2.36 2.35 2.26 2.04 1.86 1.82 1.73 1.69 1.60 1.55 1.43 1.39 1.35 1.31 1.30 1.28 1.26 1.23 1.21 1.18 1.16 1.15 1.11 1.08
O
O
N
O
Me
Cy
N
H
O
3j1
1.13 2.72 6.02 2.05 1.00 2.07 1.18 0.99 1.16 1.17 2.09 3.10 2.15 8.38
f1 (ppm)


173.4 173.2 171.6 169.7 169.2 137.2 131.8 131.1 130.8 129.1 128.6 128.4 128.2 127.2 127.0 126.9 124.0 93.2 86.5 85.7 79.8 77.4 77.2 77.0 76.6 60.0 58.0 54.4 52.7 47.9 39.2 35.5 32.9 32.7 32.3 29.5 28.6 25.6 24.6 24.5 23.6 22.7 19.2 16.7 16.3
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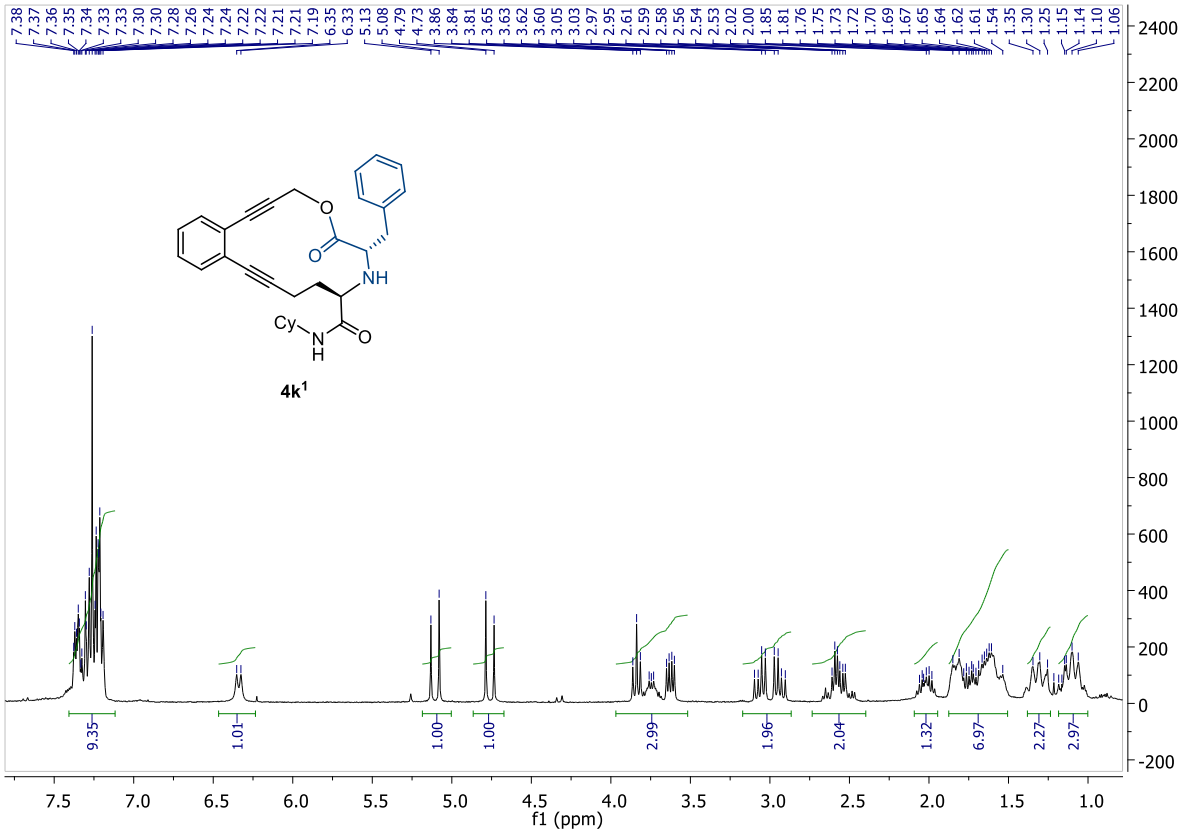
O
Me
Cy
N
H
O
3j1
f1 (ppm)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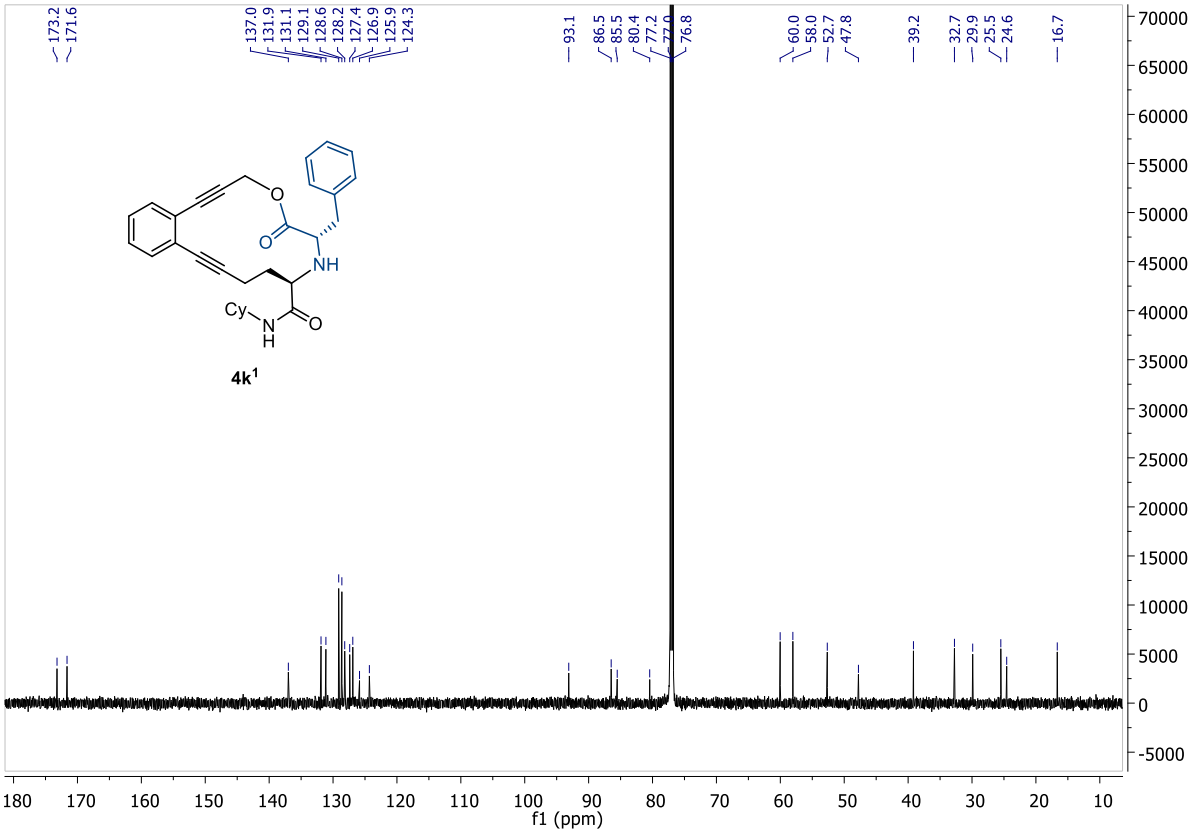
5.13 5.08 4.79 4.73 3.86 3.84 3.81 3.65 3.63 3.62 3.60 3.05 3.03 2.97 2.95 2.61 2.59 2.58 2.56 2.54 2.53 2.02 2.00 1.85 1.81 1.76 1.75 1.73 1.72 1.70 1.69 1.67 1.65 1.64 1.62 1.61 1.54 1.35 1.30 1.25 1.15 1.14 1.10 1.06
O
O
NH
Cy
N
H
O
4k1
9.35
1.01
1.00
1.00
2.99
1.96
2.04
1.32
6.97
2.27
2.97
f1 (ppm)


173.2 171.6
137.0 131.9 131.1 129.1 128.6 128.2 127.4 126.9 125.9 124.3
93.1 86.5 85.5 80.4 77.2 77.0 76.8
60.0 58.0 52.7 47.8
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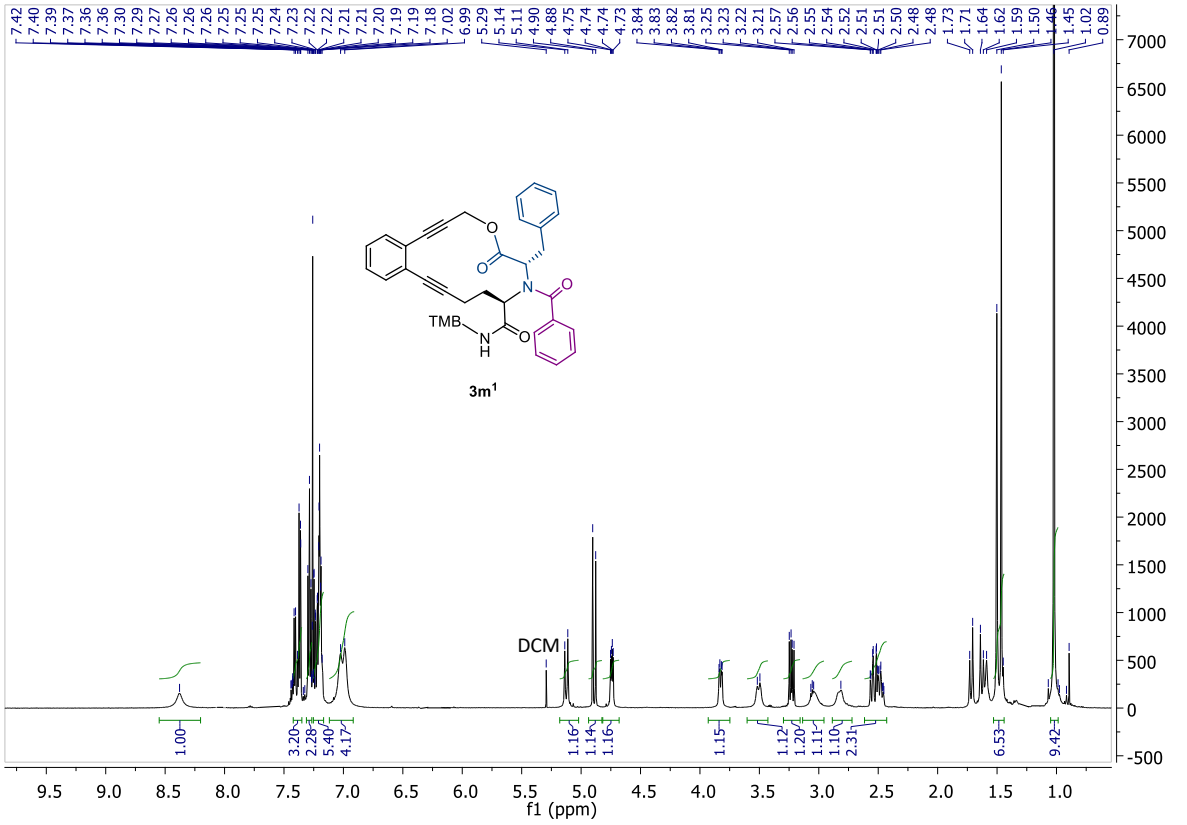
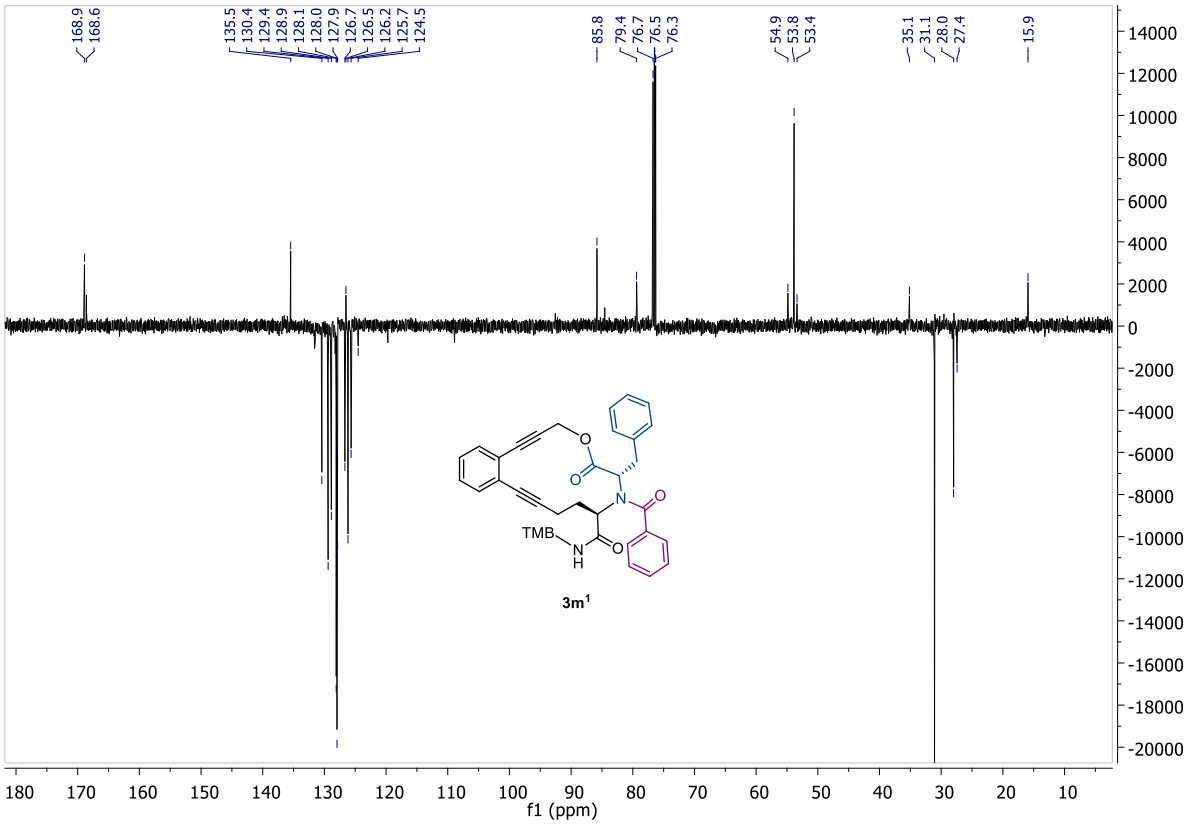
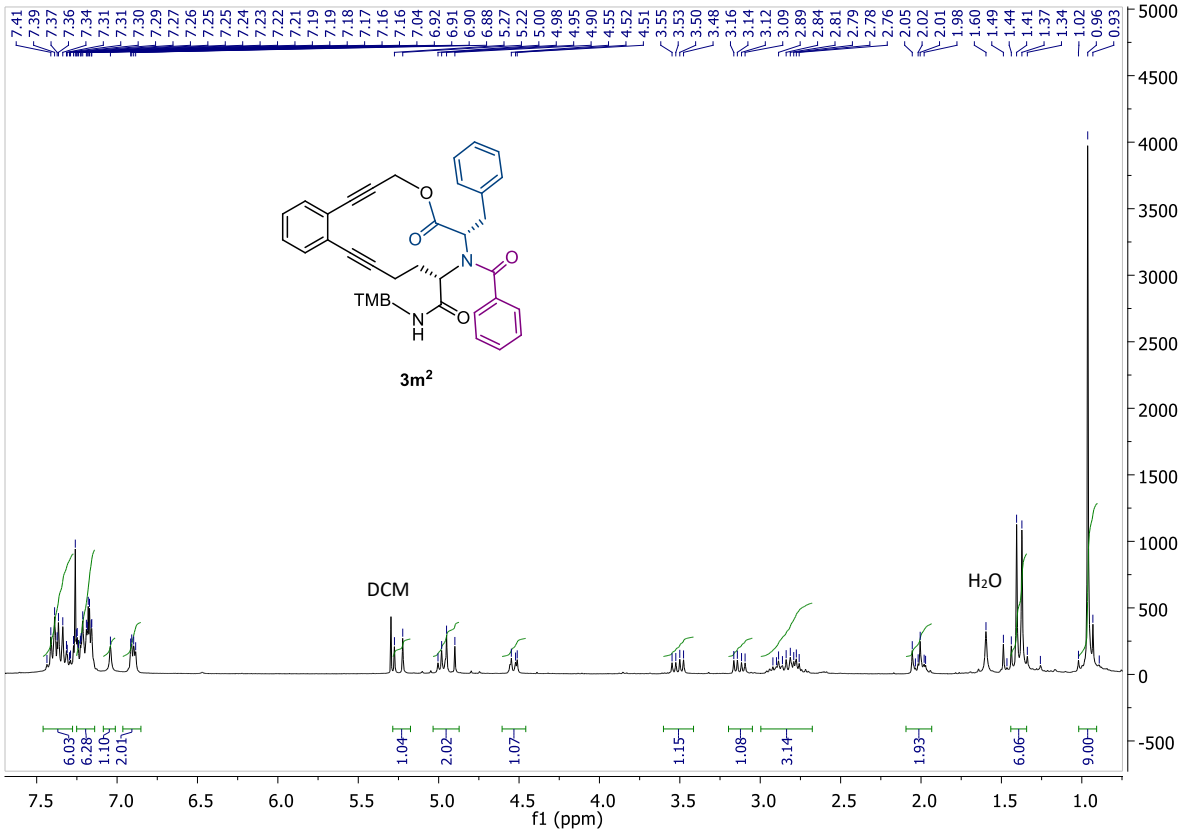
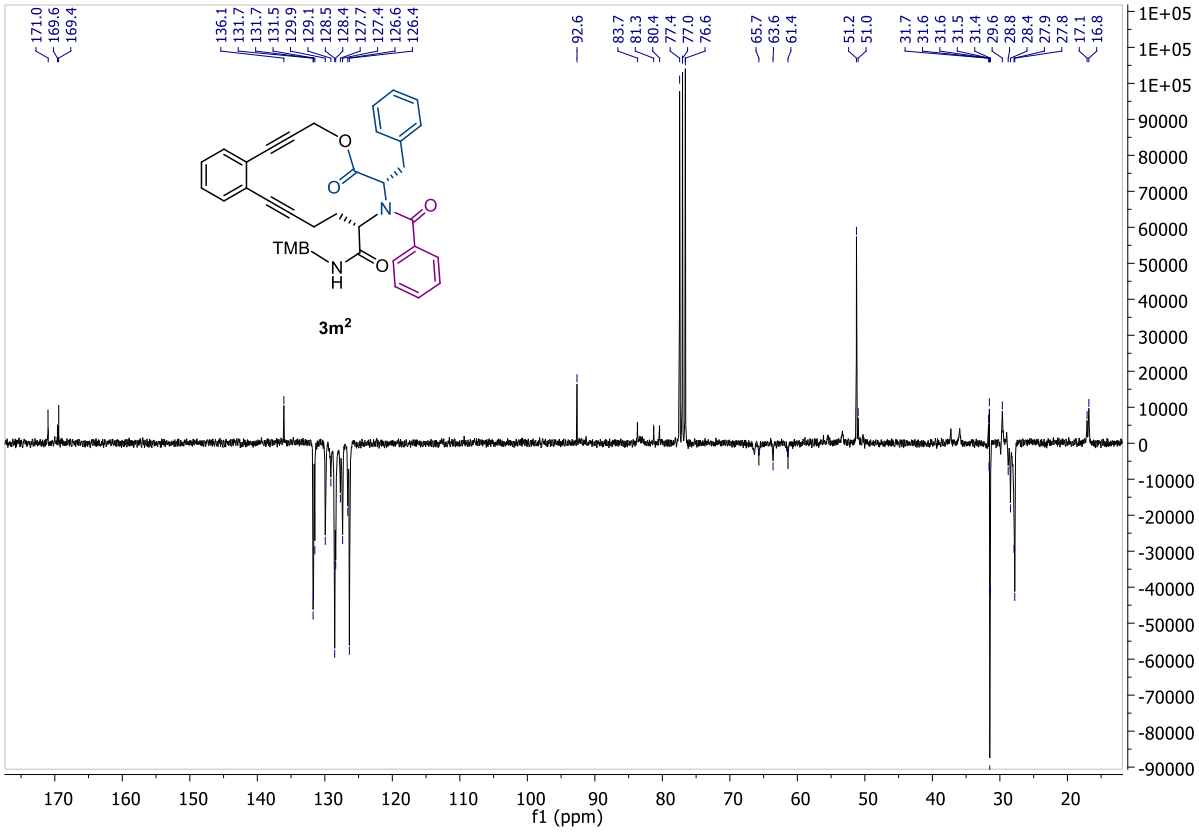
O
NH
Cy
N
H
O
4k1
f1 (ppm)

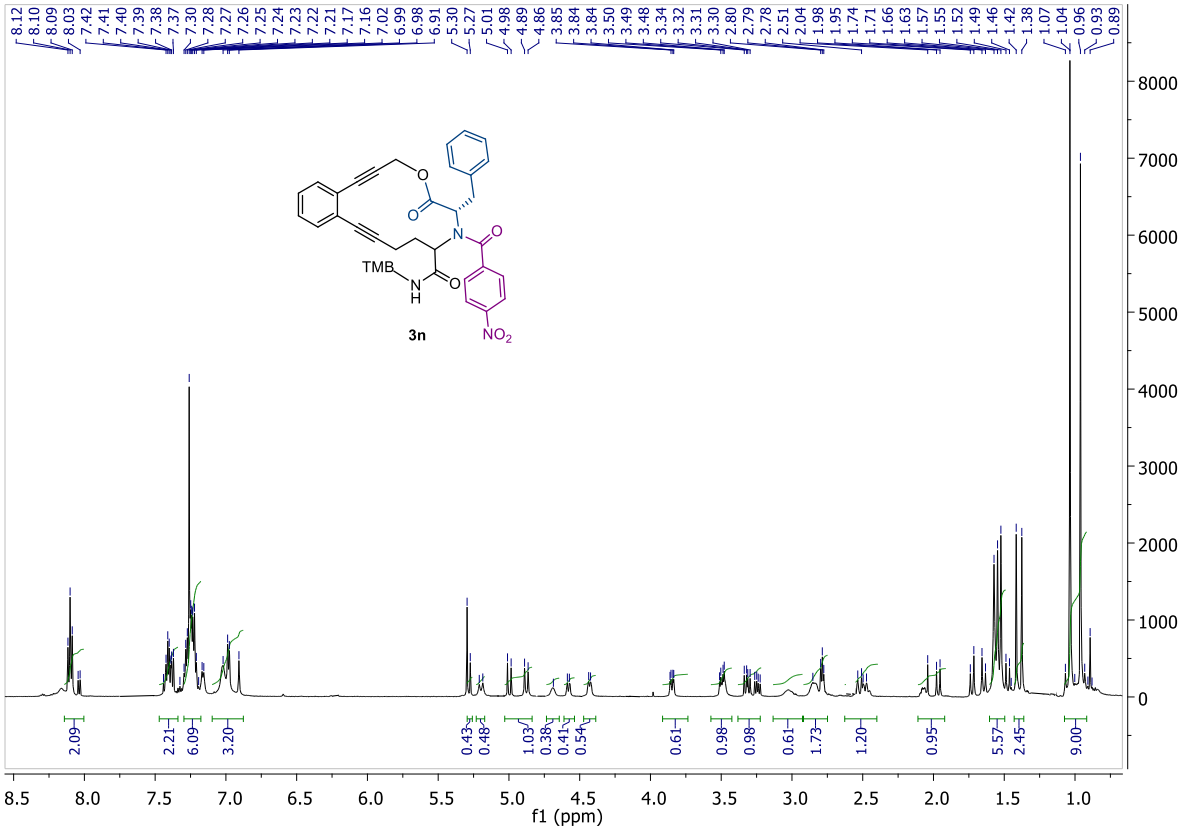

DCM
TMB
3m1
f1 (ppm)

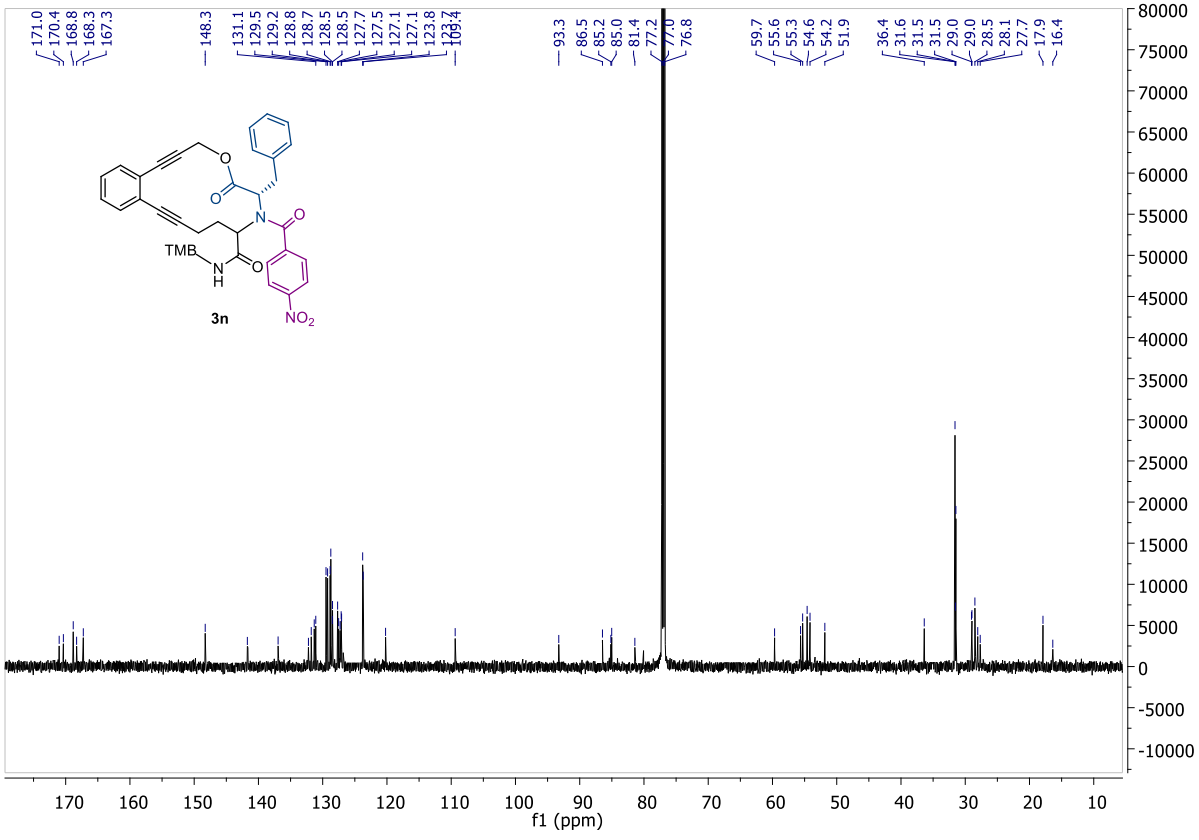

TMB
3m1
f1 (ppm)

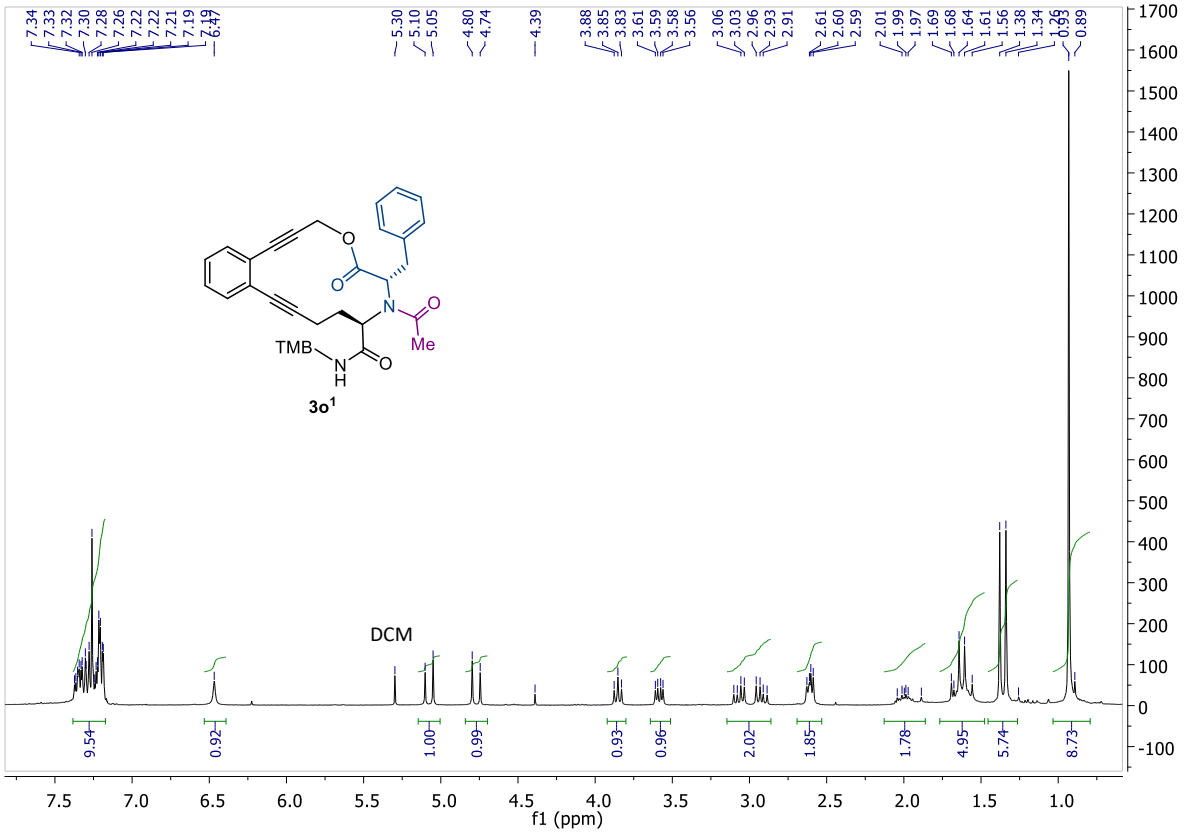

DCM
H2O
TMB
N
H
O
3m2
f1 (ppm)


TMB
N
H
O
3m2
f1 (ppm)

Macrocycle **3n** is isolated as a mixture of diastereoisomers



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6.47
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5.10
5.05
4.80
4.74
4.39
3.88
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3.59
3.58
3.56
3.06
3.03
2.96
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2.91
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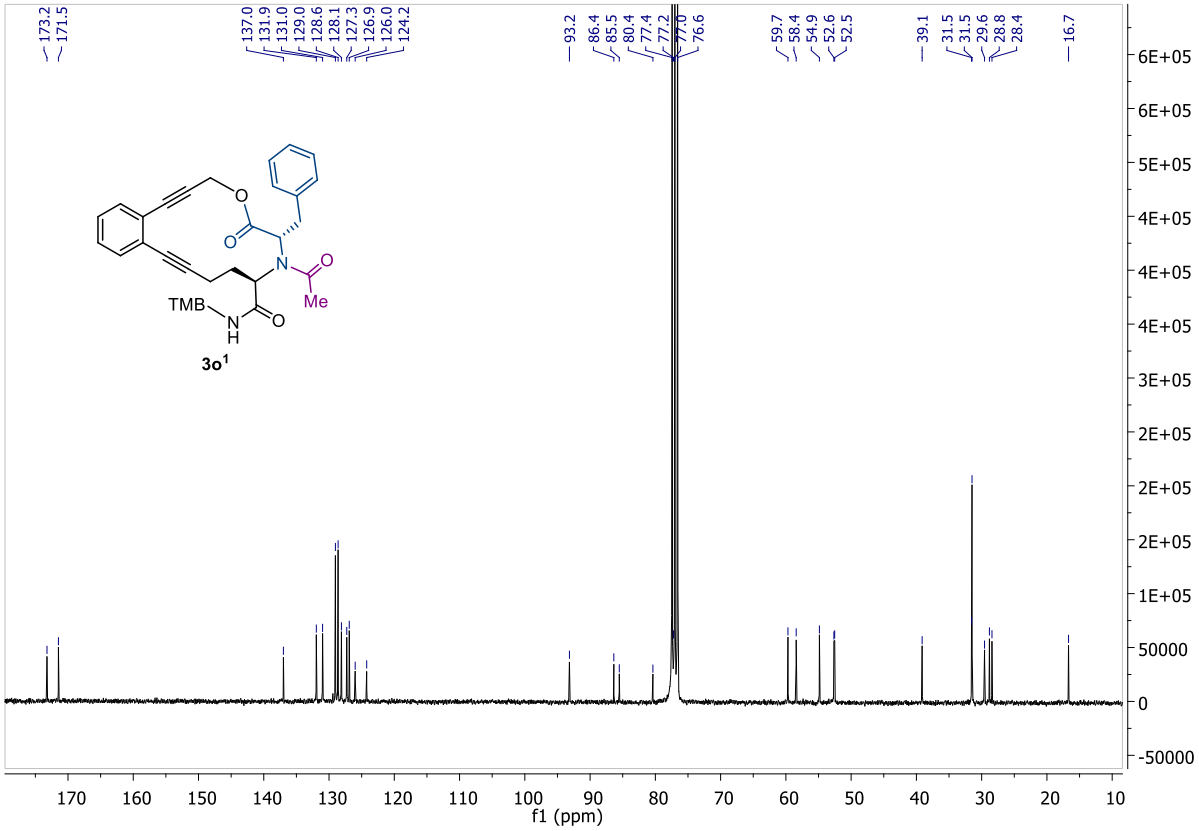
2.60
2.59
2.01
1.99
1.97
1.69
1.68
1.64
1.61
1.56
1.38
1.34
1.26
0.93
0.89
DCM
9.54
0.92
1.00
0.99
0.93
0.96
2.02
1.85
1.78
4.95
5.74
8.73
f1 (ppm)
TMB
Me
3o1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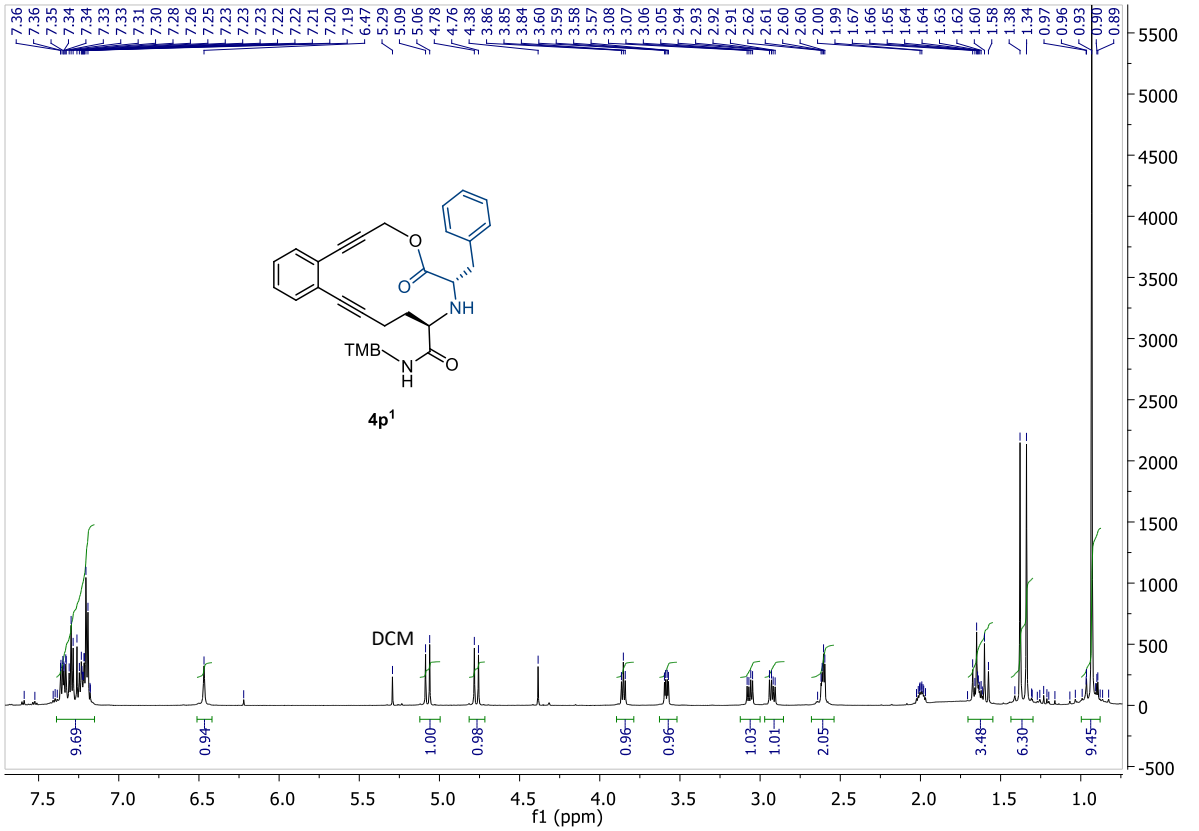
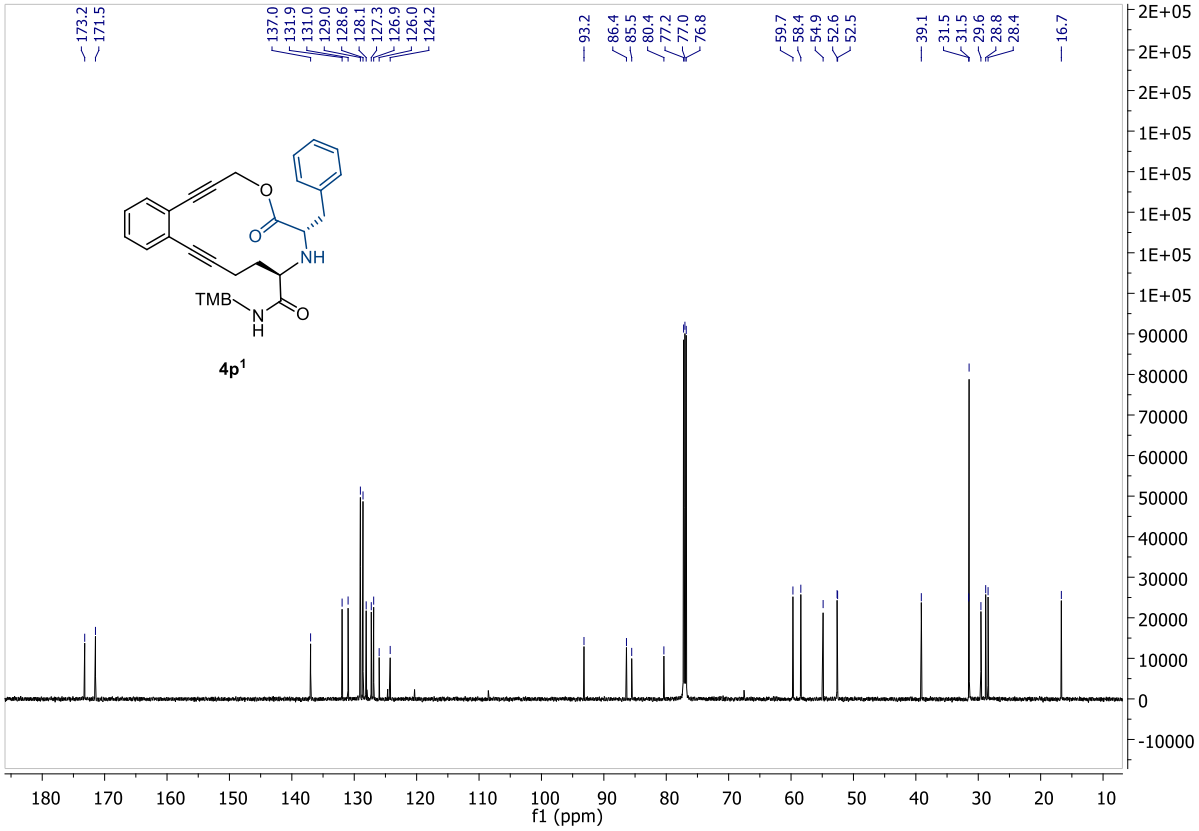
129.0
128.6
128.1
127.3
126.9
126.0
124.2
93.2
86.4
85.5
80.4
77.4
77.2
77.0
76.6
59.7
58.4
54.9
52.6
52.5
39.1
31.5
31.5
29.6
28.8
28.4
16.7
f1 (ppm)
TMB
Me
3o1


7.36 7.36 7.35 7.34 7.34 7.33 7.33 7.31 7.30 7.28 7.26 7.25 7.23 7.23 7.23 7.22 7.22 7.21 7.20 7.19 6.47 5.29 5.09 5.06 4.78 4.76 4.38 3.86 3.85 3.84 3.60 3.59 3.58 3.57 3.08 3.07 3.06 3.05 2.94 2.93 2.92 2.91 2.62 2.61 2.60 2.60 2.00 1.99 1.67 1.66 1.65 1.64 1.64 1.63 1.62 1.60 1.58 1.38 1.34 0.97 0.96 0.93 0.90 0.89
TMB
NH
O
N
H
4p1
DCM
9.69
0.94
1.00
0.98
0.96
0.96
1.03
1.01
2.05
3.48
6.30
9.45
f1 (ppm)

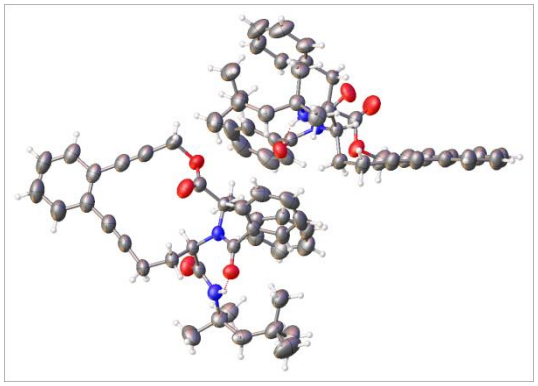

173.2 171.5 137.0 131.9 131.0 129.0 128.6 128.1 127.3 126.9 126.0 124.2 93.2 86.4 85.5 80.4 77.2 77.0 76.8 59.7 58.4 54.9 52.6 52.5 39.1 31.5 31.5 29.6 28.8 28.4 16.7
TMB
NH
O
N
H
4p1
f1 (ppm)

8. X-RAY DATA

Single crystals of compounds **3m**[1] and **4p**[1] were obtained by slow evaporation (at room temeprature) of solutions (3 mg dissolved in 200 µL ethyl acetate).

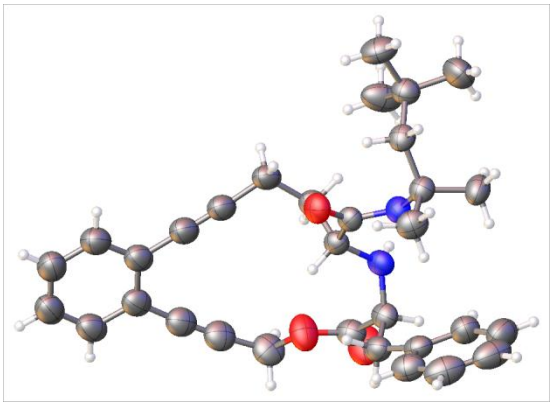
The data on single crystals of compounds **3m**[1] and **4p**[1] were collected on a Xcalibur Nova single crystal diffractometer equipped with Ruby CCD detector, using Cu Kα X-ray radiation with wavelength of λ=1.5412 Å. The crystals were kept at room temperature during data collection. Using Olex2,[2] the structures were solved with the ShelXT[3] structure solution program using Intrinsic Phasing and refined with the ShelXL refinement package using Least Squares minimisation.

Crystal structure determination of 3m[1]



Crystal Data for 3m[1]: chemical formula $C_{39}H_{42}N_2O_4$ (M=602.74 g/mol): triclinic, space group *P* 1, *a*= 11.4922(4) Å, *b* = 11.9793(4) Å, *c* = 12.3100(4) Å, *α* = 91.779(2)°, *β* = 96.053(3)°, *γ* = 91.867(3)°, V = 1683.34(10) Å^3, Z = 2, T = 293(2) K, µ(CuKα) = 0.605 mm^{-1}, D_{calc} = 1.189 g cm^{-3}, 33048 reflections measured (7.388° ≤ 2Θ ≤ 151.972°), 12807 unique (R_{int} = 0.0569, R_{sigma} = 0.0598) which were used in all calculations. The final R_1 was 0.0749 ($I > 2\sigma(I)$) and wR_2 was 0.2190 (all data). **Structure is deposited in the CCDC, Deposition Number 1967883**.

Crystal structure determination of 4p[1]



Crystal Data for 6q: chemical formula $C_{32}H_{38}N_2O3$ (M =498.64 g/mol): orthorhombic, space group *P* $2_1 2_1 2_1$ (no. 19), *a* = 5.8525(2) Å, *b* = 13.9095(4) Å, *c* = 34.5620(8) Å, V = 2813.53(14) Å^3, Z = 4, T = 293(2) K, µ(CuKα) = 0.591 mm^{-1}, Dcalc = 1.177 g cm^{-3}, 13625 reflections measured (6.85° ≤ 2Θ ≤ 151.968°), 5803 unique (R_{int} = 0.0404, R_{sigma} = 0.0503) which were used in all calculations. The final R_1 was 0.0468 ($I > 2\sigma(I)$) and wR_2 was 0.1166 (all data). **Structure is deposited in the CCDC, Deposition Number 1967885.**

[1] (a) Mossman, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Meth* **1983,** *65*, 55-63. (b) Boyd MR, Kenneth DP. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. *Drug Dev Res* **1995**, 34:91-109. (c) www.dtp.nci.nih.gov

[2] Dolomanov, O.V., Bourhis, L.J., Gildea, R.J, Howard, J.A.K. & Puschmann, H. (2009), J. Appl. Cryst. 42, 339-341.

[3] Sheldrick, G.M. (2015). Acta Cryst. A71, 3-8.