Azlactone-based heterobifunctional linkers with orthogonal clickable groups: Efficient tools for bioconjugation with complete atom economy

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Electronic Supporting Information

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1. Materials and methods

Unless otherwise stated, all chemicals were obtained from commercial sources and used as received. Column chromatography was carried out using silica gel 60 (0.040-0.063 mm) from Merck.

NMR spectra were recorded on an Advance DPX 200 or Bruker AC-400 spectrometer for ^{1}H and ^{13}C). NMR chemical Shift are reported in ppm downfield from tetramethylsilane and referenced to solvent resonances (7.26 and 77.16 ppm for ^{1}H and ^{13}C in $CDCl_3$, 2.05 and 29.84 ppm for ^{1}H and ^{13}C in $(CD_3)_2CO$, 2.50 and 39.52 ppm for ^{1}H and ^{13}C in $(CD_3)_2SO$), and 2.08 ppm for ^{1}H in toluene-d_8. The following abbreviations are used in reporting NMR data: s (singlet), d (doublet), t (triplet), q (quartet), qui (quintet), m (multiplet), br (broad). ^{1}H NMR signals are given followed by multiplicity, coupling constants *J* in Hertz and integration in parentheses.

FT-IR spectra were obtained on a Perkin Elmer Spectrum One spectrometer on a single-reflection diamond ATR unit.

High resolution mass spectra were recorded on a Waters Micromass GCT Premier spectrometer and Bruker MicroTOF QIII spectrometer.

Matrix-Assisted Laser Desorption and Ionization Time Of Flight (MALDI-TOF) mass spectrometry analysis was performed on a Bruker UltraFlex II MALDI-TOF instrument equipped with a nitrogen laser operating at 337 nm, a 2 GHz sampling rate digitizer, a pulsed ion extraction source and a reflectron. The laser pulse width is 3 ns and the maximum power is 200 mJ. Spectra were recorded in the linear mode with an acceleration voltage of 19 kV and a delay of 200 ns. 100 single shot acquisitions were summed to give the spectra and the data were analyzed using Bruker FlexAnalysis and Polytools softwares.

SDS-PAGE was performed with a Bio-Rad system electrophoresis using 4-15% polyacrylamide gels run at 200 V constant voltage for 40 min and 1X glycerine/SDS-buffer. Samples were dissolved in pure water (10 µL, $[lysozyme]_0 = 4\ mg.mL^{-1}$), mixed 10 µL Laemmli sample buffer (containing 5% of β-mercaptoethanol) and heated at 90°C for 5 min before loading. The gel was stained with Coomassie blue for 60 min.

2-(2-bromopropionylamino)-2-methyl-propanoic acid **1** and 2-(1-bromoethyl)-4,4-dimethyl-4H-oxazolin-5-one **2** were prepared according to the previously reported procedures.[1]

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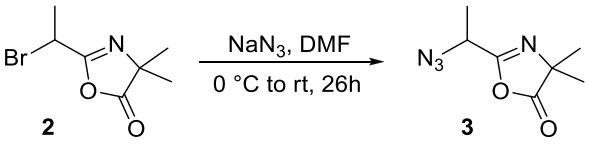
[1] Ho, H. T.; Leroux, F.; Pascual, S.; Montembault, V.; Fontaine, L. *Macromol. Rapid Commun.* **2012**, *33*, 1753–1758.

2. Synthesis of 2-(1-azidoethyl)-4,4-dimethyloxazol-5(4H)-one (3)



Procedure: To a suspension of sodium azide (1.3 g, 0.02 mol, 1 eq) in anhydrous DMF (15 mL) at 0 °C under argon was added dropwise a solution of 2-(1-bromoethyl)-4,4-dimethyloxazol-5(4*H*)-one **2** (4.4 g, 0.02 mol, 1 eq) in dry DMF (4 mL). Then the reaction mixture was allowed to warm up to room temperature and stirred for 16 h. After concentration under reduced pressure the residue was dissolved in EtOAc, filtrated and the solvent was removed under *vacuum* to afford a yellow oil (3.41 g, 86%).

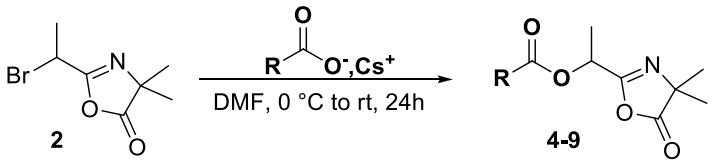
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.26 (q, *J* = 7.0 Hz, 1H, CH), 1.54 (d, *J* = 7.0 Hz, 3H, CH_3), 1.44 (s, 6H, $2CH_3$). (**Figure 1**)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 180.2 $(C=O)_{az}$, 161.6 $(C=N)_{az}$, 65.7 (C), 53.8 (CH), 24.6 (CH_3), 24.5 (CH_3), 16.4 (CH_3). (**Figure 2**)

FT-IR: υ (cm^{-1}) 2100, 1822, 1675.

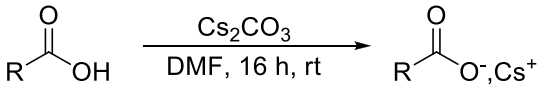
HRMS (TOF MS CI$^+$): $[M+H]^+$: $C_7H_{11}N_4O_2$, calculated: 183.0882, experimental: 183.0888.

3. General procedure for the synthesis of azlactones 4-9



General procedure: To an ice-cooled solution of cesium salt (1.1 eq) in dry DMF (2 mL per mmol) was added dropwise a solution of 2-(1-bromoethyl)-4,4-dimethyl-4H-oxazolin-5-one **2** (1 eq) in anhydrous DMF (1 mL per mmol). Then the solution was stirred at rt during 24h under argon. The solvent was removed under reduced pressure and the residue was filtrated on a short path of silica gel.

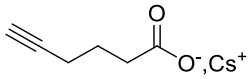
3.1. General procedure for the synthesis of cesium carboxylates



General procedure: To a solution of carboxylic acid (2 eq) in dry DMF (0.75 mL/mmol) was added slowly cesium carbonate (1 eq) at room temperature. The reaction mixture was stirred

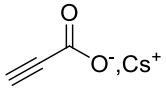
under argon for 16 hours. After filtration, the solvent was evaporated under reduced pressure and Et_2O was added to the residue. The cesium salt was filtrated, dried at 40 °C under vacuum and was used in the next step without further purification.

3.1.1. Cesium salt of hex-5-ynoic acid



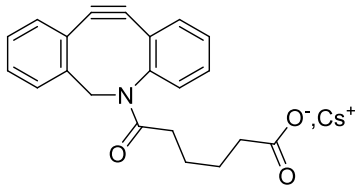
Following the general procedure hex-5-ynoic acid (2.8 g, 25 mmol, 2 eq) and cesium carbonate (4.1 g, 12.5 mmol, 1 eq) were used. Cesium hex-5-ynoate was obtained as a brown beige powder (6.1 g, 80%).

3.1.2. Cesium salt of propiolic acid



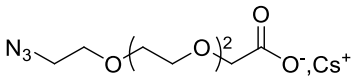
Following the general procedure propiolic acid (2.31 g, 33 mmol, 2 eq) and cesium carbonate (3.26 g, 10 mol, 1 eq) were used. Cesium propiolate was obtained as a brown solid (2.87 g, 71%).

3.1.3. Cesium salt of DBCO-acid



Following the general procedure DBCO-acid (100 mg, 0.3 mmol, 2 eq) and cesium carbonate (49 mg, 0.15 mmol, 1 eq) were used. The cesium salt was obtained as a brown solid (133 mg, 95%).

3.1.4. Cesium 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate



Following the general procedure 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetic acid (1.75 g, 7.5 mmol, 2 eq)[2] and cesium carbonate (1.22 g , 3.75 mmol, 1 eq) were used. Cesium 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate was obtained as a brown oil (1.90 g, 69%).

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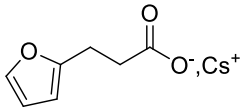
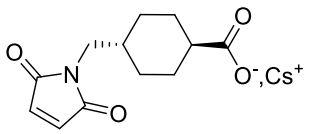
[2] a) 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol was prepared according to the following procedure: Khiar, N.; Leal, M. P.; Baati, R.; Ruhlmann, C.; Mioskowski, C.; Schultz, P.; Fernandez, I. *Chem Commun* **2009**, 27, 4121–

3.1.5. Cesium 3-(furan-2-yl)propanoate



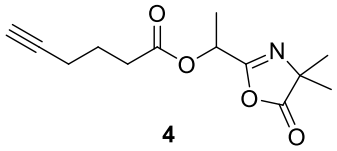
Following the general procedure 3-(2-Furyl)propionic acid (615 mg, 4.4 mmol, 2 eq) and cesium carbonate (716 mg, 2.2 mmol, 1 eq) were used. Cesium 3-(2-furyl)propanoate was obtained as a brown solid (702 mg, 59%).

3.1.6. Cesium (1R,4R)-4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)-cyclohexanecarboxylate



Following the general procedure (1*R*, 4*R*)-4-(aminomethyl)cyclohexanecarboxylic acid (2.88 g, 12 mmol, 2 eq) and cesium carbonate (1.97 g, 6 mmol, 1 eq). Cesium (1*R*, 4*R*)-4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)cyclohexanecarboxylate was obtained as a pink powder (3.38 g, 76%).

3.2. 1-(4,4-Dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl hex-5-ynoate (4)



4

Following the general procedure cesium salt of hex-5-ynoic acid (1.21 g, 5 mmol, 1.1 eq) and 2-(1-bromoethyl)-4,4-dimethyl-4H-oxazolin-5-one **2** (0.99 g, 4.5 mmol, 1 eq) were used. Azlactone **4** was obtained as colourless oil (0.844 g, 74%) after silica gel filtration (Et_2O).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.53 (q, 1H, J = 6.8 Hz, CH), 2.54 (dt, 2H, J_t = 7.2 Hz, J_d = 1.5 Hz, CH_2), 2.28 (dt, 2H, J_t = 7.2 Hz, J_d = 2.6 Hz, CH_2), 1.97 (t, 1H, J = 2.7 Hz, HC≡), 1.87 (qui, 2H, J = 7.2 Hz, CH_2), 1.56 (d, 3H, J = 6.8 Hz, CH_3), 1.43 (s, 3H, CH_3), 1.42 (s, 3H, CH_3) (**Figure 3**).

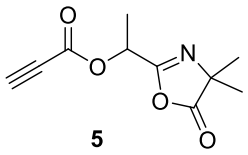
4123. b) 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetic acid was prepared according to the following procedure : Liao, L.; Liu, J.; Dreaden, E. C.; Morton, S. W.; Shopsowitz, K. E.; Hammond, P. T.; Johnson, J. A. *J. Am. Chem. Soc.* **2014**, *136*, 5896–5899.

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 180.5 $(C=O)_{az}$, 172.1 $(C=N)_{az}$, 161.9 (C=O), 83.2 (C≡), 69.4 (CH), 65.6 (HC≡), 65.1 (C), 32.7 (CH_2), 24.5 ($2CH_3$), 23.6 (CH_2), 17.9 (CH_2), 17.1 (CH_3) (**Figure 4**).

FT-IR: υ (cm^{-1}) 3292, 1825, 1744, 1679.

HRMS (TOF MS CI$^+$): $[M+H]^+$: $C_{13}H_{18}NO_4$, calculated: 252.1236, experimental: 252.1238

3.3. 1-(4,4-Dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl propiolate (5)



5

Following the general procedure cesium propiolate (0.55 g, 2.75 mmol, 1.1 eq) and 2-(1-bromoethyl)-4,4-dimethyloxazol-5(4*H*)-one **2** (0.55 g, 2.5 mol, 1 eq) were used. 1-(4,4-dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl propiolate **5** was obtained as a brown oil (366 mg, 70%) after silica gel filtration (Et_2O).

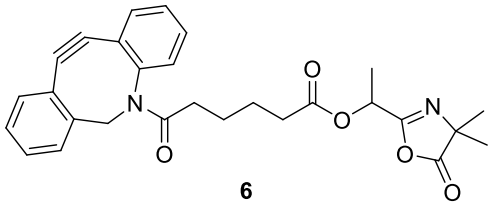
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.60 (q, *J* = 6.8 Hz, 1H, CH), 3.00 (s, 1H, ≡CH), 1.62 (d, *J* = 6.8 Hz, 3H, CH_3), 1.44 (s, 3H, CH_3), 1.43 (s, 3H, CH_3) (**Figure 5**).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 180.2 $(C=O)_{az}$, 160.7 $(C=N)_{az}$, 151.4 (C=O), 76.7 (≡CH), 74.0 (C≡), 66.9 (CH), 65.7 (C), 24.4 ($2CH_3$), 16.9 (CH_3) (**Figure 6**).

FT-IR: υ (cm^{-1}) 3445, 2112, 1826, 1724, 1673.

HRMS (TOF MS CI$^+$): $[M+H]^+$: $C_{10}H_{12}NO_4$, calculated: 210.0764, experimental: 210.0766.

3.4. DBCO-derived azlactone (Azlactone-DBCO, 6)



6

Following the general procedure cesium salt of DBCO-acid (0.133 g, 0.3 mmol, 1.1 eq) and 2-(1-bromoethyl)-4,4-dimethyloxazol-5(4*H*)-one **2** (0.060 g, 0.27 mmol, 1 eq) were used. Azlactone **6** was obtained as a yellow viscous oil (87 mg, 68%) after column chromatography (SiO_2, pentane/EtOAc: 1/1 (v/v), Rf = 0.45).

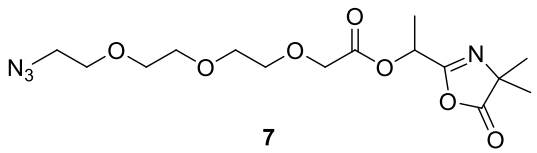
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.67 (d, 1H, *J* = 7.5 Hz, Harom), 7.42 - 7.21 (m, 7H, Harom), 5.44 (dq, 1H, *J* = 6.8 Hz, *J* = 2.0 Hz, CH), 5.14 (d, 1H, *J* = 13.8 Hz, CH_2), 3.63 (d, 1H, *J* = 13.8 Hz, CH_2), 2.27 - 2.14 (m, 3H, CH_2), 1.94 - 1.83 (m, 1H, CH_2), 1.51 - 1.33 (m, 13H, $3CH_3$ and $2CH_2$) (**Figure 7**).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 180.6 $(C=O)_{az}$, 173.0 (C=O), 172.3 (C=O), 162.0 $(C=N)_{az}$, 152.0 (Carom), 148.3 (Carom), 132.5 (CHarom), 129.5 (CHarom), 128.5 (CHarom), 128.5 (CHarom), 128.2 (CHarom), 127.9 (CHarom), 127.3 (CHarom), 125.6 (CHarom), 123.2 (Carom), 122.8 (Carom), 115.3 (C≡), 108.0 (C≡), 65.6 (CH), 64.9 (C), 55.5 (CH_2), 34.5 (CH_2), 33.7 (CH_2), 24.8 (CH_2), 24.5 ($2CH_3$), 24.2 (CH_2), 17.2 (CH_3) (**Figure 8**).

FT-IR: υ (cm^{-1}) 3030, 2868, 1823, 1748, 1660, 1480, 1453, 1364, 1233, 1210, 1053, 752, 698.

HRMS (Q-TOF ESI$^+$): $[M+H]^+$: $C_{28}H_{29}N_2O_5$, calculated: 473.2071, experimental: 473.2081.

3.5. 1-(4,4-Dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (7)



7

Following the general procedure cesium 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (286 mg, 0.78 mmol, 1 eq) and 2-(1-bromoethyl)-4,4-dimethyloxazol-5(4*H*)-one **2** (172 mg, 0.78 mmol, 1 eq) were used. Azlactone **7** was obtained as brown oil (183 mg, 63%) after silica gel filtration (EtOAc).

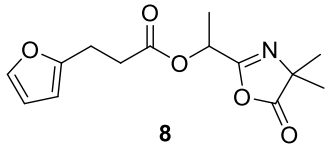
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 5.60 (q, 1H, *J* = 6.8 Hz, CH), 4.30 - 4.18 (m, 2H, CH_2), 3.77 - 3.73 (m, 2H, CH_2), 3.72 - 3.64 (m, 8H, $4CH_2$), 3.41 - 3.36 (m, 2H, CH_2) 1.58 (d, 3H, *J* = 6.8 Hz, CH_3), 1.44 (s, 3H, CH_3), 1.43 (s, 3H, CH_3) (**Figure 9**).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 180.4 $(C=O)_{az}$, 169.5 (C=O), 161.5 $(C=N)_{az}$, 71.1 (CH), 70.8 ($2CH_2$), 70.8 ($2CH_2$), 70.2 (CH_2), 68.5 (CH_2), 65.6 (C), 65.5 (CH_2), 50.8 (CH_2), 24.5 (CH_3), 24.5 (CH_3), 17.1 (CH_3) (**Figure 10**).

FT-IR: υ (cm^{-1}) 2870, 2101, 1823, 1762, 1681, 1119.

HRMS (TOF MS CI$^+$): $[M+H]^+$: $C_{15}H_{25}N_4O_7$, calculated: 373.1723, experimental: 373.1722.

3.6. 1-(4,4-Dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl 3-(furan-2-yl) propanoate (8)



8

Following the general procedure cesium salt of 3-(2-furyl)propionic acid (700 mg, 2.6 mmol, 1.1 eq) and 2-(1-bromoethyl)-4,4-dimethyl-4H-oxazolin-5-one (515 mg , 2.3 mmol, 1 eq) were used. Azlactone **8** was obtained as a brown solid (583 mg, 89%) after silica gel filtration (EtO_2).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.31 - 7.29 (m, 1H, CH), 6.29 - 6.25 (m, 1H, CH), 6.05 - 6.02 (m, 1H, CH), 5.55 (q, 1H, J = 6.8 Hz, CH), 3.00 (t, 2H, J = 7.5 Hz, H2), 2.81 - 2.69 (m, 2H, CH_2), 1.55 (d, 3H, J = 6.8 Hz, CH_3), 1.43 (s, 3H, CH_3), 1.42 (s, 3H, CH_3) (**Figure 11**).

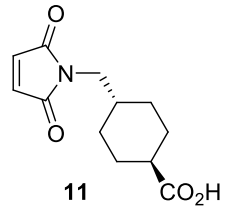
^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 180.6 $(C=O)_{az}$, 171.5 (C=O), 161.8 $(C=N)_{az}$, 153.8, 141.5 (CH), 110.3 (CH), 105.6 (CH), 65.6 (C), 65.3 (CH), 32.6 (CH_2), 24.5 ($2CH_3$), 23.4 (CH_2), 17.1 (CH_3) (**Figure 12**).

FT-IR: υ (cm^{-1}) 2984, 2937, 1824, 1746, 1681, 1202, 1151.

HRMS (TOF MS CI$^+$): $[M+H]^+$: $C_{14}H_{18}NO_4$, calculated: 280.1185, experimental: 280.1176.

3.7. (1*R*,4*R*)-1-(4,4-Dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl 4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)cyclohexanecarboxylate (9)

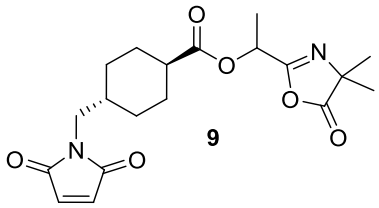
Firstly, (1*R*,4*R*)-4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)cyclohexane carboxylic acid **11** was obtained following the procedure described in the literature.[3] The spectral data were in agreement with literature values.



11

^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ (ppm) 12.05 (bs, 1H, CO_2H), 7.01 (s, 2H, HC=), 3.25 (d, 2H, J = 6.8 Hz, CH_2), 2.11 (tt, 1H, J = 11.7 Hz, J = 3.5 Hz, CH), 1.96 - 1.77 (m, 2H, CH_2), 1.71 - 1.38 (m, 3H, CH and CH_2), 1.22 (dq, 2H, J_q = 12.5 Hz, J_d = 2.5 Hz, CH_2), 0.92 (dq, 2H, J_q = 12.5 Hz, J_d = 3.2 Hz, CH_2).

[3] Christie, R. J.; Anderson, D. J.; Grainger, D. W. *Bioconjugate Chem.* **2010**, *21*, 1779–1787.



9

Following the general procedure cesium (1*R*,4*R*)-4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)methyl)cyclohexane carboxylate (2.49 g, 7 mmol, 1.1 eq) and 2-(1-bromoethyl)-4,4-dimethyl-4H-oxazolin-5-one **2** (1.35 g, 6.1 mmol, 1 eq) were used. Azlactone **9** was obtained as white powder (0.9 g, 39%) after column chromatography (SiO_2, *n*-hexane/EtOAc: 50/50 (*v/v*), Rf = 0.5).

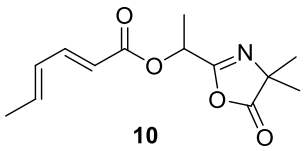
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 6.69 (s, 2H, HC=), 5.50 (q, 1H, *J* = 6.8 Hz, CH), 3.36 (d, 2H, *J* = 7.0 Hz, CH_2), 2.31 (tt, 1H, *J* = 12.2 Hz, *J* = 3.6 Hz, CH), 2.07 - 1.96 (m, 2H, CH2), 1.79 - 1.63 (m, 3H, CH and CH_2), 1.53 (d, 3H, *J* = 6.8 Hz, CH_3), 1.47 - 1.33 (m, 8H), 1.06 - 0.93 (m, 2H, CH_2) (**Figure 13**).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 180.6 (C=O), 174.6 (C=O), 171.1 (2C=O), 162.0 (C=N), 134.1 (2CH=), 65.6 (Cq), 64.9 (CH), 43.7 (CH), 42.8 (CH_2), 36.4 (CH), 29.7 (CH_2), 29.7 (CH_2), 28.2 (CH_2), 28.2 (CH_2), 24.5 ($2CH_3$), 17.1 (CH_3) (**Figure 14**).

FT-IR: υ (cm^{-1}) 2934, 1824, 1737, 1703, 1681, 1409.

HRMS (Q-TOF ESI$^+$): $[M+Na]^+$: $C_{19}H_{24}N_2NaO_6$, calculated: 399.1527, experimental: 399.1519.

4. 1-(4,4-Dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl hexa-2,4-dienoate (10)



10

Procedure: A mixture of potassium sorbate (2.41 g, 0.016 mol, 1.1 eq) and 2-(1-bromoethyl)-4,4-dimethyloxazol-5(4*H*)-one **2** (3.21 g, 0.015 mol, 1 eq) in 30 ml of anhydrous DMF was heated at 60 °C under argon for 17 h. DMF was then evaporated under vacuum. Azlactone **10** was obtained as yellow oil that crystallized upon standing (1.83 g, 49%) after silica gel filtration (acetone).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.36 - 7.29 (m, 1H, =CH), 6.28 - 6.13 (m, 2H, =CH), 5.83 (d, 1H, *J* = 15.0 Hz, =CH), 5.61 (q, 1H, *J* = 6.8 Hz, CH), 1.91 - 1.85 (m, 3H, CH_3), 1.60 (d, 1H, *J* = 6.8 Hz, CH_3), 1.45 (s, 3H, CH_3), 1.44 (s, 3H, CH_3) (**Figure 15**).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 180.7 $(C=O)_{az}$, 166.1 (C=O), 162.2 $(C=N)_{az}$, 146.8 (CH), 140.8 (CH), 129.8 (CH), 117.7 (CH), 65.6 (C), 64.9 (CH), 24.5 ($2CH_3$), 18.9 (CH_3), 17.3 (CH_3) (**Figure 16**).

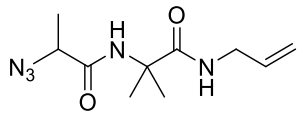
FT-IR: υ (cm^{-1}) 2985, 2939, 1826, 1720, 1683.

HRMS (TOF MS CI$^+$): $[M+H]^+$: $C_{13}H_{18}NO_4$, calculated: 252.1236, experimental: 252.1239.

5. Reaction of azido-azlactone 3 with amines

General procedure: 0.6 mmol of azido azlactone **3** was mixed with 0.6 mmol of primary amine (respectively: allylamine, propargylamine and benzylamine) in $CDCl_3$ (0.5 mL). The ^{1}H NMR spectrum (200 MHz) of the mixture was recorded after 16 h of reaction (**Figure 17**, **Figure 18** and **Figure 19**, respectively). In these conditions the corresponding products were quantitatively obtained.

5.1. 2-(2-Azidopropanamido)-2-methyl-*N*-vinylpropanamide (12)

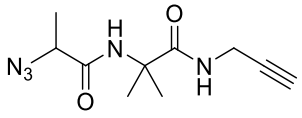


12

^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.03 (bs, 1H, NH), 6.51 (bs, 1H, NH), 5.94 - 5.71 (m, 1H, HC=), 5.26 - 5.07 (m, 2H, H_2C=), 4.02 (dq, 1H, J_q = 7.0 Hz, J_d = 1.2 Hz, CH), 3.94 - 3.82 (m, 2H, CH_2), 1.58 (s, 6H, $2CH_3$), 1.52 (dd, 3H, J = 7.0 Hz, J = 1.2 Hz, CH_3) (**Figure 17**).

HRMS (Q-TOF ESI$^+$): $[M+Na]^+$: $C_{10}H_{17}N_5NaO_2$, calculated: 262.1274, experimental: 262.1295.

5.2. 2-(2-Azidopropanamido)-2-methyl-*N*-(prop-2-ynyl)propanamide (13)



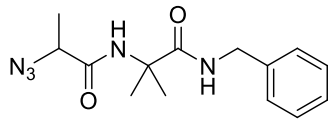
13

^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 6.90 (bs, 1H, NH), 6.73 (bs, 1H, NH), 4.04 (q, 1H, J = 6.9 Hz, CH), 4.03 (dd, H, J = 5.3 Hz, J = 2.5 Hz, CH_2), 2.23 (t, 1H, J = 2.6 Hz, HC≡), 1.57 (s, 6H, $2CH_3$), 1.53 (d, 3H, J = 6.9 Hz, CH_3) (**Figure 18**).

Using the general procedure in D_2O solution, the ^{1}H NMR after 16 h showed similar signals at δ (ppm) = 4.05 (CH), 3.95 (CH_2), 1.55 ($2CH_3$), 1.50 (CH_3).

HRMS (Q-TOF ESI$^+$): $[M+Na]^+$: $C_{10}H_{15}N_5NaO_2$, calculated: 260.1116, experimental: 260.1120.

5.3. 2-(2-Azidopropanamido)-*N*-benzyl-2-methylpropanamide (14)

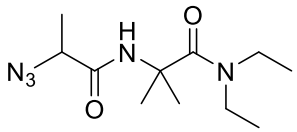


14

^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.38 - 7.21 (m, 5H, Harom), 7.02 (bs, 1H, NH), 6.69 (bs, 1H, NH), 4.45 (d, 2H, J = 5.7 Hz, CH_2), 4.01 (q, 1H, J = 7.0 Hz, CH), 1.61 (s, 3H, CH_3), 1.60 (s, 3H, CH_3), 1.50 (d, 3H, J = 7.0 Hz, CH_3) (**Figure 19**).

HRMS (Q-TOF ESI$^+$): $[M+Na]^+$: $C_{14}H_{19}N_5NaO_2$, calculated: 312.1431, experimental: 312.1424.

5.4. 2-(2-Azidopropanamido)-*N*,*N*-diethyl-2-methylpropanamide (15)



15

Procedure: To a solution of azido azlactone **3** (23 mg, 0.125 mmol, 1 eq), diethylamine (9 mg, 0.125 mmol, 1 eq) in CH_2Cl_2 (0.125 mL) was added a solution of DIPEA (0.05 eq) in CH_2Cl_2 (0.125 mL). The resulting solution was stirred overnight at rt. Then volatiles were removed under reduced pressure. **15** was obtained as white solid (28 mg, 88 %) after silica gel filtration (CH_2Cl_2/MeOH: 98/2).

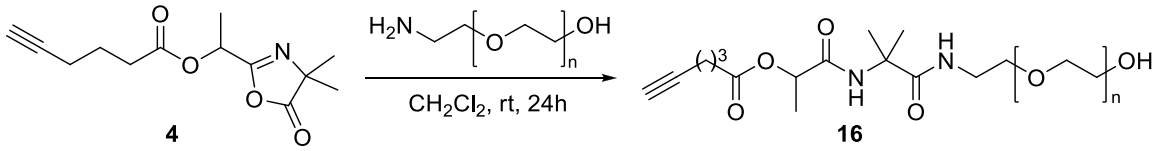
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.98 (bs, 1H, NH), 3.97 (q, 1H, J = 7.0 Hz, CH), 3.53 - 3.32 (m, 4H, CH_2), 1.66 (s, 3H, CH_3), 1.65 (s, 3H, CH_3), 1.50 (d, 3H, J = 7.0 Hz, CH_3), 1.15 (t, 6H, J = 6.5 Hz, CH_3). (**Figure 20**)

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 172.5 (CO), 168.1 (CO), 59.7 (CH_2), 57.2 (Cq), 42.1 ($2CH_2$), 24.1 (CH_3), 24.1 (CH_3), 17.2 (CH_3), 13.7 (CH_3), 12.9 (CH_3).

HRMS (Q-TOF ESI$^+$): $[M+Na]^+$: $C_{11}H_{21}N_5NaO_2$, calculated: 278.1587, experimental: 278.1588.

FT-IR: υ (cm^{-1}) 3279, 3071, 2978, 2936, 2107, 2085, 1653, 1625, 1542, 1422, 1239, 1198, 1124.

6. Reaction of the alkyne-azlactone 4 with α-amino ω-hydroxyl PEO



Procedure: To an ice-cooled solution of α-amino ω-hydroxyl PEO[4] (2.23 g, 0.74 mmol, 1 eq, Mw: 3000 $g.mol^{-1}$) in CH_2Cl_2 (15 mL) was added dropwise a solution of azlactone **4** (206 mg, 0.82 mmol, 1.1 eq) in CH_2Cl_2 (5 mL) under argon and the resulting mixture was stirred 24 h at 25 °C. Then half of the solvent (≈10 mL) was removed under reduced pressure and the product was precipitated in cold Et_2O. After filtration and drying under reduced pressure, α-alkynyl-functionalized PEO **16** was obtained as a white solid (2.39 g, 99%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.16 (bs, 1H, NH), 6.67 (bs, 1H, NH), 5.09 (q, 1H, J = 6.8 Hz, CH), 3.70 - 3.60 (m, 256H, CH_2), 2.56 (dt, 2H, J_t = 7.3 Hz, J_d = 0.9 Hz, CH_2), 2.32 - 2.24 (m, 2H, CH_2), 1.99 (t, 1H, J = 2.6 Hz, HC≡), 1.96 - 1.83 (m, 2H, CH_2), 1.60 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 1.45 (d, 3H, J = 6.8 Hz, CH_3) (**Figure 21**).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 174.6 (C=O), 172.0 (C=O), 169.8 (C=O), 83.2 (C≡), 72.7 (CH), 70.7 (CH_2, 2n CH_2), 70.4 (CH_2), 69.6 (CH_2), 69.5 (HC≡), 61.8 (CCH_2), 56.9 (C), 39.8 (CH_3), 33.0 (CH_2), 24.9 (CH_3), 24.8 (CH_3), 23.5 (CH_2), 17.8 (CH_3), 17.8 (CH_3) (**Figure 22**).

[4] Commercially available: Rapp Polymer GmbH.

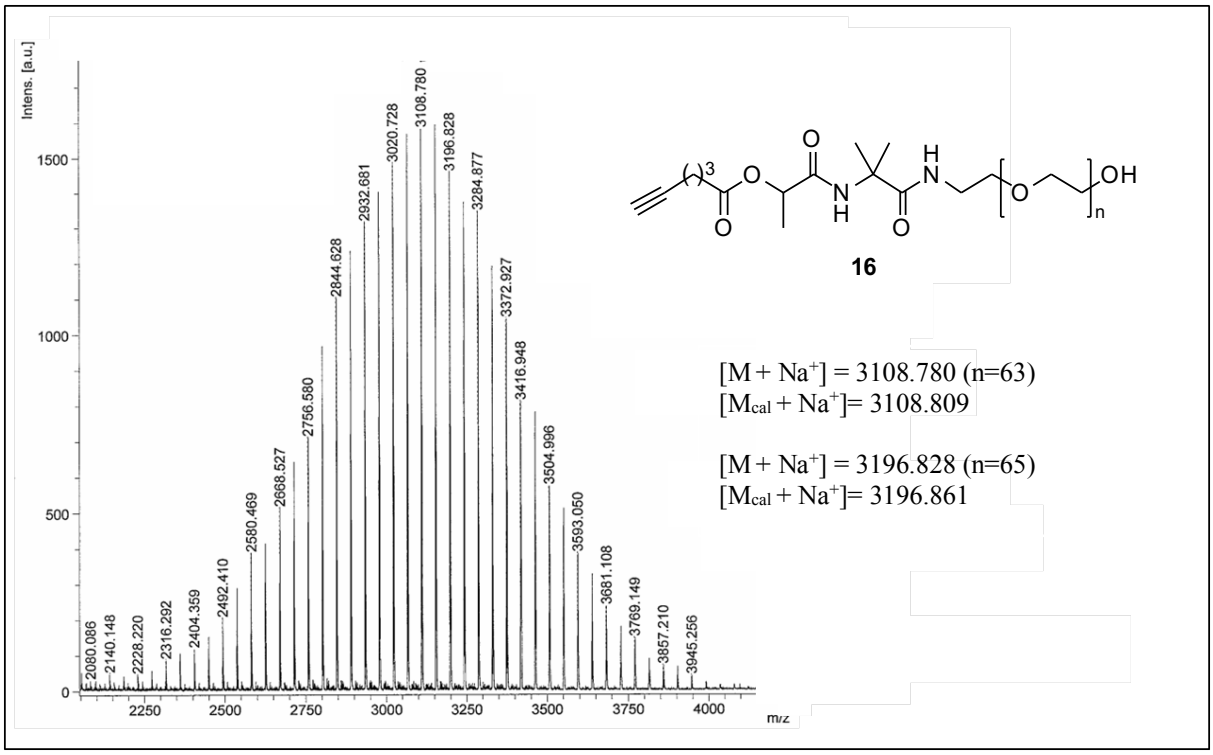


Figure S1: MALDI-TOF mass spectrum of **16** (matrix: trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile, DCTB + sodium trifluoroacetate (NaTFA)).

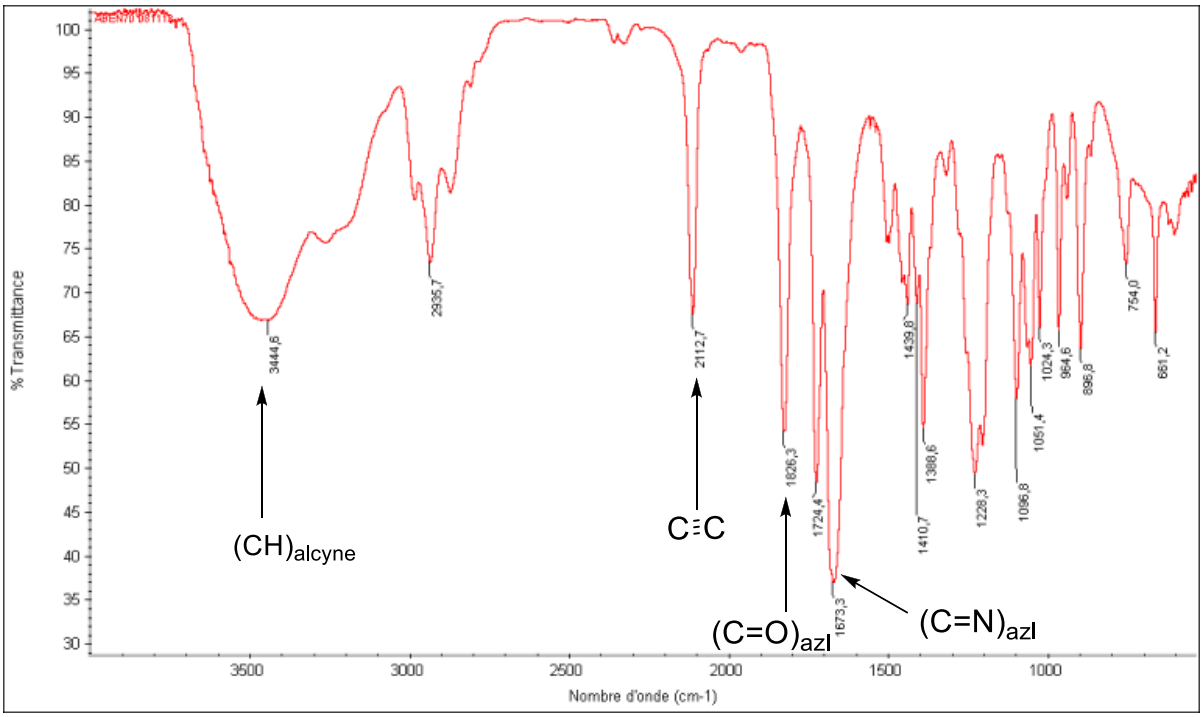
7. Reaction of azlactones with thiols

General procedure: 0.6 mmol of azlactone (**3**, **4** or **5**, 1 eq) was mixed with the thiol (dodecanethiol or benzyl mercaptan, 1 or 2 eq) in $CDCl_3$ (0.5 mL). The ^1H NMR spectrum (200 MHz) and FT-IR spectra of the mixture was recorded after 16 h of reaction at rt or 35 °C.

- Reaction between azlactones **3** or **4** and dodecanethiol or benzyl mercaptan: After 16 h of reaction at rt or 35 °C, no sign of reaction was observed on NMR spectra for the both reactions. These results were confirmed on the FT-IR spectra.

- Reaction between azlactone **5** and benzyl mercaptan: After 16h at rt no significant sign of reaction was observed on NMR and FT-IR spectra with 1 or 2 equivalents of thiol.

- Reaction between azlactone **5**, benzyl mercaptan (1 eq), and Et_3N (1 eq): After 16 h at room temperature in $CDCl_3$ significant changes were observed on the NMR spectra: the characteristic signal of alkynyl proton (3 ppm) disappeared and new signals =CH appeared at (7.8 and 5.9 ppm). On the FT-IR spectra (see Figure S2 below) the characteristic band of azlactone (1825 cm^{-1}) is still present on the spectra, nevertheless the characteristics signals of the alkyne function disappeared (C≡C: 2120 cm^{-1}, ≡CH: 3444 cm^{-1}) and a new band was observed at 1580 cm^{-1}.



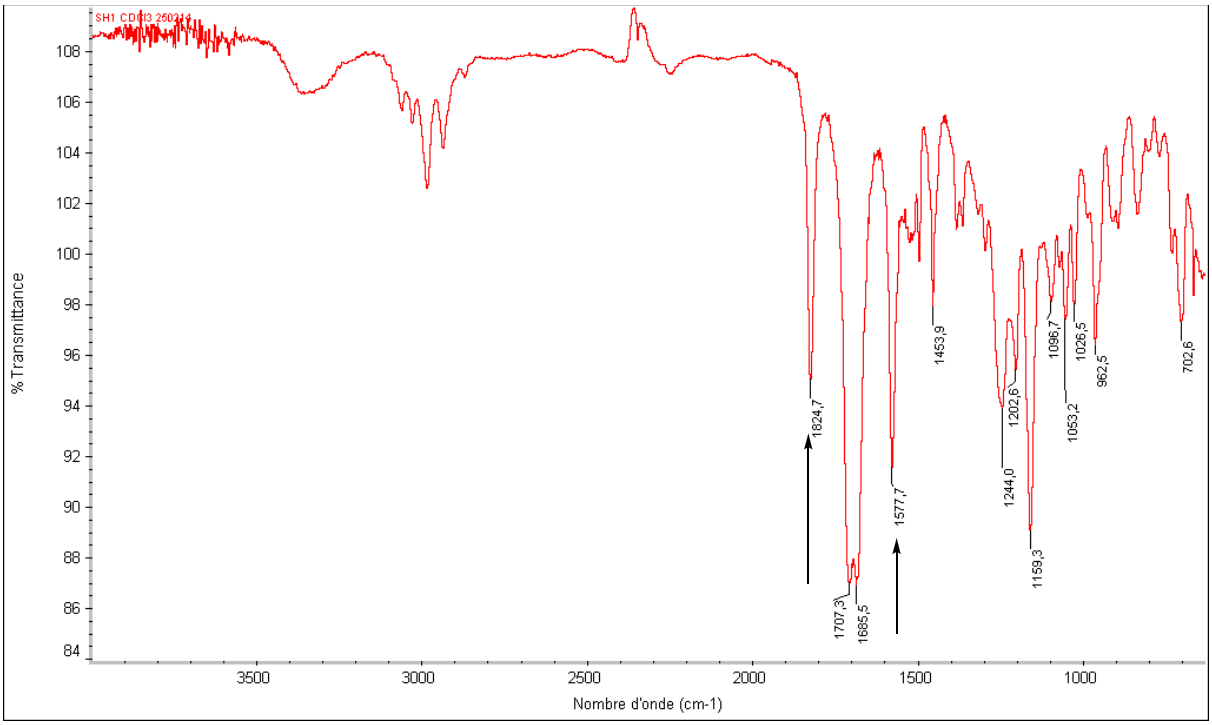
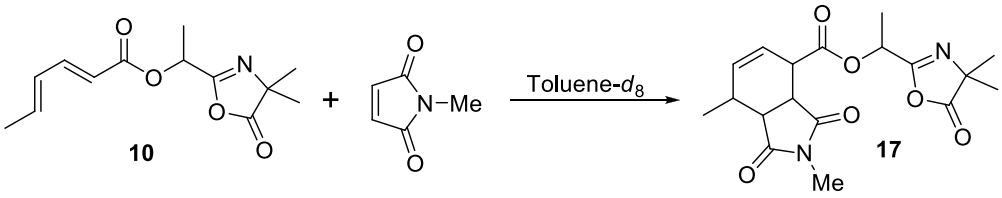


Figure S2: FT-IR spectra of azlactone **5** (top) and of the mixture **5** + 1 eq. of benzyl mercaptan + 1 eq. Et_3N after 16 h in $CDCl_3$ at rt (bottom).

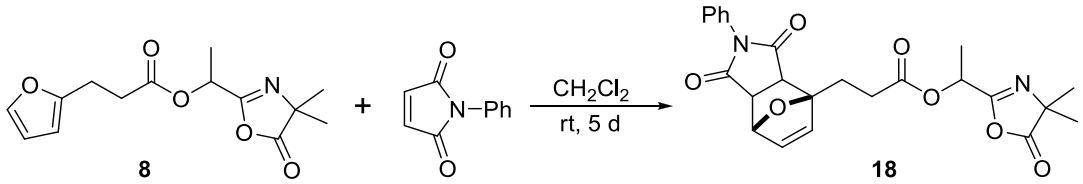
8. Diels-Alder reaction using functionalized azlactones

8.1. Diels-Alder reaction between azlactone 10 and *N*-methylmaleimide



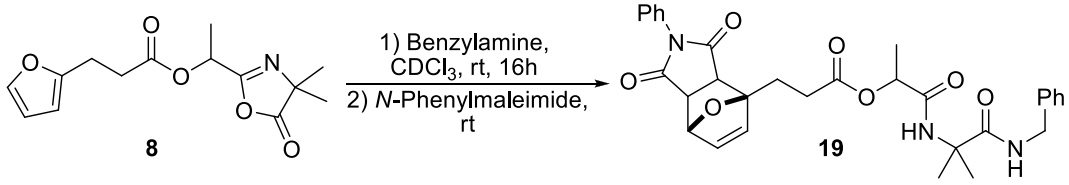
Procedure: In a NMR tube was prepared a solution of azlactone **10** (20 mg, 0.080 mmol, 1 eq), *N*-methylmaleimide (9 mg, 0.080 mmol, 1 eq) in toluene-d_8 (0.5 mL). After 5 days at rt, the reaction mixture was heated at 110 °C during 24h. A 94 % conversion was calculated by ^{1}H NMR analysis of the crude mixture (**Figure 23** (t0), **Figure 24** (final time), 200 MHz).

8.2. Diels-Alder reaction between azlactone 8 and *N*-phenylmaleimide



Procedure: A solution of *N*-phenylmaleimide (66 mg, 0.38 mmol, 1.2 eq) and azlactone **8** (89 mg, 0.31 mmol, 1 eq) in CH_2Cl_2 (2 mL) was prepared. The resulting solution was stirred 5 days at rt. Then the mixture was concentrated under reduced pressure. A 85 % conversion was calculated by ^{1}H NMR analysis of the crude mixture (**Figure 25**). The formation of cycloadducts **18** was confirmed by HRMS.

HRMS (Q-TOF ESI$^+$): $[M+Na]^+$: $C_{24}H_{24}N_2O_8$, calculated: 475.1476, experimental: 475.1477.



Procedure: To a solution of azlactone **8** (35.7 mg, 0.13 mmol, 1 eq) in $CDCl_3$ (0.5 mL) was added a solution of benzylamine (13.7 mg, 0.13 mmol, 1 eq) in $CDCl_3$ (0.5 mL). After 16 h at rt, *N*-phenylmaleimide (26.6 mg, 0.15 mmol, 1.2 eq) was added to the reaction mixture and the evolution of the reaction was monitored by ^{1}H NMR analysis (Table S1). The formation of the cycloadducts **19** was confirmed by HRMS. 77 % of conversion was observed after 60 days by ^{1}H NMR analysis (**Figure 26**).

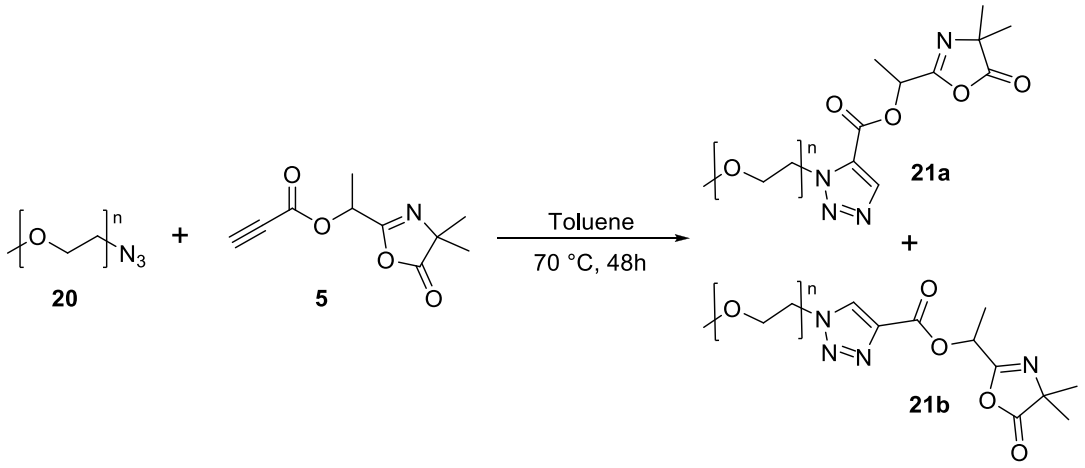
HRMS (Q-TOF ESI$^+$): [M+Na]: $C_{31}H_{33}N_3NaO_7$, calculated: 582.2211, experimental: 582.2196.

Table S1: Kinetics of the formation of cycloadduct 19 through DA reaction

Duration	16 hours	7 days	15 days	60 days
Conversion (%)	21	57	69	77

9. Azide-alkyne cycloadditions

9.1. Azide-alkyne cycloaddition between azlactone 5 and PEO-N_3



Procedure: A stirring solution of PEO-N_3[5] **20** (0.862 g, 0.43 mmol, 1 eq) and 1-(4,4-dimethyl-5-oxo-4,5-dihydrooxazol-2-yl) ethyl propiolate **5** (0.891 g, 4.3 mmol, 10 eq) in dry toluene (20 mL) was heated at 70 °C under argon. Then the solvent was eliminated under reduced pressure and the polymer was purified by precipitation in cold diethyl ether (200 mL).

The following data are selected from the ^{1}H NMR analyses of the mixtures (**21a**/**21b**, ratio: 15/85) in $CDCl_3$ (**Figure 27**). Signals (-OC**H$_2$**-C**H$_2$**O) from the PEG fragment have been omitted.

^{1}H NMR 21b (400 MHz, $CDCl_3$): δ (ppm) 8.37 (s, 1H, HC=), 5.80 (q, 1H, *J* = 6.8 Hz, CH), 4.63 - 4.56 (m, 2H, CH_2), 3.92 - 3.85 (m, 2H, CH_2), 3.36 (s, 3H, CH_3), 1.70 (d, 3H, *J* = 6.8 Hz), 1.42 (s, 6H).

^{1}H NMR 21a (400 MHz, $CDCl_3$): δ (ppm) 8.17 (s, 1H, HC=), 5.72 (q, 1H, *J* = 6.8 Hz, CH), 4.63 - 4.56 (m, 2H, CH_2), 3.92 - 3.85 (m, 2H, CH_2), 3.36 (s, 3H, CH_3), 1.70 (d, 3H, *J* = 6.8 Hz), 1.42 (s, 6H).

FT-IR: υ (cm^{-1}) 2881, 1824, 1732, 1465, 1341, 1101, 959, 841.

[5] PEO-N_3 (Mw: 2000 g.mol^{-1}) was prepared according to the following procedure: Gao, H.; Matyjaszewski, K. *J. Am. Chem. Soc.* **2007**, *129*, 6633–6639.

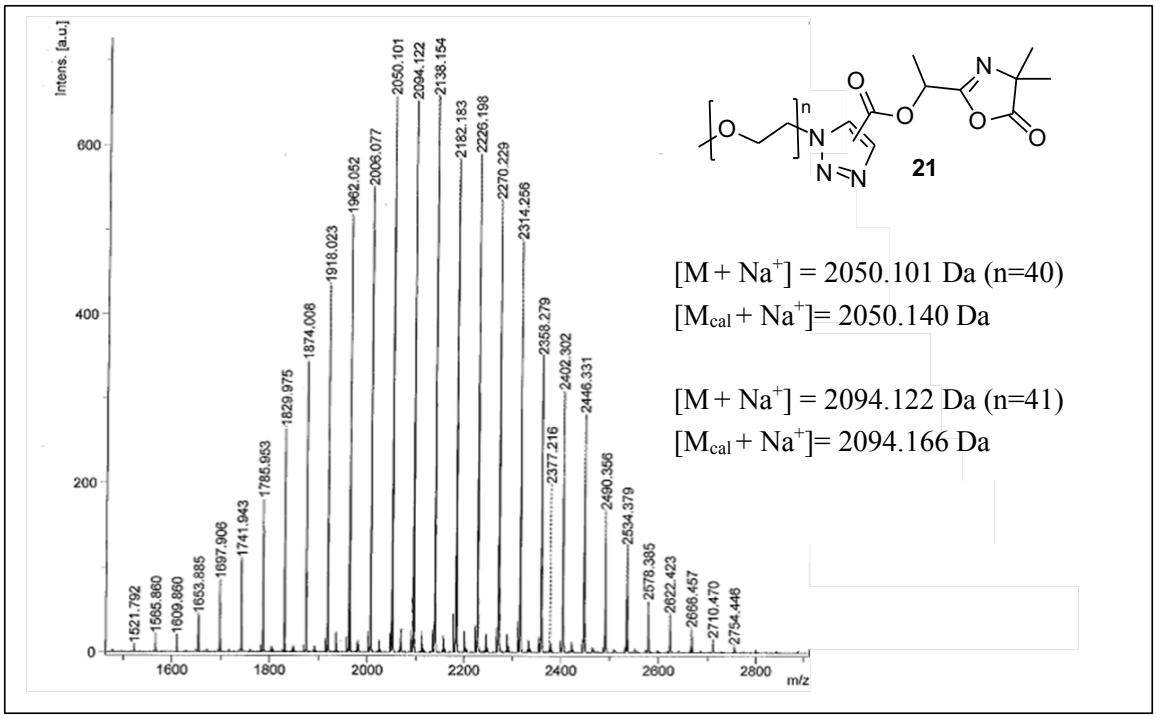
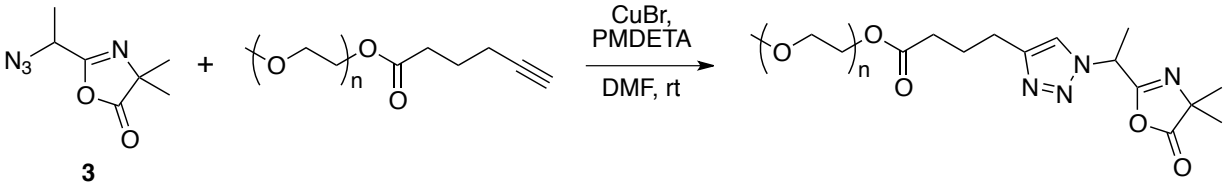


Figure S3: MALDI-TOF mass spectrum of **21** (matrix: trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile, DCTB + sodium trifluoroacetate (NaTFA)).

9.2. Copper-catalyzed azide-alkyne cycloaddition (CuAAC) between azido-azlactone 3 and alkynyl-PEO



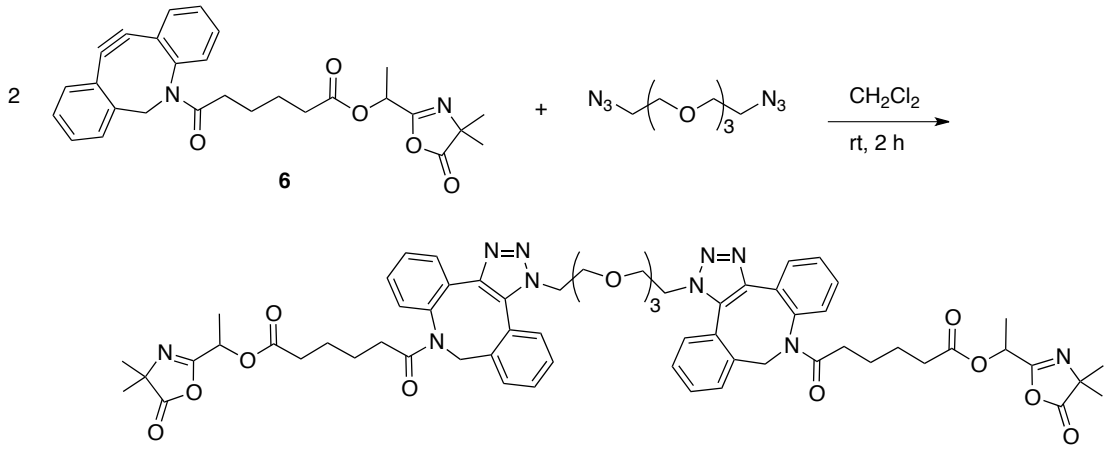
3

Procedure: In a Schlenk tube, a solution of alkynyl-PEO **26** (80 mg, 0.035 mmol, 1 eq) and azido-azlactone **3** (24 mg, 0.13 mmol, 3.5 eq) in anhydrous DMF (5 mL) was degassed by argon bubbling during 30 min at rt. The solution was transferred in a second flask containing CuBr (5 mg, 0.035 mmol, 1 eq). To this suspension was added degassed PMEDTA (8 µl, 0.035 mmol, 1 eq) and the resulting mixture was stirred overnight at room temperature. The crude mixture was filtrated though an alumina column and the solvent removed under reduced pressure. Then THF (few ml) was added to the residue and the PEO-polymer was precipitated by dropwise addition of *n*-hexane. The resulting solid was dried under reduced pressure.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) (signals from the PEG fragment (-OC**H$_2$**-C**H$_2$**O) have been omitted) 7.50 (s, 1H, HC=), 5.63 (q, 1H, *J* = 7.2 Hz, CH), 4.23 (m, 2H, CH_2), 3.86 - 3.52

(m, 2H, CH_2), 3.38 (s, 3H, CH_3), 2.80 (t, 2H, *J* = 7.5 Hz, CH_2), 2.43 (t, 2H, *J* = 7.5 Hz, CH_2), 2.03 (qui, 2H, *J*= 7.5 Hz, CH_2), 1.90 (d, 3H, *J* = 7.2 Hz, CH_3), 1.45 (s, 3H, CH_3), 1.44 (s, 3H, CH_3).

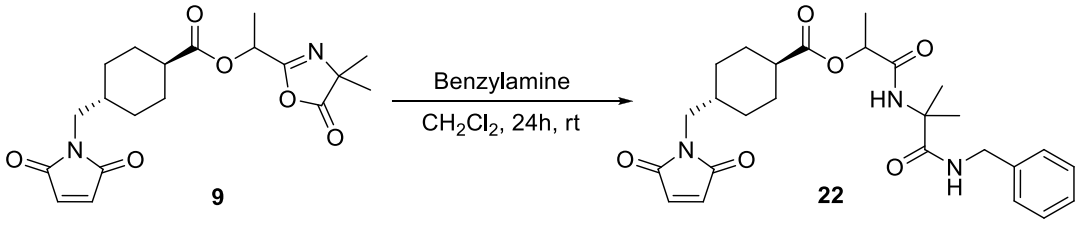
9.3. Strain-promoted azide-alkyne cycloaddition (SPAAC) using azlactone 6



Procedure: To a solution of azlactone **6** (27 mg, 0.06 mmol, 1 eq) in CH_2Cl_2 (0.5 mL) was added 1-azido-2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethane (7 mg, 0.03 mmol, 0.5 eq). The resulting solution was stirred 2 hours at rt. Then the solvent was removed under reduced pressure to give a white solid. After hydrolysis, the structure of the product was confirmed by HRMS.

HRMS (Q-TOF ESI$^+$): [M+Na]: $C_{64}H_{76}N_{10}NaO_{15}$, calculated: 1247.5384, experimental: 1247.5407.

10. Ligations with *N*-acetylcysteine



Procedure: To a solution of **9** (214 mg, 0.57 mmol, 1 eq) in CH_2Cl_2 (5 mL) was added at rt a solution of benzylamine (61 mg, 0.57 mmol, 1 eq) in CH_2Cl_2 (1 mL). After 24 h at rt the solvent was eliminated under reduced pressure. **22** was obtained as white powder (201 mg, 73%) after column chromatography (SiO_2, *n*-hexane/ethyl acetate: 30/70 (*v/v*), Rf = 0.3).

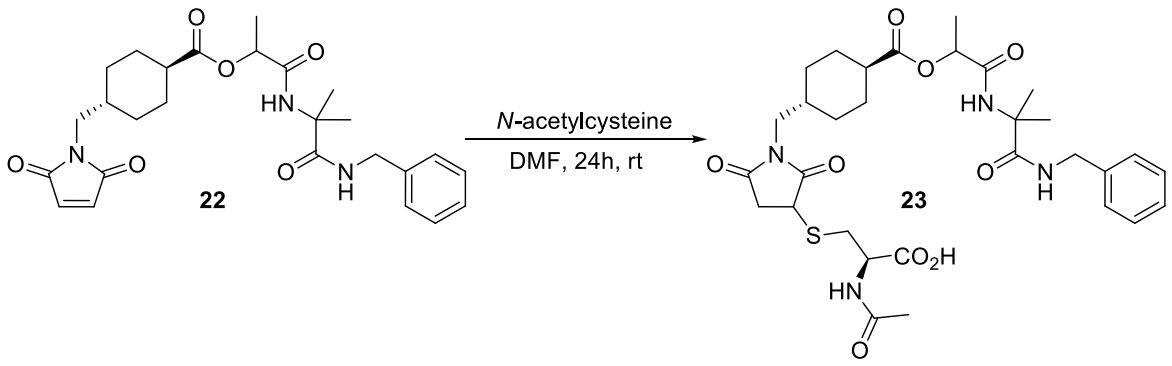
^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.35 - 7.23 (m, 5H, Harom), 6.70 (s, 2H, HC=), 6.66 (bs, 1H, NH), 6.65 (bs, 1H, NH), 5.00 (q, 1H, *J* = 6.8 Hz, CH), 4.52 - 4.37 (m, 2H, CH_2), 3.37 (d, 2H, *J* = 7.0 Hz, CH_2), 2.27 (tt, 1H, *J* = 12.2 Hz, *J* = 3.6 Hz, CH), 2.03 - 1.93 (m, 2H, CH_2),

1.78 - 1.42 (m, 9H), 1.42 (d, 3H, J = 6.8 Hz, CH_3), 1.39 - 1.23 (m, 2H, CH_2), 1.08 - 0.93 (m, 2H, CH_2) (**Figure 28**).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 174.9 (C=O), 174.1 (C=O), 171.1 (2C=O), 170.4 (C=O), 138.3 (Carom), 134.2 (2HC=), 128.8 (2CHarom), 127.7 (2CHarom), 127.6 (CHarom), 70.9 (CH), 57.4 (C), 43.9 (CH_2), 43.7 (CH), 42.9 (CH_2), 36.4 (CH), 29.7 (CH_2), 29.6 (CH_2), 28.3 (CH_2), 28.1 (CH_2), 25.6 (CH_3), 25.1 (CH_3), 17.6 (CH_3) (**Figure 29**).

FT-IR: υ (cm^{-1}) 3305, 2931, 1701, 1651, 1515, 1408, 1361, 1152, 1044, 828, 732, 695.

HRMS (Q-TOF ESI$^+$): $[M+H]^+$: $C_{26}H_{34}N_3O_6$, calculated: 484.2442, experimental: 484.2459.



Procedure: To a solution of **22** (201 mg, 0.42 mmol, 1 eq) in DMF (10 mL) was added dropwise a solution of *N*-acetylcysteine (67.8 mg, 0.42 mmol, 1 eq) in DMF (2 mL) at rt. After 48 hours of stirring, the solvent was removed under reduced pressure. The residue was dissolved in $CHCl_3$ was and precipitated by addition of Et_2O.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.33 - 7.20 (m, 5H, CHarom), 6.97 - 6.75 (m, 3H, NH), 4.99 (dq, 1H, J_q = 6.8 Hz, J_d = 2.0 Hz, CH), 4.85 - 4.79 (m, 1H, CH), 4.49 - 4.35 (m, 2H, CH_2), 3.93 - 3.76 (m, 1H, CH), 3.52 - 3.32 (m, 3H), 3.21 - 3.02 (m, 2H), 2.58 - 2.43 (m, 1H), 2.30 - 2.20 (m, 1H, CH), 2.09 - 1.90 (m, 5H), 1.73 - 1.63 (m, 3H), 1.60 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.44 - 1.23 (m, 5H), 1.05 - 0.95 (m, 2H, CH_2) (**Figure 30**).

^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 177.8, 177.8, 177.2, 177.2, 175.0, 174.9, 174.9, 174.7, 174.7, 171.9, 171.8, 171.6, 171.5, 171.5, 171.4, 171.3, 170.7, 137.9, 137.9, 128.8, 127.6, 127.6, 70.8, 57.3, 52.8, 51.7, 44.8, 44.6, 44.0, 42.8, 42.8, 40.9, 40.8, 39.2, 39.2, 36.5, 35.9, 35.7, 35.7, 35.7, 34.8, 34.8, 34.7, 33.9, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 28.2, 28.0, 27.9, 25.5, 25.5, 24.8, 23.0, 23.0, 17.6, 17.6.

HRMS (Q-TOF ESI$^+$): $[M+Na]^+$: $C_{31}H_{42}N_4NaO_9S$, calculated: 669.2565, experimental: 669.2561.

FT-IR: υ (cm^{-1}) 2928, 1627, 1435, 1282, 1245, 1220, 1016, 908, 727.

11. Lysozyme bioconjugates

Lysozyme from chicken egg white was purchase from Aldrich® (≈100000 U/mg, 14.3 kDa) and used as received.

Alkynyl-PEO (α-hex-5-ynoate ω-methoxy PEO) **26** was prepared as follows: To an ice-cooled solution of PEO-OH (4 g, 2 mmol, 1 eq, Mw: 2000 g.mol^{-1}), DMAP (49 mg, 0.4 mmol, 0.2 eq) in CH_2Cl_2 (30 mL) was added dropwise hex-5-ynoic acid (0.45 g, 4 mmol, 2 eq). After homogenization of the reaction mixture, a solution of DCC (0.83 g, 4 mmol, 2 eq) in CH_2Cl_2 (20 mL) was added dropwise and the resulting mixture was stirred 8 days at rt. Then the reaction mixture was filtrated and concentrated under reduced pressure. The polymer was dissolved in a minimum of CH_2Cl_2 and precipitated with cold Et_2O.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.26 - 4.18 (m, 2H), 3.36 (s, 3H), 2.47 (t, 2H, J = 7.2 Hz), 2.25 (dt, 2H, J_t = 7.2 Hz, J_d = 2.6 Hz), 1.96 (t, 1H, J = 2.6 Hz), 1.84 (qui, 2H, J = 7.2 Hz). Signals from the PEG fragment (-OC**H$_2$**-C**H$_2$**O) have been omitted (**Figure 31**).

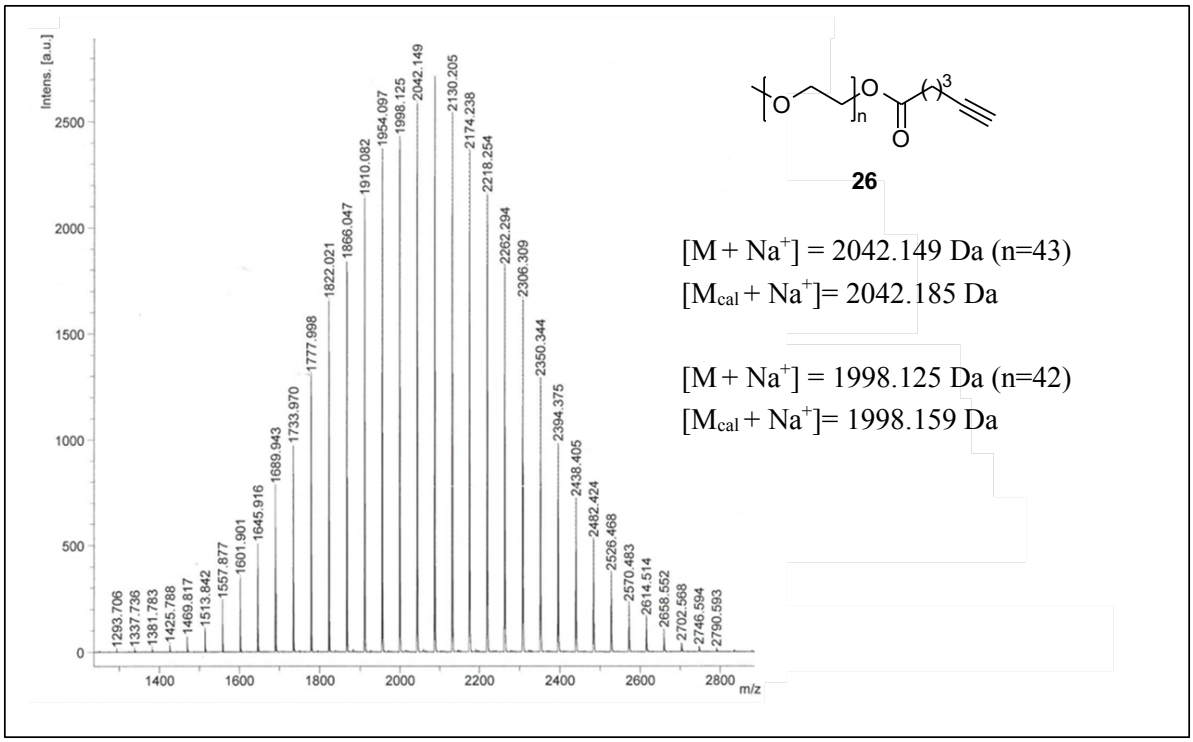
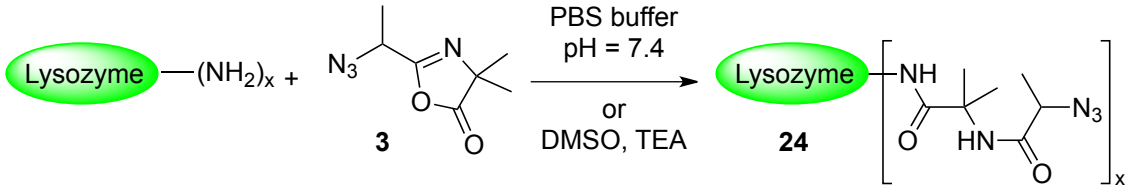


Figure S4: MALDI-TOF mass spectrum of alkynyl-PEO **26** (matrix: trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene]malononitrile, DCTB + sodium trifluoroacetate (NaTFA)).

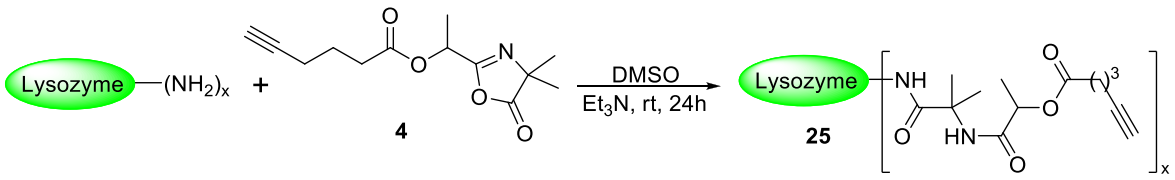
- Bioconjugation of lysozyme using azido-azlactone 3



In PBS buffer: A solution of azlactone **3** (7 mg, 40 µmol, 10 eq) in DMF (10 µL per µmol) was added dropwise to a solution of lysozyme (57.1 mg, 4 µmol) in phosphate buffer (PBS[6], 2.5 mL per µmol) under stirring. Then the mixture was stirred for 16 hours at 23 °C. The product was obtained after dialysis in water/methanol (80/20, v/v) with a membrane (MWCO = 3500) for 24 hours and the product (white powder) was obtained after lyophilisation.

In the presence of TEA: To a solution of lysozyme (128.4 mg, 8 µmol) in DMSO (10 mL) was added an excess Et_3N (TEA, 0.4 mL, 2.98 mmol, 186 eq). After 15 minutes of stirring at rt, a solution of azlactone **3** (0.252 g, 1.38 mmol, 172 eq) in DMSO (4 mL) was added slowly. The solution was stirred for 24 hours at rt. Then Millipore water (3.75 mL per µmol of lysozyme) and concentrated HCl (0.125 mL per µmol of lysozyme) was added. The mixture was stirred for 1 hour at rt. A white precipitated was formed. The mixture was dialyzed in water/methanol (80/20, v/v) with a membrane (MWCO = 3500) for 24 hours and the product (white powder) was obtained after lyophilization.

- Bioconjugation of lysozyme using alkynyl-azlactone 4

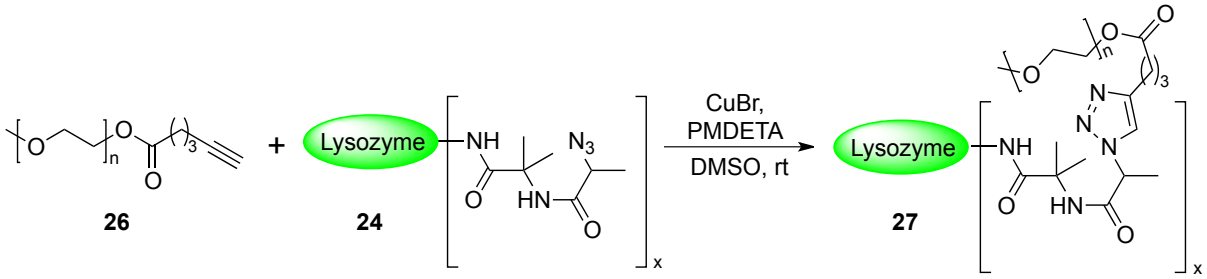


To a solution of lysozyme (128.4 mg, 8 µmol) in DMSO (10 mL) was added an excess Et_3N (0.4 mL, 2.98 mmol, 186 eq). After 15 minutes of stirring at rt, a solution of azlactone **4** (0.695 g, 2.76 mmol, 172 eq) in DMSO (4 mL) was added slowly. The solution was stirred for 24 hours at rt. Then Millipore water (3.75 mL per µmol of lysozyme) and concentrated HCl (0.125 mL per µmol of lysozyme) was added. The mixture was stirred for 1 hour at rt. A white precipitated was formed. The mixture was dialyzed in water/methanol (80/20, v/v) with a

[6] The PBS buffer solution 1X was prepared as follows: NaCl (8 g), KCl (0.2 g), Na_2HPO_4 (1.44 g) and KH_2PO_4 (0.24 g) were dissolved in distilled water (800 mL). Then the pH was adjusted to 7.4 with HCl and distilled water was added to reach the total volume of the solution (1 L).

membrane (MWCO = 3500) for 24 hours and the product (white powder) was obtained after lyophilization.

- Azide-alkyne cycloaddition between azido-lysozyme (24) and alkynyl-PEO (26)

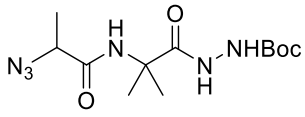


26 **24** **27**

A solution of azido-lysozyme **24** (10 mg, 0.6 µmol, 1 eq) and alkynyl-PEO **26** (Mw=2000, 25.5 mg, 11.7 µmol, 20 eq) in DMSO (5 mL) was prepared. Then the solution was degassed under argon for 30 minutes. To this solution was respectively added CuBr (1.7 mg, 11.7 µmol, 20 eq) and PMDETA (10 µl, 11.7 µmol, 20 eq) under deoxygenated atmosphere. The mixture was stirred for 16 hours at rt. After filtration on Al_2O_3 basic, the mixture was dialyzed in water/methanol (80/20, v/v) with a membrane (MWCO = 3500) for 24 h and the product was obtained after lyophilisation. The product was analyzed by Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) (see Figure 2).

12. Reaction of azido-azlactone 3 with hydrazine derivatives

12.1. *t*-Butyl 2-(2-(2-azidopropanamido)-2-methylpropanoyl)hydrazine-1-carboxylate (28)



28

Procedure: To a solution of azido azlactone **3** (91 mg, 0.5 mmol, 1 eq) in CH_2Cl_2 (0.5 mL) was added a solution of tert-butyl carbazate (66 mg, 05 mmol, 1 eq) in CH_2Cl_2 (0.5 mL). The resulting solution was stirred overnight at rt. Then volatiles were removed under reduced pressure. **28** was obtained as white solid (141 mg, 90 %) after silica gel filtration (CH_2Cl_2/MeOH: 97/3).

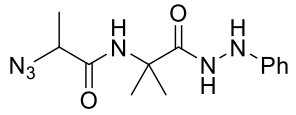
^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 3.90 (q, 1H, *J* = 6.9 Hz, CH), 1.53 (s, 3H, CH_3), 1.52 (s, 3H, CH_3), 1.46 (s, 9H, CH_3), 1.43 (d, 3H, *J* = 6.9 Hz, CH_3). (**Figure 32**)

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 176.2 (CO), 172.4 (CO), 157.6 (CO), 81.7 (Cq), 59.1 (CH), 57.3 (Cq), 28.5 ($3CH_3$), 25.3 (CH_3), 25.1 (CH_3), 17.0 (CH_3). (**Figure 33**)

HRMS (Q-TOF ESI$^+$): $[M+Na]^+$: $C_2H_{22}N_6NaO_4$, calculated: 337.1595, experimental: 337.1583.

FT-IR: υ (cm^{-1}) 3293, 2980, 2931, 2108, 1667, 1515, 1367, 1245, 1158, 1012.

12.2. 2-Azido-N-(2-methyl-1-oxo-1-(2-phenylhydrazinyl)propan-2-yl)propanamide (29)



29

Procedure: To a solution of azido azlactone **3** (91 mg, 0.5 mmol, 1 eq) in CH_2Cl_2 (0.5 mL) was added a solution of phenyl hydrazine (54 mg, 05 mmol, 1 eq) in CH_2Cl_2 (0.5 mL). The resulting solution was stirred overnight at rt. Then volatiles were removed under reduced pressure. **29** was obtained as white solid (133 mg, 92 %) after silica gel filtration (CH_2Cl_2/MeOH: 97/3).

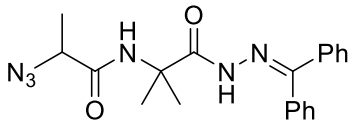
^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 7.21 - 7.12 (m, 2H, Harom), 6.89 - 6.82 (m, 2H, Harom), 6.78 (tt, 1H, *J* = 7.5 Hz, *J* = 1.1 Hz, Harom), 3.31 (q, 1H, *J* = 6.9 Hz, CH), 1.55 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 1.46 (d, 3H, *J* = 6.9 Hz, CH_3). (**Figure 34**)

^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 176.6 (C=O), 172.5 (C=O), 150.1 (Cq), 129.8 (2CH), 120.9 (CH), 114.3 (2CH), 59.1 (Cq), 57.3 (CH), 25.5 (CH_3), 25.4 (CH_3), 17.0 (CH_3). **Figure 35**)

HRMS (Q-TOF ESI$^+$): $[M+Na]^+$: $C_{13}H_{18}N_6NaO_6$, calculated: 313.1370, experimental: 313.1375.

FT-IR: υ (cm^{-1}) 3281, 3054, 2984, 2935, 2106, 1661, 1602, 1495, 1385, 1235, 1100, 753, 692.

12.3. 2-Azido-*N*-(1-(2-(diphenylmethylene)hydrazinyl)-2-methyl-1-oxopropan-2-yl)propenamide (30)



30

Procedure: To a solution of azido azlactone **3** (23 mg, 0.125 mmol, 1 eq) in CH_2Cl_2 (0.125 mL) was added a solution of benzophenone hydrazone (25 mg, 0.125 mmol, 1 eq) in CH_2Cl_2 (0.125 mL). The resulting solution was stirred overnight at rt. Then volatiles were removed

under reduced pressure. **30** was obtained as white solid (42 mg, 89 %) after silica gel filtration (CH_2Cl_2/MeOH: 97/3).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.66 - 7.57 (m, 2H, Harom), 7.50 - 7.31 (m, 6H, Harom), 7.24 - 7.15 (m, 2H, Harom), 5.60 (q, 1H, *J* = 6.8 Hz, CH), 5.35 (bs, H, NH), 1.63 (d, 3H, *J* = 6.8 Hz, CH_3), 1.43 (s, 3H, CH_3), 1.37 (s, 3H, CH_3). (**Figure 36**)

^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 174.1 (CO), 163.2 (CO), 162.2 (CN), 137.9 (Cq), 136.3 (Cq), 130.2 (CH), 129.0 (CH), 128.8 (2CH), 128.5 (2CH), 128.4 (2CH), 128.3 (2CH), 59.6 (Cq), 55.1 (CH), 26.6 (CH_3), 25.0 (CH_3), 19.2 (CH_3). (**Figure 37**)

HRMS (Q-TOF ESI$^+$): $[M+Na]^+$: $C_{20}H_{22}N_6NaO_2$, calculated: 401.1696, experimental: 401.1684.

FT-IR: υ (cm^{-1}) 3373, 2926, 2124, 2094, 1720, 1602, 1508, 1236, 1220, 1163 1024, 774, 691.

13. NMR spectra

Figure 1: ^{1}H NMR spectrum of 2-(1-azidoethyl)-4,4-dimethyloxazol-5(4*H*)-one 3

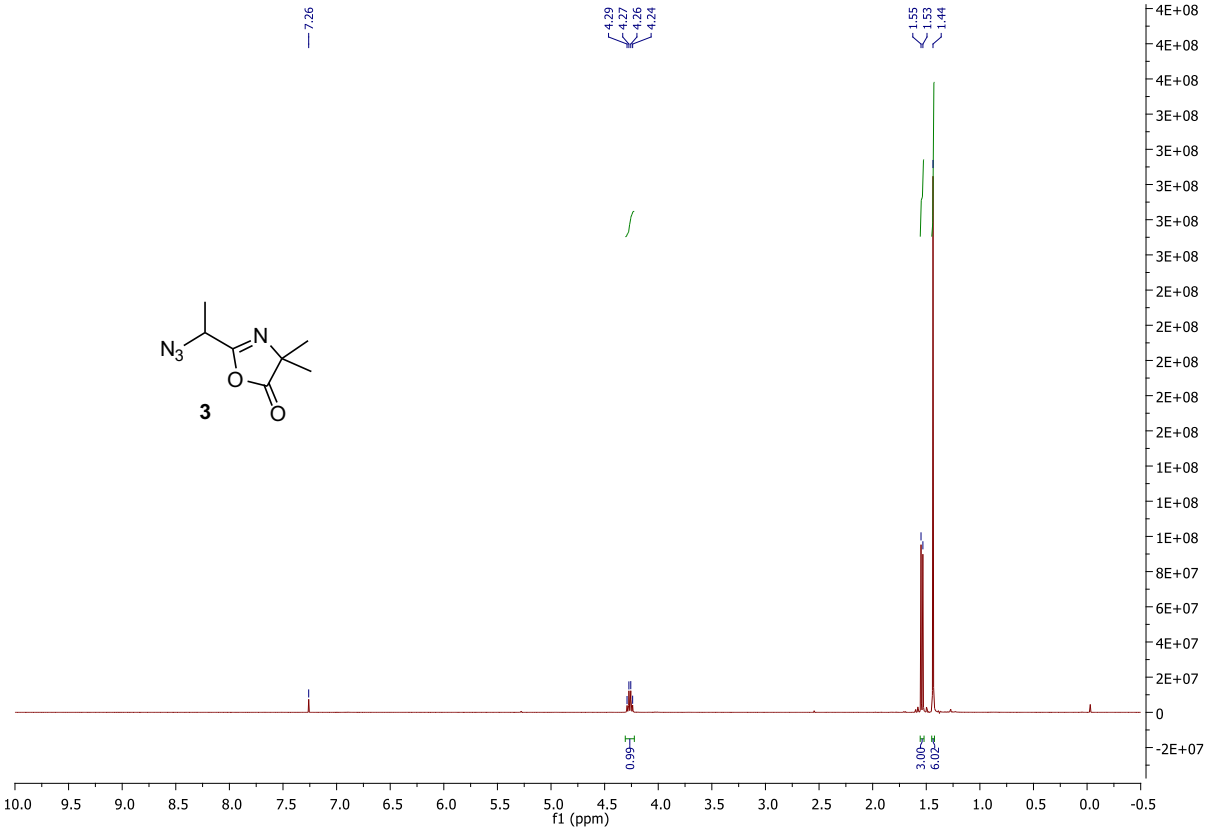


Figure 2: ^{13}C NMR spectrum of 2-(1-azidoethyl)-4,4-dimethyloxazol-5(4*H*)-one 3

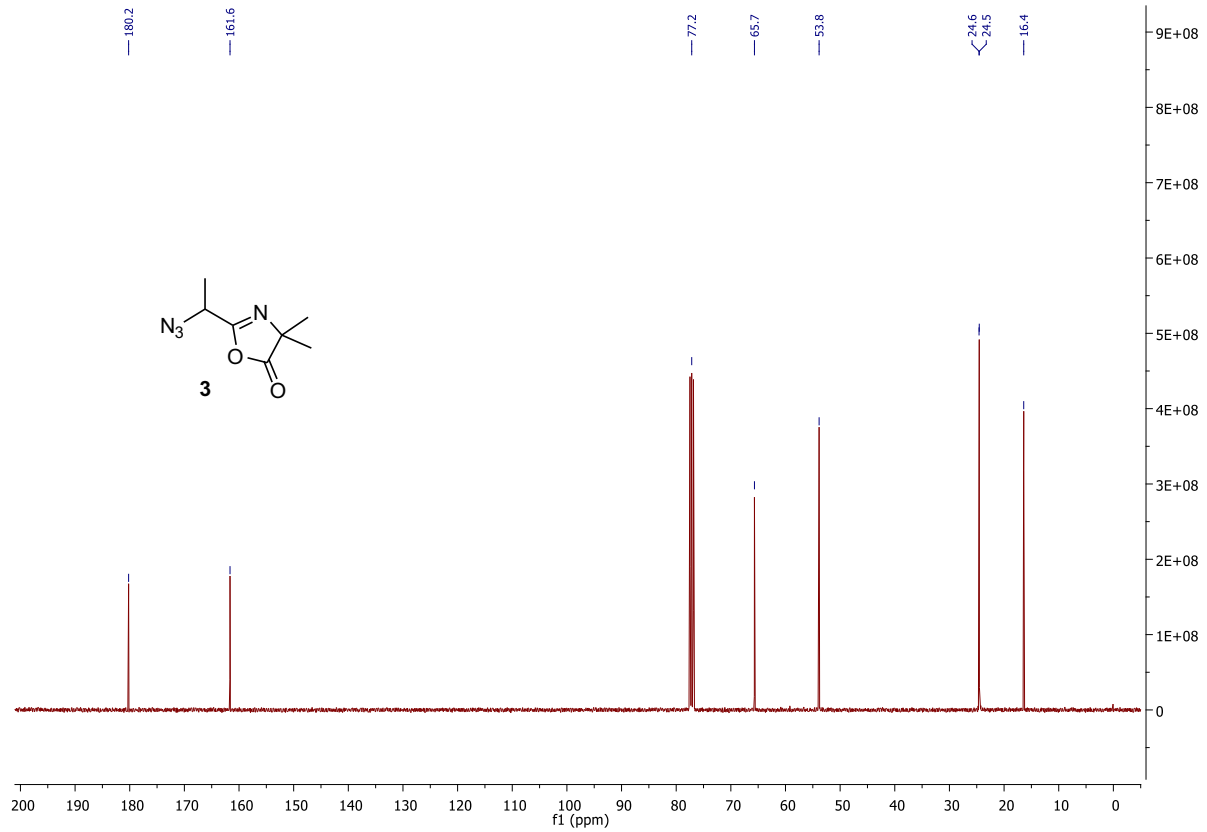


Figure 3: ^{1}H NMR spectrum of 1-(4,4-dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl hex-5-ynoate 4

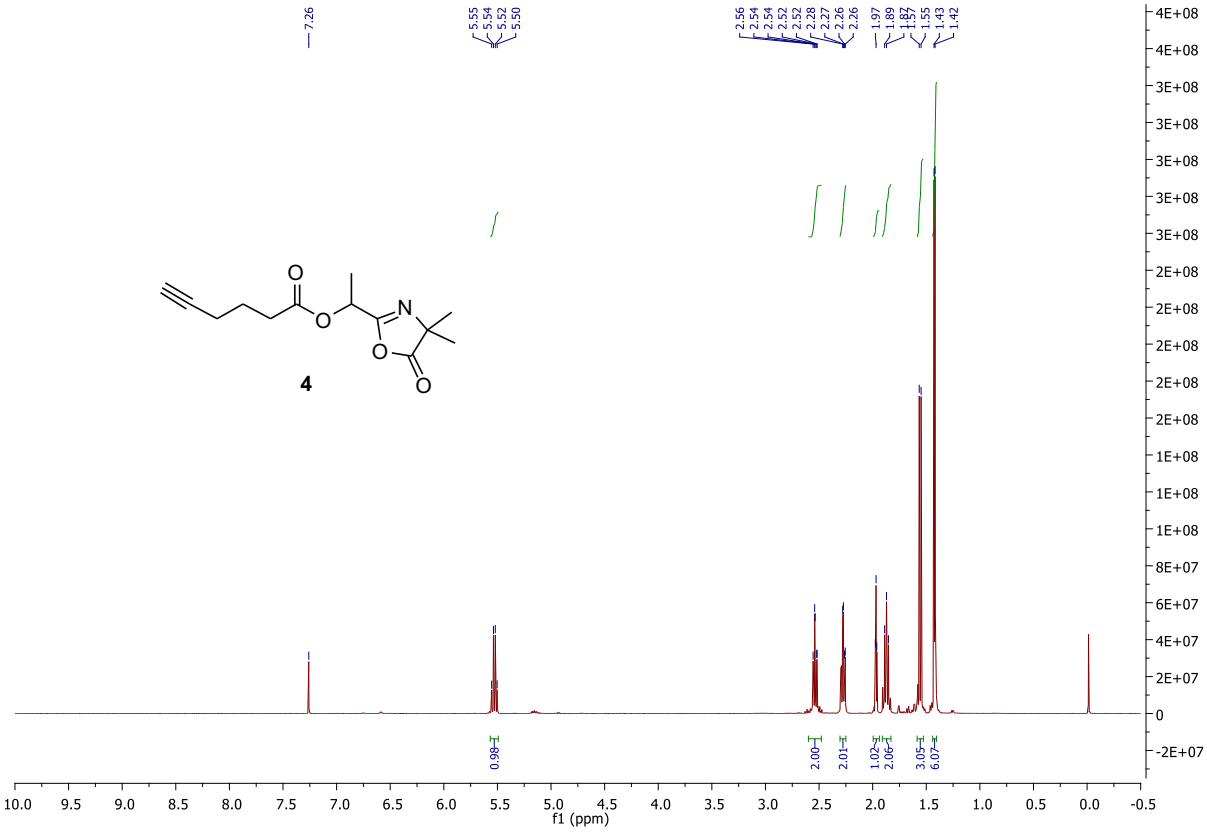


Figure 4: ^{13}C NMR spectrum of 1-(4,4-dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl hex-5-ynoate 4

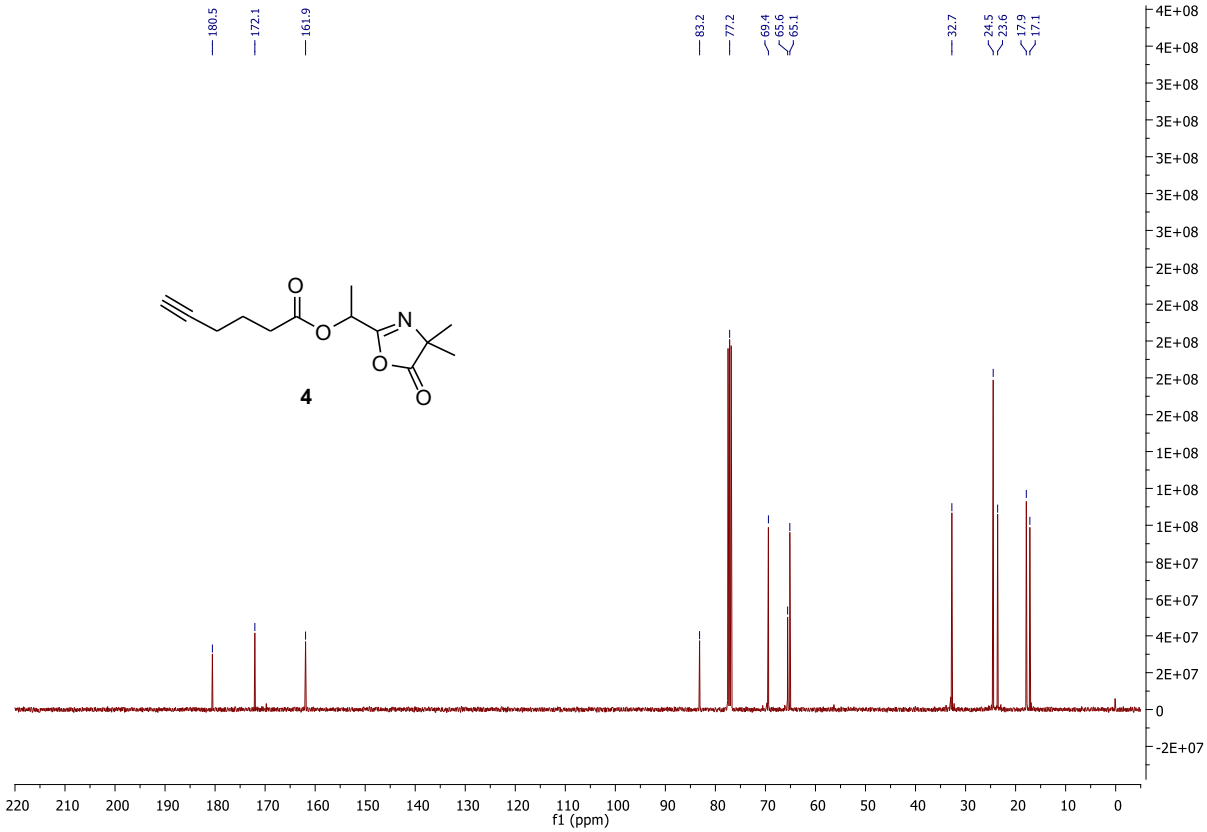


Figure 5: ^{1}H NMR spectrum of 1-(4,4-dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl propiolate 5

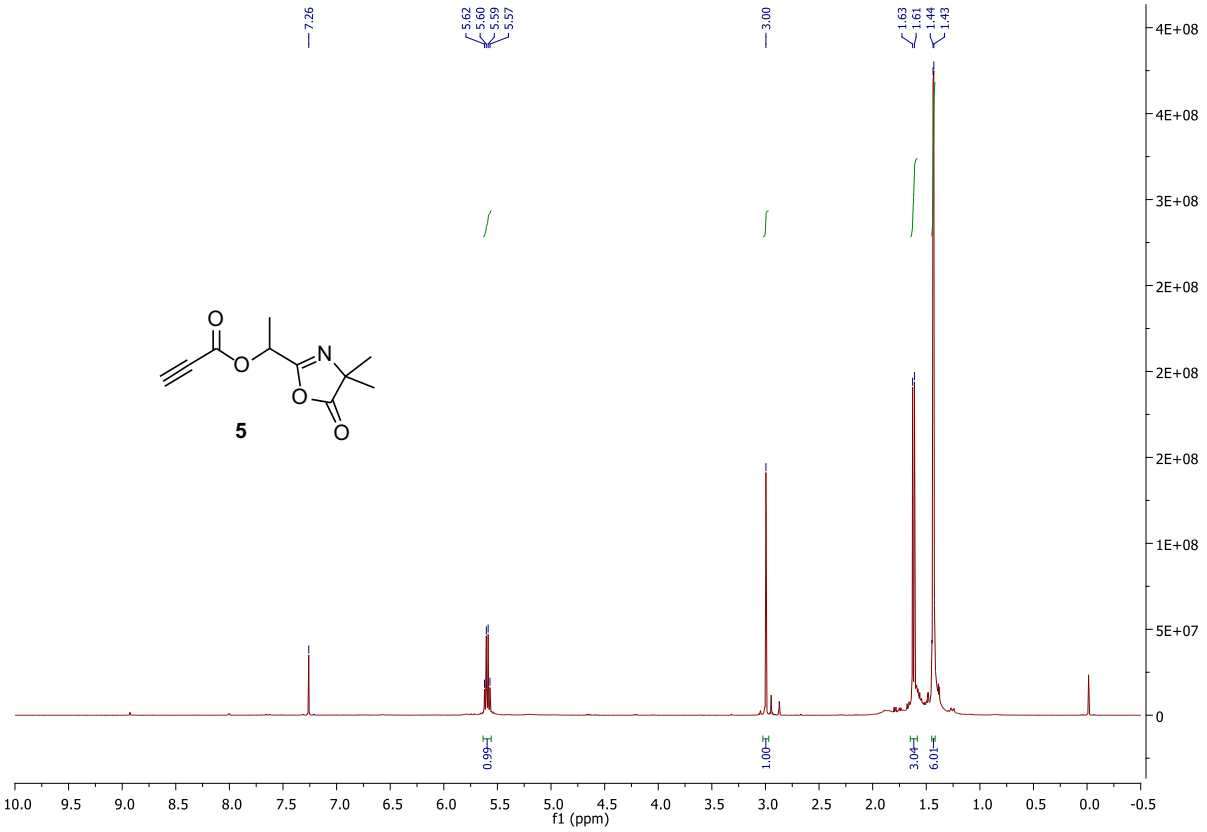


Figure 6: ^{13}C NMR spectrum of 1-(4,4-dimethyl-5-oxo-4,5-dihydrooxazol-2-yl)ethyl propiolate 5

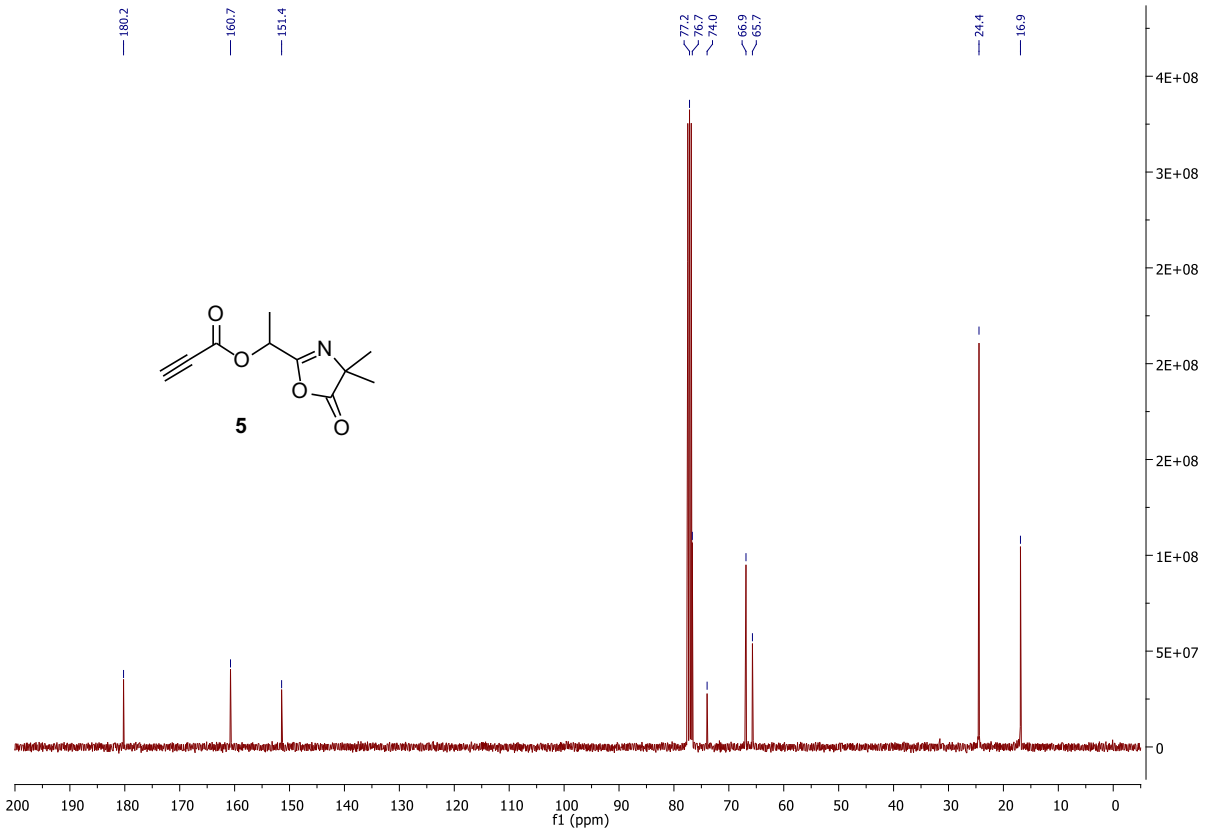


Figure 7: ^{1}H NMR spectrum of Azl-DBCO 6

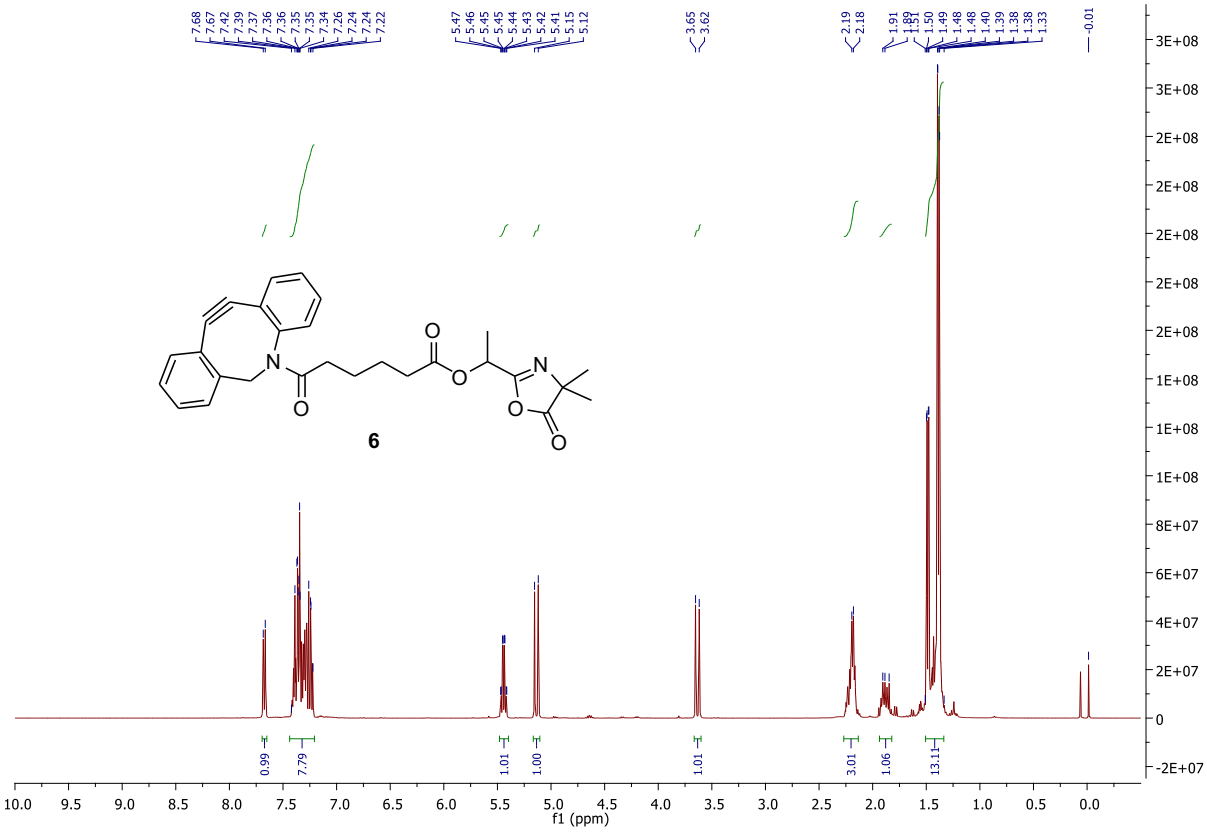


Figure 8: ^{13}C NMR spectrum of Azl-DBCO 6

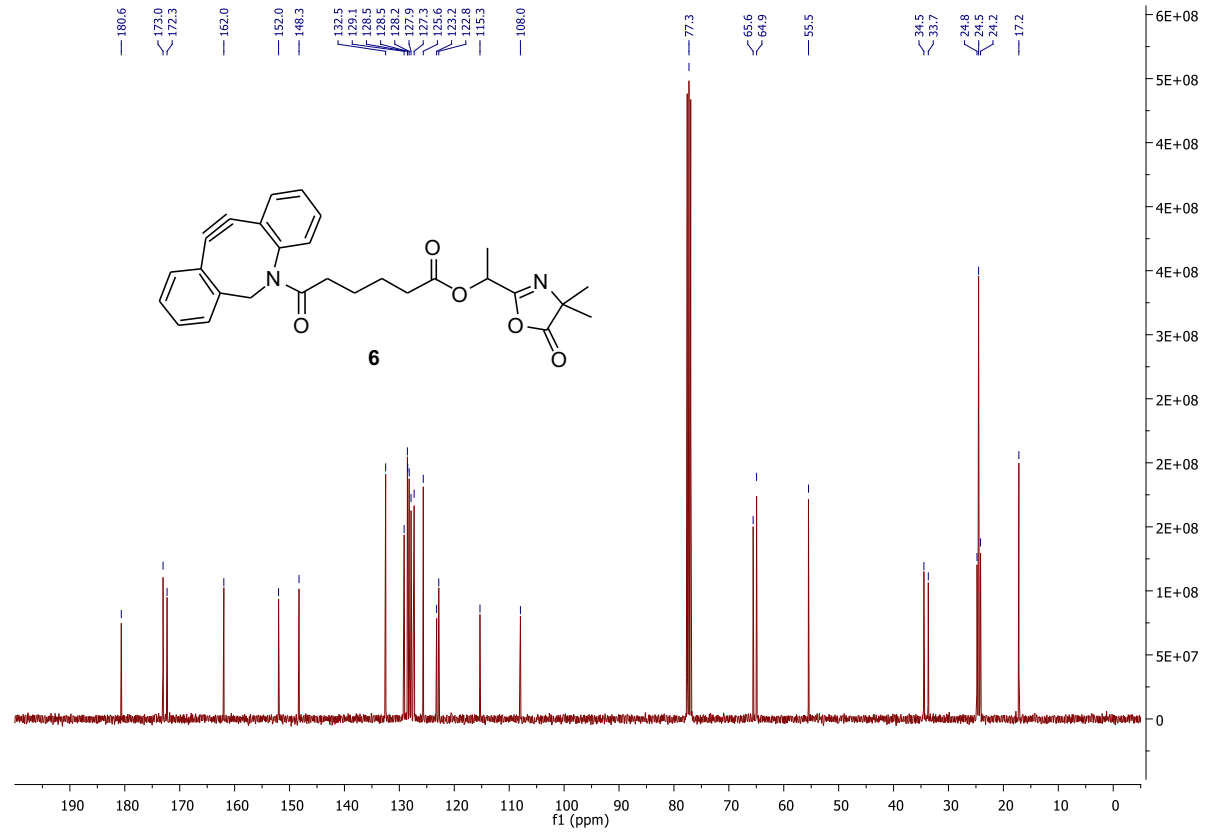


Figure 9: ^{1}H NMR spectrum of azlactone 7

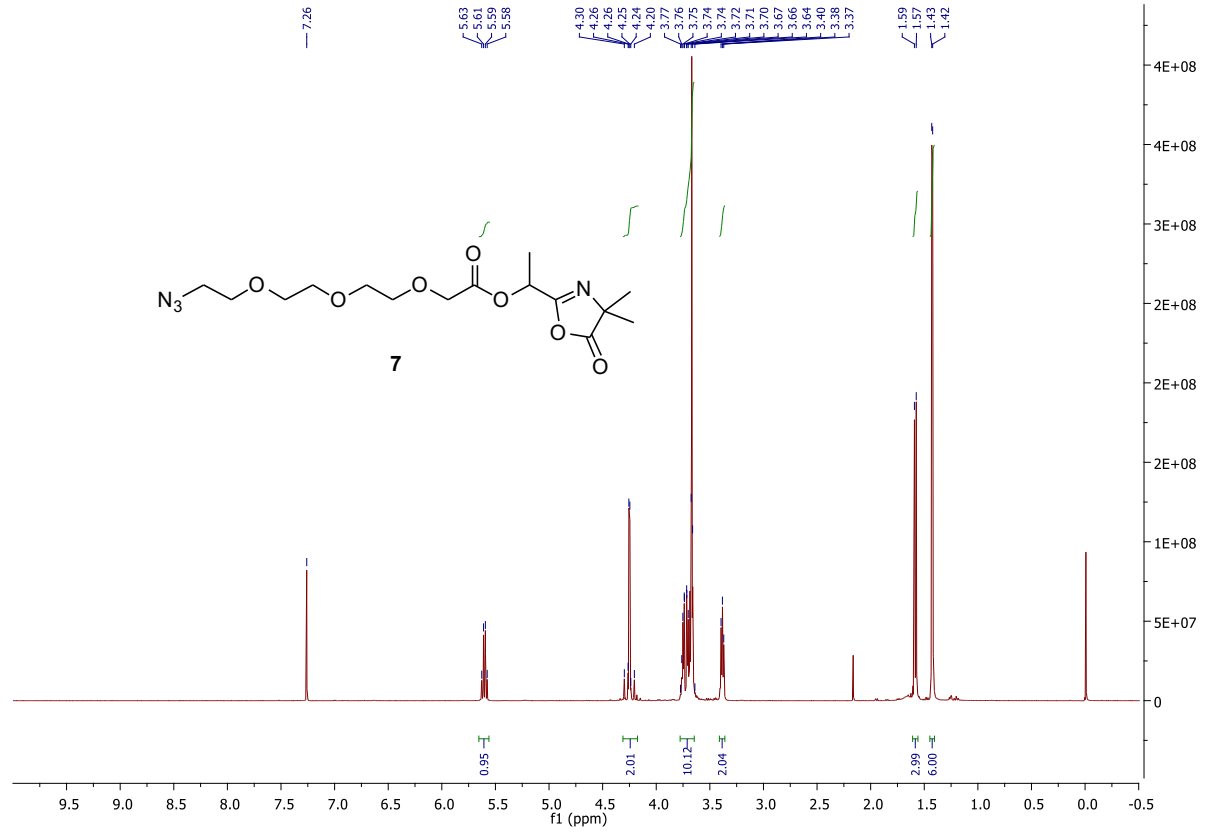


Figure 10: ^{13}C NMR spectrum of azlactone 7

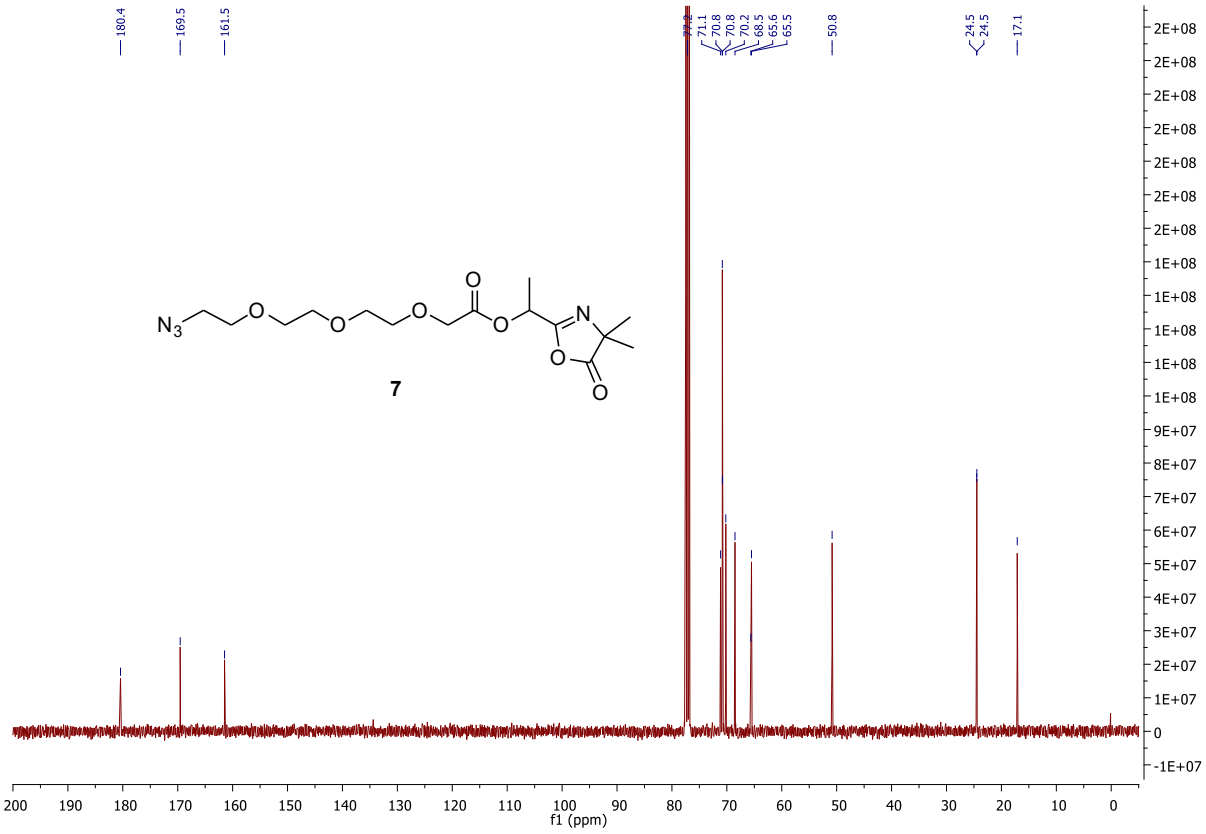


Figure 11: ^{1}H NMR spectrum of azlactone 8

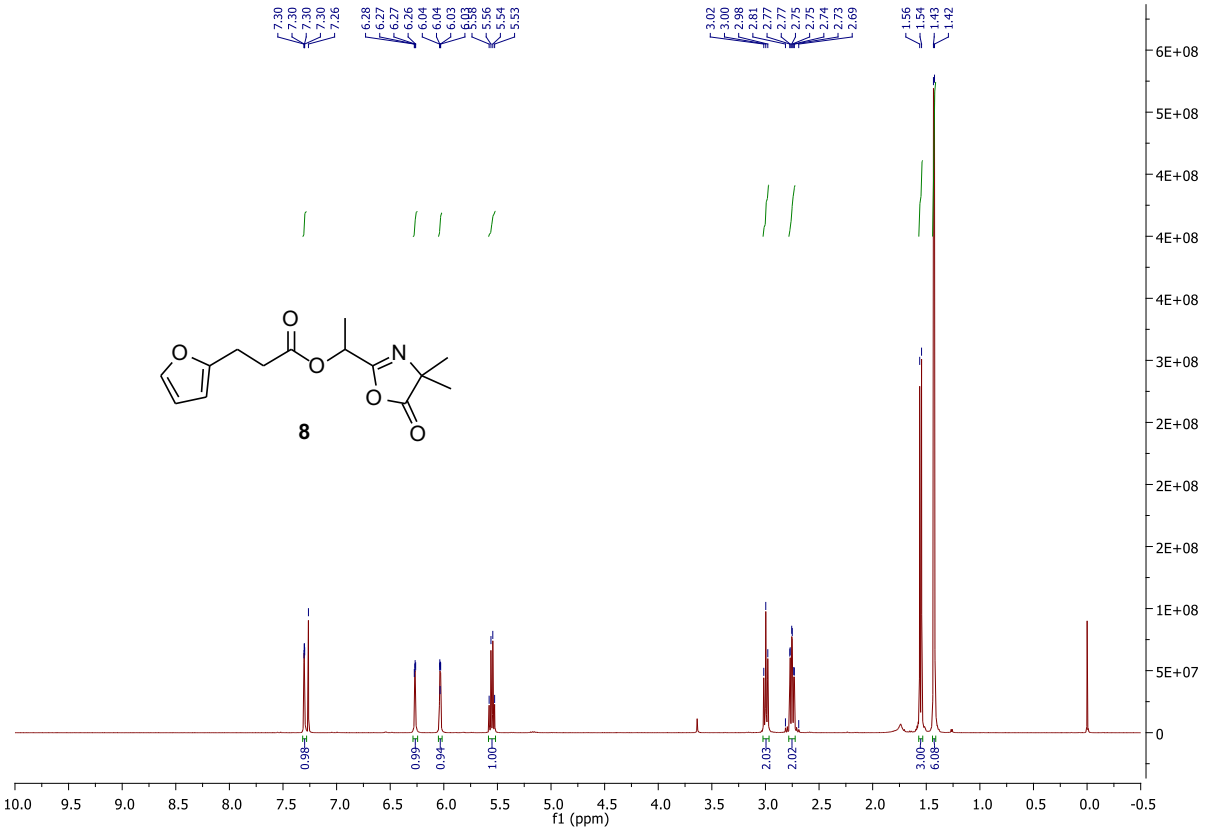


Figure 12: ^{13}C NMR spectrum of azlactone 8

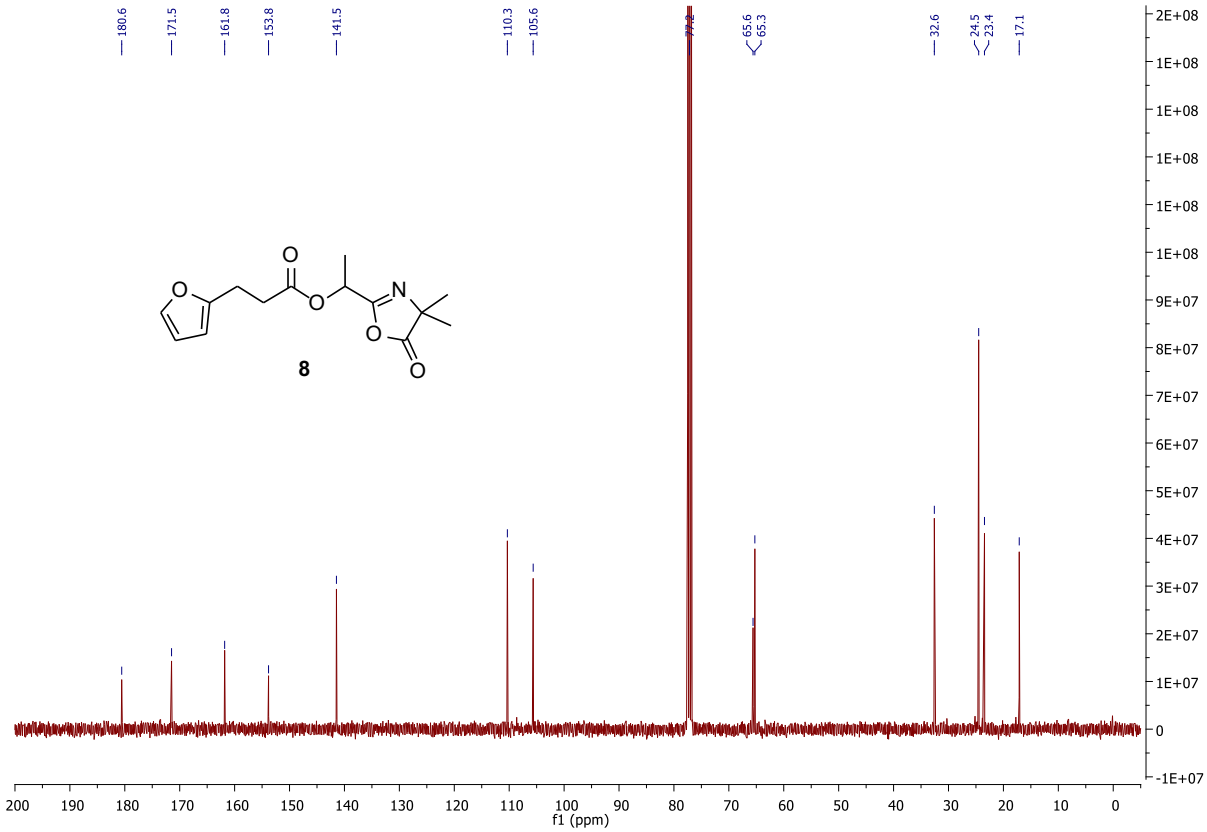


Figure 13: ^{1}H NMR spectrum of azlactone 9

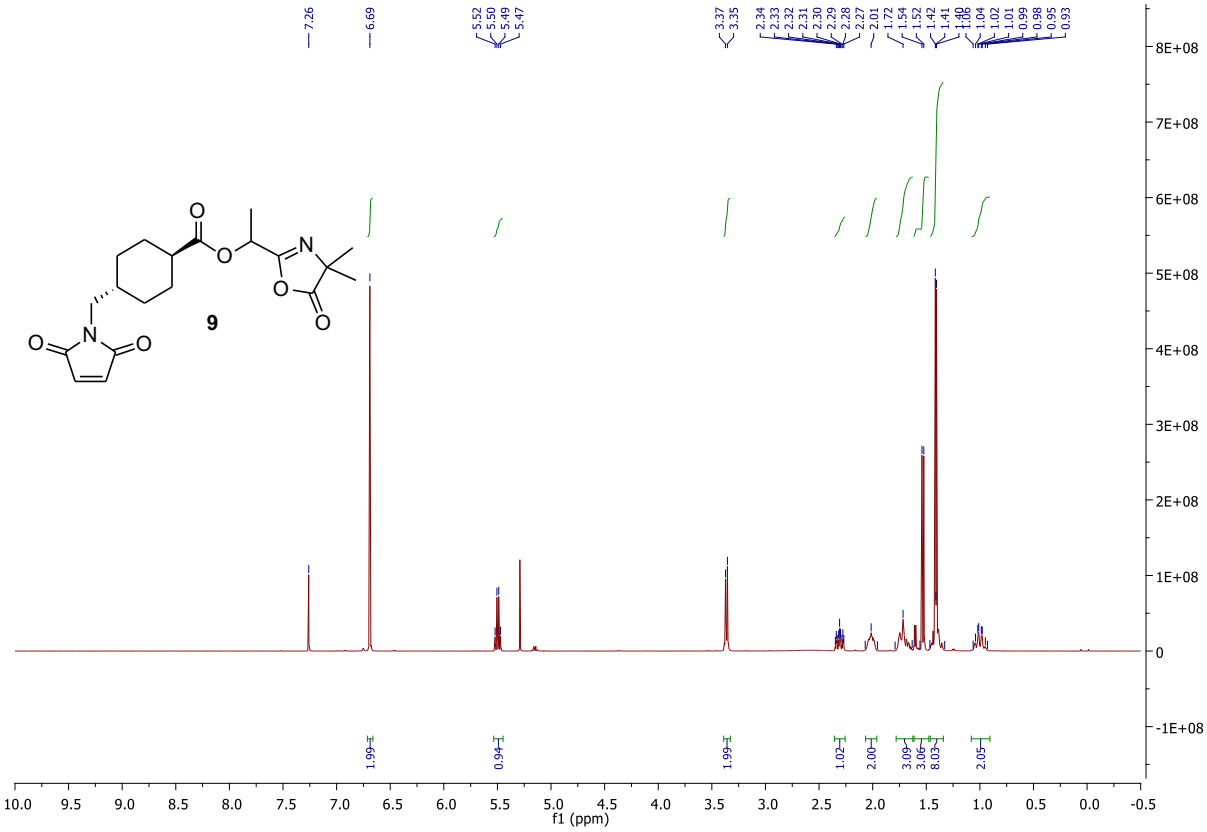


Figure 14: ^{13}C NMR spectrum of azlactone 9

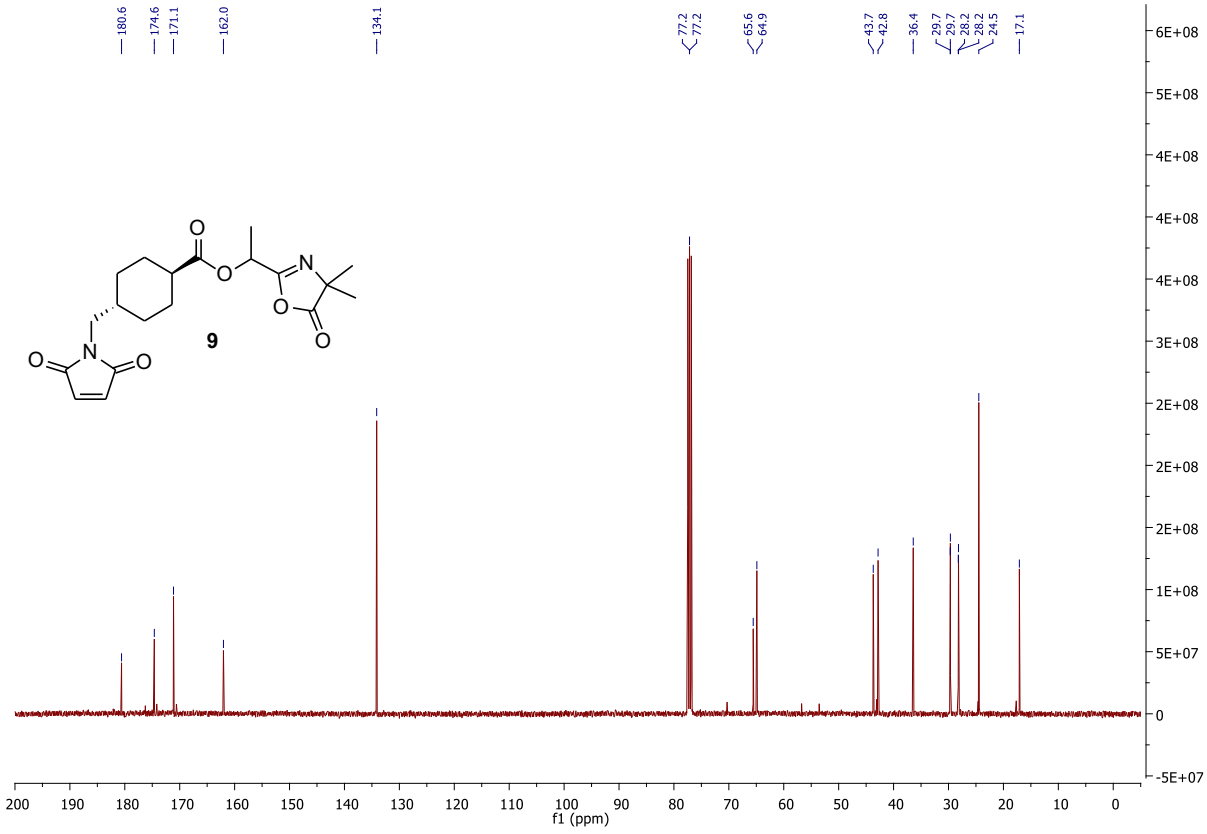


Figure 15: ^{1}H NMR spectrum of azlactone 10

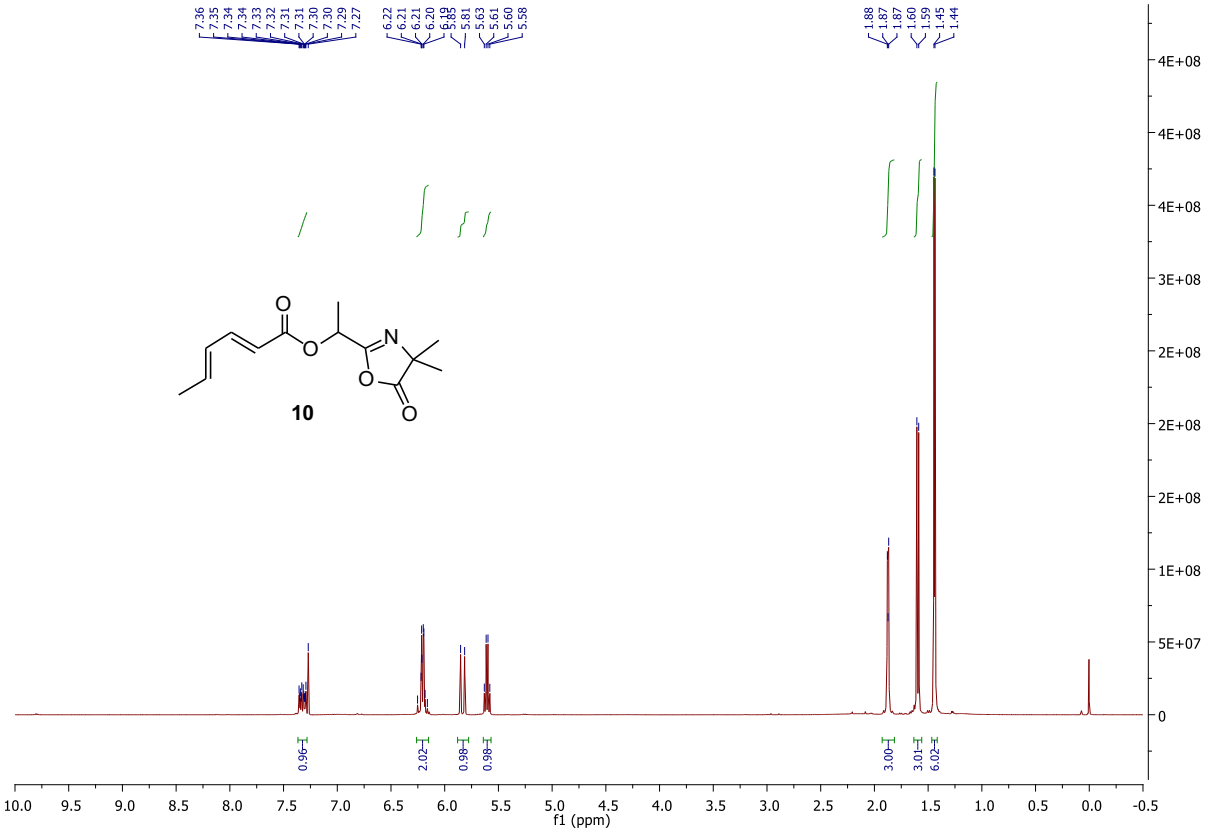


Figure 16: ^{13}C NMR spectrum of azlactone 10

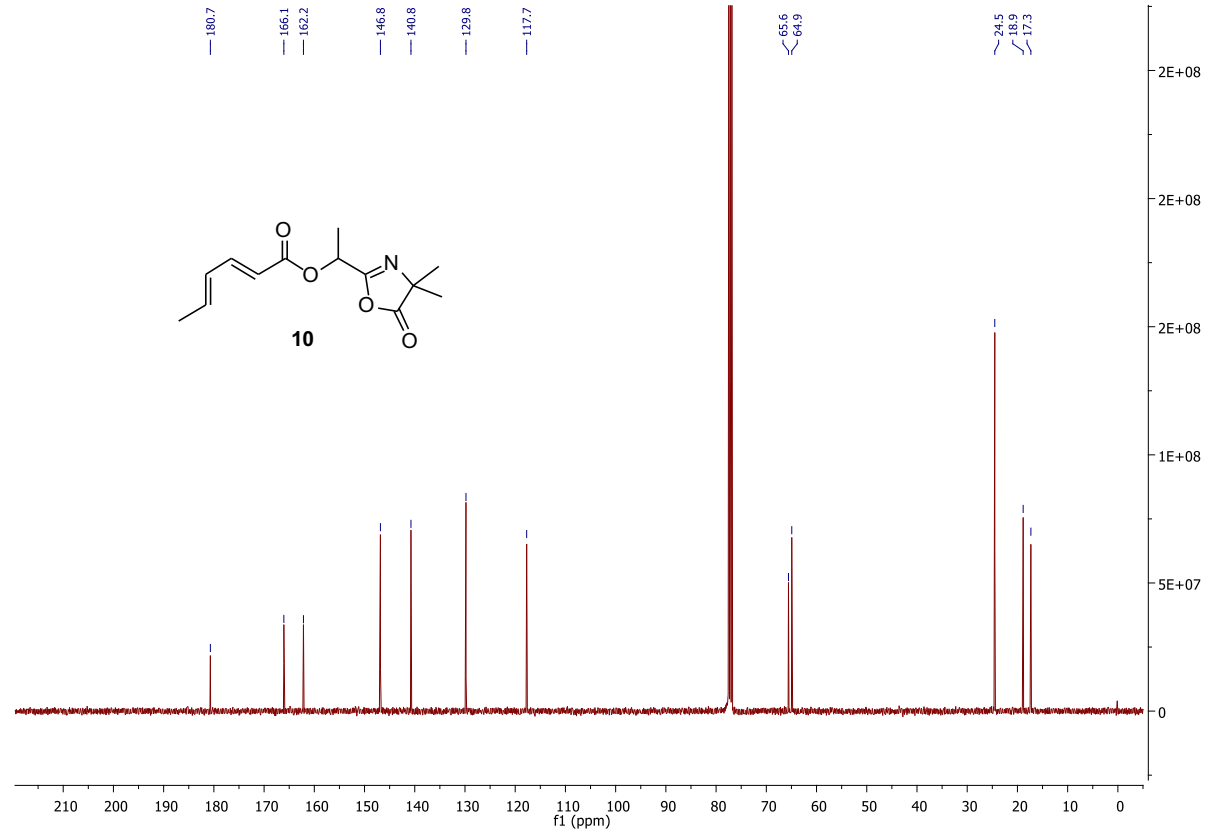


Figure 17: ^{1}H NMR spectrum of reaction between azido-azlactone 3 with allylamine after 16 h at rt

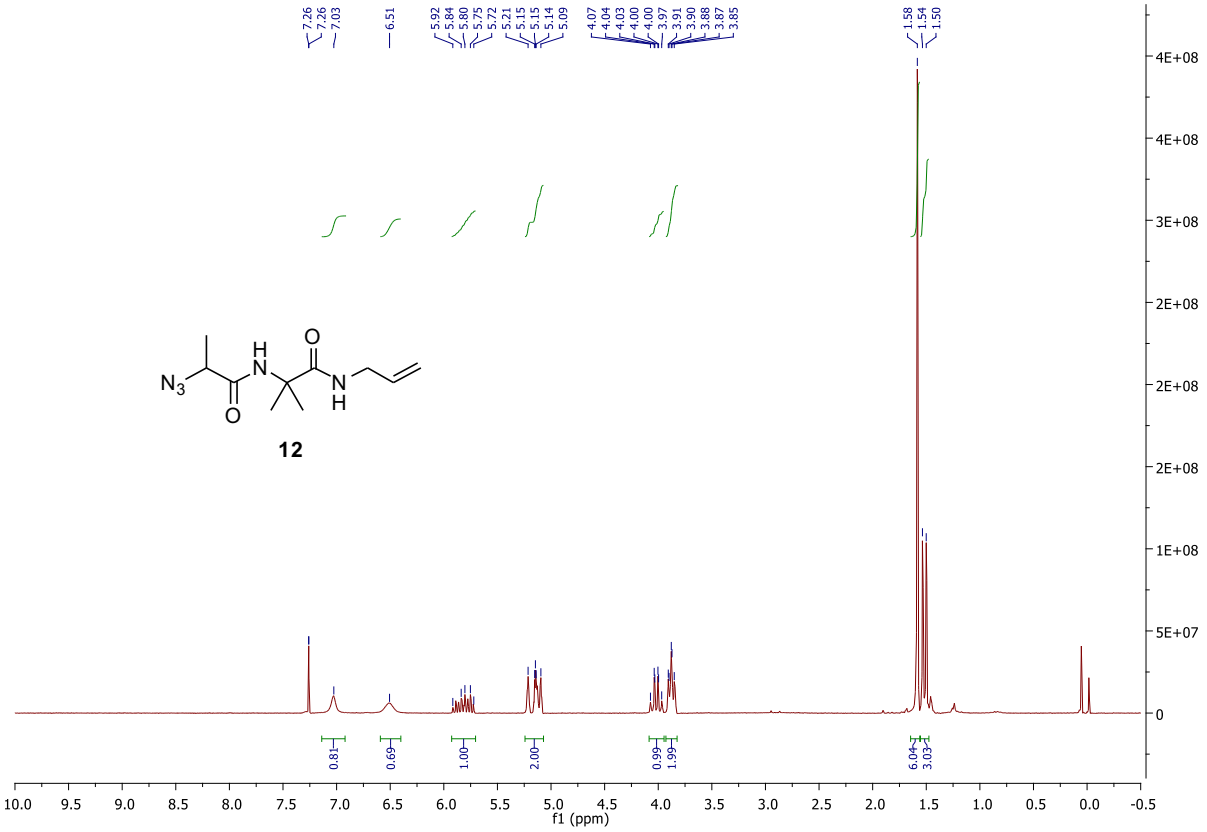


Figure 18: ^{1}H NMR spectrum of reaction between azido-azlactone 3 with propargylamine after 16 h at rt

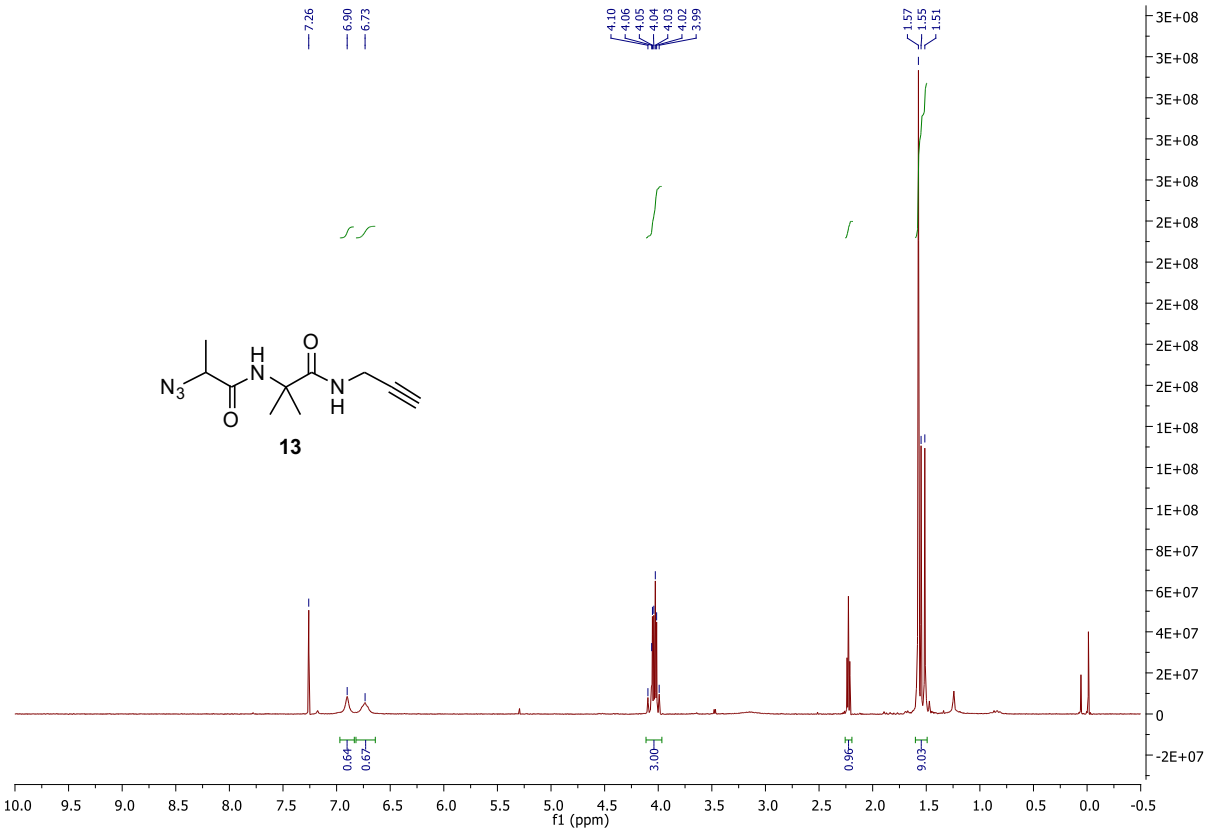


Figure 19: ^{1}H NMR spectrum of reaction between azido-azlactone 3 with benzylamine after 16 h at rt

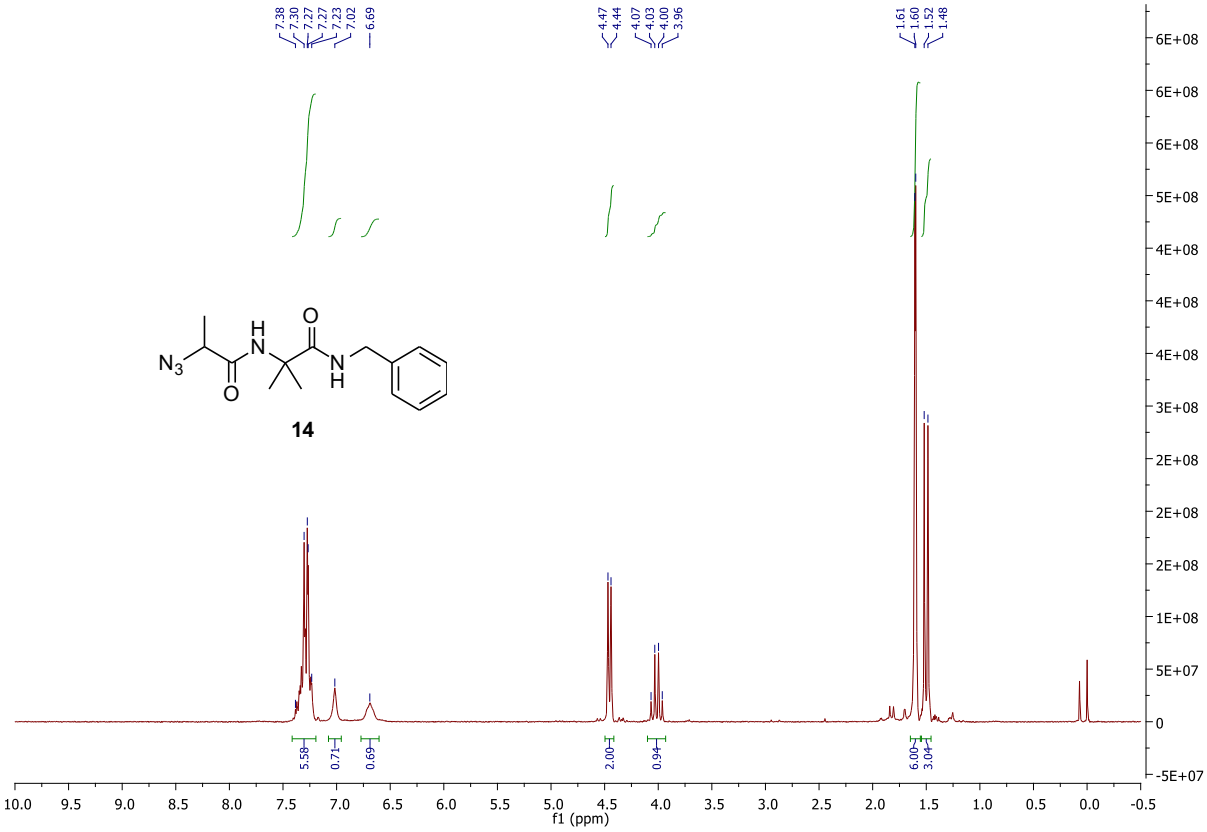


Figure 20: ^{1}H NMR spectrum of reaction between azido-azlactone 3 with diethylamine after 16 h at rt

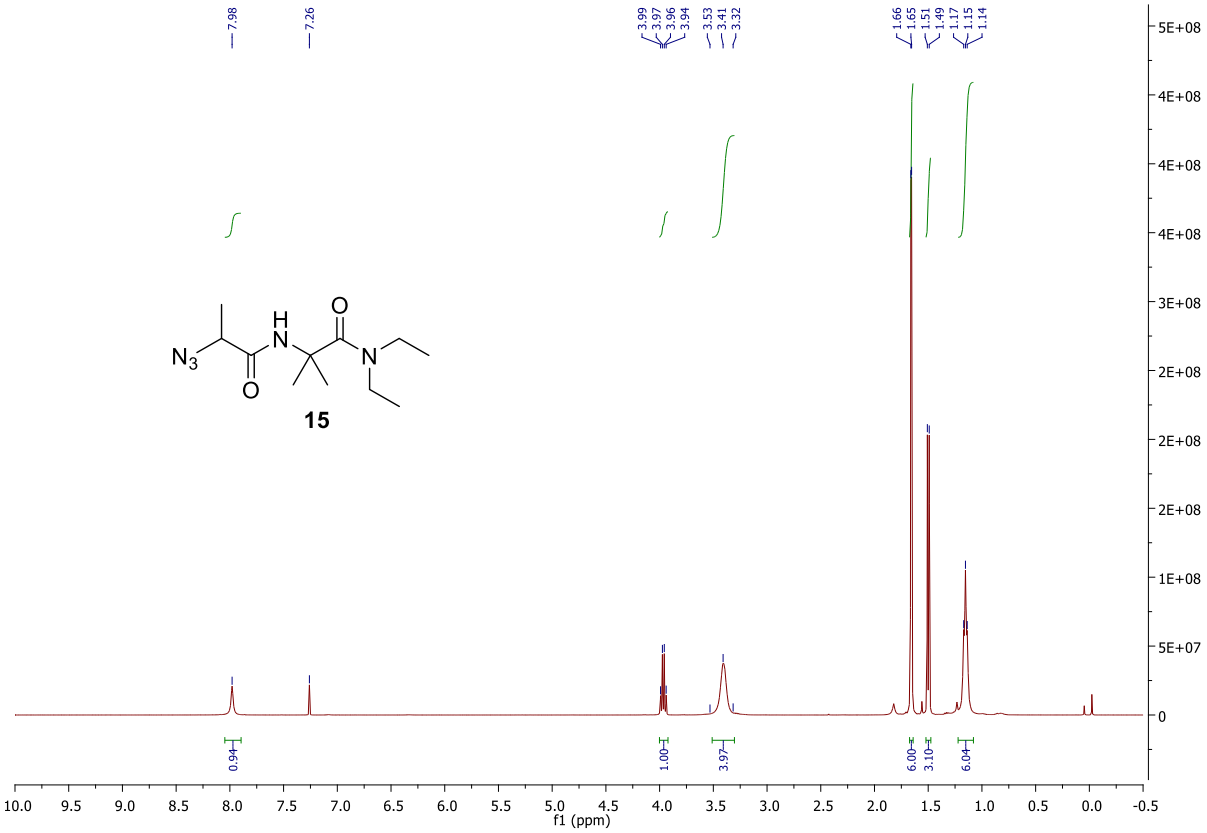


Figure 21: ^{1}H NMR spectrum of POE 16

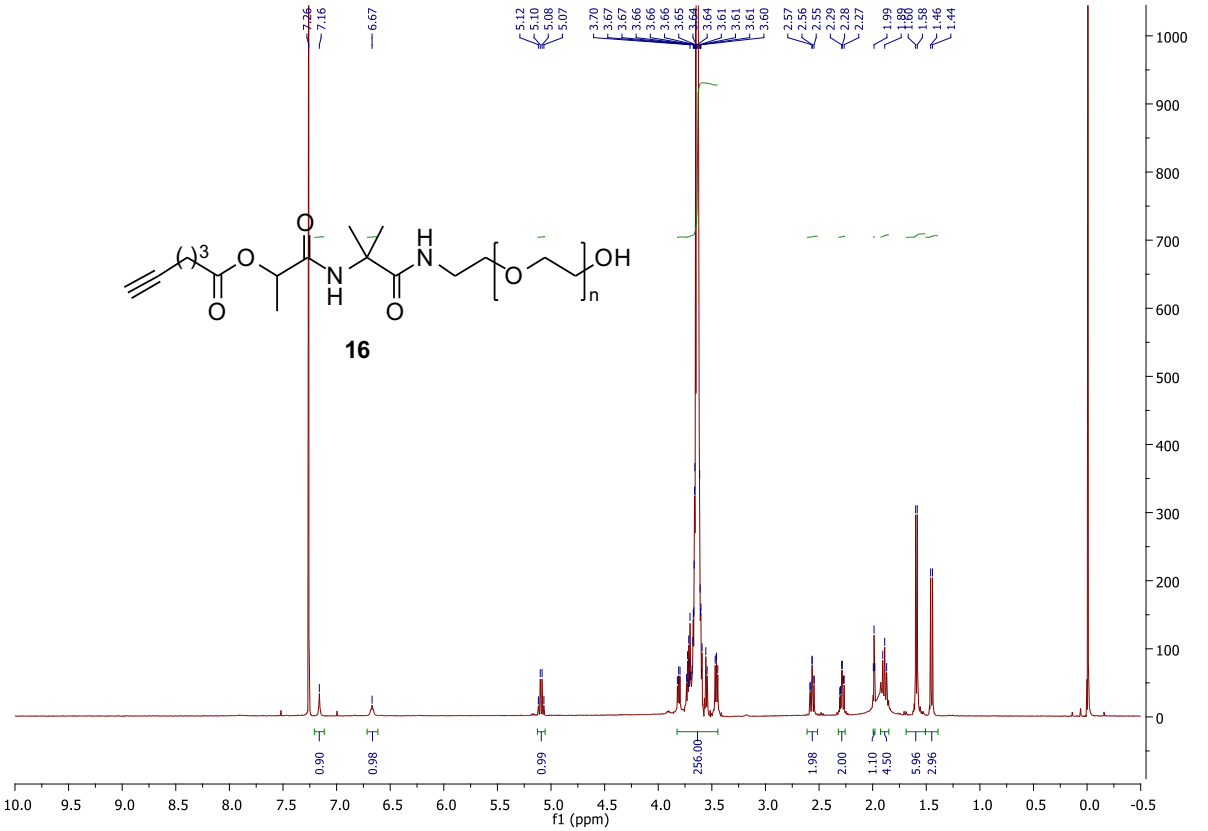


Figure 22: ^{13}C NMR spectrum of POE16

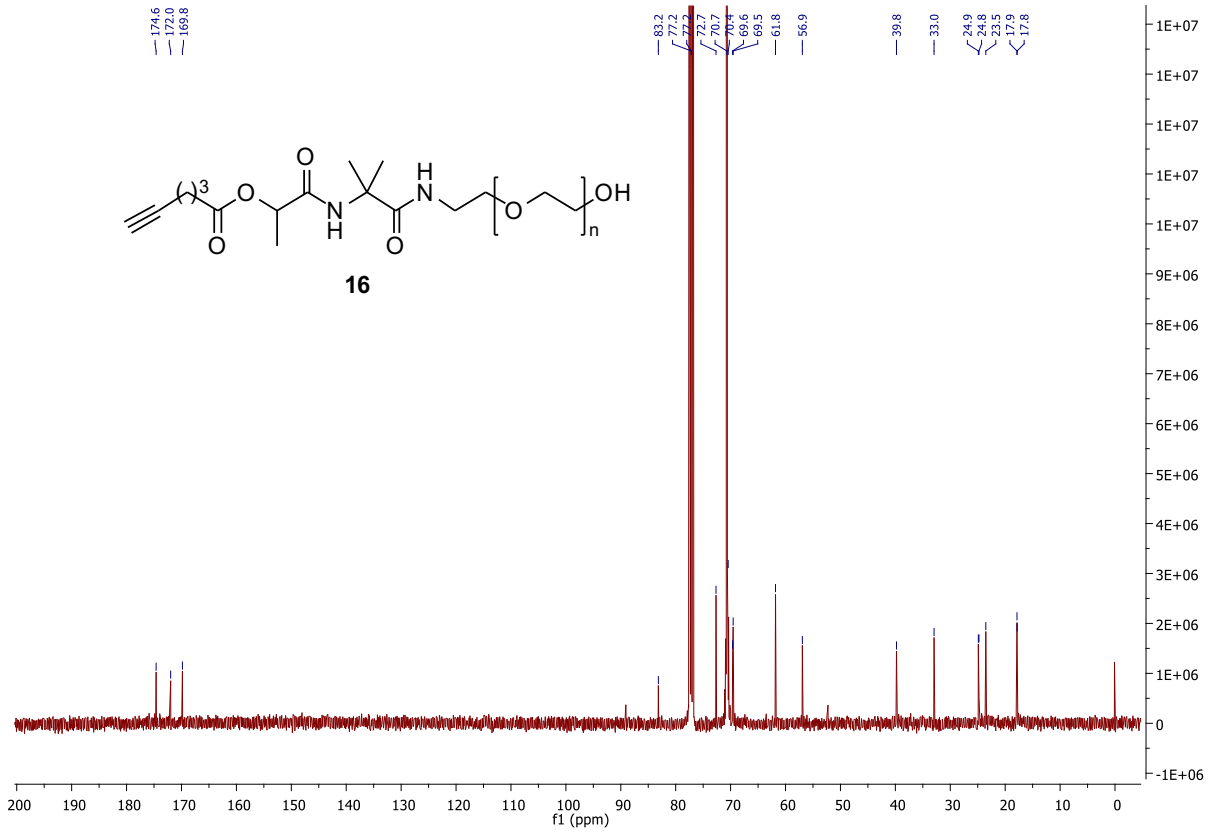


Figure 23: ^{1}H NMR spectrum of DA reaction between 10 and *N*-methylmaleimide (initial time)

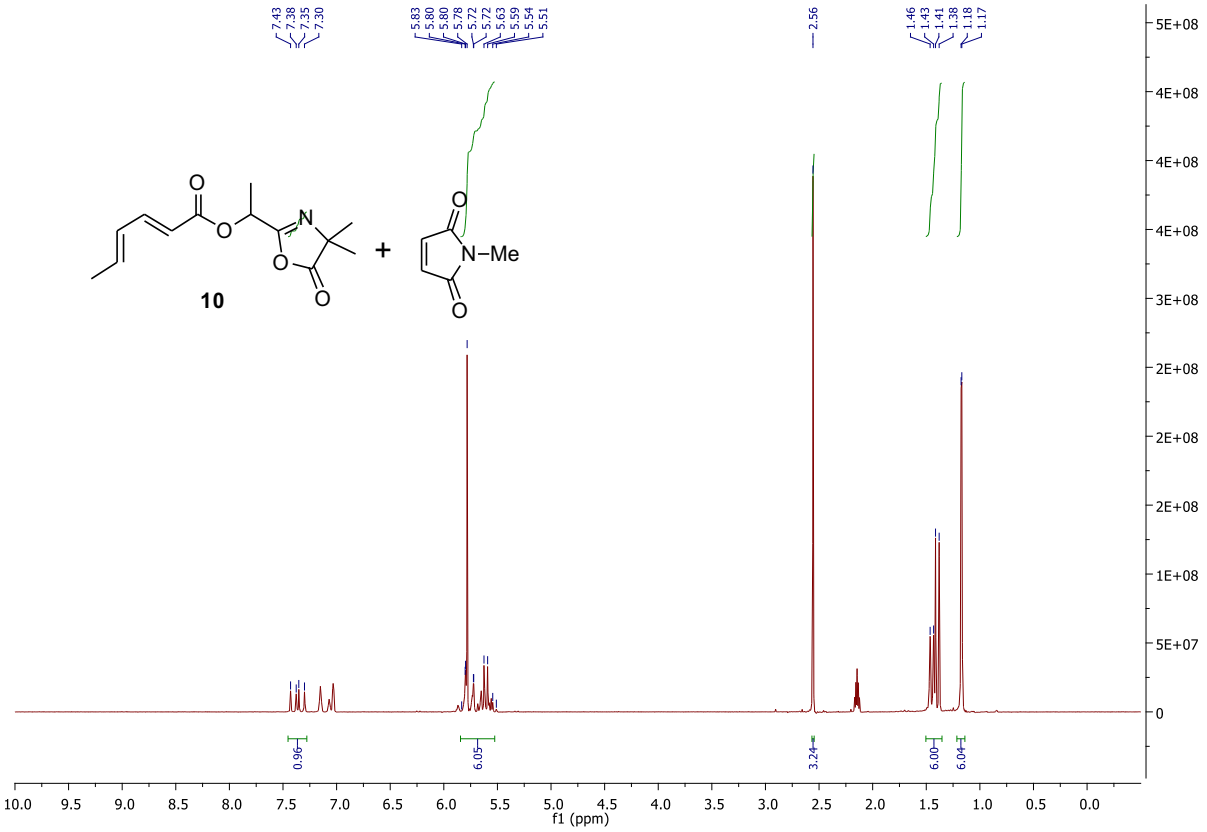


Figure 24: ^{1}H NMR spectrum of DA reaction between 10 and *N*-methylmaleimide (final time)

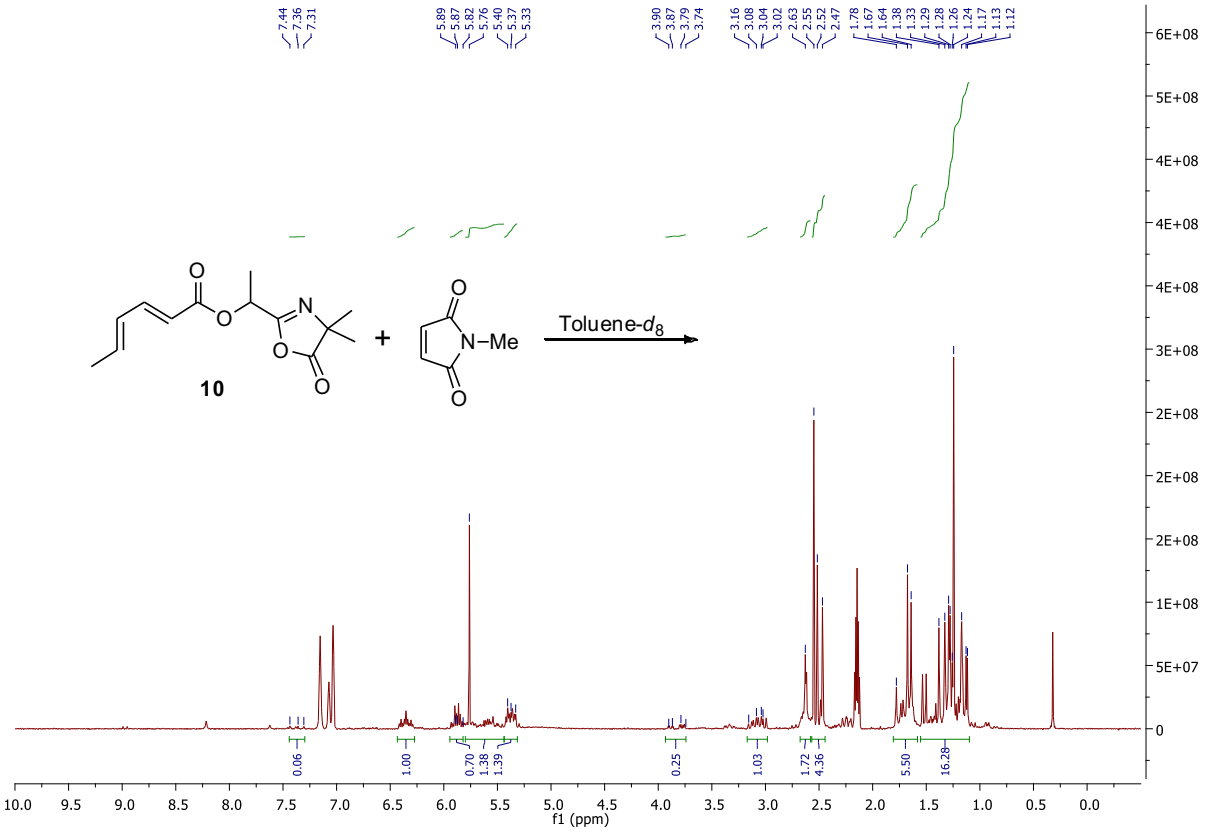


Figure 25: ^{1}H NMR spectrum of DA reaction between 10 and *N*-phenylmaleimide (t=5 days)

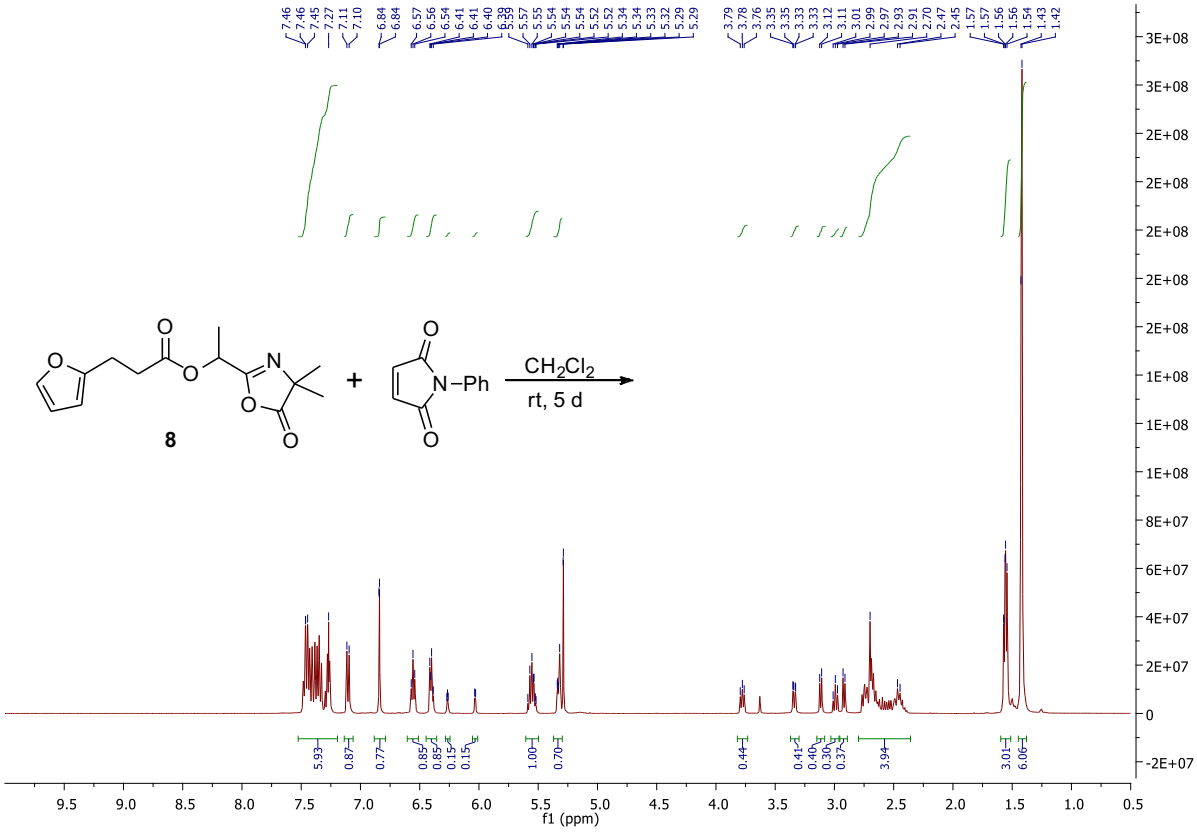


Figure 26: ^{1}H NMR spectrum of DA reaction

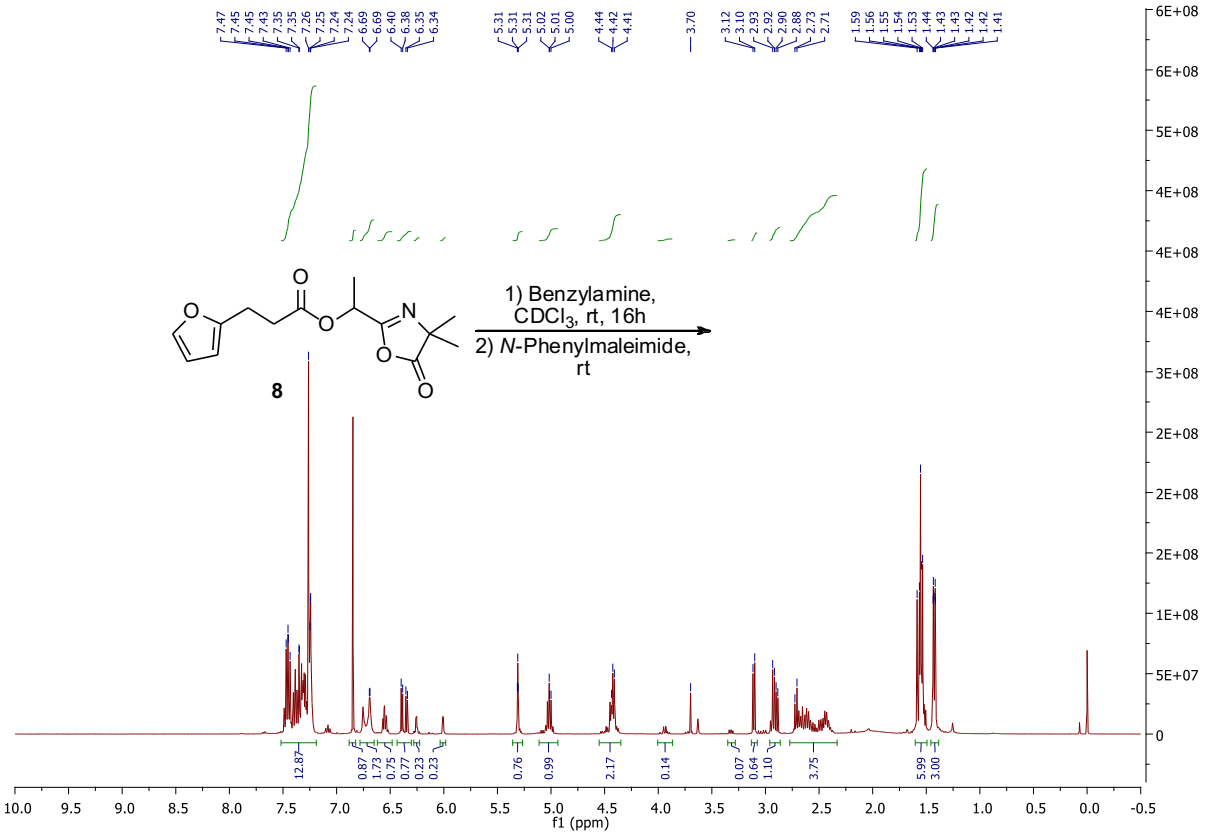


Figure 27: ^{1}H NMR spectrum of triazole 21 (2 isomers)

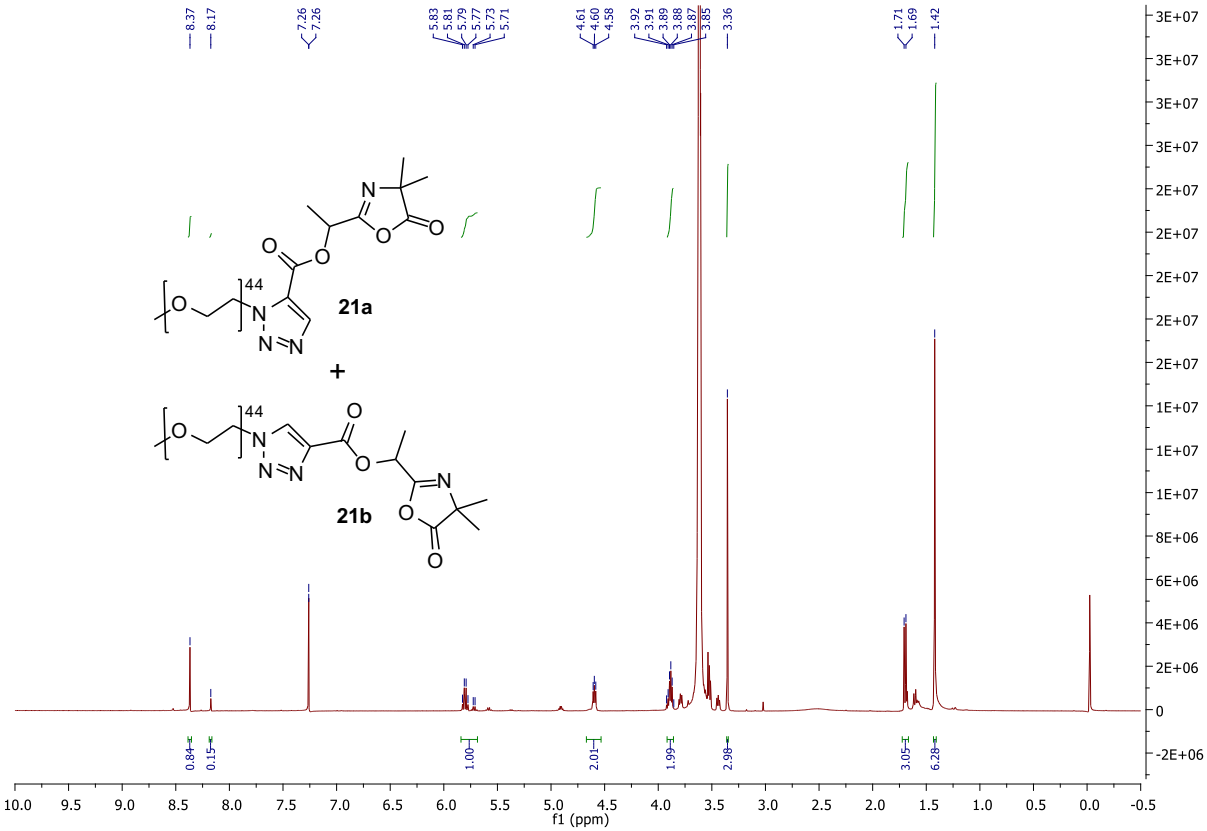


Figure 28: ^{1}H NMR spectrum of 23

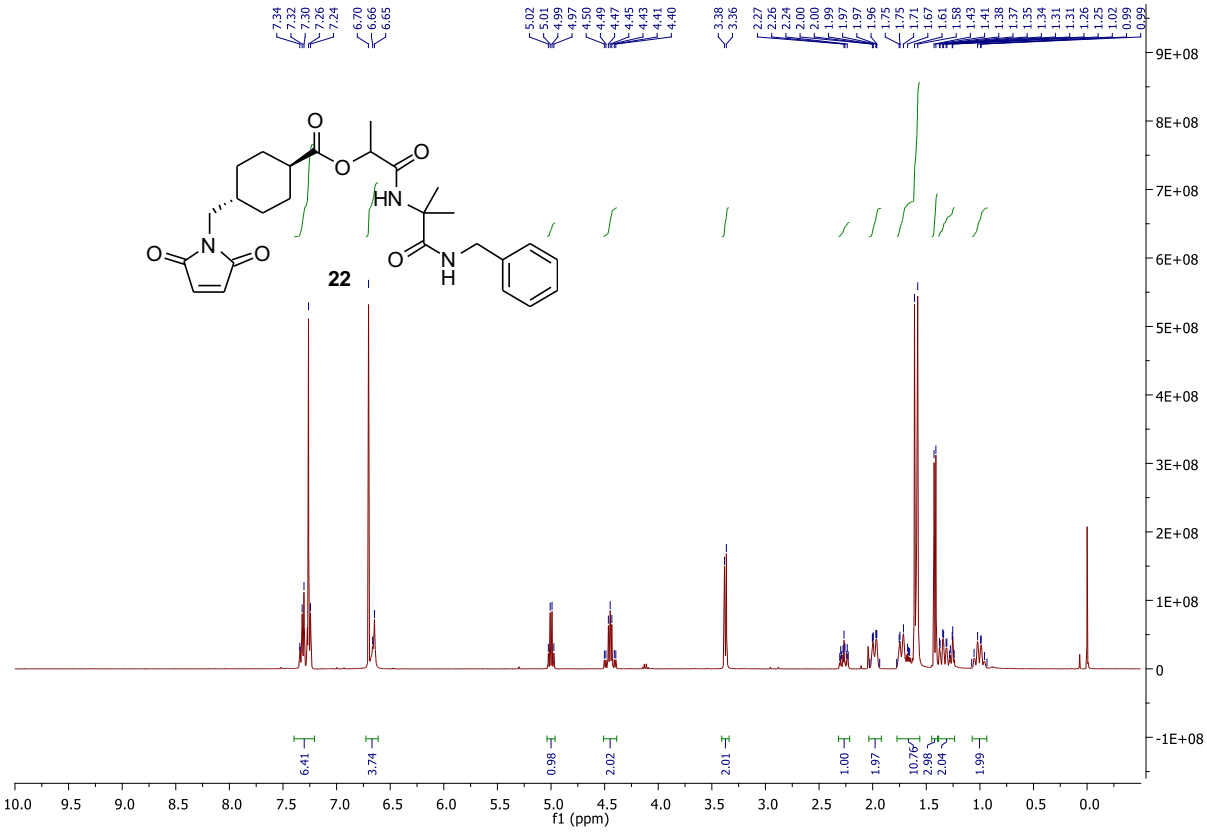


Figure 29: ^{13}C NMR spectrum of 23

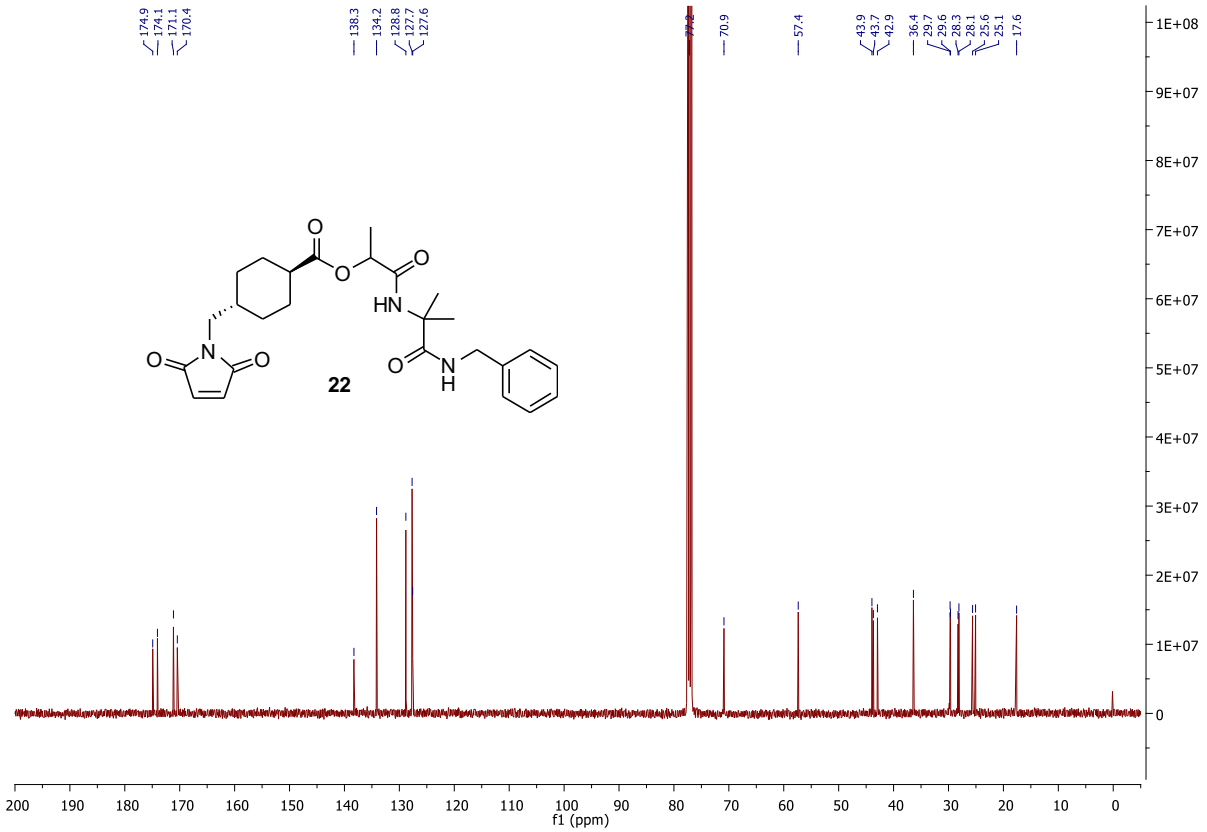


Figure 30: ^{1}H NMR spectrum of 24

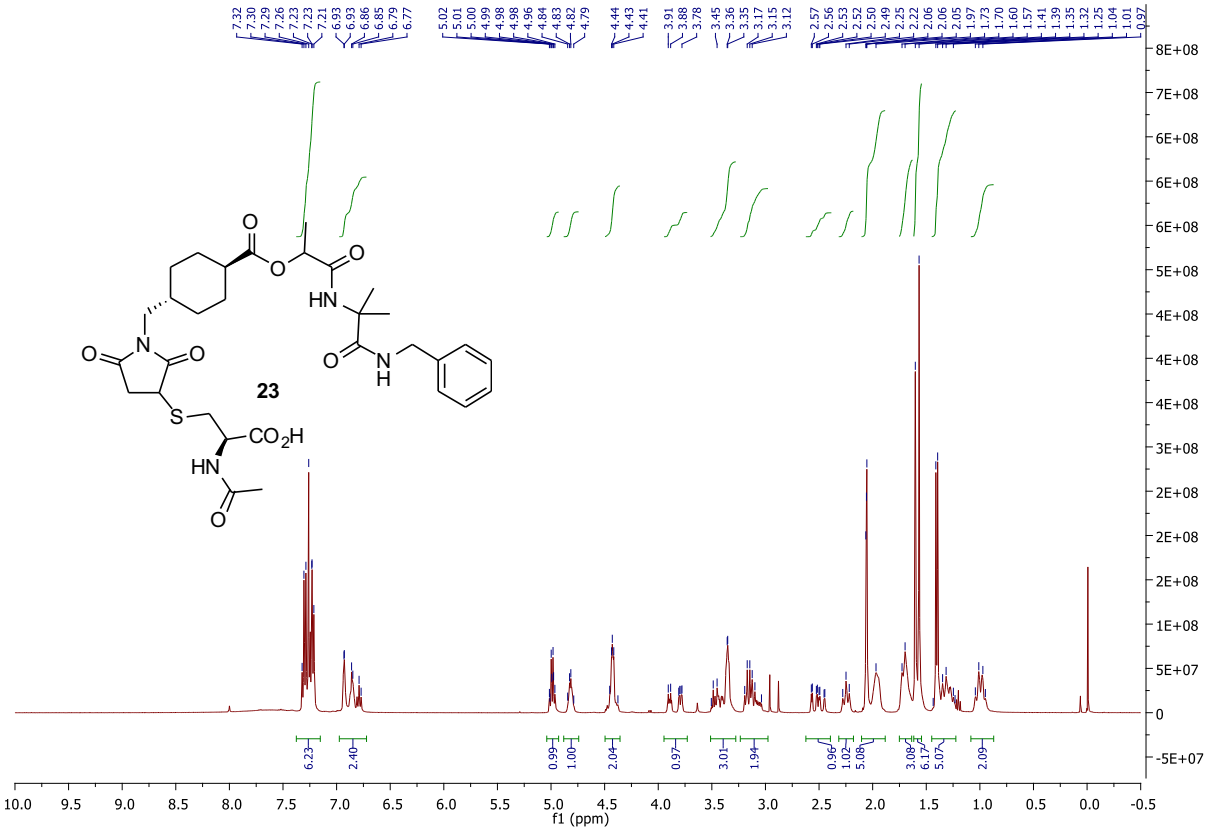


Figure 31: ^{1}H NMR spectrum of PEO 26

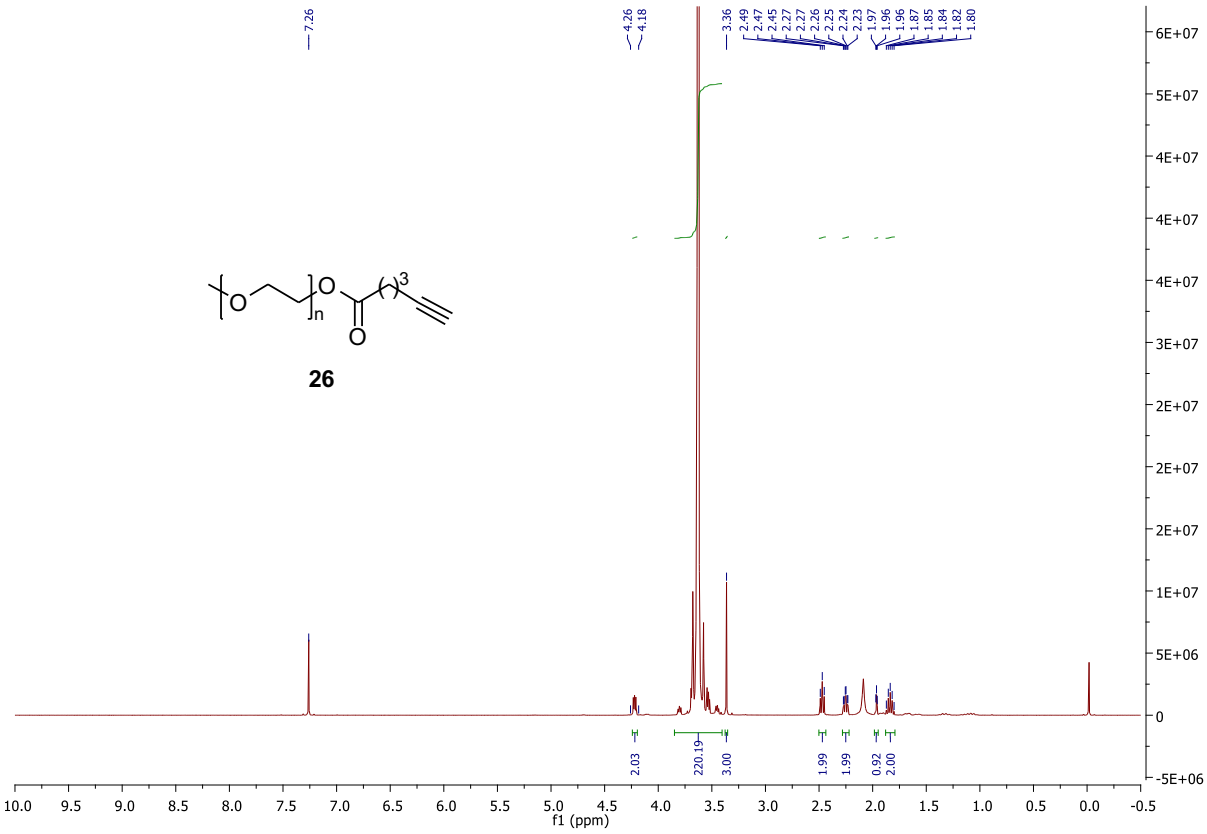


Figure 32: ^{1}H NMR spectrum of 28

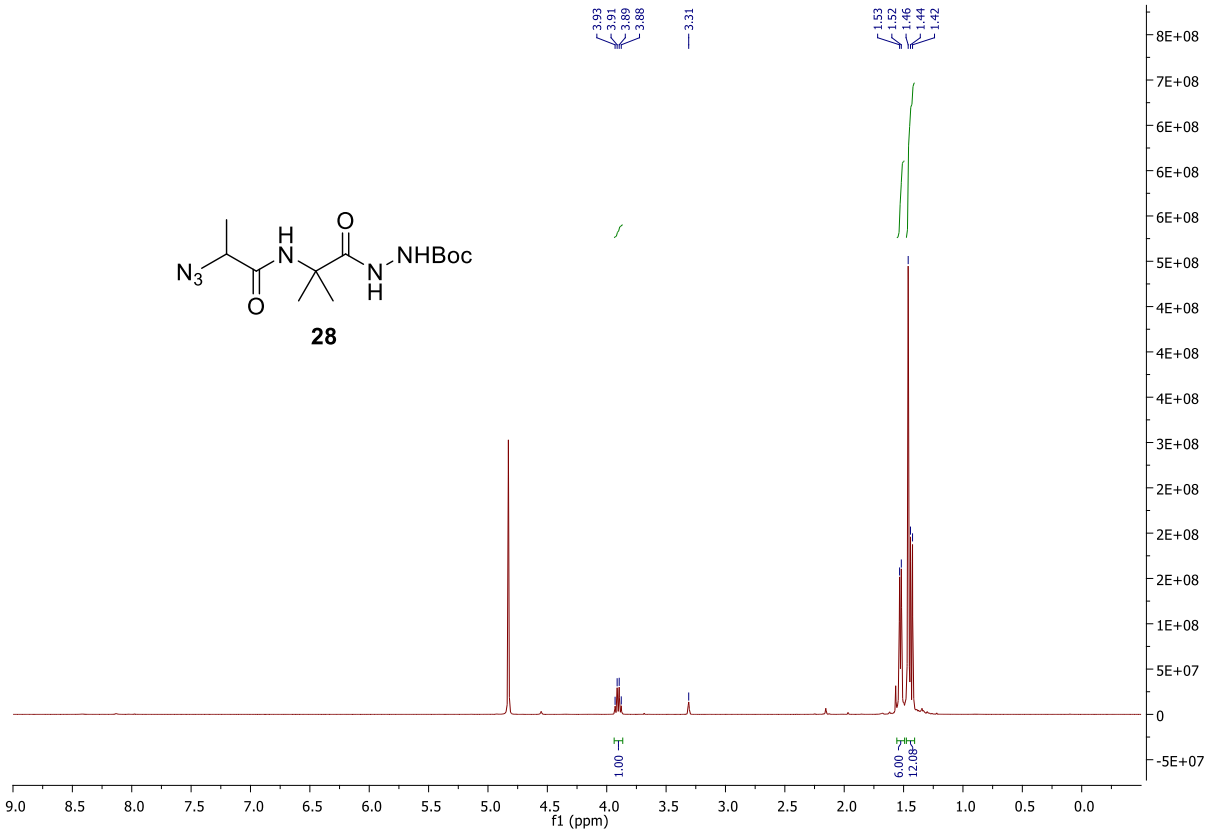


Figure 33: ^{13}C NMR spectrum of 28

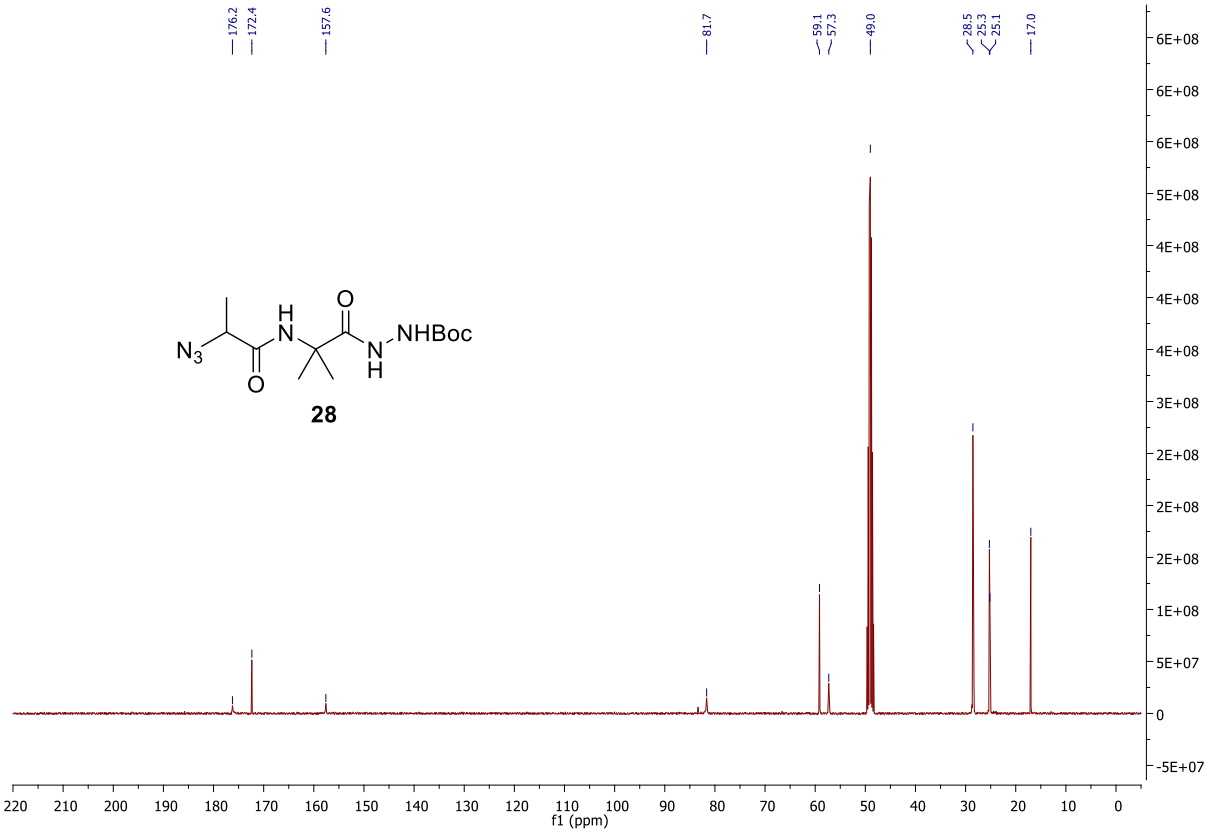


Figure 34: ^{1}H NMR spectrum of 29

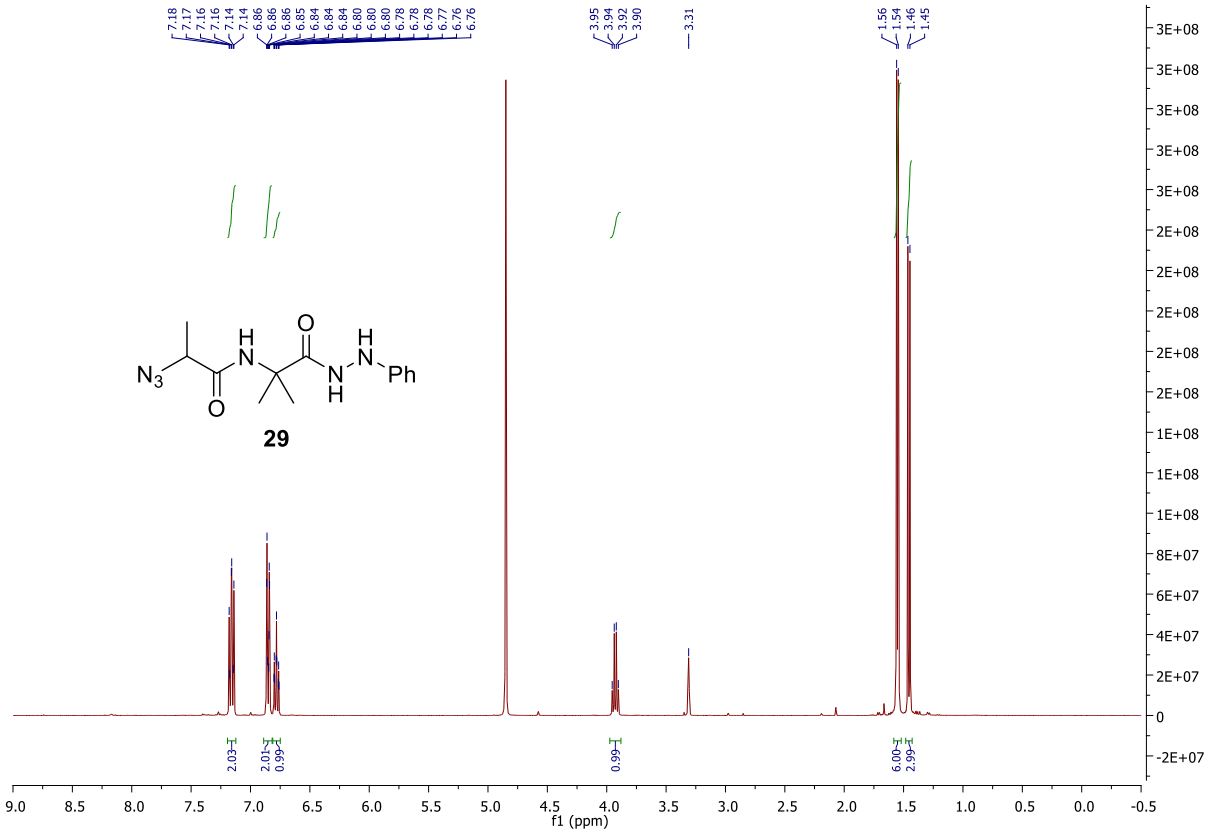


Figure 35: ^{13}C NMR spectrum of 29

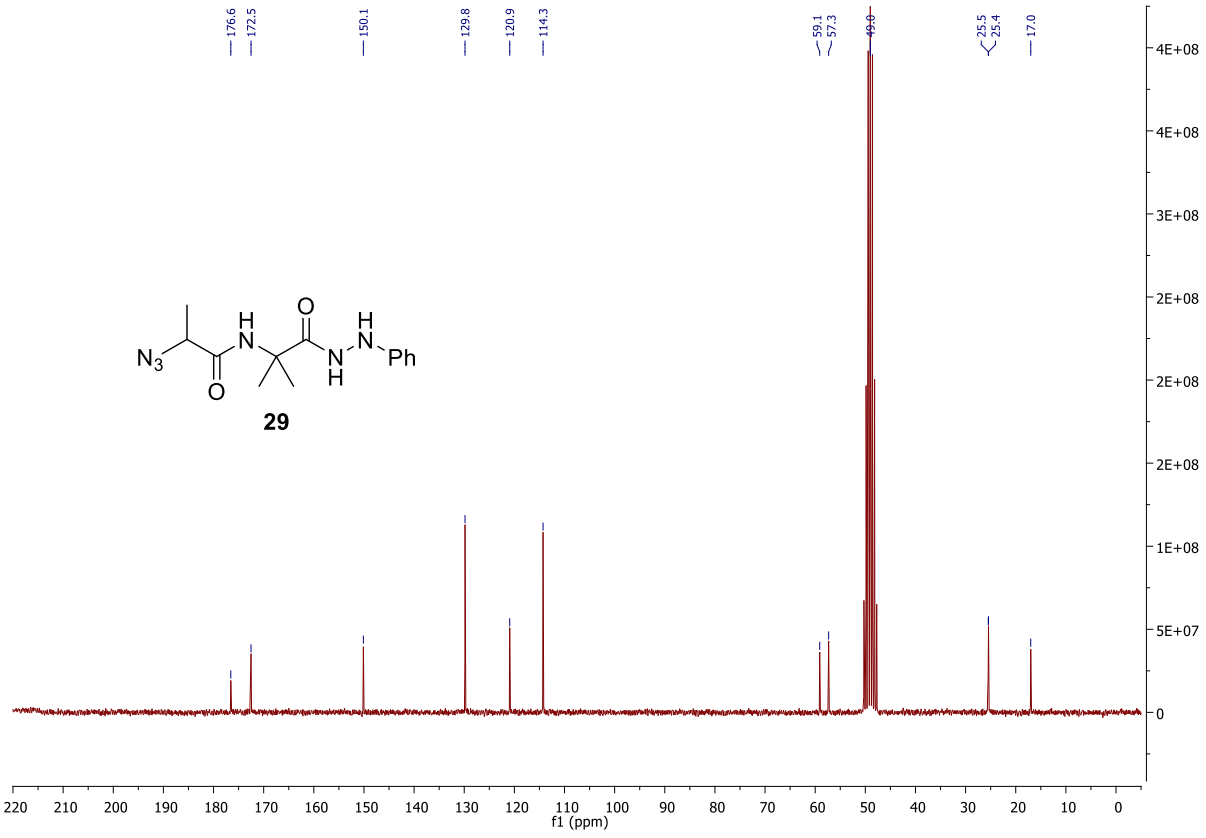


Figure 36 : ^{1}H NMR spectrum of 30

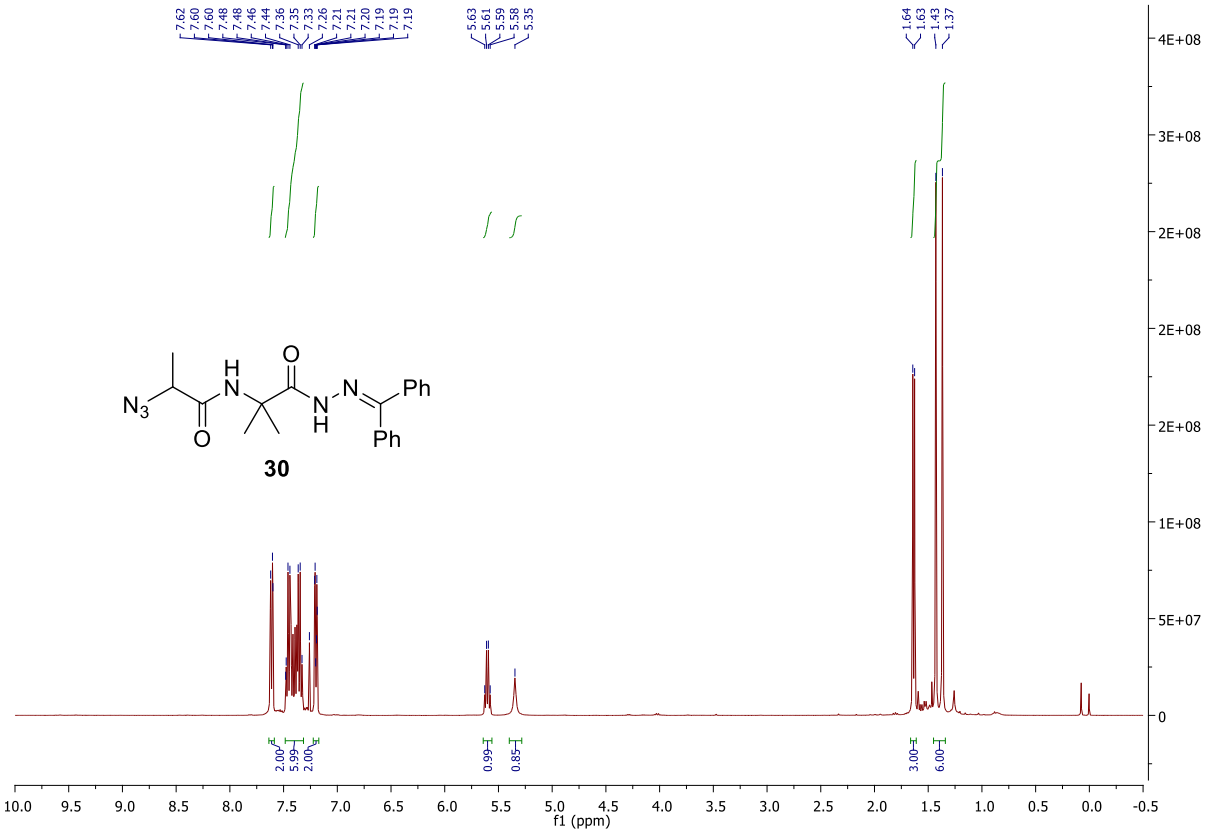


Figure 37: ^{13}C NMR spectrum of 30