General Information

All photoinduced reactions were carried out under ambient conditions using oven-dried quartz test tubes with magnetic stirring. Solvents and chemicals were purchased from commercial sources and used directly without further purification. Flash chromatography was performed with SiliCycle P60 silica gel (40-63 μm, 60Å). ^{1}H NMR spectra were recorded with Inova-300, -400 or -500 MHz spectrometers and chemical shifts were reported in ppm using either TMS or deuterated solvents as internal standards (TMS, 0.00; $CDCl_3$, 7.26; C_6D_6, 7.15; DMSO-d_6, 2.50). Multiplicity was reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. ^{13}C NMR spectra were recorded at 75.4 MHz, and chemical shifts were reported in ppm using the deuterated solvents as internal standards ($CDCl_3$, 77.0; DMSO-d_6, 39.5; C_6D_6, 128.0). Absorption spectra were recorded using 1-cm quartz cuvette on a HP-8452 Diode Array Spectrometer. Fluorescence spectra were recorded using 1-cm plastic cuvette on a JY Fluorolog Spectrofluorimeter at 20 °C. All fluorescence image acquisitions were carried out using a Zeiss LSM-710 confocal microscope equipped with a continuous laser and fluorescence lifetime (FLIM) detectors.

Experimental Procedures and Characterization Data

Ethyl 2-diazopropanoate (**1a**) [S1]

A mixture of sodium azide (6.5 g, 100 mmol), 2 M sodium hydroxide solution (200 mL, 40 mmol), tetrabutylammonium bromide (TBAB) (80 mg, 0.25 mmol) in 100 mL hexanes (100 mL) was stirred vigorously at 0 °C in an open 1000-mL round-bottom flask. To the stirred solution was added Tf_2O (8.2 mL, 50 mmol) dropwise through a syringe. After 10 min, a solution of ethyl 2-methylacetoacetate (3.53 mL, 25 mmol) in 100 mL acetonitrile was added into the reaction flask through a funnel, followed by additional 10 mL acetonitrile to complete the transfer. The initially colorless reaction mixture immediately turned yellow. After stirring at 0 °C for 30 min, the mixture was diluted successively with ice-water (100 mL) and chilled Et_2O (100 mL) and transferred to a separation funnel. The organic layer was separated the aqueous layer was further extracted with cold Et_2O (100 mL × 2). The organic fractions were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated to afford the titled compound as a bright yellow oil (3.0 g, 84% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.21 (q, J = 7.2 Hz, 2H), 1.96 (s, 3H), 1.27 (t, J = 7.2 Hz, 3H).

Ethyl 1-methyl-2-(trimethylsilyl)cycloprop-2-enecarboxylate (**1b**)

A 250-mL round-bottom flask was charged with $Rh_2(TPA)_4$ (29 mg, 0.02 mmol), DCM (80 mL), and trimethylsilylacetylene (5.7 mL, 40 mmol). To this mixture at room temperature was added a solution of ethyl 2-diazopropanoate **1a** (2.84 g, 20 mmol) in DCM (15 mL) at a rate of 0.6 mL/hr via a syringe pump. After the addition was completed, the mixture was concentrated under a reduced pressure. The residue was applied to silica gel based flash chromatography using 10% ether/hexanes as an eluent to afford the titled compound as a pale yellow oil (1.9 g, 46% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (s, 1H), 4.11-4.01 (m, 2H), 1.34 (s, 3H), 1.19 (t, J = 4.2 Hz, 3H), 0.20 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.90, 120.50, 119.84, 60.19, 21.90, 14.39, -1.45; HRMS (ESI-FTICR) calcd for $C_{10}H_{19}O_2Si$ [M + H^+] 199.1149, found 199.1153.

1-Methylcycloprop-2-enecarboxylic acid (**1c**)

To a stirred solution of ester **1b** (0.594 g, 3.0 mmol) in 15 mL methanol at 0 °C was added a 20-mL solution of 2 M KOH in water. The mixture was stirred at room temperature overnight before methanol was removed under reduced pressure. The resulting aqueous solution was

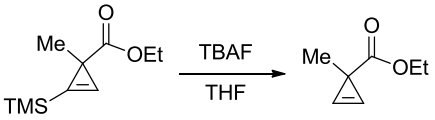
adjusted to pH 1–2 with 6 M HCl, extracted with DCM three times. The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and concentrated to provide the titled compound as a white solid (0.217 g, 74% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (s, 2H), 1.38 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.85, 109.34, 109.32, 21.60, 21.14.

2, 5-Dioxopyrrolidin-1-yl 1-methylcycloprop-2-enecarboxylate (**1d**)

To a stirred solution of acid **1c** (0.196 g, 2 mmol) and *N*-hydroxysuccinimide (0.242 g, 2.1 mmol) in 24 mL ethyl acetate/dioxane (1:1) at 0 °C was added DCC (0.433 g, 2.1 mmol) in one portion. The resulting mixture was stirred at room temperature for 5 hours. The precipitate was then filtered off and the filtrate was concentrated under reduced pressure. The residue was applied to silica gel column chromatography with hexanes/ethyl acetate (1:1) as the eluent to afford the titled compound (0.337 g, 84% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (s, 2H), 2.83 (d, J = 5 Hz, 4H) 1.51 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.50, 169.40, 109.07, 109.06, 25.61, 25.60, 21.26, 20.42.

(S)-2-amino-6-(1-methylcycloprop-2-enecarboxamido)hexanoic acid (**1**)

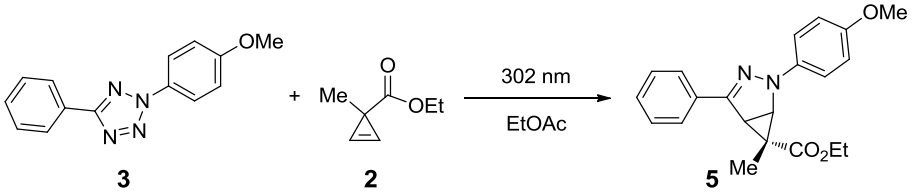
To a stirred solution of Fmoc-lysine-OH·HCl (0.689 g, 1.7 mmol) and DIPEA (0.490 g, 3.8 mmol) in 8 mL DMF was added dropwise a solution of **1d** (331 mg, 1.7 mmol) in 3 mL DMF over 5 min at room temperature. After 5 hours, the reaction mixture was concentrated under reduced pressure to produce an oily residue. The residue was re-dissolved in 50 mL ethyl acetate and the solution was washed sequentially with concentrated citric acid (25 mL × 2), water (25 mL), and brine (25 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated to give the crude product **1e** (0.500 g). The crude materials were dissolved in 10 mL DCM and treated with 5 mL diethyl amine. After 5 hours, the mixture was concentrated under reduced pressure. The resulting solid was washed with diethyl ether (20 mL × 2) and dried to afford the titled compound (0.180 g, 88% yield): ^{1}H NMR (500 MHz, D_2O) δ 7.05 (s, 2H), 3.58 (s, 1H), 3.04 (s, 2H), 1.81-1.61 (m, 2H), 1.46-1.32 (m, 2H), 1.32-1.16 (m, 5H); ^{13}C NMR (125 MHz, D_2O) δ 181.16, 174.68, 110.48, 54.59, 39.19, 30.02, 28.23, 21.92, 21.61, 20.47; HRMS calcd for $C_{11}H_{19}N_2O_3$, 227.1390 [M + H^+], found 227.1387.



1b **2**

Ethyl 1-methylcycloprop-2-enecarboxylate (**2**)

To a stirred solution of ester **1b** (99 mg, 0.5 mmol) in THF (1 mL) at 0 °C was added TBAF (0.6 mL, 1 M in THF), and the resulting mixture was stirred at room temperature for 1 hour. The mixture was then concentrated under reduced pressure, and the residue was applied to silica gel column chromatography using 10% diethyl ether in hexanes as an eluent to afford the desired product as pale yellow oil (37 mg, 59% yield): ^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.04 (s, 2H), 4.09 (q, J = 7.0 Hz, 2H), 1.39 (s, 3H), 1.24 (t, J = 7 Hz, 3H); ^{13}C NMR (75 MHz, CD_2Cl_2) δ 176.65, 109.81, 60.35, 21.58, 21.48, 14.07. HRMS (ESI-FTICR) calcd for $C_8H_{17}O_4$ 177.1121 [M + MeOH+ H_2O+H^+], found 177.1121.

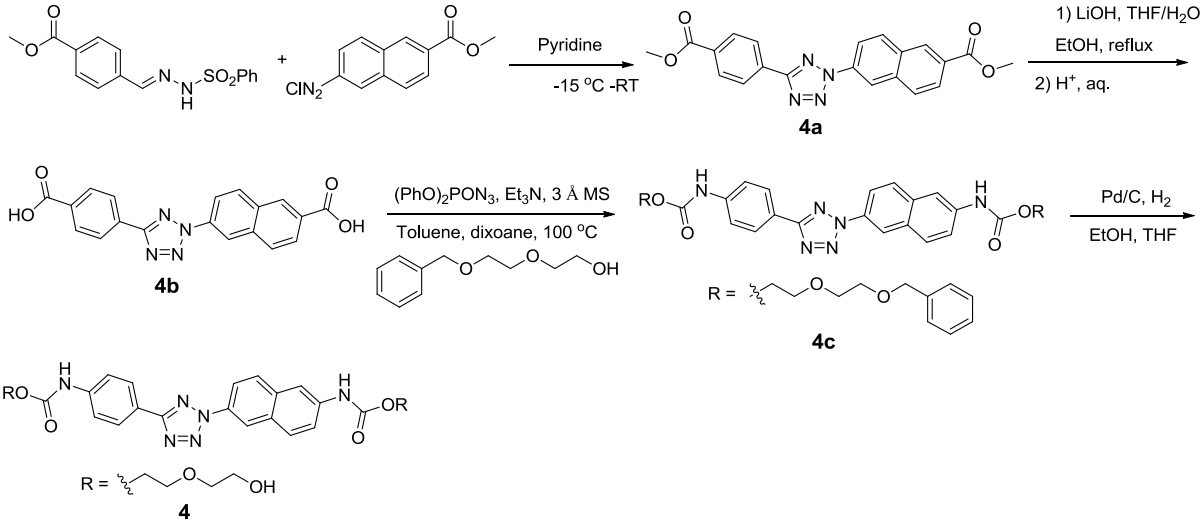


3 **2** **5**

Ethyl 2-(4-methoxyphenyl)-6-methyl-4-phenyl-2,3-diazabicyclo[3.1.0]hex-3-ene-6

-carboxylate (5)

A solution of tetrazole **3** (20 mg, 0.08 mmol) and cyclopropene **2** (0.16 mmol) in 160 mL ethyl acetate was irradiated with a handheld 302 nm UV lamp for 2 hours. The solvent were removed under reduced pressure. The residue was applied to preparative reverse-phase HPLC equipped with a C_{18} column (5 μm, 250 × 10.00 mm, a gradient of 90:10 water/acetonitrile to 10:90 water/acetonitrile with 0.1% formic acid added), and the correct fractions were pooled and lyophilized to give the desired pyrazoline product **5** (17 mg, 61% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.76 (m, 2H), 7.40-7.32 (m, 3H), 7.25 (d, *J* = 8.8 Hz, 2H) 6.90 (d, *J* = 9.2 Hz, 2H), 4.65 (d, *J* = 7.2 Hz, 1H), 4.24 (q, *J* = 6.8 Hz, 2H), 3.79 (s, 3H), 3.71 (d, *J* = 7.2 Hz, 1H), 1.33 (t, *J* = 6.8 Hz, 3H), 0.78 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.69, 154.61, 146.15, 137.01, 132.74, 128.57, 128.44, 125.69, 114.92, 114.64, 61.43, 55.65, 55.32, 40.42, 16.47, 14.30, 7.11; HRMS (EI) calcd for $C_{21}H_{22}N_2O_3$, 350.1625; found 350.1630.



6-(5-(4-Carboxyphenyl)-*2H*-tetrazol-2-yl)-2-naphthoic acid (4b)

Tetrazole **4b** was synthesized according to the literature procedure[S2] using (*E*)-methyl 4-((2-(phenylsulfonyl)-hydrazono)methyl)benzoate and 6-(methoxycarbonyl)-naphthalene- 2-diazonium chloride in pyridine to form tetrazole **4a** as a red solid, followed by the hydrolysis with LiOH in EtOH:THF:H_2O mixed solvent, affording the desired naphthalene tetrazoledicarboxylic acid **4b** as a pink powder: ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.3 (s, 2H), 8.87 (d, *J* = 2.0 Hz, 1H), 8.75 (s, 1H), 8.47-8.45 (m, 1H), 8.40-8.38 (dd, *J* = 9.0, 2.0 Hz, 1H), 8.36-8.31 (m, 3H), 8.20-8.17 (m, 2H), 8.12 (dd, *J* = 8.5, 1.5 Hz, 1H); HRMS (ESI-FTICR) calcd for $C_{19}H_{13}N_4O_4$ 361.0931 [M+H$^+$], found 361.0932.

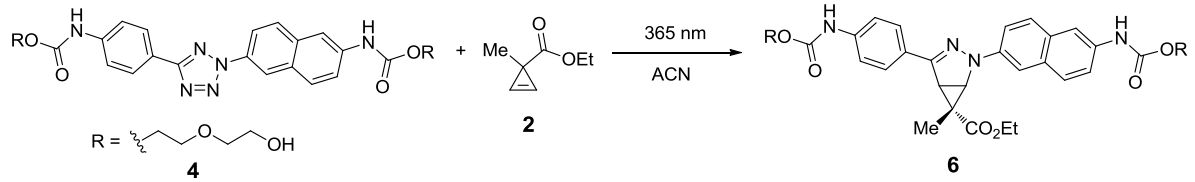
(6-(5-(4-(((2-(2-(Benzyloxy)ethoxy)ethoxy)carbonyl)amino)phenyl)-*2H*-tetrazol-2-yl)naphthalen-2-yl)carbamic acid 2-(2-(benzyloxy)ethoxy)ethyl ester (4c)

A solution of naphthalene tetrzoledicarboxylic acid **4b** (88.5 mg, 0.246 mmol), triethylamine (83.5 μL, 0.6 mmol) and 3 Å molecular sieve (0.1 g) in a mixed solvent of 2-(2-(benzyloxy) ethoxy)ethanol (0.3 mL), toluene (2.7 mL), dioxane (3.0 mL) was treated with diphenylphosphoryl azide (130 μL, 0.6 mmol) under argon protection was vigorously stirred and refluxed at 100 °C. After the reaction was complete based on TLC monitoring, the molecular sieve was removed via filtration through a thin layer of Celite. The solvent was evaporated under reduced pressure, and the residue was applied to silica gel flash chromatography (EtOAc/hexanes = 1:2) to give the desired dibenzyloxyethoxyethyl-amino naphthalene tetrazole **4c** as a pink solid (85 mg, 51% yield): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.53 (d, *J* = 2.5 Hz, 1H), 8.25 (dd, *J* = 8.5, 1.5 Hz, 1H), 8.20-8.18 (m, 2H), 8.06 (br, 1H), 7.92-7.88 (m, 2H), 7.53-7.52 (m, 2H), 7.43 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.36-7.26 (m, 10H),

7.00 (br, 1H), 6.95 (br, 1H), 4.59 (s, 4H), 4.42-4.37 (m, 4H), 3.82-3.78 (m, 4H), 3.75-3.71 (m ,4H), 3.69-3.66 (m ,4H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 164.74, 153.27, 153.10, 139.89, 138.09, 136.66, 133.99, 133.29, 129.55, 129.52, 129.20, 128.37, 127.95, 127.72, 127.65, 122.15, 120.53, 118.55, 118.50, 117.84, 114.60, 73.31, 70.65, 69.39, 64.51, 64.45; HRMS (ESI-FTICR) calcd for $C_{41}H_{42}N_6NaO_8$ 769.2956 [M+Na$^+$], found 769.2953.

(6-(5-(4-(((2-(2-Hydroxyethoxy)ethoxy)carbonyl)amino)phenyl)-2H-tetrazol-2-yl)naphthalen-2-yl)carbamic acid 2-(2-hydroxyethoxy)ethyl ester (4)

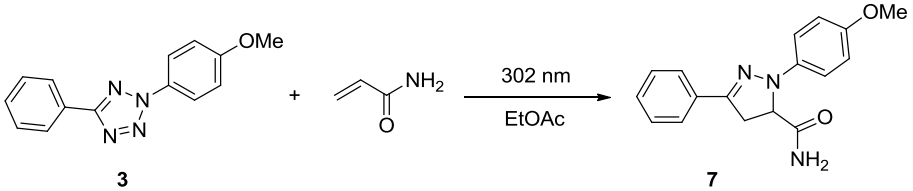
A solution of tetrazole **4c** (75 mg, 0.1 mmol) in ethanol/THF (1:1; 2 ml) with 20 µL 2 M HCl was treated with 10 % palladium on carbon (7.5 mg, 10 wt %) under argon at room temperature and then placed under 1 atm hydrogen gas. After 12 hours, the mixture was filtered through a layer of Celite to remove the catalyst and the filtrate was evaporated to dryness in vacuum. The white residue was further purified through recrystallization in EtOH to give the titled compound as a white crystal (47 mg, 83% yield): ^{1}H NMR (DMSO-d_6, 500 MHz) δ), 10.19 (br, 1H), 10.14 (br, 1H), 8.64 (d, *J* = 2.0 Hz, 1H), 8.24 (br, 1H), 8.21 (dd, *J* = 9.0, 2.5 Hz, 1H), 8.14-8.12 (m, 3H), 8.10-8.09 (m, 1H), 7.73-7.71 (m, 2H), 7.69 (dd, *J* = 8.5, 2.0 Hz, 1H), 4.64 (t, *J* = 5.5 Hz, 2H), 4.29-4.24 (m, 4H), 3.71-3.67 (m, 4H), 3.65-3.51 (m ,4H), 3.50-3.46 (m ,4H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 164.81, 154.00, 153.84, 142.08, 138.90, 134.19, 132.72, 129.92, 129.79, 129.13, 127.90, 121.42, 120.77, 118.85, 118.72, 118.45, 113.85, 72.72, 69.01, 64.33, 60.63; HRMS (ESI) calcd for $C_{27}H_{31}N_6O_8$ 567.2198 [M+H$^+$], found 567.2198.



4 2 6

Ethyl 2-(6-(((2-(2-hydroxyethoxy)ethoxy)carbonyl)amino)naphthalen-2-yl)-4-(4-(((2-(2-hydroxyethoxy)ethoxy)carbonyl)amino)phenyl)-6-methyl-2,3-diazabicyclo[3.1.0]hex-3-ene-6-carboxylate (6)

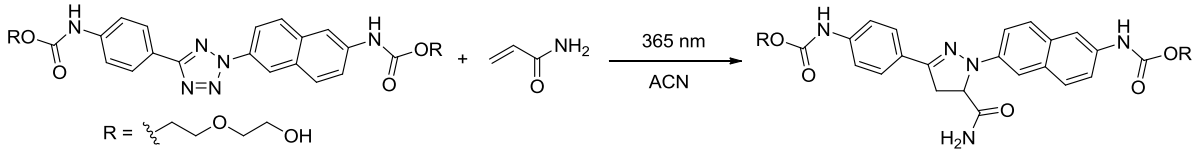
A solution of tetrazole **4** (8 mg, 0.014 mmol) and cyclopropene **2** (1.4 mmol) in 100 mL acetonitrile was irradiated with a handheld 365 nm UV lamp for 5 hours. The solvent was then removed under reduced pressure. The residue was applied to preparative reverse-phase HPLC equipped with a C_{18} column (5 µm, 250 × 10.00 mm, a gradient of 90:10 water/acetonitrile to 10:90 water/acetonitrile with 0.1% formic acid added), and the correct fractions were pooled and lyophilized to give the desired pyrazoline product **6** (5.4 mg, 58% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (br, 1H), 7.76-7.73 (m, 2H), 7.72-7.65 (m, 4H), 7.43-7.42 (m, 2H), 7.35-7.33 (m, 2H), 6.99 (br, 1H), 6.95 (br, 1H), 4.74 (d, *J* = 7.5 Hz, 1H), 4.40-4.35 (m, 4H), 4.28 (q, *J* = 7.0 Hz, 2H), 3.80-3.76 (m, 8H), 3.69 (d, *J* = 7.0 Hz, 1H), 3.68-3.64 (m, 4H), 1.37 (t, *J* = 7.0 Hz, 3H), 1.08 (t, *J* = 7.0 Hz, 1H), 0.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.49, 153.13, 146.73, 139.94, 138.24, 133.14, 131.10, 129.38, 128.73, 127.89, 127.62, 126.78, 125.37, 118.55, 116.53, 107.68, 72.44, 69.49, 69.40, 64.25, 64.16, 61.73, 61.56, 54.40, 40.16, 17.40, 14.30, 7.12; HRMS (ESI-FTICR) calcd for $C_{34}H_{41}N_4O_{10}$ 665.2817 [M+H$^+$], found 665.2817.



3 7

1-(4-Methoxyphenyl)-3-phenyl-4,5-dihydro-*1H*-pyrazole-5-carboxamide (7)

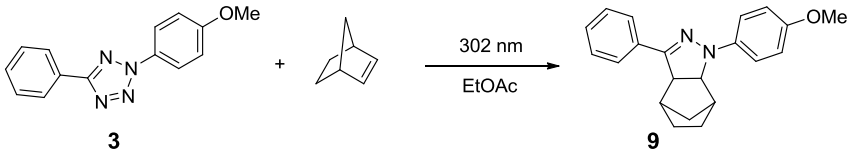
A solution of tetrazole **3** (30 mg, 0.12 mmol) and acrylamide (85 mg, 1.2 mmol) in 100 mL ethyl acetate was irradiated with a handheld 302 nm UV lamp for 1.5 hours. The solvent was then removed under reduced pressure. The residue was recrystallized in EtOAc to give the desired pyrazoline product **7** as a yellow solid (25 mg, 71% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.73-7.70 (m, 3H), 7.44-7.41 (m, 2H), 7.37-7.34 (m, 1H), 7.30 (br, 1H), 6.99-6.98 (m, 2H), 6.89-6.87 (m, 2H), 4.54 (dd, *J* = 12.0, 8.5 Hz, 1H), 3.71 (dd, *J* = 18.0, 12.5 Hz, 1H), 3.70 (s, 3H), 3.21 (dd, *J* = 17.0, 8.0 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.41, 153.32, 147.08, 139.74, 132.56, 129.05, 128.95, 126.00, 114.91, 114.31, 63.83, 55.74, 38.98; HRMS (ESI-FTICR) calcd for $C_{17}H_{18}N_3O_2$ 296.1394 [M+H$^+$], found 296.1394.



4 **8**

(6-(5-Carbamoyl-3-(4-(((2-(2-hydroxyethoxy)ethoxy)carbonyl)amino)phenyl)-4,5-dihydro-*1H*-pyrazol-1-yl)naphthalen-2-yl)carbamic acid 2-(2-hydroxyethoxy)ethyl ester (8)

A solution of tetrazole **4** (30 mg, 0.053 mmol) and acrylamide (71 mg, 1.0 mmol) in 100 mL acetonitrile was irradiated with a handheld 365 nm UV lamp for 5 hours. The solvent was then removed under reduced pressure. The residue was recrystallized in ACN/EtOAc (3:1) to give the desired pyrazoline product **8** as a brown solid (27 mg, 84% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.95 (br, 1H), 9.79 (br, 1H), 7.93 (br, 1H), 7.82 (br, 1H), 7.71-7.68 (m, 3H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.56-7.54 (m, 2H), 7.52 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.45 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.33 (br, 1H), 7.06 (d, *J* = 1.5 Hz, 1H), 4.72 (dd, *J* = 13.0, 8.0 Hz, 1H), 4.64-4.62 (m, 2H), 4.24-4.21 (m, 4H), 3.73 (dd, *J* = 17.0, 13.0 Hz, 1H), 3.68-3.66 (m, 4H), 3.55-3.50 (m ,4H), 3.49-3.46 (m, 4H), 3.25 (dd, *J* = 17.5, 7.5 Hz, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.26, 154.05, 153.81, 147.87, 142.11, 140.18, 134.38, 130.85, 128.45, 128.26, 127.13, 126.92, 126.53, 118.42, 116.86, 109.98, 106.09, 72.71, 69.11, 69.03, 64.19, 64.01, 62.78, 60.62, 39.11; HRMS (ESI-FTICR) calcd for $C_{30}H_{36}N_5O_9$ 610.2508 [M+H$^+$], found 610.2508.

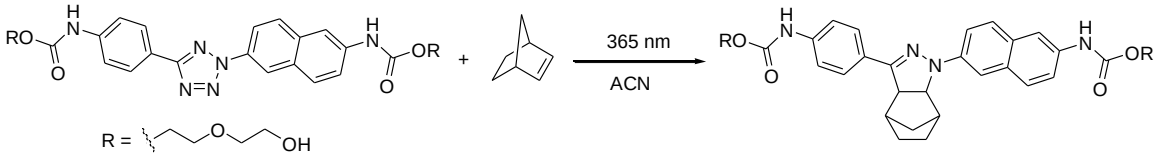


3 **9**

1-(4-Methoxyphenyl)-3-phenyl-3a,4,5,6,7,7a-hexahydro-1*H*-4,7-methanoindazole (9)

A solution of tetrazole **3** (30 mg, 0.119 mmol) and norbornene (112 mg, 1.19 mmol) in 150 mL ethyl acetate was irradiated with a handheld 302 nm UV lamp for 1 hour. The solvent was then removed under reduced pressure. The crude mixture was purified by silica gel flash column using Et_2O/hexanes (1:5) as an eluent. The green solid was then recrystallized in hexanes/EtOAc (8:1) to give the desired pyrazoline product **9** as a yellow solid (32 mg, 84% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.72 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (t, *J* = 7.5 Hz, 1H), 7.05-7.02 (m, 2H), 6.89-6.85 (m, 2H) 4.13 (d, *J* = 9.5 Hz, 1H), 3.70 (s, 3H), 3.68 (d, *J* = 9.5 Hz, 1H), 2.64 (s, 1H), 2.58 (s, 1H), 1.57-1.46 (m, 3H), 1.42-1.36 (m ,1H), 1.26-1.24 (m, 1H), 1.15-1.13 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ152.81, 148.72, 139.00, 132.63, 129.06, 128.37, 125.85, 115.09, 113.60, 68.67, 55.76, 54.64, 41.71,

33.07, 27.76, 24.15; HRMS (ESI-FTICR) calcd for $C_{21}H_{23}N_2O$ 319.1805 [M+H$^+$], found 319.1806.

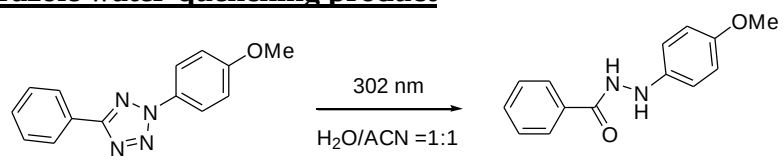


4 **10**

(6-(3-(4-(((2-(2-Hydroxyethoxy)ethoxy)carbonyl)amino)phenyl)-3a,4,5,6,7,7a-hexahydro-1*H*-4,7-methanoindazol-1-yl)naphthalen-2-yl)carbamic acid 2-(2-hydroxyethoxy)ethyl ester (10)

A solution of tetrazole **4** (12 mg, 0.021 mmol) and norbornene (200 mg, 2.12 mmol) in 150 mL ethyl acetate/ACN (1:3) was irradiated with a handheld 365 nm UV lamp for 1 hour. The solvent was the removed under reduced pressure. The crude product was recrystallized in hexanes/EtOAc (8:1) to give the desired pyrazoline product **10** as a dark green solid (12 mg, 88% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.92 (br, 1H), 9.77 (br, 1H), 7.90 (br, 1H), 7.71-7.66 (m, 4H), 7.57-7.53 (m, 3H), 7.43 (dd, *J* = 8.5, 1.5 Hz, 1H), 7.16 (d, *J* = 2.0 Hz, 1H), 4.63 (td, *J* = 5.0, 1.5 Hz, 2H), 4.26 (d, *J* = 10.0 Hz, 1H), 4.24-4.21 (m, 4H), 3.71 (d, *J* = 9.5 Hz, 1H), 3.68-3.65 (m, 4H), 3.54-3.50 (m, 4H), 3.49-3.46 (m, 4H), 2.76 (s, 1H), 2.54 (s, 1H), 1.59-1.55 (m, 2H), 1.52-1.43 (m, 2H), 1.28-1.27 (m, 1H), 1.16-1.14 (m, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.05, 153.82, 149.82, 141.39, 139.76, 134.09, 131.16, 128.54, 127.91, 127.15, 126.81, 126.55, 118.59, 116.54, 114.69, 105.46, 72.71, 69.12, 69.04, 67.96, 64.16, 63.99, 60.63, 54.77, 41.39, 33.04, 27.75, 24.26; HRMS (ESI-FTICR) calcd for $C_{34}H_{41}N_4O_8$ 633.2919 [M+H$^+$], found 633.2917.

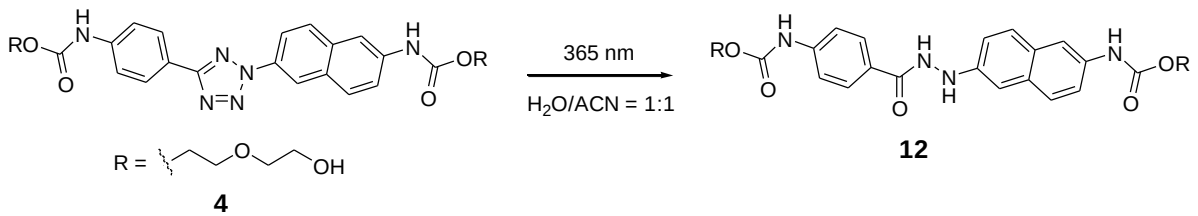
Synthesis of tetrazole water-quenching product



3 **11**

***N′*-(4-methoxyphenyl)benzohydrazide (11)**

A solution of tetrazole **3** (30 mg, 0.12 mmol) in 100 mL H_2O/ACN (1:1) was irradiated with a handheld 302 nm UV lamp for 1.5 hours. The solvent was then removed under reduced pressure. The residue was applied to silica gel column chromatography to give the desired hydrazide product **11** as a brown solid (18 mg, 62% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (br, 1H), 7.81 (d, *J* = 7.5 Hz, 2H), 7.55-7.52 (m, 1H), 7.44-7.41 (m, 2H), 6.90-6.87 (m, 2H), 6.79-6.77 (m, 2H), 5.98 (br, 1H), 3.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.73, 154.89, 141.36, 132.24, 132.18, 128.73, 127.13, 115.89, 114.60, 55.61; HRMS (ESI-FTICR) calcd for $C_{14}H_{14}N_2NaO_2$ 265.0948 [M+Na$^+$], found 265.0947.

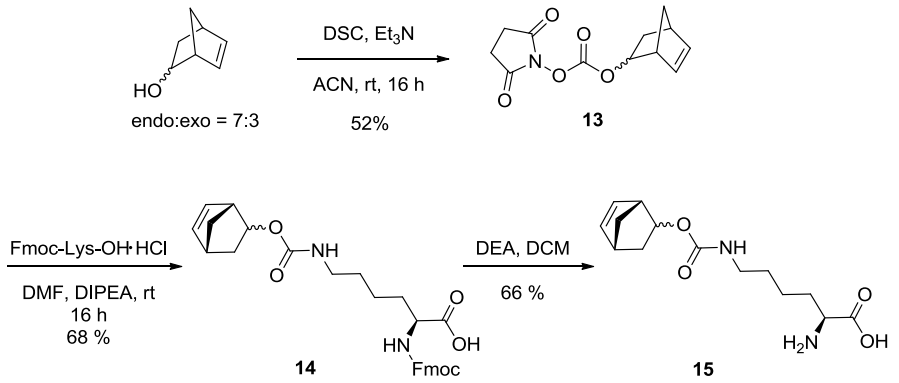


4 **12**

(6-(2-(4-(((2-(2-Hydroxyethoxy)ethoxy)carbonyl)amino)benzoyl)hydrazinyl)naphthalen-2-yl)carbamic acid 2-(2-hydroxyethoxy)ethyl ester (12)

A solution of tetrazole **4** (30 mg, 0.053 mmol) in 100 mL H_2O/acetonitrile (1:1) was irradiated with a handheld 365 nm UV lamp for 5 hours. The solvent was then removed under reduced pressure. The residue was applied to preparative reverse-phase HPLC equipped with a C_{18} column (5 μm, 250 × 10.00 mm, a gradient of 90:10 water/acetonitrile to 10:90 water/acetonitrile with 0.1% formic acid added), and the correct fractions were pooled and lyophilized to give the desired hydrazine product **12** as a colorless solid (20 mg, 68% yield): ^{1}H NMR (500 MHz, CD_3OD-d_4) δ 8.19 (br, 1H), 8.00 (br, 1H), 7.92 (br, 1H), 7.87 (br, 1H), 7.78-7.73 (m, 4H), 7.66 (d, *J* = 9.0 Hz, 2H), 7.52-7.46 (m, 4H), 7.41 (dd, *J* = 8.5, 2.5 Hz, 1H), 4.23-4.20 (m, 4H), 4.67-3.64 (m, 4H), 3.59-3.56 (m, 4H), 3.51-3.48 (m, 4H); ^{13}C NMR (75 MHz, CD_3OD-d_4) δ 166.84, 154.46, 154.00, 129.67, 128.62, 128.57, 128.52, 128.48, 128.36, 128.28, 127.45, 125.16, 122.55, 117.59, 117.42, 113.93, 113.90, 72.22, 72.19, 69.02, 68.91, 63.92, 60.77; HRMS (ESI-FTICR) calcd for $C_{27}H_{33}N_4O_9$ 557.2242 [M+H$^+$], found 557.2242.

Synthesis of norbornene-lysine[S3]



(1*R*,4*R*)-bicyclo[2.2.1]hept-5-en-2-yl (2,5-dioxopyrrolidin-1-yl) carbonate (**13**)
The titled compound was prepared according to the reported procedure[S3] with some minor modifications. Disuccinimide carbonate (DSC, 3.95 g, 15.4 mmol) was added to a solution of (1*R*,4*R*)-5-norbornene-2-ol (endo/exo mixture, 1.0 g, 9.1 mmol) and triethylamine (3.9 mL, 26.3 mmol) in dry acetonitrile (25 mL) at room temperature. The resulting mixture was stirred overnight and then concentrated under vacuum. The product was purified by silica gel column chromatography (DCM/Hexane/EtOAc = 5:5:1) to yield compound **13** as a white solid (1.20 g, 7:3 *endo/exo*, 52% yield): R_f = 0.2 (DCM/Hexane/EtOAc = 5:5:1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.39 and 6.29 (dd$_{endo}$, dd$_{exo}$, J_{endo} = 5.5, 3.0 Hz, J_{exo} = 6.0, 3.0 Hz, 1H), 6.02 and 5.96 (dd$_{endo}$, dd$_{exo}$, J_{endo} = 5.5, 3.0 Hz, J_{exo} = 6.0, 3.5 Hz, 1H), 5.36 and 4.74 (m$_{endo}$, d$_{exo}$, J_{exo} = 7.0 Hz, 1H), 3.27 and 3.08 (m$_{endo}$, m$_{exo}$, 1H), 2.91 (s, 1H), 2.82-2.84 (s$_{endo}$, s$_{exo}$, 4H), 2.22-2.17 and 1.82-1.77 (m$_{endo}$, m$_{exo}$, 1H), 1.71 and 1.34 (m$_{exo}$, m$_{endo}$, 1H), 1.68-1.65 and 1.55-1.52 (m$_{exo}$, m$_{endo}$, 1H), 1.63-1.60 and 1.16-1.12 (m$_{exo}$, m$_{endo}$, 1H); ESI-MS calcd for $C_{13}H_{18}NO_6$ [M + MeOH + H$^+$] 284.1, found 284.2.

(2*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-6-((((1*R*,4*R*)-bicyclo[2.2.1]hept-5-en-2-yloxy)carbonyl)amino)hexanoic acid (14)
Compound **13** (600 mg, 2.39 mmol) was added to a stirred solution of Fmoc-Lys-OH•HCl (968 mg, 2.39 mmol) and DIPEA (425 μL, 2.39 mmol) in dry dimethylformamide (20 mL). The pH of the reaction mixture was kept at 8-9 via adding three portions of DIPEA (425 μL, 2.39 mmol). Then the reaction was allowed to proceed overnight at room temperature. The solvent was removed under vacuum and the residue was diluted in saturate citric acid (50 mL in water) and extracted with ethyl acetate (50 mL x 3). The combined organic layers were

washed twice with water (50 mL each) and brine (75 mL). The resulting organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum to dryness. The desired product was purified via silica gel column chromatography (EtOAc/EtOH = 10:1−1:1) to yield the titled compound as an off-white foam (816 mg, 68% yield): ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.52 (s, br, 1H), 7.90 (d, J = 8.0 Hz, 2H), 7.73 (d, J = 7.5 Hz, 2H), 7.62-7.60 (m, 1H), 7.42 (t, J = 7.5 Hz, 2H), 7.33 (d, J = 7.5 Hz, 2H), 7.07 and 6.93 (t, br_{exo}, J = 5.5 Hz, d, br_{endo}, J = 5.5 Hz, 1H), 6.30 and 6.24 (m_{endo}, m_{exo}, 1H), 5.94 and 5.98 (m_{endo}, m_{exo}, 1H), 5.10 and 4.45 (m_{endo}, m_{exo}, 1H), 4.29-4.21 (m, 3H), 3.94-3.88 (m, 1H), 3.04-2.77 (m, 5H), 2.06-2.03 (m_{endo}, 0.7H), 1.75-1.30 (m, 7H), 0.79 (d, J = 12.5 Hz, 1H); ESI-MS calcd for $C_{29}H_{33}N_2O_6$ [M + H^+] 505.2, found 505.3.

(2*S*)-2-amino-6-((((1*R*,4*R*)-bicyclo[2.2.1]hept-5-en-2-yloxy)carbonyl)amino)hexanoic acid (15)

Compound **14** (725 mg, 1.44 mmol) was dissolved in 10 mL DCM and treated with 6 mL diethyl amine. After 15 hours, the mixture was concentrated under reduced pressure. The resulting solid was resuspended in 10 mL DCM/diethyl ether = 1:1, filtered, washed with diethyl ether (20 mL × 2) and dried to give the titled compound (267 mg, 66% yield): ^{1}H NMR (500 MHz, D_2O) δ 6.27 and 6.18 (dd_{endo}, dd_{exo}, J_{endo} = 6.0, 3.0 Hz, J_{exo} = 5.5, 3.0 Hz, 1H), 5.89-5.87 (m, 1H), 5.03-5.00 and 4.36-4.33 (m_{endo}, d_{exo}, J_{exo} = 6.0 Hz, 1H), 3.59-3.56 (m, 1H), 3.00-2.90 (m, 3H), 2.72 (s, 1H), 2.01-1.96 (m_{endo}, 0.7H), 1.80-1.62 (m, 2H), 1.55-1.48 (m_{endo}, 0.7H), 1.41-1.20 (m, 5H), 0.75 (d, J = 12.5 Hz, 1H); ESI-MS (ESI) calcd for $C_{14}H_{23}N_2O_6$ [M + H^+] 283.2, found 283.3.

X-Ray data collection and structural refinement

Data collection, structure solution, and structure refinement were conducted at the X-ray Crystallographic Facility at the Department of Chemistry of University of Rochester. A crystal (0.36 x 0.28 x 0.24 mm) was placed onto the tip of a 0.1 mm diameter glass capillary tube or fiber and mounted on a Bruker SMART APEX II CCD Platform diffractometer for a data collection at 100.0(1) K. A preliminary set of cell constants and an orientation matrix were calculated from reflections harvested from three orthogonal wedges of reciprocal space. The full data collection was carried out using MoKα radiation (graphite monochromator) with a frame time of 25 seconds and a detector distance of 3.98 cm. A randomly oriented region of reciprocal space was surveyed: six major sections of frames were collected with 0.50º steps in ω at six different ϕ settings and a detector position of -38º in 2θ. The intensity data were corrected for absorption. Final cell constants were calculated from the xyz centroids of 3960 strong reflections from the actual data collection after integration. See Table 1 for additional crystal and refinement information.

The structure was solved using SIR97 and refined using SHELXL-97. The space group *Pn*m*a* was determined based on systematic absences and intensity statistics. A direct-methods solution was calculated which provided most non-hydrogen atoms from the E-map. Full-matrix least squares/difference Fourier cycles were performed which located the remaining non-hydrogen atoms. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were found from the difference Fourier map and their positional and isotropic displacement parameters were refined independently from those of all other atoms. The final full matrix least squares refinement converged to $R1$ = 0.0374 (F^2, $I > 2\sigma(I)$) and $wR2$ = 0.1138 (F^2, all data).

Table S1. Crystal data and structure refinement for cyclopropene **1c**.

Identification code	cyclopropene **1c**	
Empirical formula	$C_5 H_6 O_2$	
Formula weight	98.10	
Temperature	100.0(1) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	*Pn*m*a*	
Unit cell dimensions	a = 11.7879(7) Å	α = 90°
	b = 6.7883(4) Å	β = 90°
	c = 12.4266(7) Å	γ = 90°
Volume	994.37(10) Å^3	
Z	8	
Density (calculated)	1.311 Mg/m^3	
Absorption coefficient	0.102 mm^{-1}	
F(000)	416	
Crystal color, morphology	colorless, needle	
Crystal size	0.36 x 0.28 x 0.24 mm^3	
Theta range for data collection	2.38 to 37.78°	
Index ranges	$-20 \le h \le 20$, $-11 \le k \le 11$, $-21 \le l \le 21$	
Reflections collected	35401	
Independent reflections	2847 [R(int) = 0.0354]	
Observed reflections	2362	
Completeness to theta = 37.78°	99.9%	
Absorption correction	Multi-scan	
Max. and min. transmission	0.9760 and 0.9644	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	2847 / 0 / 107	
Goodness-of-fit on F^2	1.052	
Final R indices [I>2sigma(I)]	R1 = 0.0374, wR2 = 0.1067	
R indices (all data)	R1 = 0.0463, wR2 = 0.1138	
Largest diff. peak and hole	0.657 and -0.226 e.Å^{-3}	

Stability studies of CpK in the presence of 10 mM glutathione (GSH) in D_2O as monitored by ^{1}H-NMR

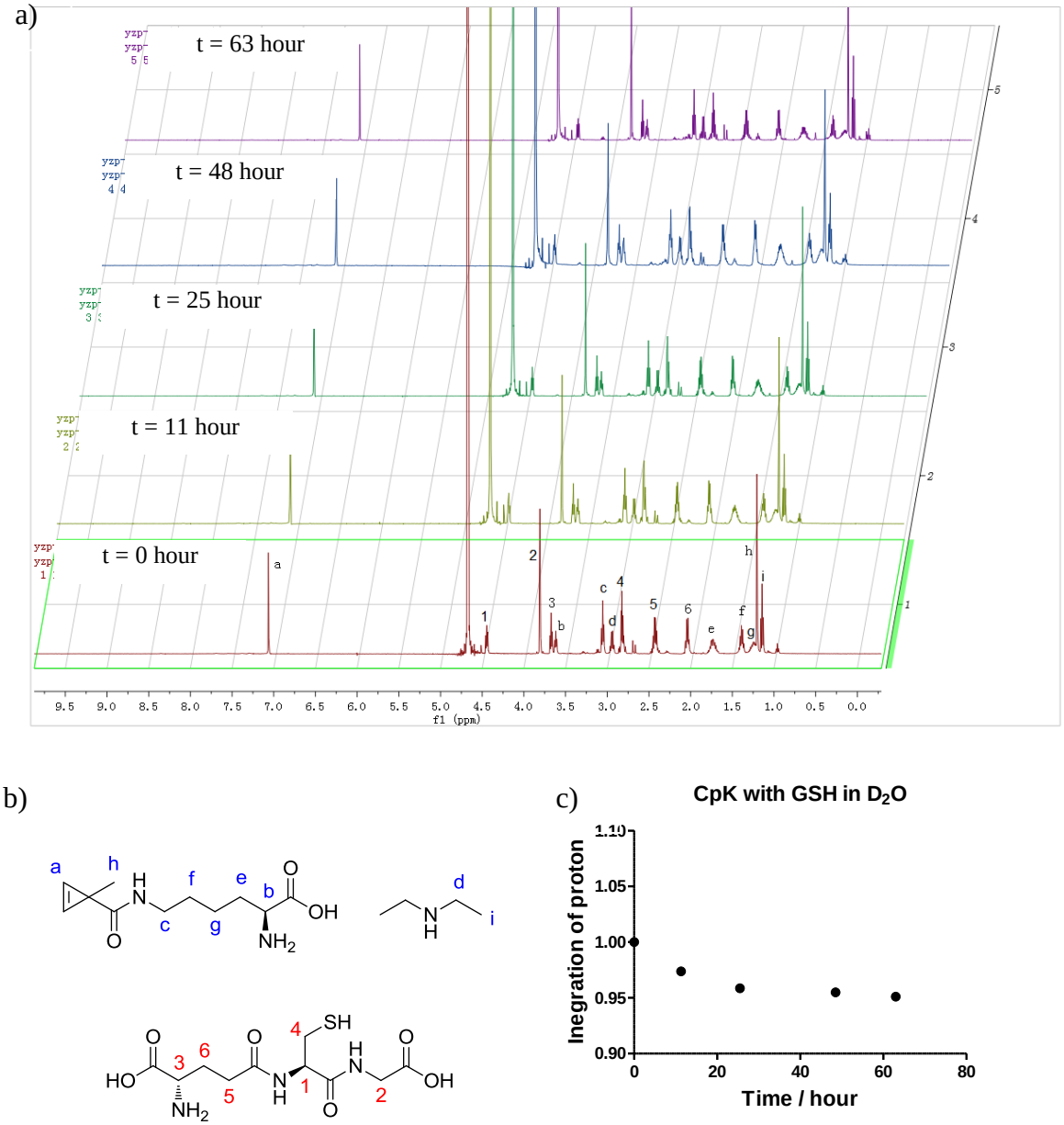
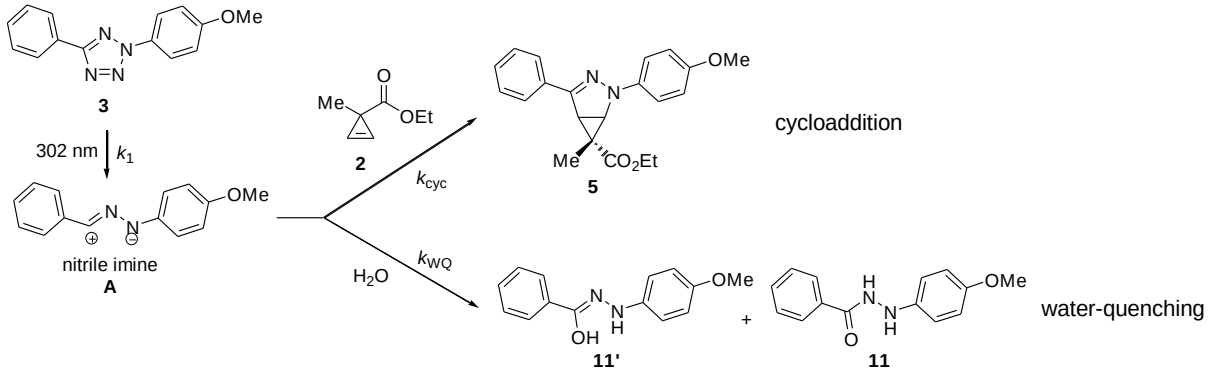


Figure S1. CpK is stable towards glutathione. **(**a) ^{1}H NMR spectra of CpK in the presence of 10 mM GSH (reduced form) in D_2O. (b) The proton assignments for CpK, diethylamine (a counter ion in the CpK salt), and the reduced form of glutathione. c) The plot of CpK decay over a period of 63 hours. The alkene-proton peaks (denoted "a") were used for quantification using peaks '2' and 'h' as reference.

Kinetic study of the photoinduced cycloaddition reaction between tetrazole 3 and ethyl 1-methylcycloprop-2-enecarboxylate (5 equiv) upon 302-nm UV irradiation

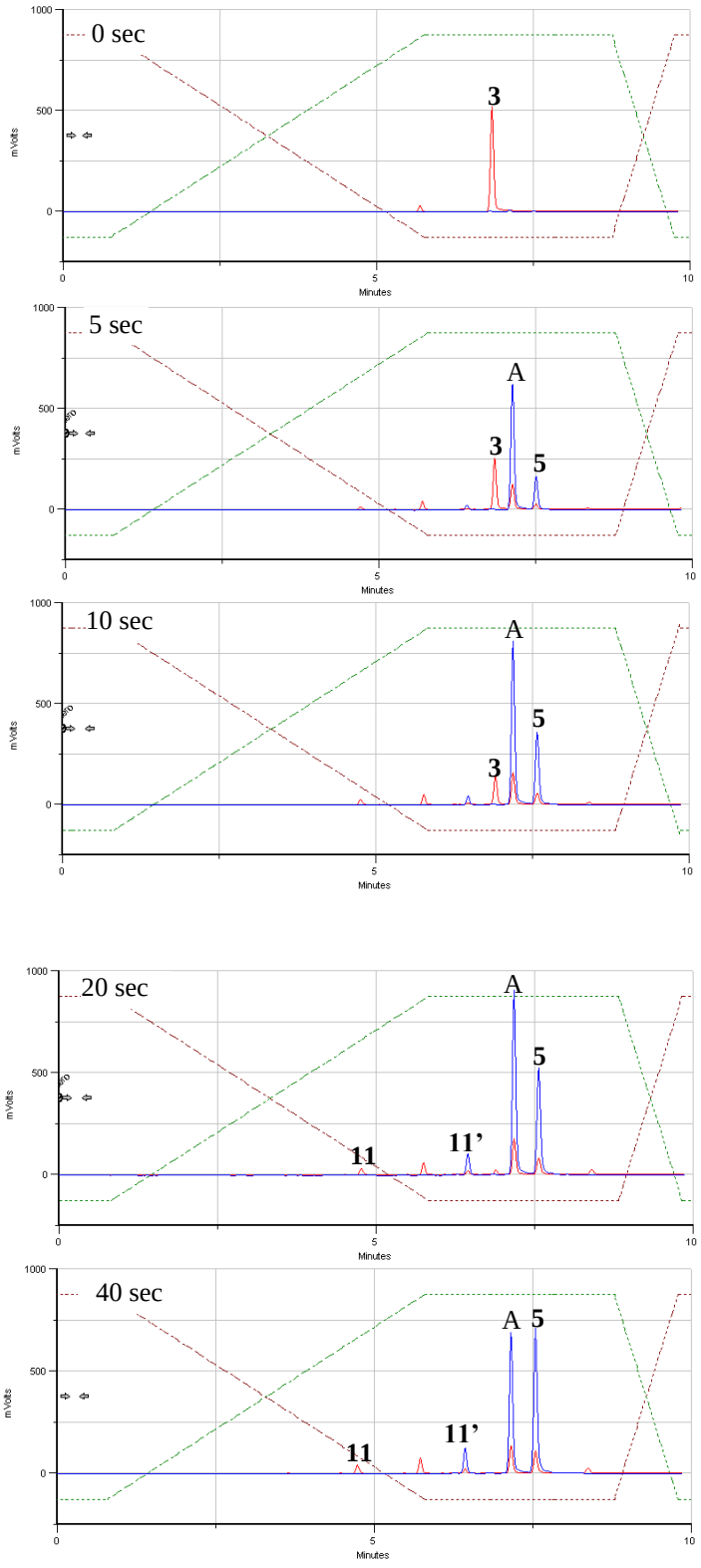


Aliquots of tetrazole **3** stock solution (100 mM in DMSO) and cyclopropene **2** stock solution (1.0 M in CH_3CN) were added into 0.5 mL CH_3CN/PBS buffer (1:1 v/v) in quartz test tubes to obtain the final tetrazole concentration of 100 μM and the final cyclopropene **2** concentration of 500 μM. Under vigorous stirring, the reaction mixtures were photoirradiated with a handheld 302-nm UV lamp for a period of 5, 10, 20, 40, 80, 160, and 320 sec, respectively. Then, aliquots of 100-μL reaction mixtures from each sample were withdrawn and injected into a reverse phase HPLC system (C_{18} column, 2.6 μm, 50 × 4.60 mm, gradient of 90:10 water/acetonitrile to 10:90 water/acetonitrile with 0.1% formic acid). The nitrile imine intermediate, cycloaddition product, and water-quenching products in the mixtures were monitored by UV absorbance at 254 nm and 370 nm (Figure S2a, b). The integrated areas at 254 nm of the cycloadduct and the water-quenching products were compared to the standard curves (Figure S2c) in order to obtain the concentrations of these products.

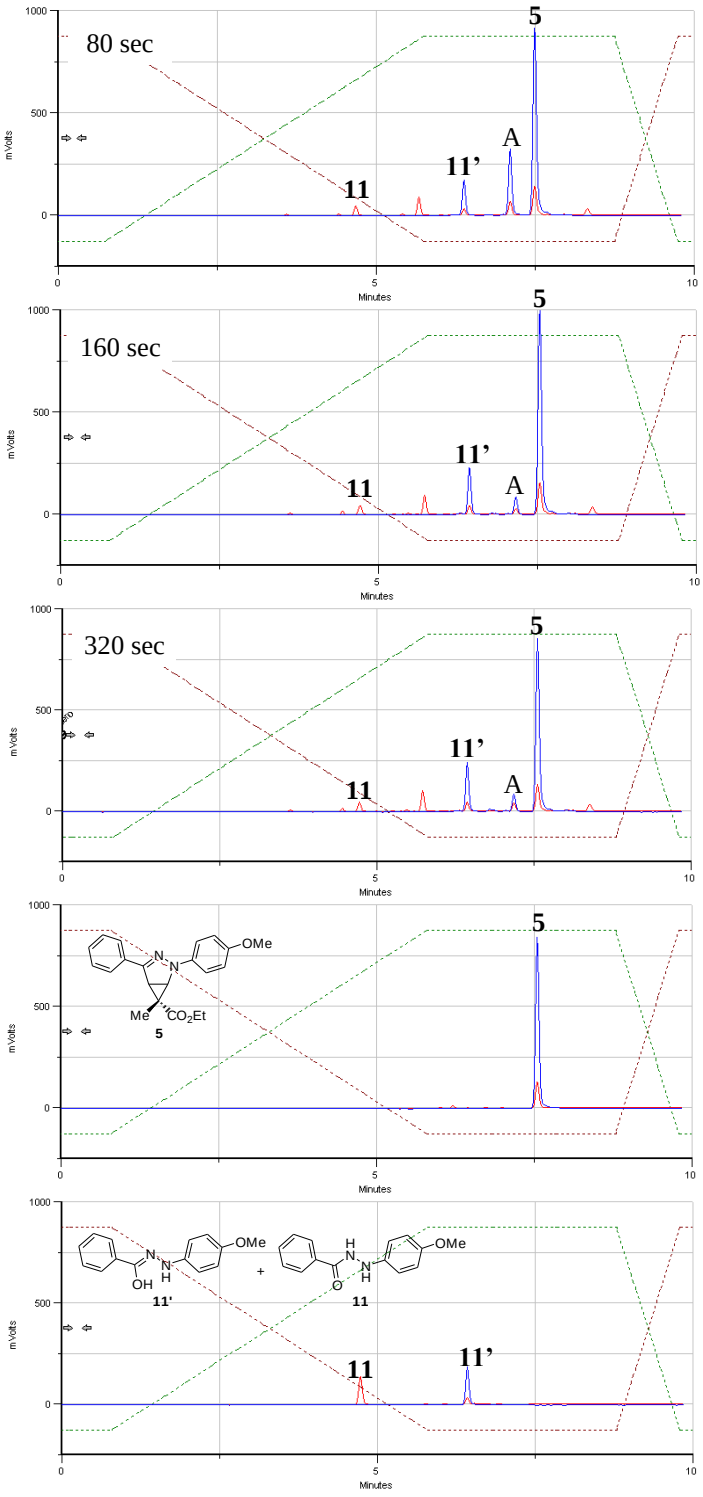
For the photoinduced ring rupture, the change in concentration for tetrazole **3** was fitted to an exponential decay equation: $y = (y_0 - a)\, e^{-kt} + a$. It was found that ring rupture was very rapid, with a first-order rate constant k_1 of 0.14 s^{-1} (Figure S1d). The ring rupture was essentially complete after 20 sec at which time the concentration of the nitrile imine intermediate reached maximum. The subsequent decay of the nitrile imine proceeds through two pathways: (a) the cycloaddition reaction with cyclopropene **2**; (b) the water-quenching reaction with bulk water. In the presence of 5 equiv of dipolarophile, the cycloaddition between tetrazole **3** and cyclopropene **2** can be treated as a pseudo-first-order reaction. Thus, the pyrazoline cycloadduct concentrations were fitted to an exponential rise to maximum equation: $y = (y_0 - a)\, e^{-k_{obs}t} + a$ (Figure 2d), to give $k_{obs} = 0.029 \pm 0.0084$ s^{-1}. The second-order rate constant of cycloaddition, k_{cyc}, was calculated to be 58 ± 16 M^{-1} s^{-1} based on the equation: $k_{cyc} = k_{obs}$/[dipolarophile].

Similarly, the competing water-quenching reaction follows the pseudo-first-order kinetics and rate constant, k_{obs}, was determined to be 0.018 ± 0.012 s^{-1} by fitting the water-quenching products to an exponential rise to maximum equation: $y = (y_0 - a)\, e^{-k_{obs}t} + a$ (Figure S2d). The corresponding second-order rate constant, k_{WQ}, was calculated to be 6.5 ± 4.3 × 10^{-4} M^{-1} s^{-1} based on the equation: $k_{WQ} = k_{obs}$/[water].

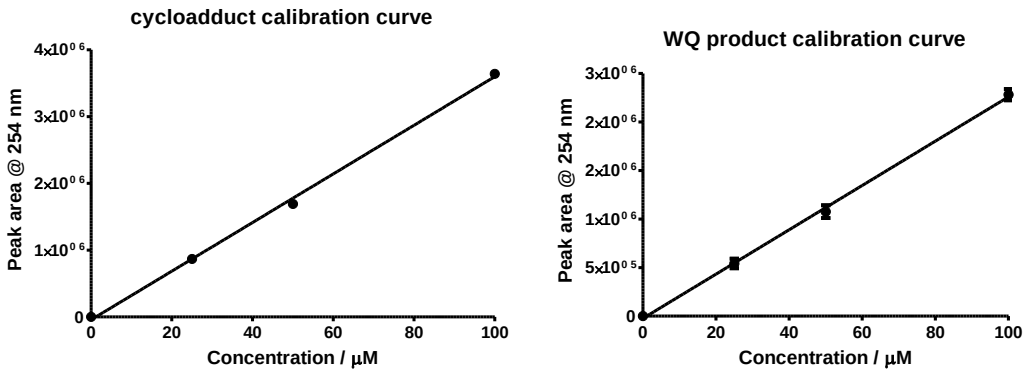
a)


0 sec
3
5 sec
A
3
5
10 sec
A
5
3
20 sec
A
5
11
11'
40 sec
A 5
11
11'
mVolts
Minutes

b)


80 sec
160 sec
320 sec
5
11'
11
A
mVolts
Minutes
OMe
Me
CO2Et
5
OH
11'
11

c)



d)

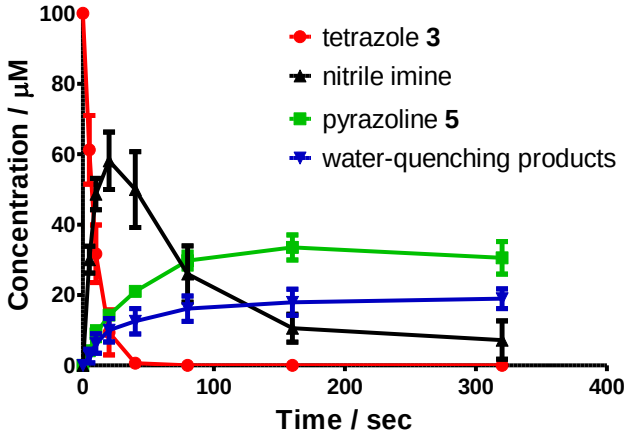
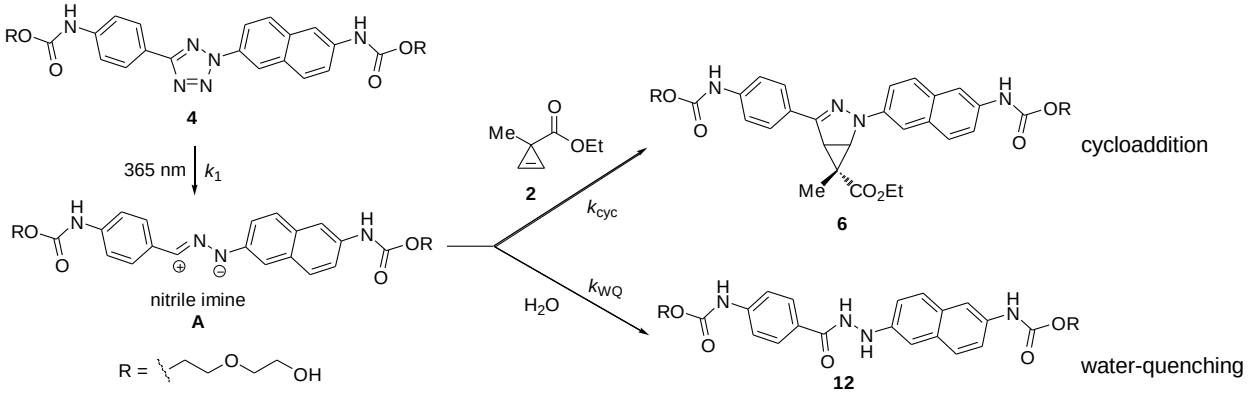
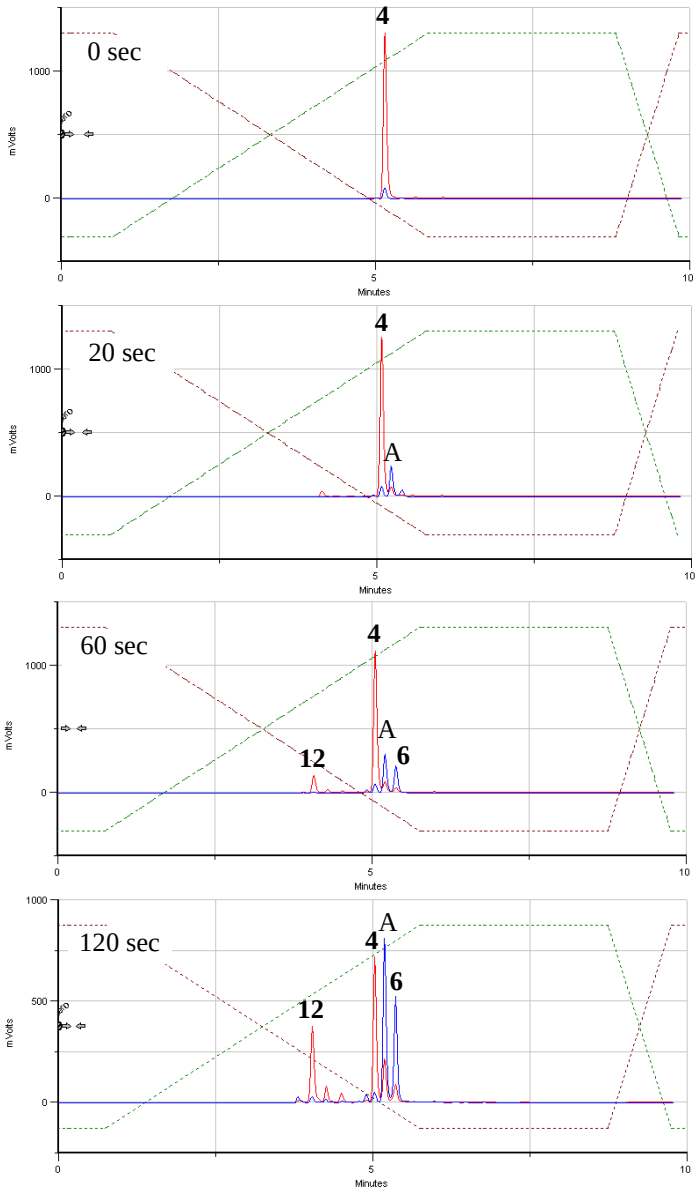


Figure S2. Kinetic studies of cyclopropene **2** in the photoinduced cycloaddition reaction with tetrazole **3** in acetonitrile/PBS buffer (1:1 v/v) under 302-nm irradiation: (a) Time course of the cycloaddition reactions at 0, 5, 10, 20, 40, 80, 160, and 320-sec intervals monitored by reverse-phase HPLC. Red traces = absorbance at 254 nm; blue traces = absorbance at 370 nm; (b) Standard traces of the purified pyrazoline cycloadduct **5** and the water-quenching product **11**; (c) Calibration curves of the cycloadduct **5** as well as the water-quenching product **11** over the concentration range of 0-100 µM; (d) Plots of the concentrations of the various species over the course of the reaction. The photoinduced decay of tetrazole **3** (red curve) was fitted to an exponential decay equation: $y = (y_0 - a)\, e^{-kt} + a$, while the formation of the pyrazoline cycloadduct (green curve) as well as the water-quenching product (blue curve) were fitted to an exponential rise to maximum equation: $y = (y_0 - a)\, e^{-k_{obs}t} + a$. The concentrations of the pyrazoline adducts as well as the water-quenching products were obtained by comparing their absorption peak areas at 254 nm to their respective standard curves. For determination of the cycloaddition rate, k_{cyc}, as well as the water-quenching rate, k_{WQ}, the data between 20 sec to 320 sec were used in the curve fitting. The time-course studies were repeated three times and the standard errors at each time point were included in the plots c and d.

Kinetic study of the photoinduced cycloaddition reaction between tetrazole 4 and ethyl 1-methylcycloprop-2-enecarboxylate (5 equiv) upon 365-nm UV irradiation



a)



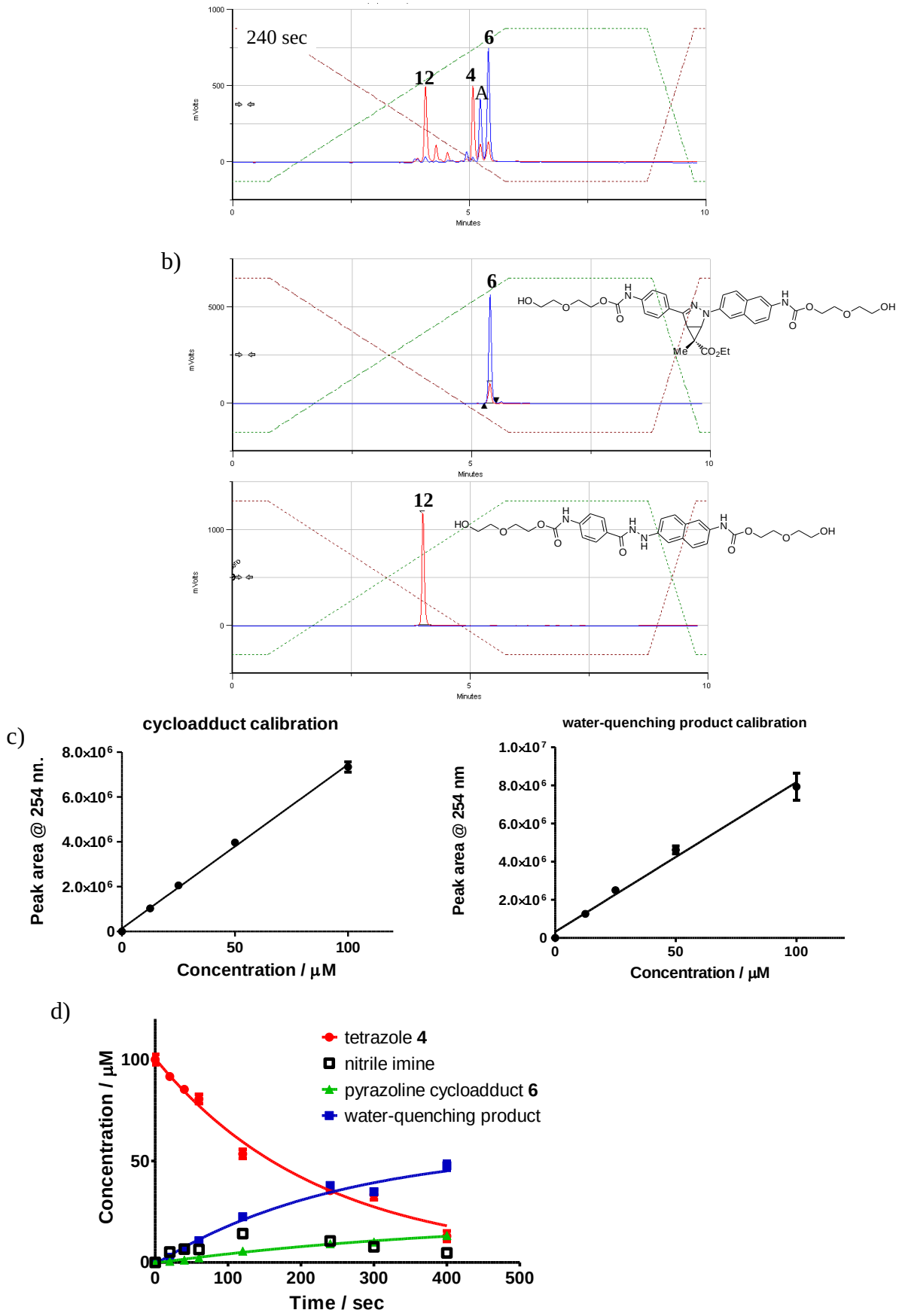
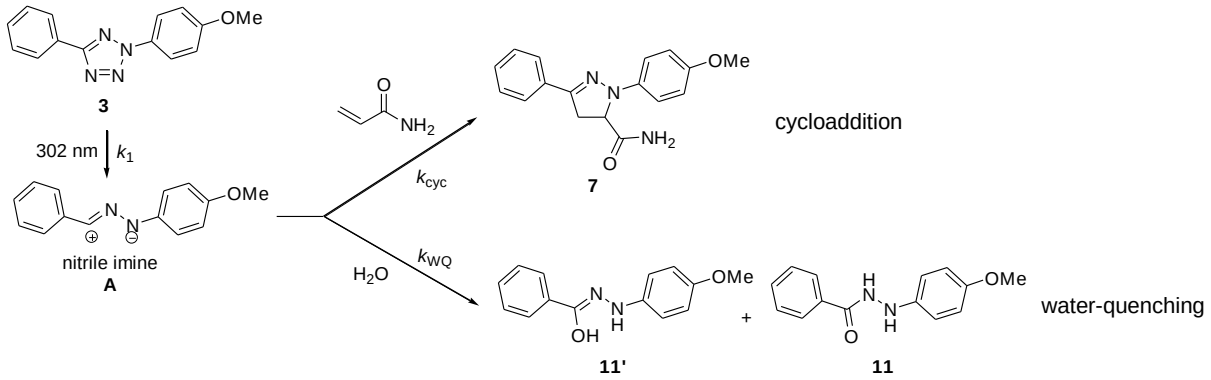


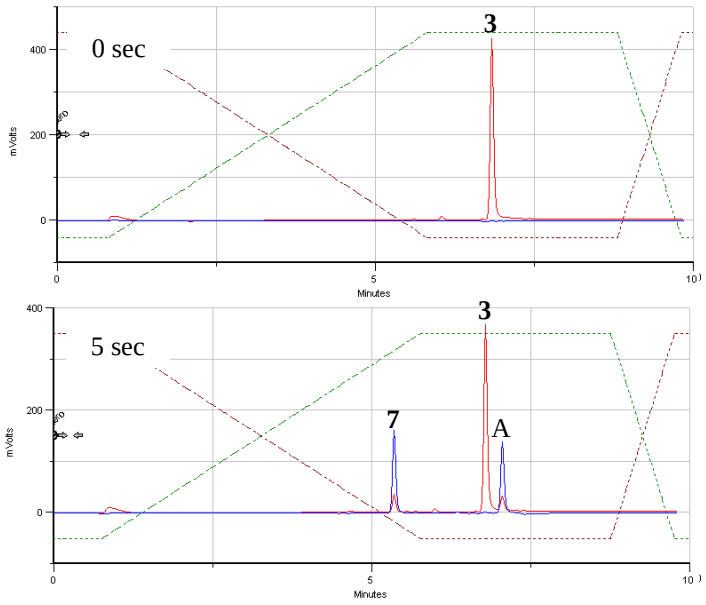
Figure S3. Kinetic studies of cyclopropene **2** in the photoinduced cycloaddition reaction with tetrazole **4** in acetonitrile/PBS buffer (1:1 v/v) under 365-nm irradiation: (a) Time course of

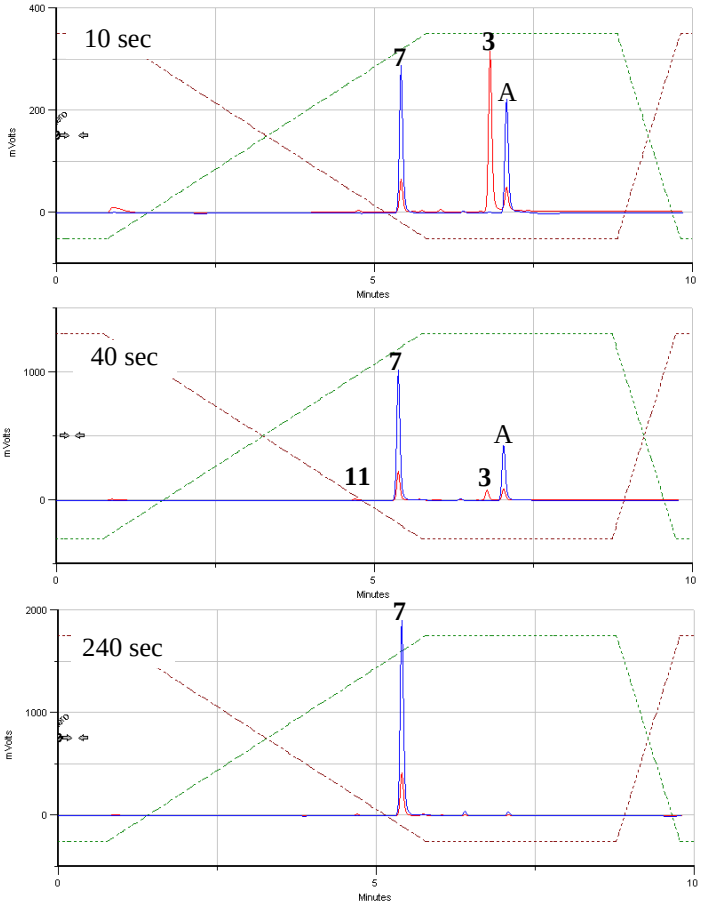
the cycloaddition reactions at 0, 20, 60, 120, and 240-sec intervals monitored by reverse-phase HPLC. Red traces = absorbance at 254 nm; blue traces = absorbance at 370 nm; (b) Standard traces of the purified pyrazoline cycloadduct **6** and the water-quenching product **12**; (c) Calibration curves of the cycloadduct as well as the water-quenching product over the concentration range of 0-100 μM; (d) Plots of the concentrations of the various species over the course of the reaction. The photoinduced decay of tetrazole **4** (red curve) was fitted to an exponential decay equation: $y = (y_0 - a)\, e^{-kt} + a$, while the formation of the pyrazoline cycloadduct (green curve) as well as the water-quenching product (blue curve) were fitted to an exponential rise to maximum equation: $y = (y_0 - a)\, e^{-k_{obs}t} + a$. The concentrations of the pyrazoline adducts as well as the water-quenching products were obtained by comparing their absorption peak areas at 254 nm to their respective standard curves. The time-course studies were repeated three times and the standard errors at each time point were included in the plots. Based on the curve fitting, $k_1 = 0.0045 \pm 0.00079\ s^{-1}$, $k_{cyc} = 4.6 \pm 1.3\ M^{-1}\ s^{-1}$, and $k_{WQ} = 1.4 \pm 0.29 \times 10^{-4}\ M^{-1}\ s^{-1}$.

Kinetic study of the photoinduced cycloaddition reaction between tetrazole 3 and acrylamide (5 equiv) upon 302-nm UV irradiation

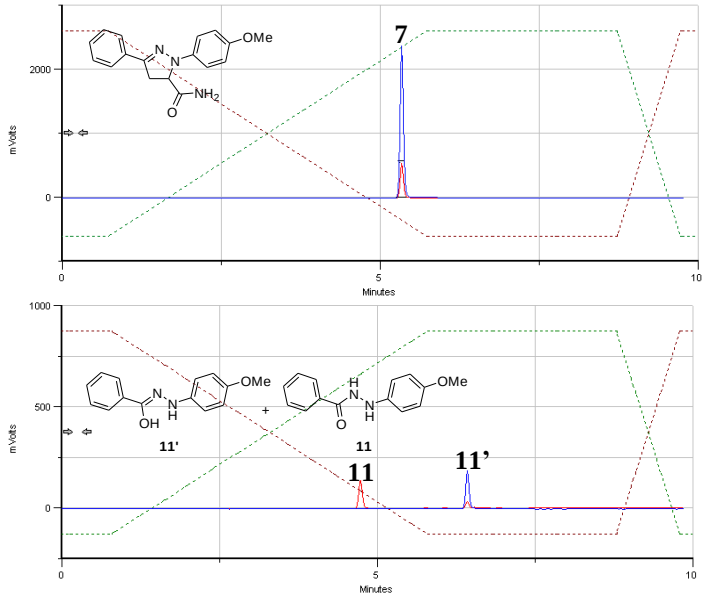


a)




10 sec
7
3
A
40 sec
7
11
A
3
240 sec
7
mVolts
Minutes

b)


7
OMe
NH2
O
11'
11
11'
mVolts
Minutes

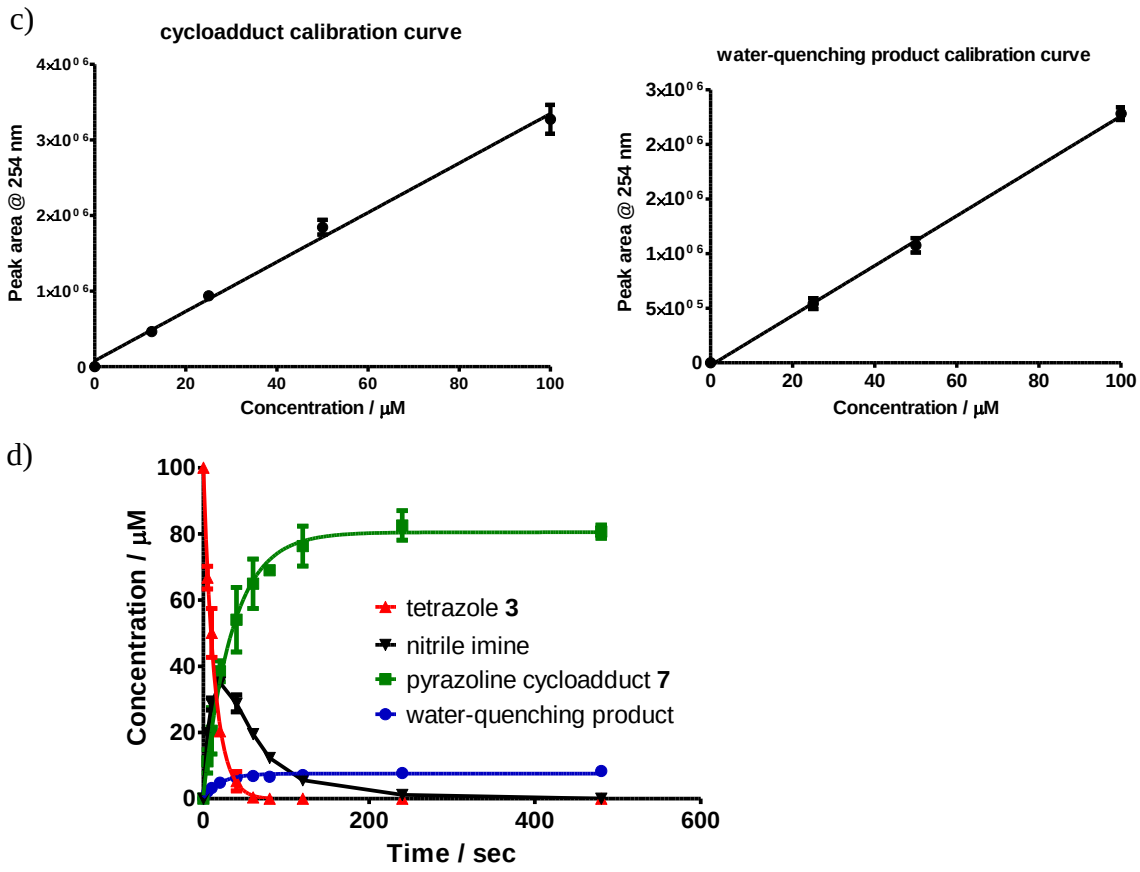
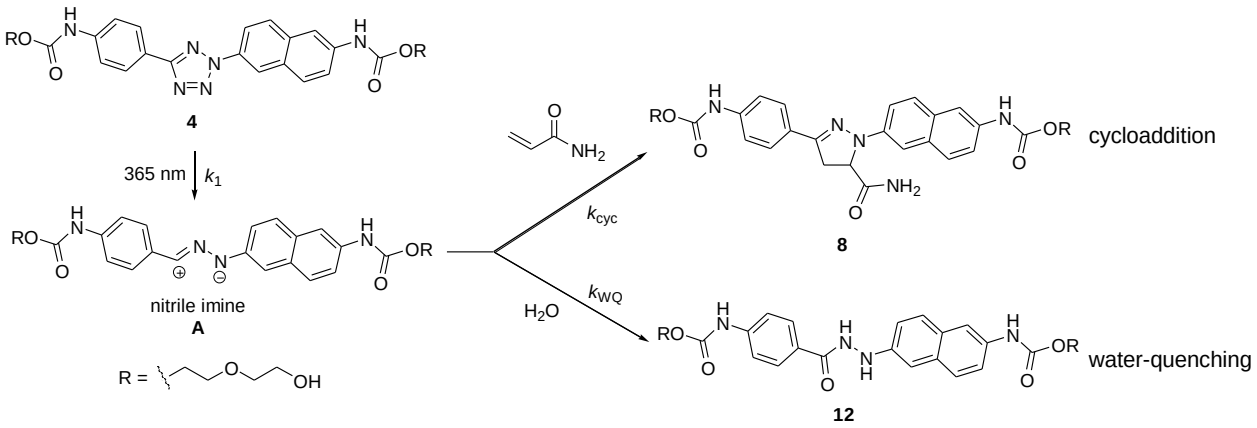
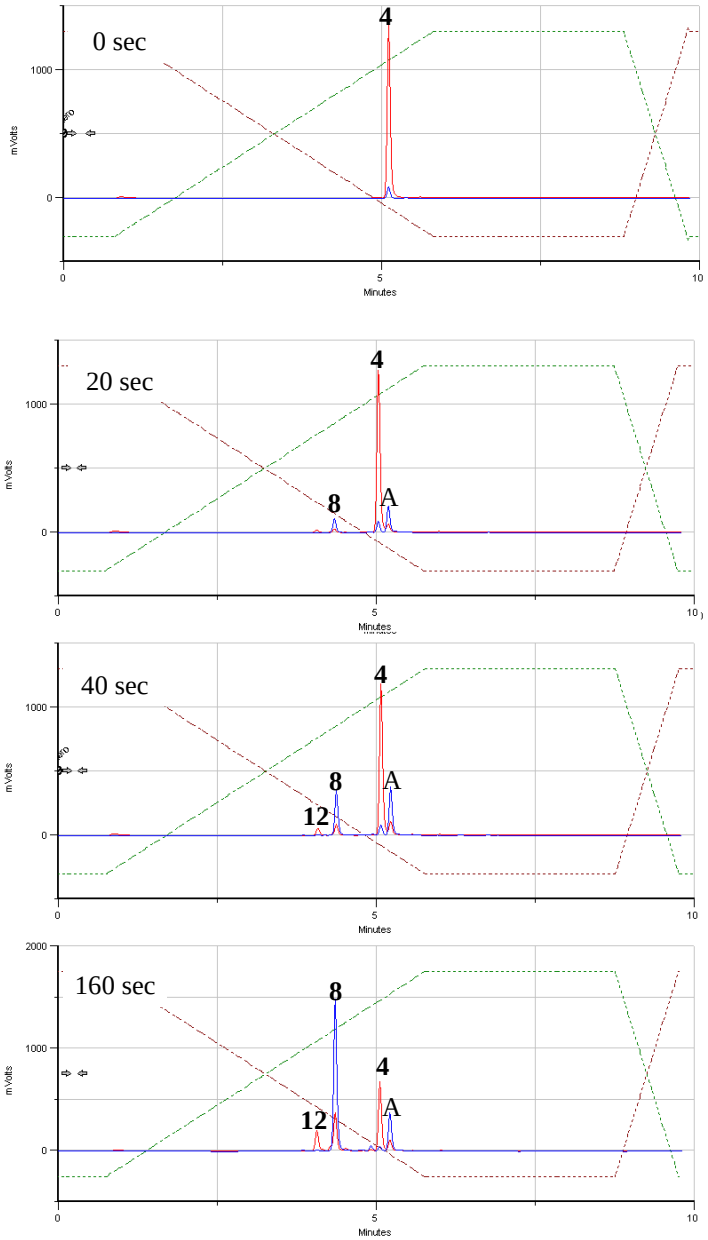


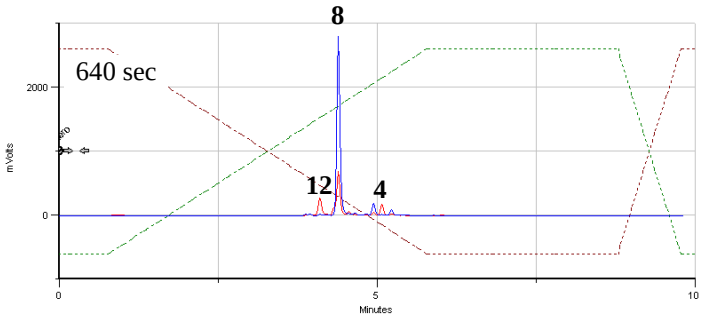
Figure S4. Kinetic studies of acrylamide in the photoinduced cycloaddition reaction with tetrazole **3** in acetonitrile/PBS buffer (1:1 v/v) under 302-nm irradiation: (a) Time course of the cycloaddition reactions at 0, 5, 10, 40, and 240-sec intervals monitored by reverse-phase HPLC. Red traces = absorbance at 254 nm; blue traces = absorbance at 370 nm; (b) Standard traces of the purified pyrazoline cycloadduct **7** and the water-quenching product **11**; (c) Calibration curves of the cycloadduct as well as the water-quenching product over the concentration range of 0-100 µM; (d) Plots of the concentrations of the various species over the course of the reaction. The photoinduced decay of tetrazole **3** (red curve) was fitted to an exponential decay equation: $y = (y_0 - a)\, e^{-kt} + a$, while the formation of the pyrazoline cycloadduct (green curve) as well as the water-quenching product (blue curve) were fitted to an exponential rise to maximum equation: $y = (y_0 - a)\, e^{-k_{obs}t} + a$. The concentrations of the pyrazoline adducts as well as the water-quenching products were obtained by comparing their absorption peak areas at 254 nm to their respective standard curves. The time-course studies were repeated three times and the standard errors at each time point were included in the plots. Based on the curve fitting, $k_1 = 0.075 \pm 0.0028\ s^{-1}$, $k_{cyc} = 46 \pm 8.6\ M^{-1}\, s^{-1}$, and $k_{WQ} = 6.1 \pm 2.2 \times 10^{-4}\ M^{-1}\, s^{-1}$.

Kinetic study of the photoinduced cycloaddition reaction between tetrazole 4 and acrylamide (5 equiv) upon 365-nm UV irradiation

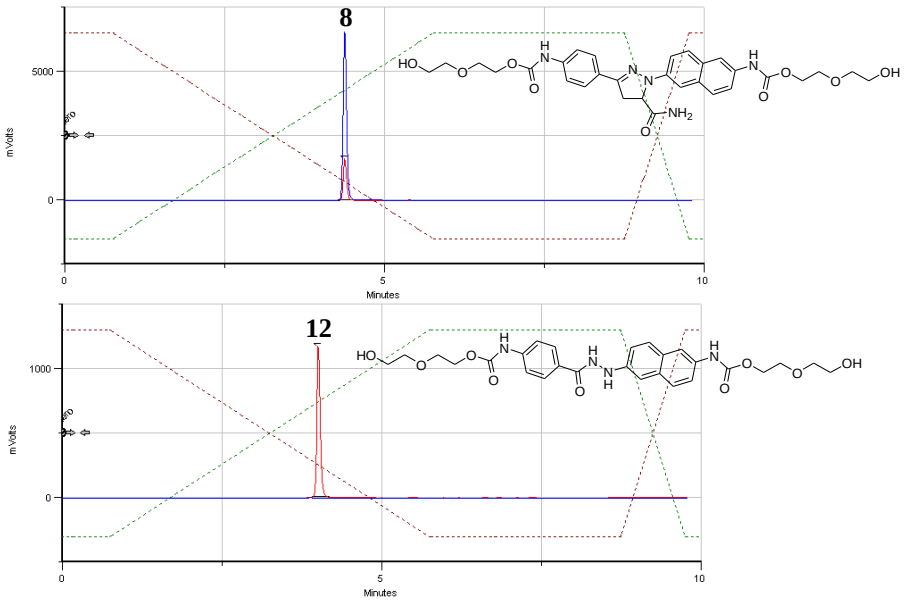

4
365 nm
k_1
nitrile imine
A
R =
k_{cyc}
k_{WQ}
H_2O
cycloaddition
8
water-quenching
12

a)

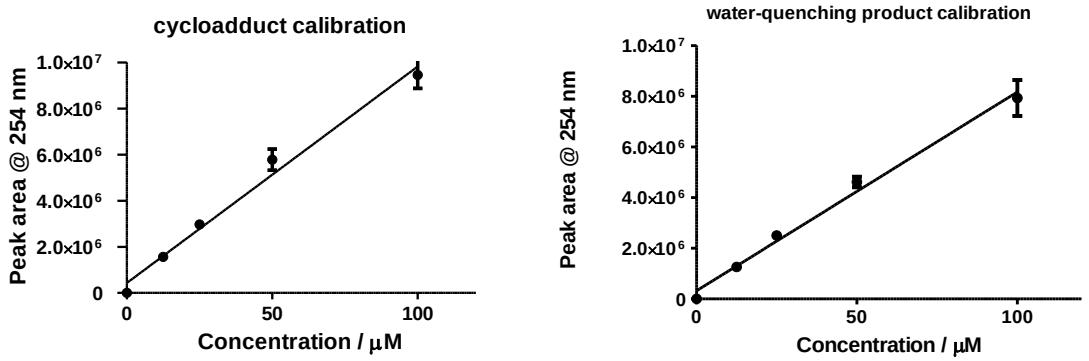

0 sec
4
20 sec
8
A
40 sec
12
160 sec
mVolts
Minutes


8
640 sec
12
4
2000
0
m Volts
%
0
5
10
Minutes

b)


8
5000
0
m Volts
%
HO
O
O
O
H
N
N
N
NH2
O
O
O
O
O
H
N
O
OH
0
5
10
Minutes
12
1000
0
m Volts
%
HO
O
O
O
H
N
O
H
N
N
H
O
H
N
O
O
O
O
OH
0
5
10
Minutes

c)


cycloadduct calibration
Peak area @ 254 nm
1.0x10⁷
8.0x10⁶
6.0x10⁶
4.0x10⁶
2.0x10⁶
0
0
50
100
Concentration / µM
water-quenching product calibration
Peak area @ 254 nm
1.0x10⁷
8.0x10⁶
6.0x10⁶
4.0x10⁶
2.0x10⁶
0
0
50
100
Concentration / µM

d)

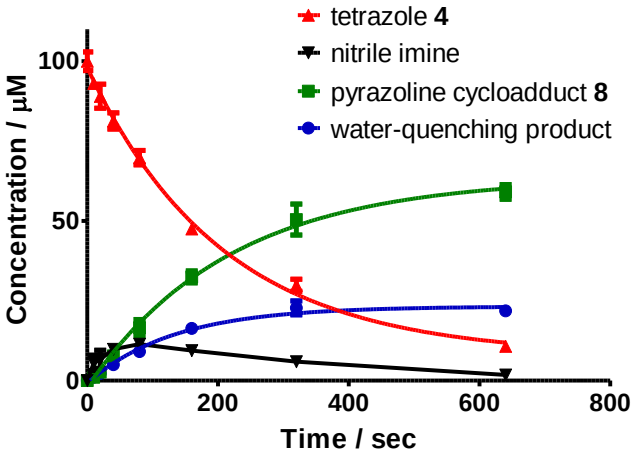
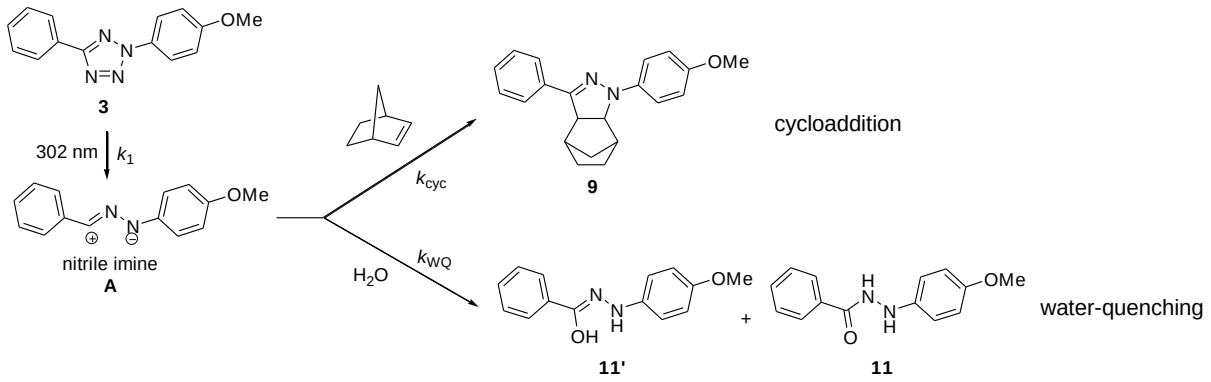
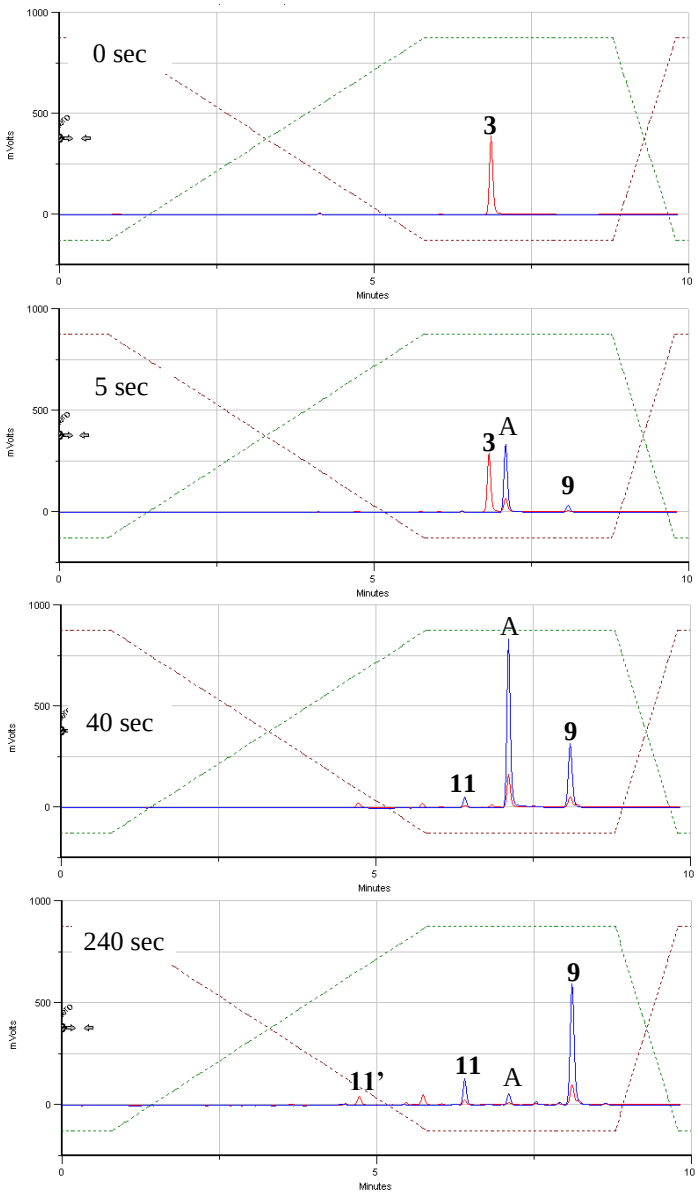

tetrazole 4
nitrile imine
pyrazoline cycloadduct 8
water-quenching product
Concentration / µM
100
50
0
0
200
400
600
800
Time / sec

Figure S5. Kinetic studies of acrylamide in the photoinduced cycloaddition reaction with tetrazole **4** in acetonitrile/PBS buffer (1:1 v/v) under 365-nm irradiation: (a) Time course of the cycloaddition reactions at 0, 20, 40, 160, and 640-sec intervals monitored by reverse-phase HPLC. Red traces = absorbance at 254 nm; blue traces = absorbance at 370 nm; (b) Standard traces of the purified pyrazoline cycloadduct **8** and the water-quenching product **12**; (c) Calibration curves of the cycloadduct **8** as well as the water-quenching product **12** over the concentration range of 0-100 μM; (d) Plots of the concentrations of the various species over the course of the reaction. The photoinduced decay of tetrazole **4** (red curve) was fitted to an exponential decay equation: $y = (y_0 - a)\, e^{-kt} + a$, while formation of the pyrazoline cycloadduct (green curve) as well as the water-quenching product (blue curve) were fitted to an exponential rise to maximum equation: $y = (y_0 - a)\, e^{-k_{obs}t} + a$. The concentrations of the pyrazoline adducts as well as the water-quenching products were obtained by comparing their absorption peak areas at 254 nm to their respective standard curves. The time-course studies were repeated three times and the standard errors at each time point were included in the plots. Based on the curve fitting, $k_1 = 0.0048 \pm 0.00029\ s^{-1}$, $k_{cyc} = 9.2 \pm 0.74\ M^{-1}\ s^{-1}$, and $k_{WQ} = 2.7 \pm 0.25 \times 10^{-4}\ M^{-1}\ s^{-1}$.

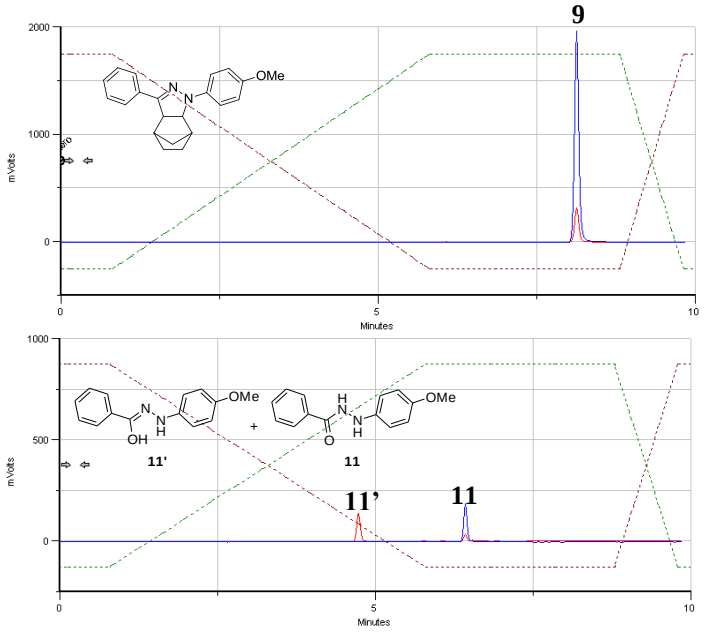
Kinetic study of the photoinduced cycloaddition reaction between tetrazole 3 and norbornene (5 equiv) under 302-nm UV irradiation



a)


0 sec
3
5 sec
3
A
9
40 sec
A
9
11
240 sec
9
11'
11
A
mVolts
Minutes

b)


9
OMe
N
N
OH
OMe
11'
11
11'
11
mVolts
Minutes

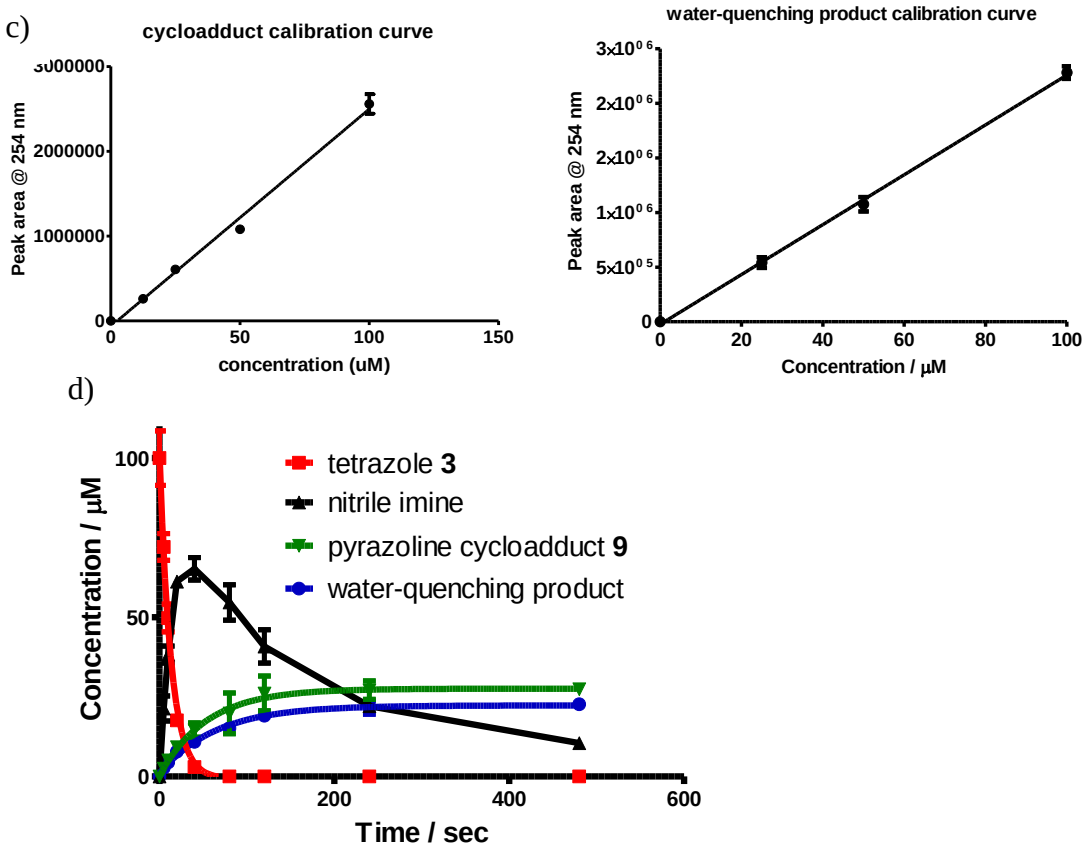
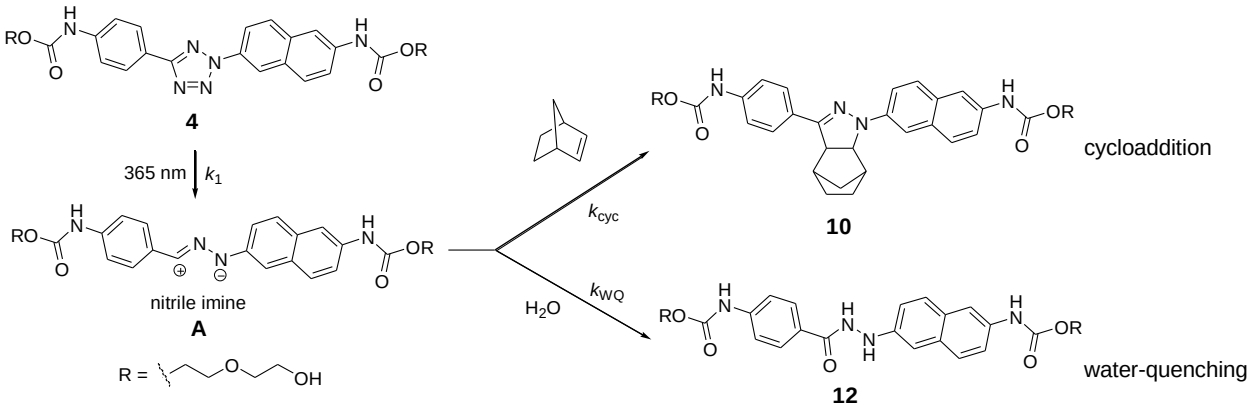
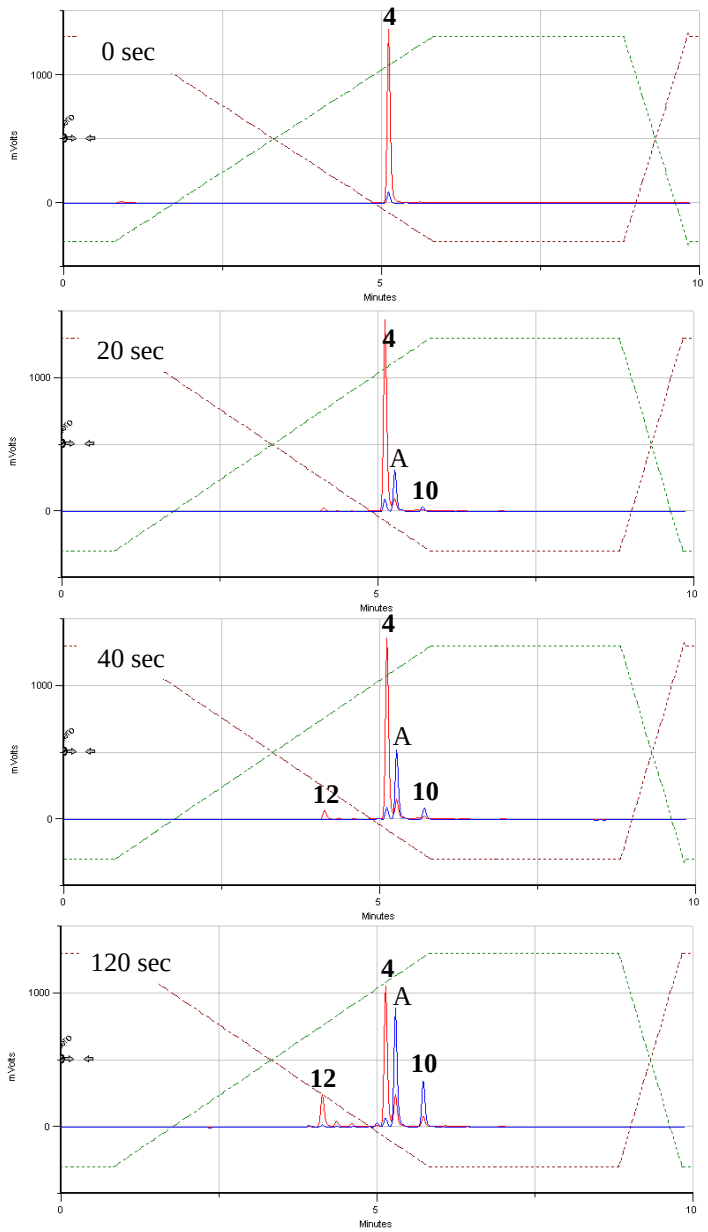


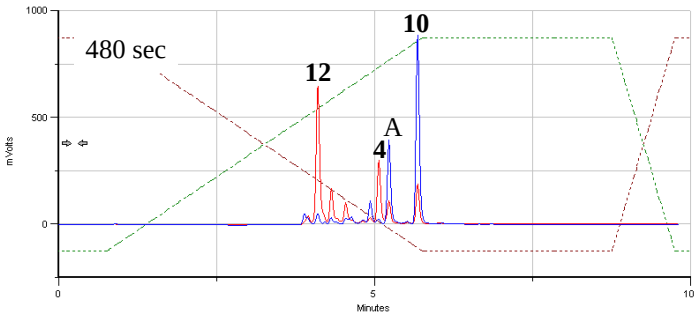
Figure S6. Kinetic studies of norbornene in the photoinduced cycloaddition reaction with tetrazole **3** in acetonitrile/PBS buffer (1:1 v/v) under 302-nm irradiation: (a) Time course of the cycloaddition reactions at 0, 5, 40, and 240-sec intervals monitored by reverse-phase HPLC. Red traces = absorbance at 254 nm; blue traces = absorbance at 370 nm; (b) Standard traces of the purified pyrazoline cycloadduct **9** and the water-quenching product **11**; (c) Calibration curves of the cycloadduct as well as the water-quenching product over the concentration range of 0-100 µM; (d) Plots of the concentrations of the various species over the course of the reaction. The photoinduced decay of tetrazole **4** (red curve) was fitted to an exponential decay equation: $y = (y_0 - a)\, e^{-kt} + a$, while the formation of the pyrazoline cycloadduct (green curve) as well as the water-quenching product (blue curve) were fitted to an exponential rise to maximum equation: $y = (y_0 - a)\, e^{-k_{obs}t} + a$. The concentrations of the pyrazoline adducts as well as the water-quenching products were obtained by comparing their absorption peak areas at 254 nm to their respective standard curves. The time-course studies were repeated three times and the standard errors at each time point were included in the plots. Based on the curve fitting, $k_1 = 0.075 \pm 0.0047\ s^{-1}$, $k_{cyc} = 32 \pm 12\ M^{-1}\, s^{-1}$, and $k_{WQ} = 6.8 \pm 0.81 \times 10^{-4}\ M^{-1}\, s^{-1}$.

Kinetic study of the photoinduced cycloaddition reaction between tetrazole 6 and acrylamide (5 equiv) under 365-nm UV irradiation

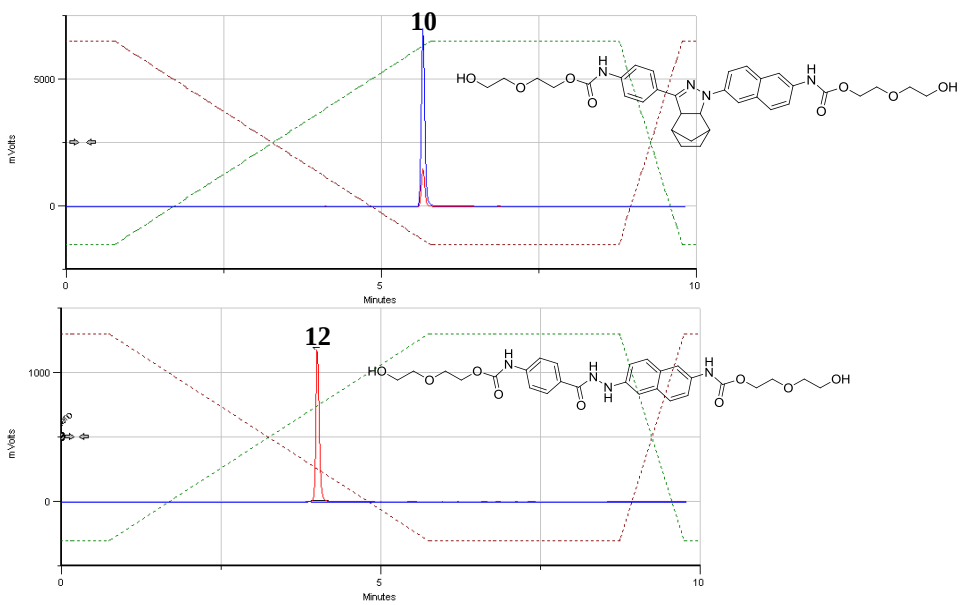

4
365 nm
k_1
nitrile imine
A
R =
k_{cyc}
k_{WQ}
H_2O
10
cycloaddition
12
water-quenching

a)

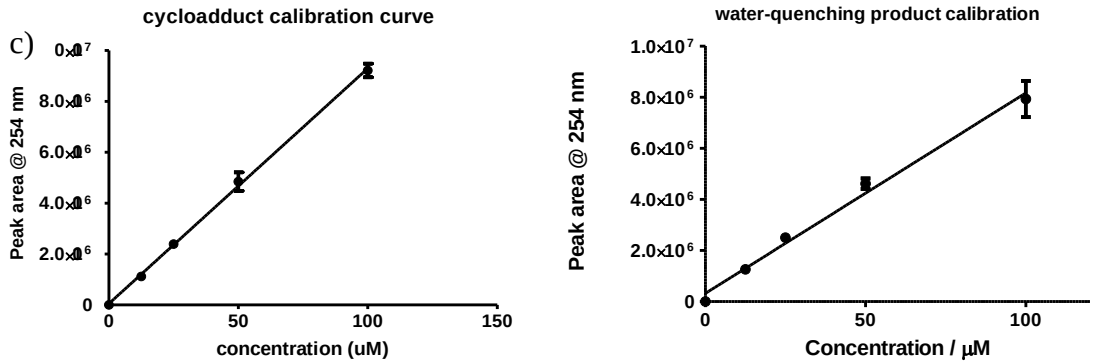

0 sec
4
20 sec
A
10
40 sec
12
120 sec
m Volts
Minutes


480 sec
10
12
A
4
m Volts
Minutes

b)


10
12
m Volts
Minutes

c)


cycloadduct calibration curve
Peak area @ 254 nm
concentration (uM)
water-quenching product calibration
Peak area @ 254 nm
Concentration / µM

d)

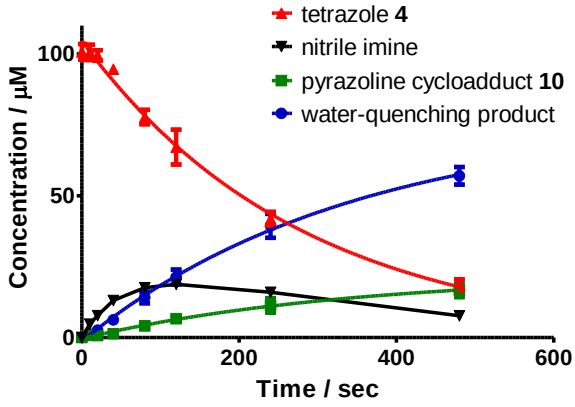

tetrazole 4
nitrile imine
pyrazoline cycloadduct 10
water-quenching product
Concentration / µM
Time / sec

Figure S7. Kinetic studies of norbornene in the photoinduced cycloaddition reaction with tetrazole **4** in acetonitrile/PBS buffer (1:1 v/v) under 365-nm irradiation: (a) Time course of the cycloaddition reactions at 0, 20, 40, 120, and 480-sec intervals monitored by reverse-phase HPLC. Red traces = absorbance at 254 nm; blue traces = absorbance at 370 nm; (b) Standard traces of the purified pyrazoline cycloadduct **10** and the water-quenching product **12**; (c) Calibration curves of the cycloadduct as well as the water-quenching product over the concentration range of 0-100 μM; (d) Plots of the concentrations of the various species over the course of the reaction. The photoinduced decay of tetrazole **4** (red curve) was fitted to an exponential decay equation: $y = (y_0 - a)e^{-kt} + a$, while the formation of the pyrazoline cycloadduct (green curve) as well as the water-quenching product (blue curve) were fitted to an exponential rise to maximum equation: $y = (y_0 - a)e^{-k_{obs}t} + a$. The concentrations of the pyrazoline adducts as well as the water-quenching products were obtained by comparing their absorption peak areas at 254 nm to their respective standard curves. The time-course studies were repeated three times and the standard errors at each time point were included in the plots. Based on the curve fitting, $k_1 = 0.0036 \pm 0.00043\ s^{-1}$, $k_{cyc} = 5.8 \pm 1.4\ M^{-1}\ s^{-1}$, and $k_{WQ} = 1.0 \pm 0.13\times10^{-4}\ M^{-1}\ s^{-1}$.

Table S2. Synthetic oligonucleotide primers used in the construction of pBK-CpK library.

Name	Sequence
jw-f1	CGAAAGGTCTCCCCCGACCNNKNNKAACTATNNKCGTAAACTGGATCGTATTCTGC
jw-r1	CGAAAGGTCTCTCGGGGCMNNCATCGGACGCAGGCACAG
jw-f2	CACCATGGTTAGTTTTNNKCAAATGGGCAGCGGCTGCACCCGTGAAAAC
jw-r2	CATTTGACTAAAMNNAACCATGGTGAATTCTTCCAGGTGTTCTTTG

Expression of CpK-Containing Myoglobin

Plasmid pBAD-pylT-Myo4TAG was co-transformed with pBK-CpK into GeneHog®-Fis *E coli* cells. Cells were amplified in 5 mL of 2YT medium containing kanamycin (50 μg/mL) and tetracycline (15 μg/mL). The starter culture (1 mL) was then used to inoculate 100 mL of LB medium supplemented with 1 mM of CpK and appropriate antibiotics. The cells were grown at 37 °C to OD_{600} of 0.5 before addition of 0.2% arabinose to induce the protein expression. After another 4-12 hours, cells were harvested by centrifugation. The myoglobin TAG4→**1** mutant protein was purified by Ni-NTA affinity chromatography under the native conditions.

Mass Spectrometric Analysis of CpK-Containing Myoglobin and Pyrazoline-Containing Myoglobin Adduct

The purified myoglobin-TAG4CpK protein was digested with trypsin, and the mixture was analyzed by a Finnigan nanoLC-LTQ tandem mass spectrometer (Thermo Electron). The tandem mass spectrum of the tryptic CpK-containing fragment of MVLK*EGEWQLVLHV-

WAK (K* denotes CpK) indicated an efficient incorporation of CpK. The CpK-containing fragemented ions (b_4^+, b_6^+, y_{14}^{2+}, and y_{16}^{2+}) showed the expected masses, which led to the unambiguous assignment of CpK incorporation at position 4. To acquire the mass of the myoglobin-TAG4CpK photoclick reaction adduct, we set up the reaction by incubating 100 μM of tetrazole **3** and 1 mg/mL of Myo-CpK in 0.5 mL PBS/ACN (1:1). The mixture was exposed to a handheld UV lamp for 8 min and then immediately subjected to MS analysis by Q-TOF Micromass spectrometer（Waters Ltd., Manchester, UK).

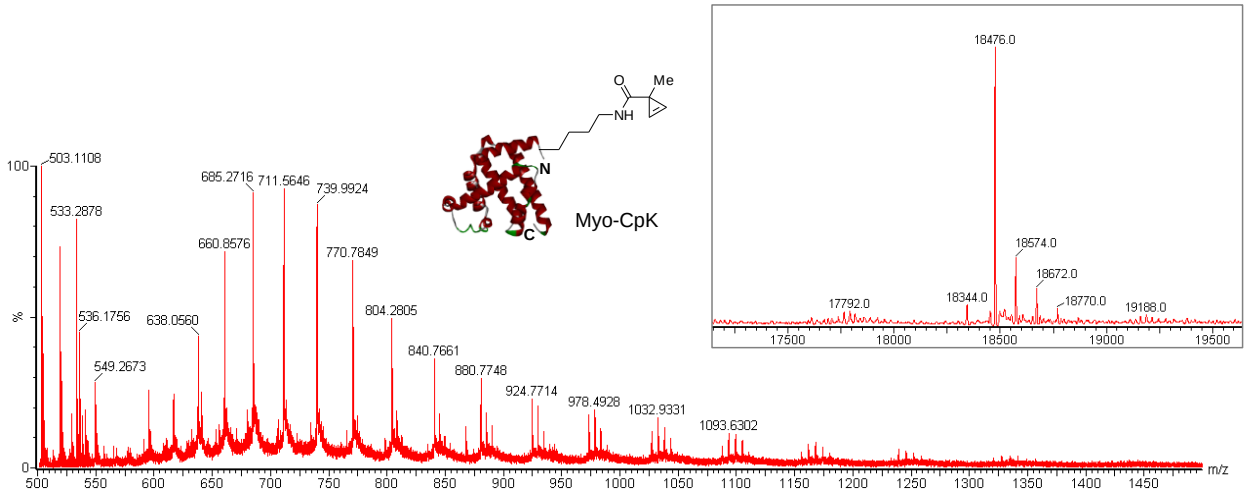


Figure S8. Charge ladder of Myo-CpK analyzed by ESI-TOF mass spectrometry. The de-convoluted intact protein mass is shown in the inert.

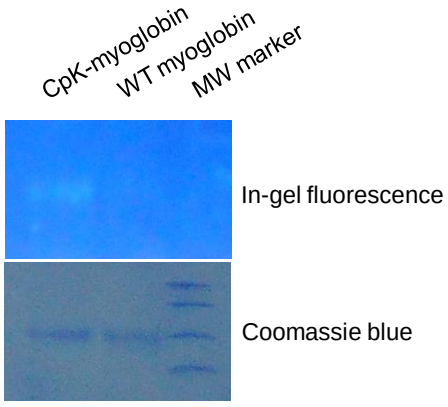


Figure S9. Selective labeling of CpK-encoded myoglobin via photoclick chemistry. A solution of CpK-myoglobin (0.5 mg/mL) or WT-myoglobin (0.5 mg/mL) and tetrazole **3** (100 μM) in PBS buffer was exposed to 302-nm photoirradiation for a period of 12 min before the mixtures were resolved by SDS-PAGE. The in-gel fluorescence (top panel) was recorded with a digital camera by illuminating the gel with 365-nm UV light. The gel was then stained with Commassie blue (bottom panel) to confirm protein size and equal loading.

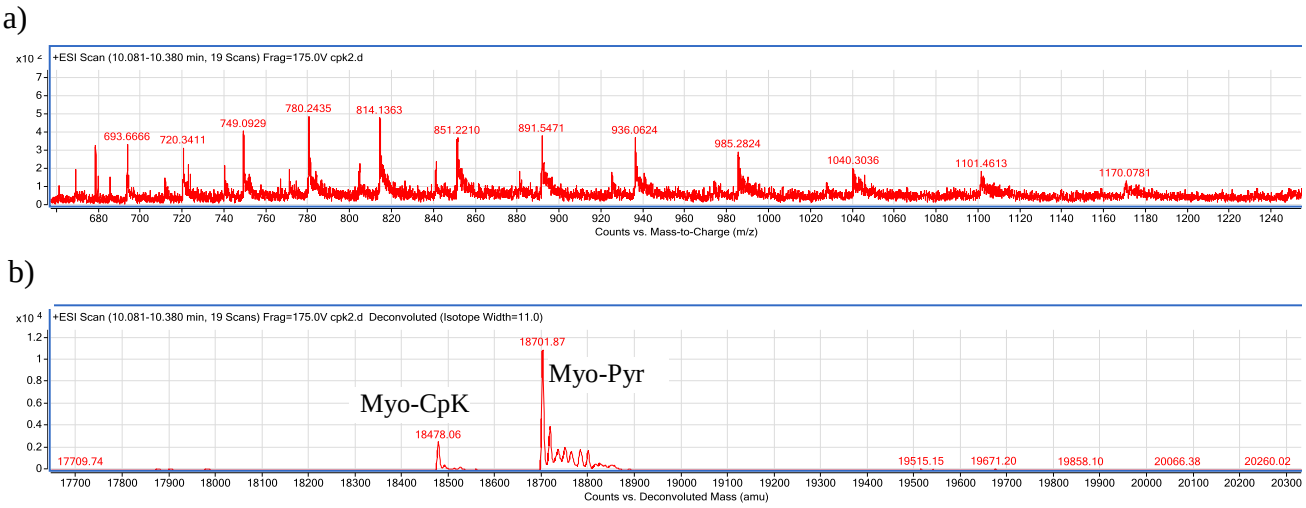


Figure S10. (a) Charge ladder of the pyrazoline-labeled myoglobin (Myo-pyr) analyzed by ESI-TOF mass spectrometry. (b) The de-convoluted intact protein mass showing greater than 85% conversion from Myo-CpK to Myo-Pyr. The small peaks with higher molecular weight correspond to the phosphate (+ 98 Da) adducts of Myo-CpK and Myo-Pyr.

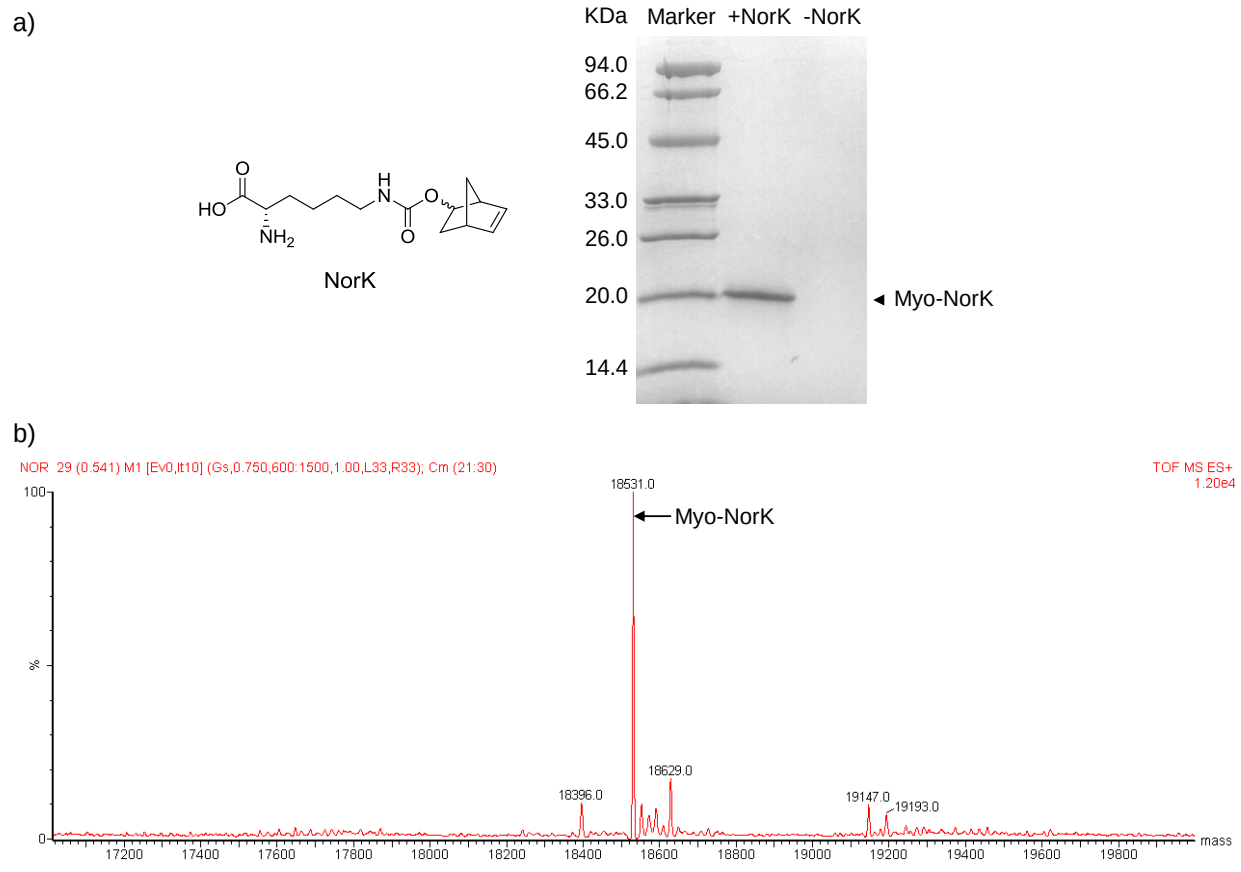


Figure S11. Incorporation of NorK site-selectively into myoglobin: (a) Coomassie blue stained gel of Myo-NorK expressed in the presence or absence of 1 mM NorK. An isolated yield of 1.0 mg/L was obtained. (b) The de-convoluted intact mass for NorK as analyzed by ESI-TOF: expected 18532.0, found 18531.0.

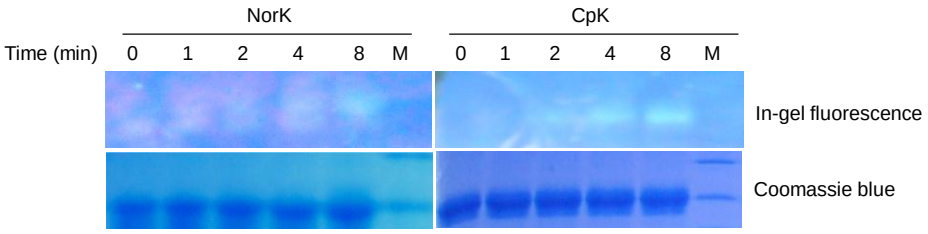
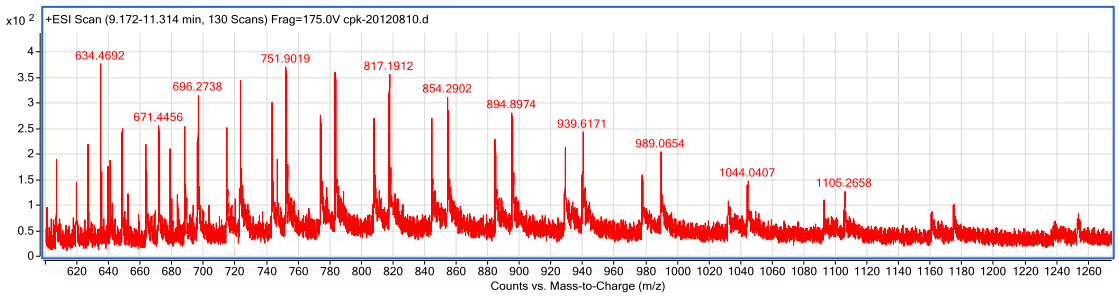


Figure S12. Comparison of the reactivity of NorK to CpK, both of which is genetically encoded at position-4 of myoglobin, in the photoinduced cycloaddition reaction with tetrazole **3**. A solution of myoglobin-NorK (0.5 mg/mL) or myoglobin-CpK (0.5 mg/mL) and tetrazole **3** (100 μM) in PBS buffer was exposed to 302-nm photoirradiation for a period of 8 min before the mixtures were resolved by SDS-PAGE. The in-gel fluorescence (top panel) was recorded with a digital camera by illuminating the gel with 365-nm UV light. The gel was then stained with Coommassie blue (bottom panel) to confirm protein size and equal loading.

a)



b)

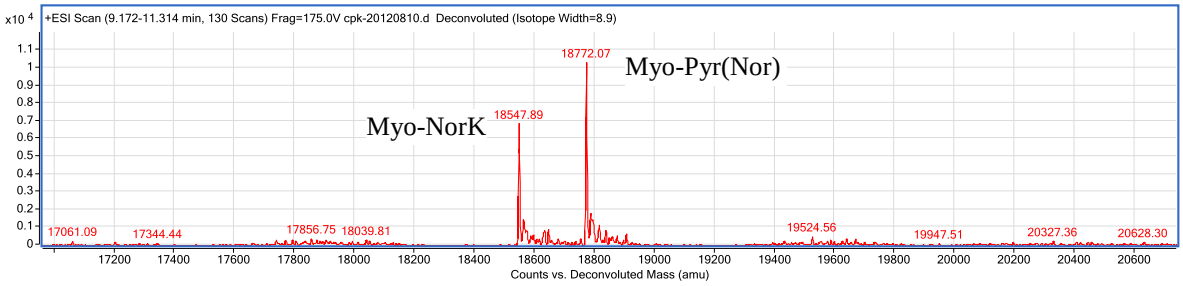


Figure S13. (a) Charge ladder of the pyrazoline cycloadduct of myoglobin-NorK analyzed by ESI-TOF mass spectrometry. (b) The de-convoluted intact mass showing greater than 60% conversion from Myo-NorK to Myo-Pyr(Nor). The apparent increase of 224.18 Da in MW after the cycloaddition reaction with tetrazole **3** matches well to the theoretic increase of 225.26 Da.

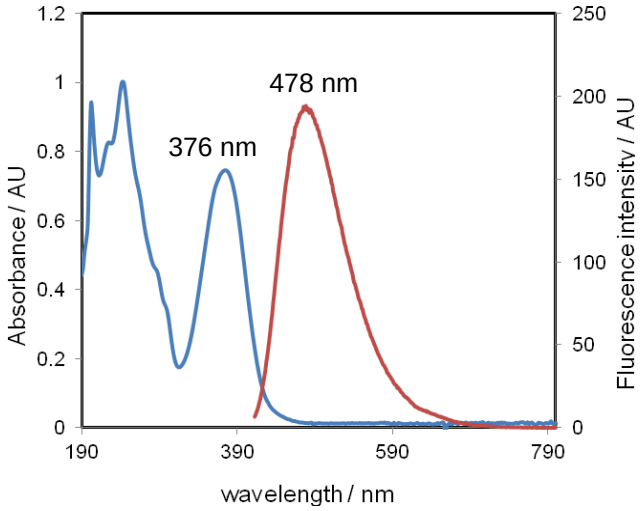


Figure S14. UV-vis (blue line) and fluorescence (red line) of pyrazoline **6** from from the photoinduced cycloaddition reaction between tetrazole **4** and cyclopropene **2**. Pyrazoline **6** was dissolved in the acetonitrile/PBS (1:1) mixed solvent at a concentration of 25 μM. For fluorescence measurement, 405 nm excitation was used.

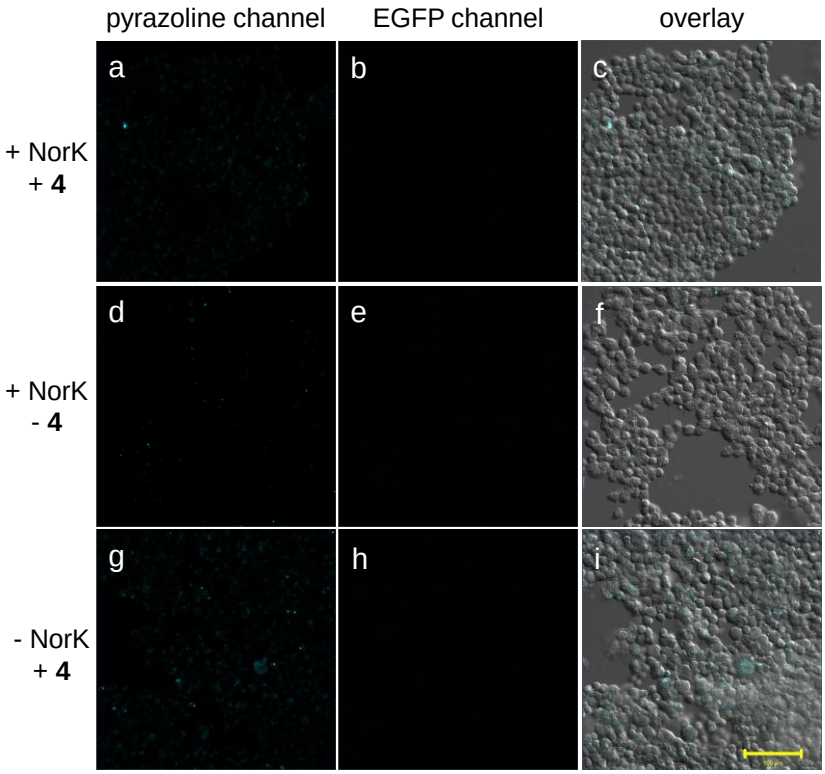


Figure S15. Confocal micrographs of human embryonic kidney 293 cells transfected with the plasmids expressing wild-type *Mb*PylRS, *Mb*tRNA$_{CUA}$ and EGFP37TAG and grown in the presence of 4 mM NorK (a-f) or the absence of NorK (g-i). The cells in Panels a-c and h-j were treated with 40 μM of tetrazole 4 for 2 hours before 365-nm photoirradiation with a handheld UV lamp for 3 minutes. Scale bar = 100 μm.

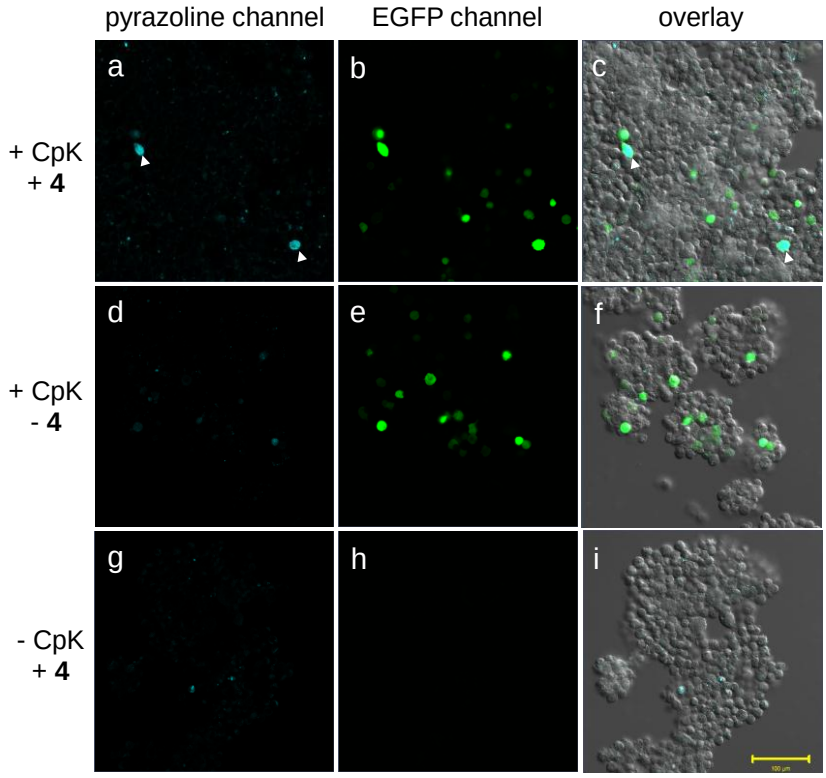


Figure S16. Confocal micrographs of human embryonic kidney 293 cells transfected with the plasmids expressing CpKRS, *Mb*tRNA$_{CUA}$ and EGFP37TAG under identical conditions as Figure S15 and grown in the presence of 4 mM CpK (a-f) or the absence of CpK (g-i). The cells in Panels a-c and h-j were treated with 40 μM of tetrazole 4 for 2 hours before 365-nm photoirradiation with a handheld UV lamp for 3 minutes. Scale bar = 100 μm.

Site-Specific Incorporation of CpK into EGFP in HEK293 Cells and Subsequent Labeling by Tetrazole 4

HEK293 cells were allowed to grow to 80-90% confluency on 35-mm tissue culture plates in 2 mL DMEM medium supplemented with 10% FBS in a humidified 37 °C, 5% CO2 incubator. The cells were then co-transfected with pCMV-CpKRS and pSwan-EGFP37TAG with transfectamine 2000 (Invitrogen) in 1-mL OPTI-MEM medium containing 8 mM CpK by following the manufacturer's instructions. Appropriate amount of pure FBS and DMEM were added to the culture medium 6 hours after transfection to return the medium conditions back to 2 mL DMEM containing 10% FBS and 4 mM CpK. The cells were incubated in the CO2 incubator for 36 hours. Then, the cells were treated with 40 μM of tetrazole **4** for 1.5 hours, washed twice with low fluorescence medium (EBSS medium supplemented with 1× essential amino acids, 1× L-glutamate, and 1× sodium pyruvate), and incubated for additional 0.5 hour. The cells in the tissue culture plate were photoirradiated with a handheld UV lamp (UVP, UVGL-25, 365 nm, 3.5 mW/cm^2) for 2 minutes and incubated for another 2 minutes before fluorescent image acquisition. The control experiments were carried out with the transfected cells either grown in the presence of CpK but without the tetrazole **4** treatment or grown in the absence of CpK but with the tetrazole **4** treatment. The image acquisition was carried out using a Zeiss LSM 710 laser scanning microscope equipped with an EC Plan-

Neofluar 10×/0.30 M27 objective and 1× plane scan zoom. The acquired images were processed using Zeiss ZEN 2009 Light Edition program.

Confocal Fluorescence Microscopy

Cells placed in low fluorescent medium were imaged using a Zeiss LSM 710 laser scanning microscope with an EC Plan-Neofluar 10x/0.30 M27 objective and using 1× plane scan zoom, averaging 2, 12-bit imaging file. The following excitation sources and filter sets were used:

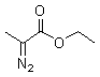
Fluorophore	Laser excitation (nm)	Emission windows(nm)	Beam splitters
Pyrazoline (track1)	405	410-498	f-MBS 405/580c
EGFP (track 2)	488	499-578	f-MBS 405/610c

The images were acquired at two tracks with single laser excitation rather than single track with two lasers exciting simultaneously to avoid fluorescence interference. Images were acquired using Zeiss ZEN 2010 and processed using the software Zeiss ZEN 2009 Light Edition. All three samples were imaged under the identical excitation and acquisition conditions and the images were processed with identical scaling factors for valid comparison.

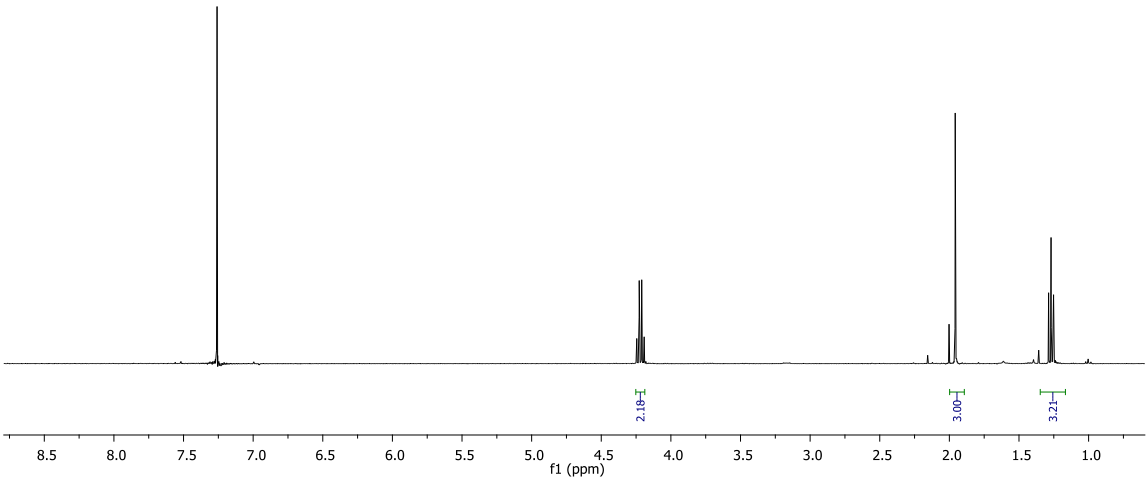
Reference:

[S1] Bachmann, S.; Fielenbach, D.; Jørgensen, K. A. *Org. Biomol. Chem.* **2004**, *2*, 3044.
[S2] Yu, Z.; Ho, L. Y.; Wang, Z.; Lin, Q. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5033.
[S3] Lang, K.; Davis, L.; Torres-Kolbus, J.; Chou, C.; Deiters, A.; Chin. J. W. *Nature Chem.* **2012**, *4*, 298.

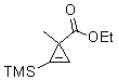
071010-2-001-1
071010-2-001-1



1a

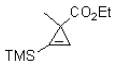


071710-B-5-1
071710-B-5-1



1b

071710-B-5-1-C13
071710-B-5-1-C13



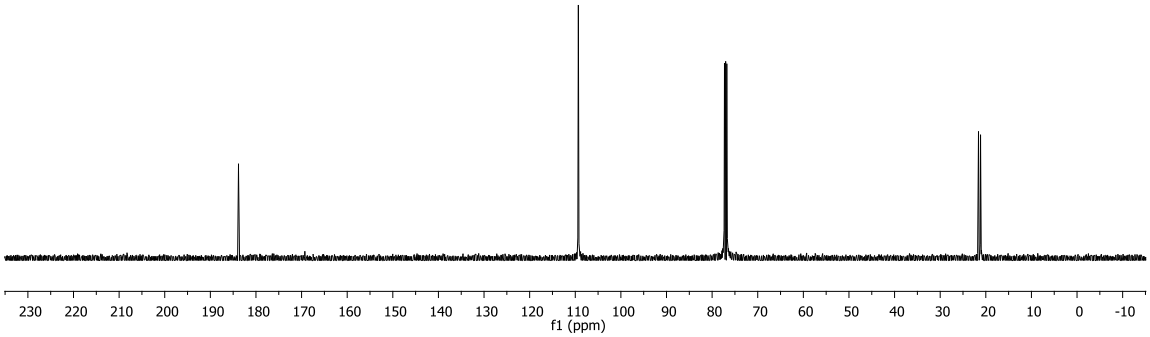
1b

073010-B-15-1
073010-B-15-1

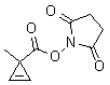
1c

073110-B15-1-C13
073110-B15-1-C13

1c

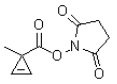


073110-B-16-1
073110-B-16-1

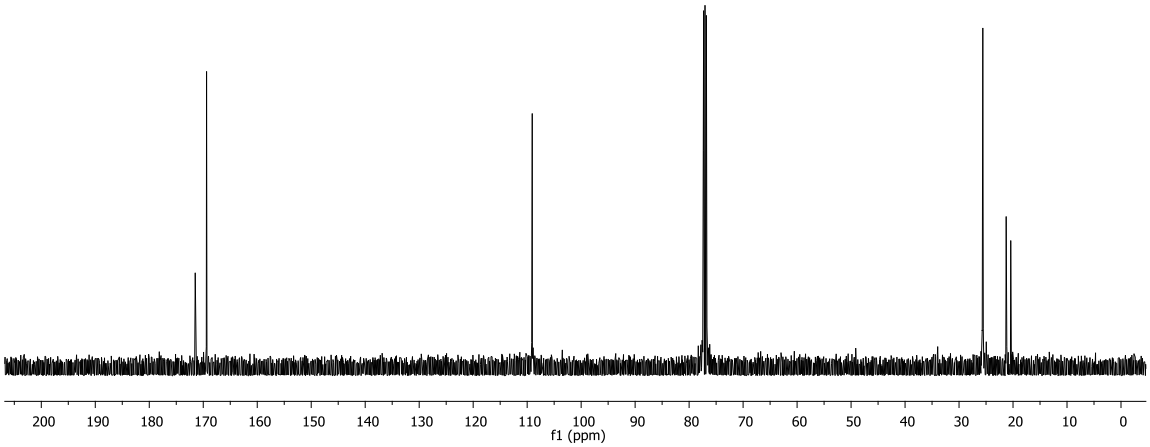


1d

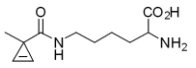
073110-B-16-1-C13
073110-B-16-1-C13



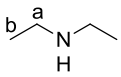
1d



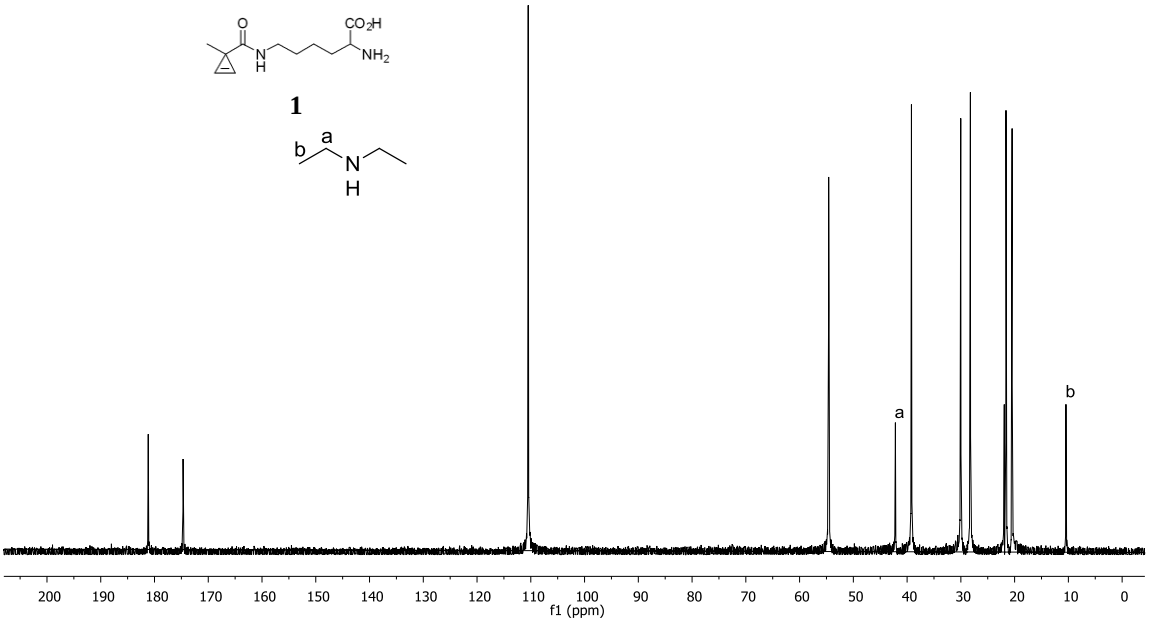
080610-B-20-D2O
080610-B-20-D2O



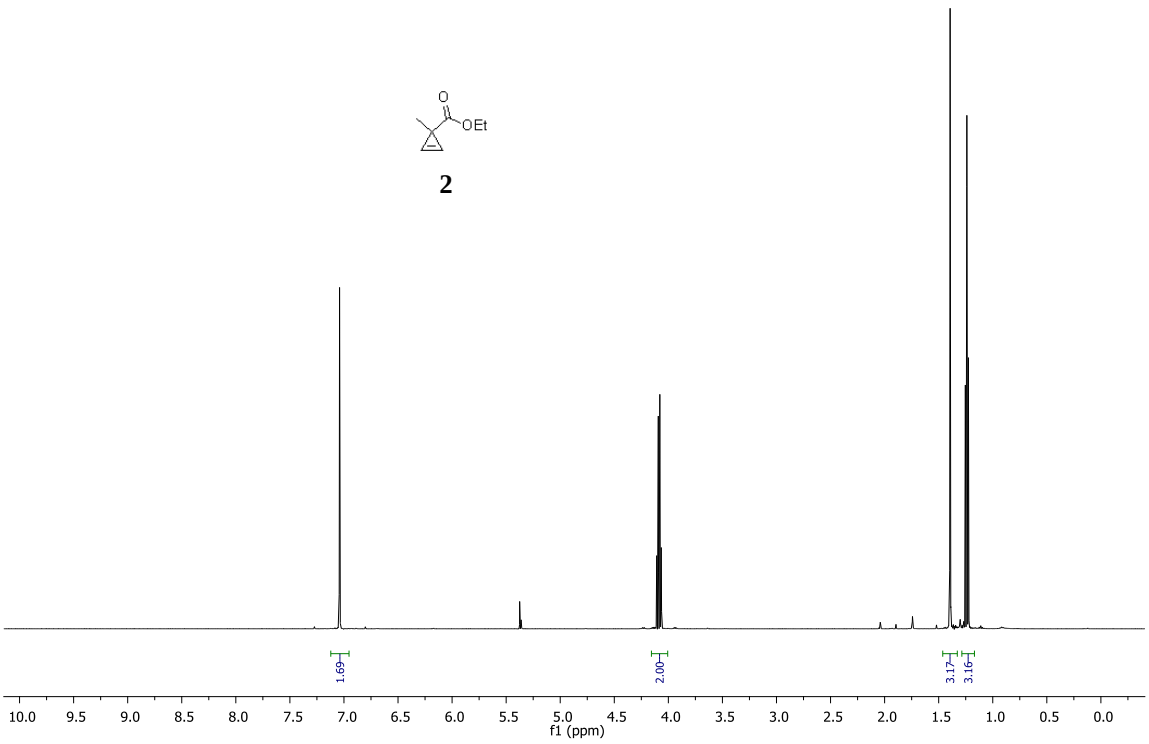
1



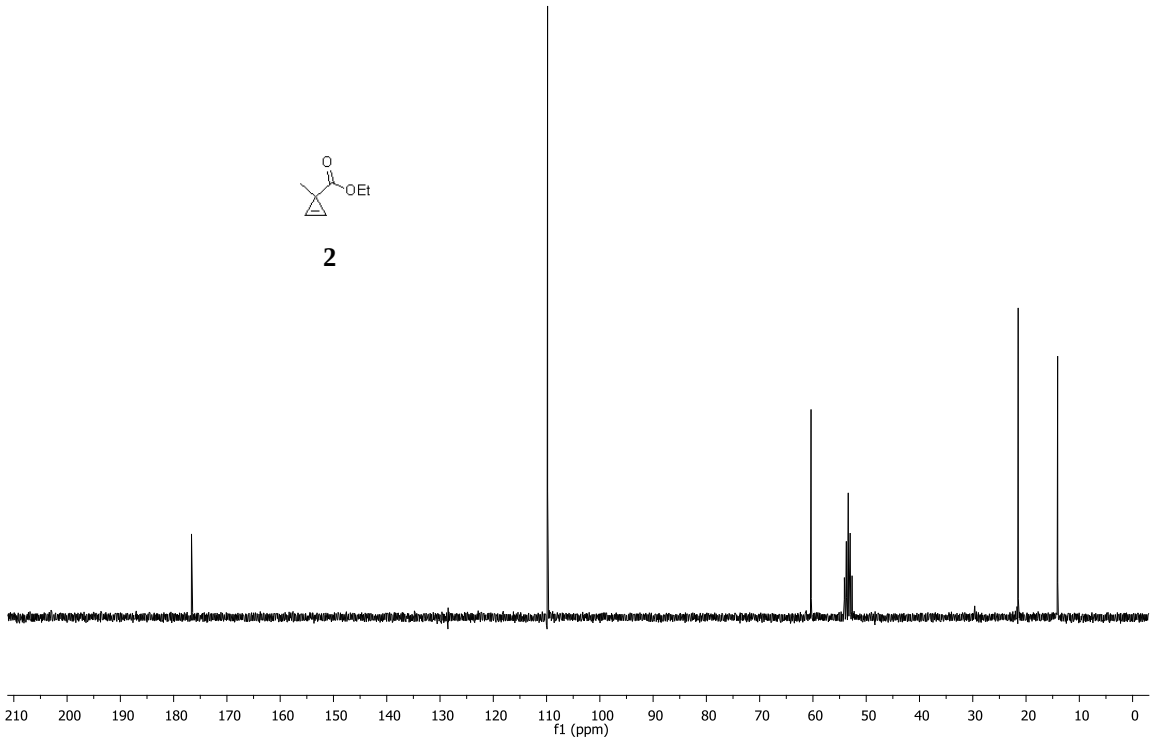
080610-B-20-D2O-C13
STANDARD CARBON PARAMETERS



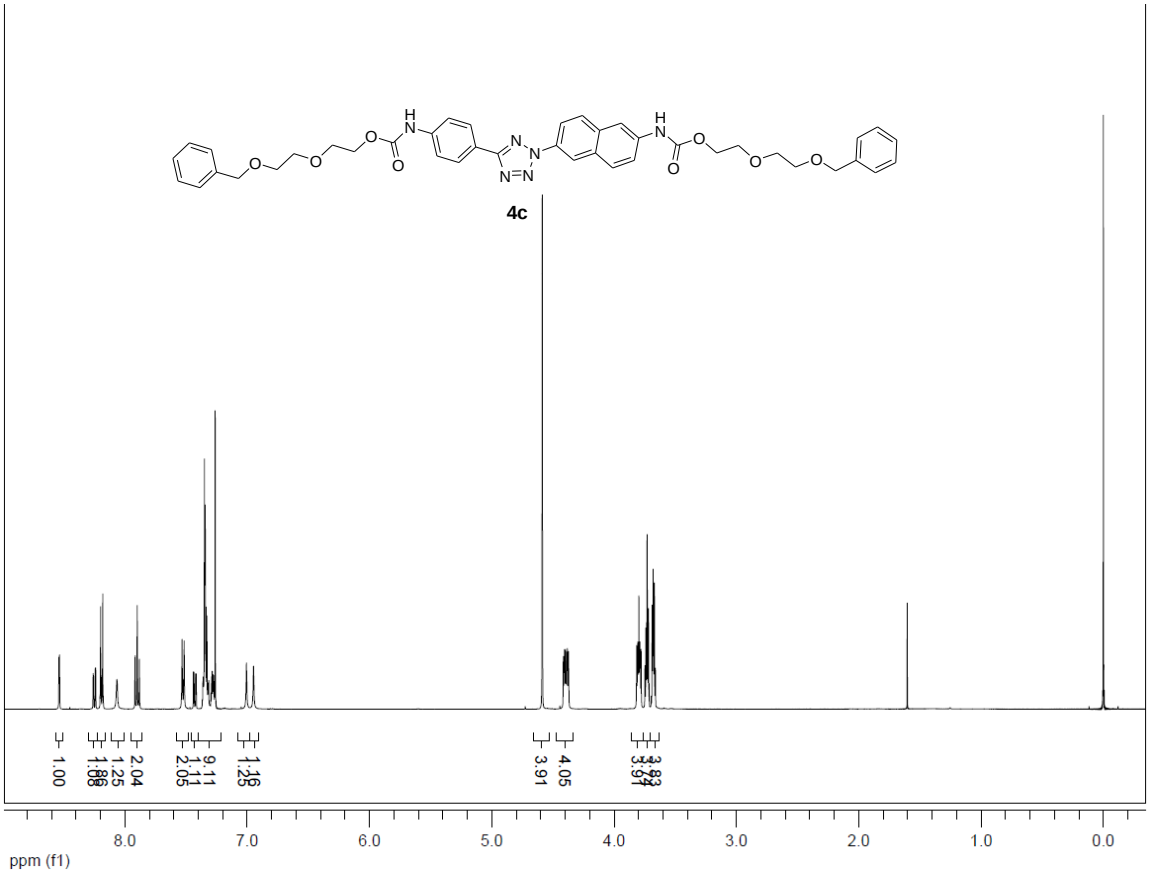
072710-B-12-1
072710-B-12-1



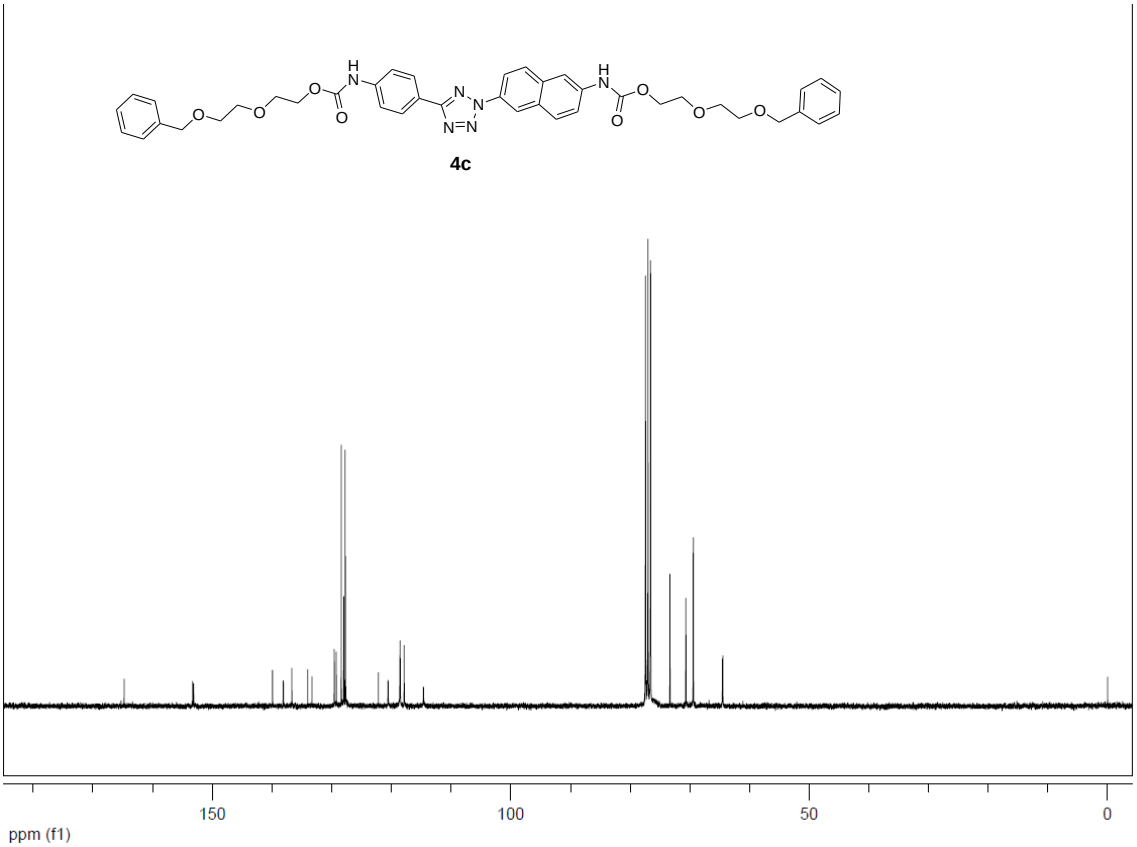
072710-B-12-1-C13
072710-B-12-1-C13



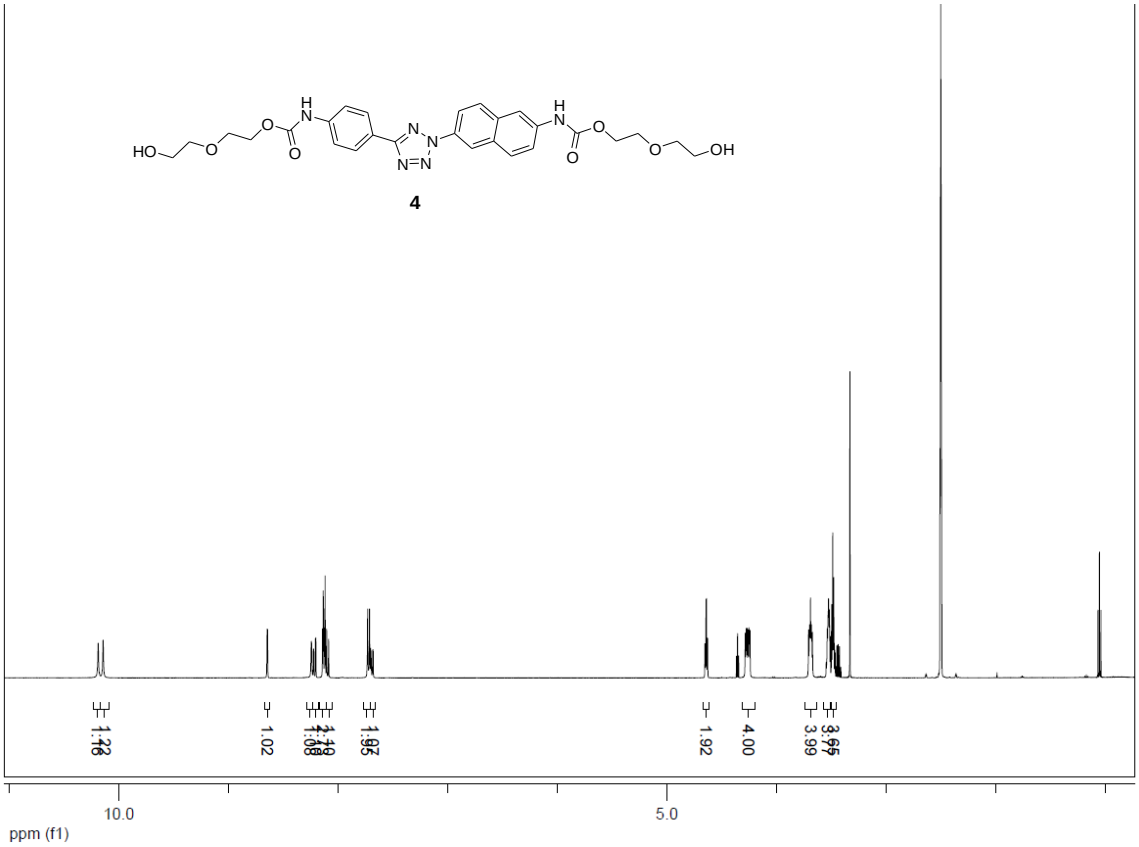
2



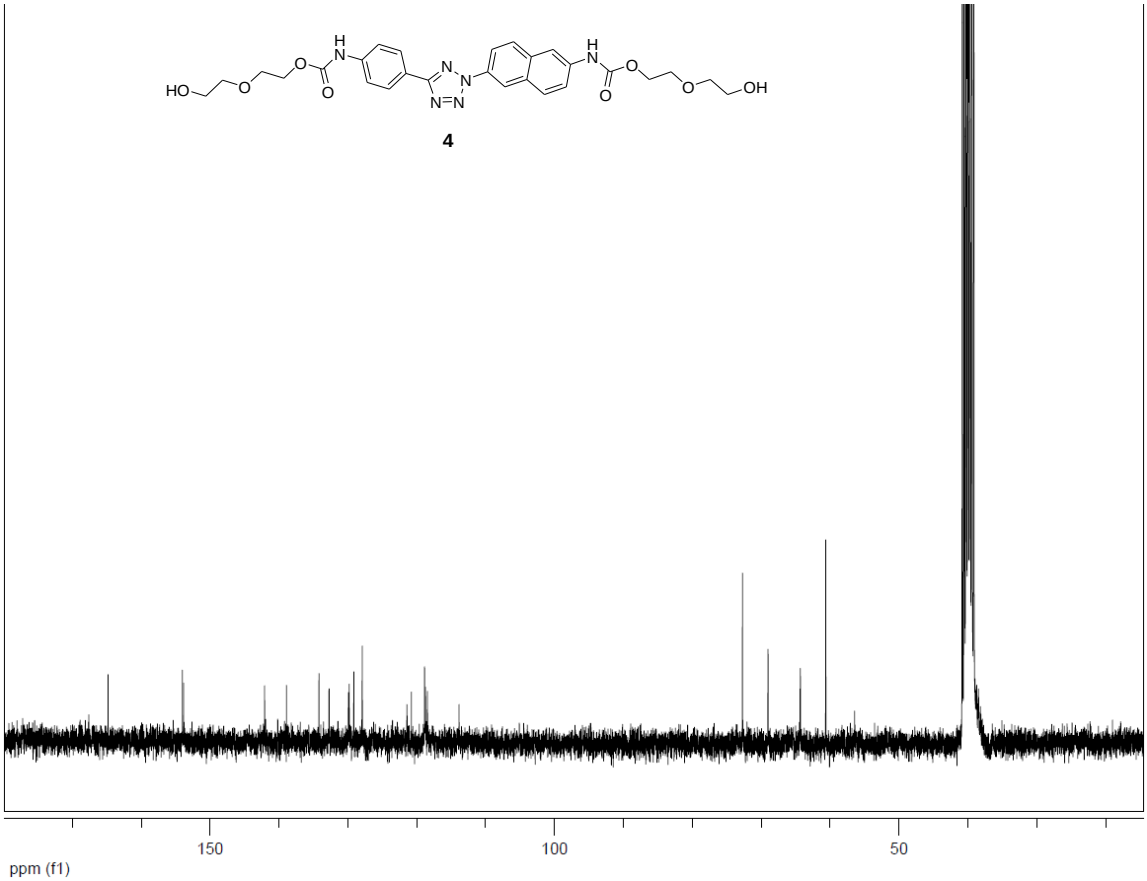
4c

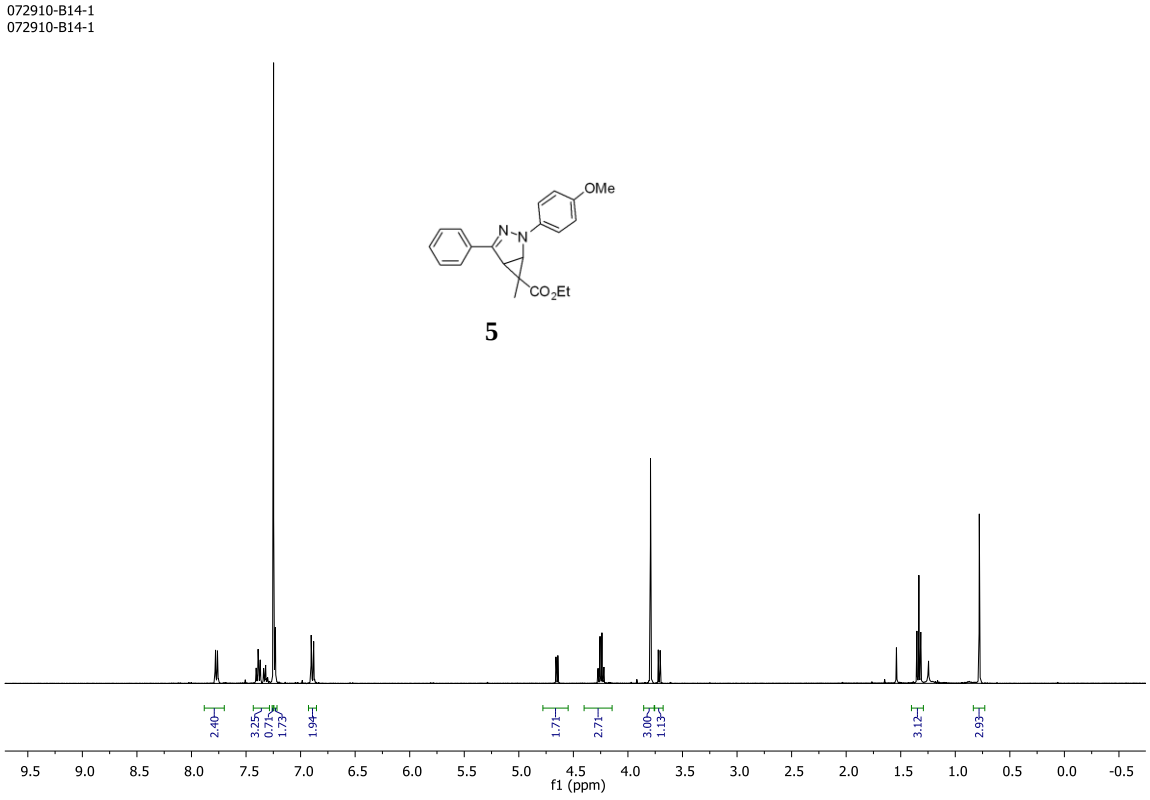


4c

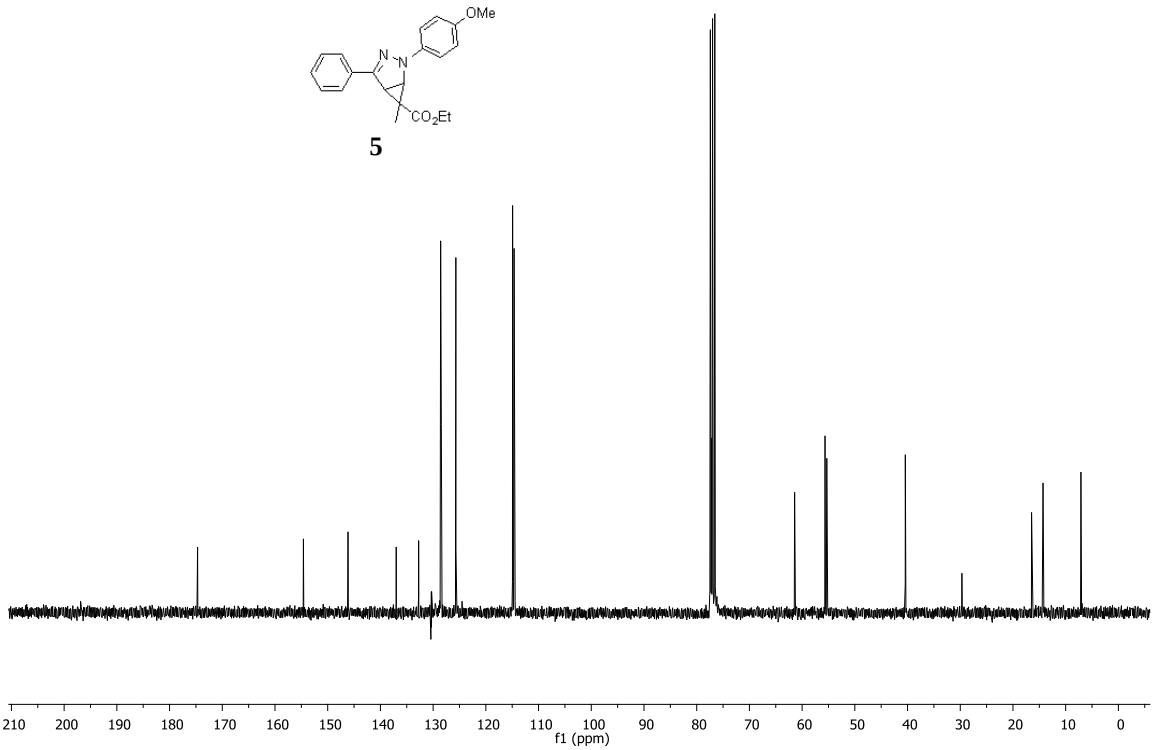


4

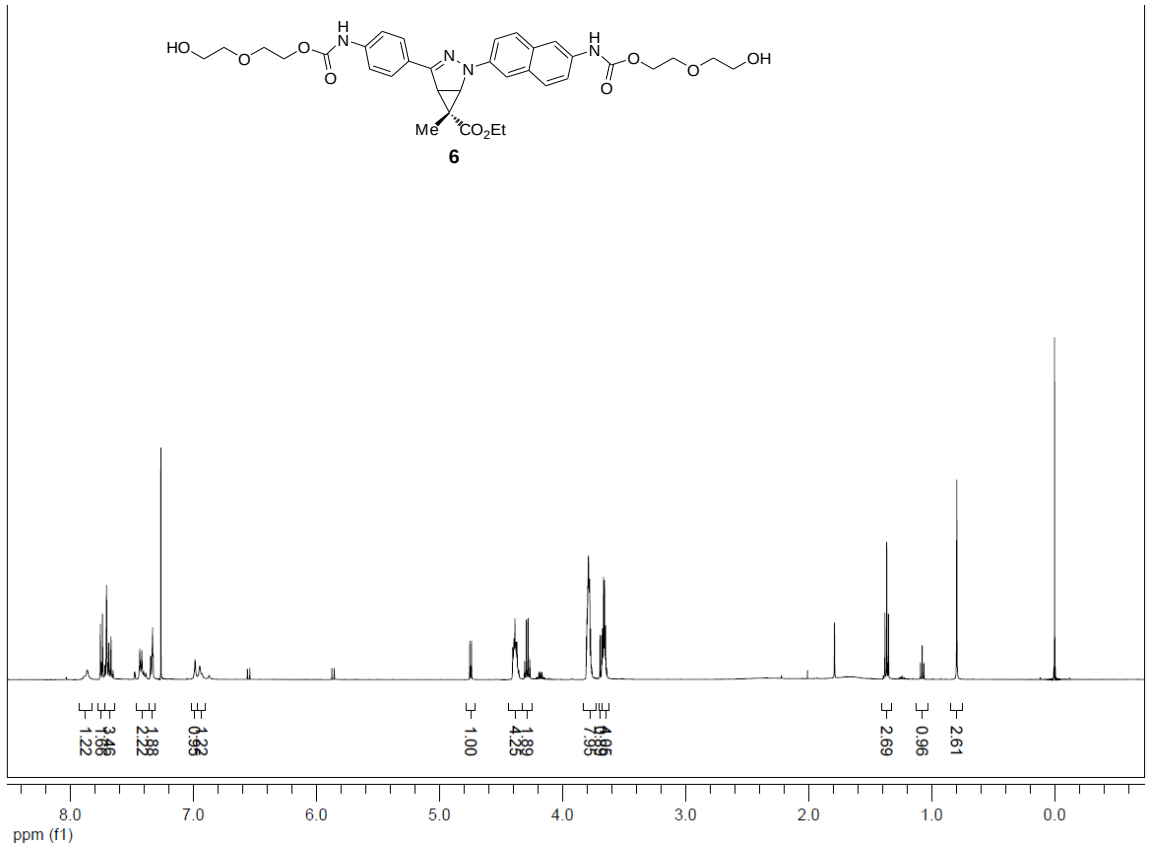

HO
O
O
O
H
N
O
N
N
N=N
H
N
O
O
O
OH
4
150
100
50
ppm (f1)


072910-B14-1
072910-B14-1
OMe
N
N
CO2Et
5
2.40
3.25
0.71
1.73
1.94
1.71
2.71
3.00
1.13
3.12
2.93
9.5
9.0
8.5
8.0
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
0.0
-0.5
f1 (ppm)

073110-B14-1-C13
073110-B14-1-C13

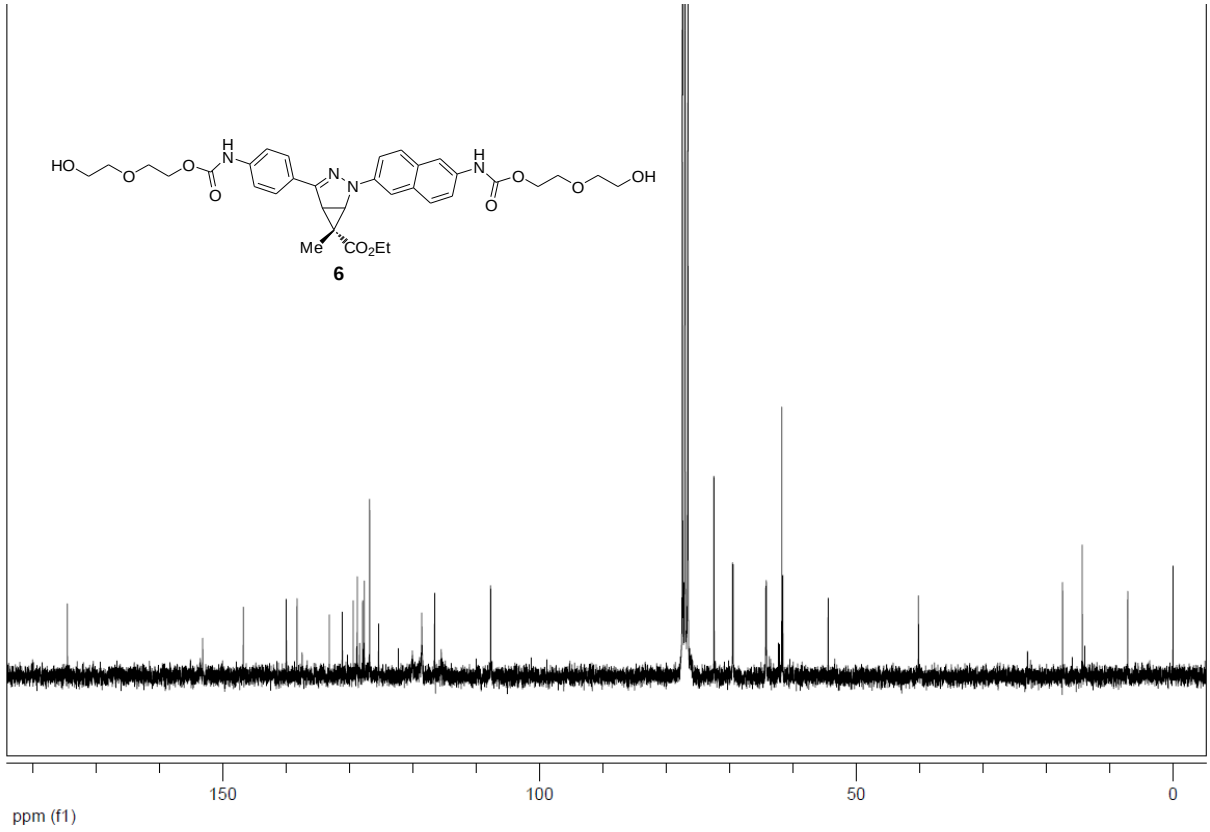


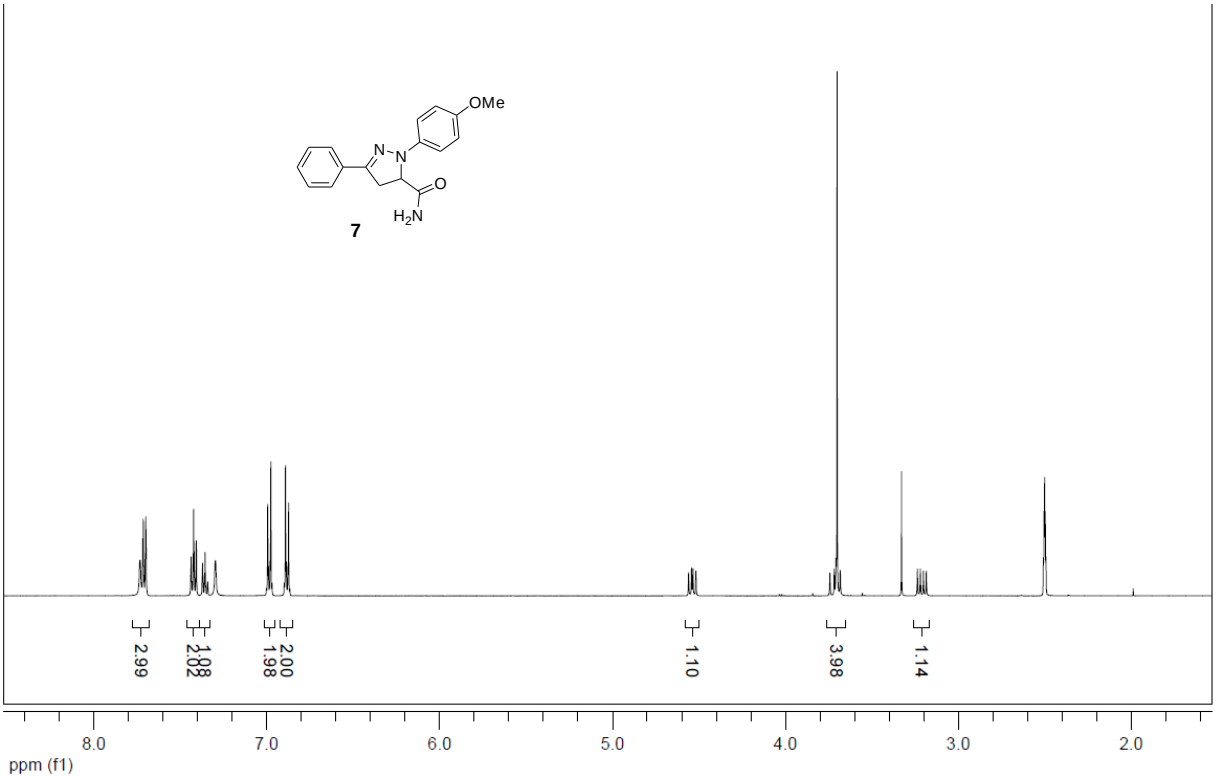
5

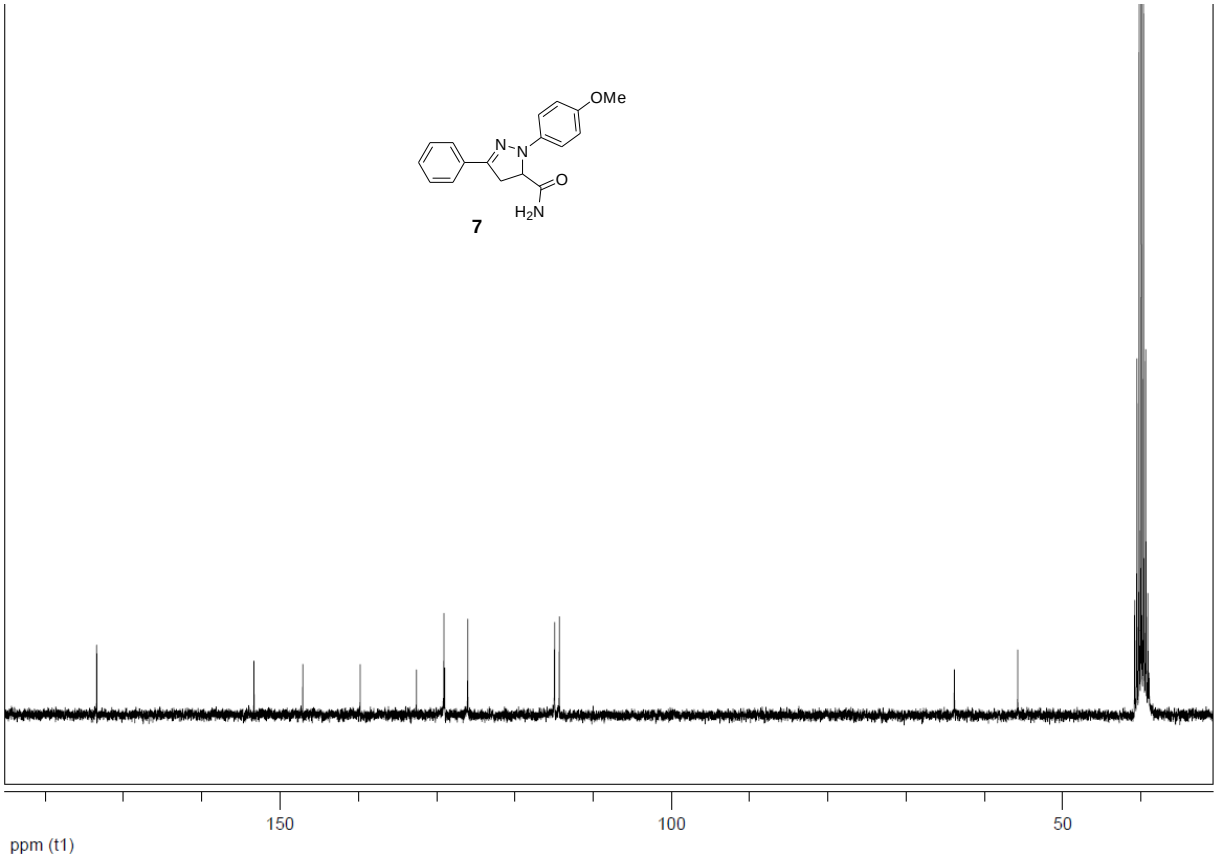


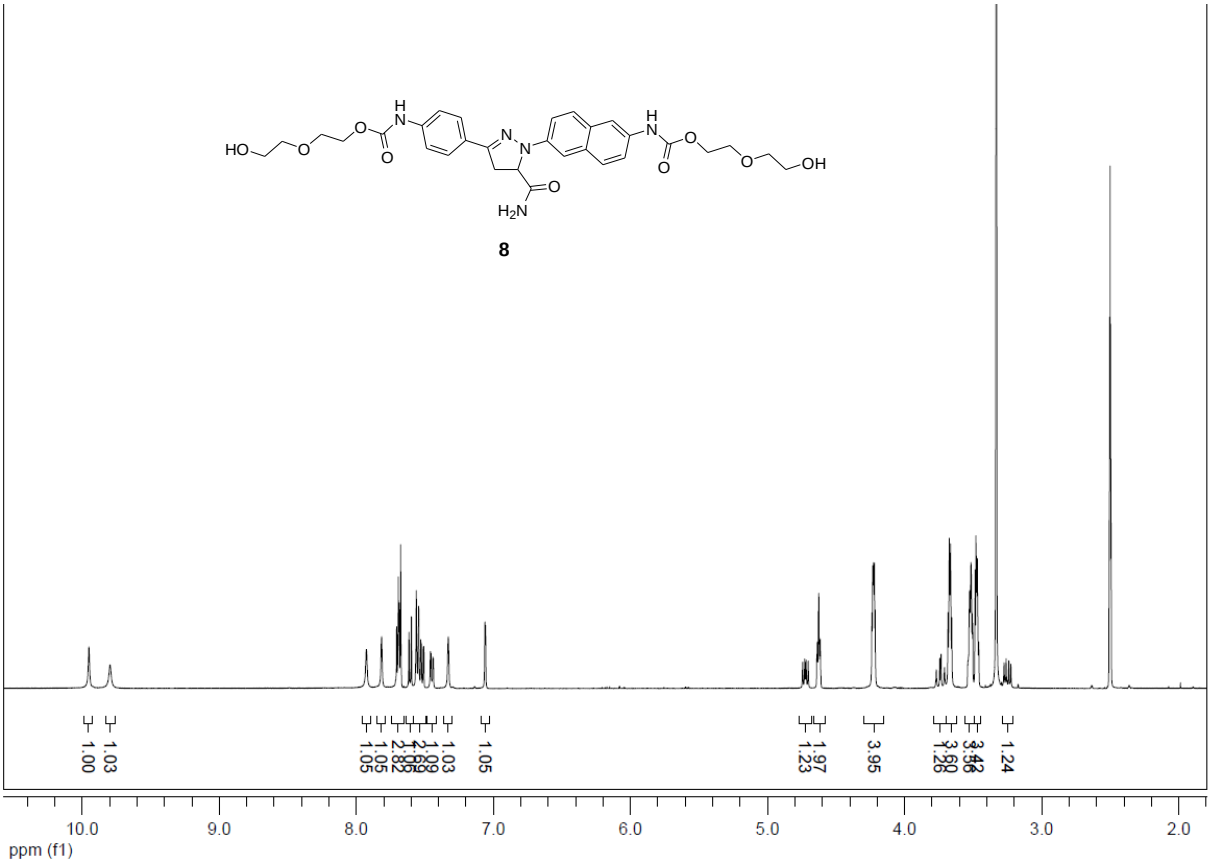
6

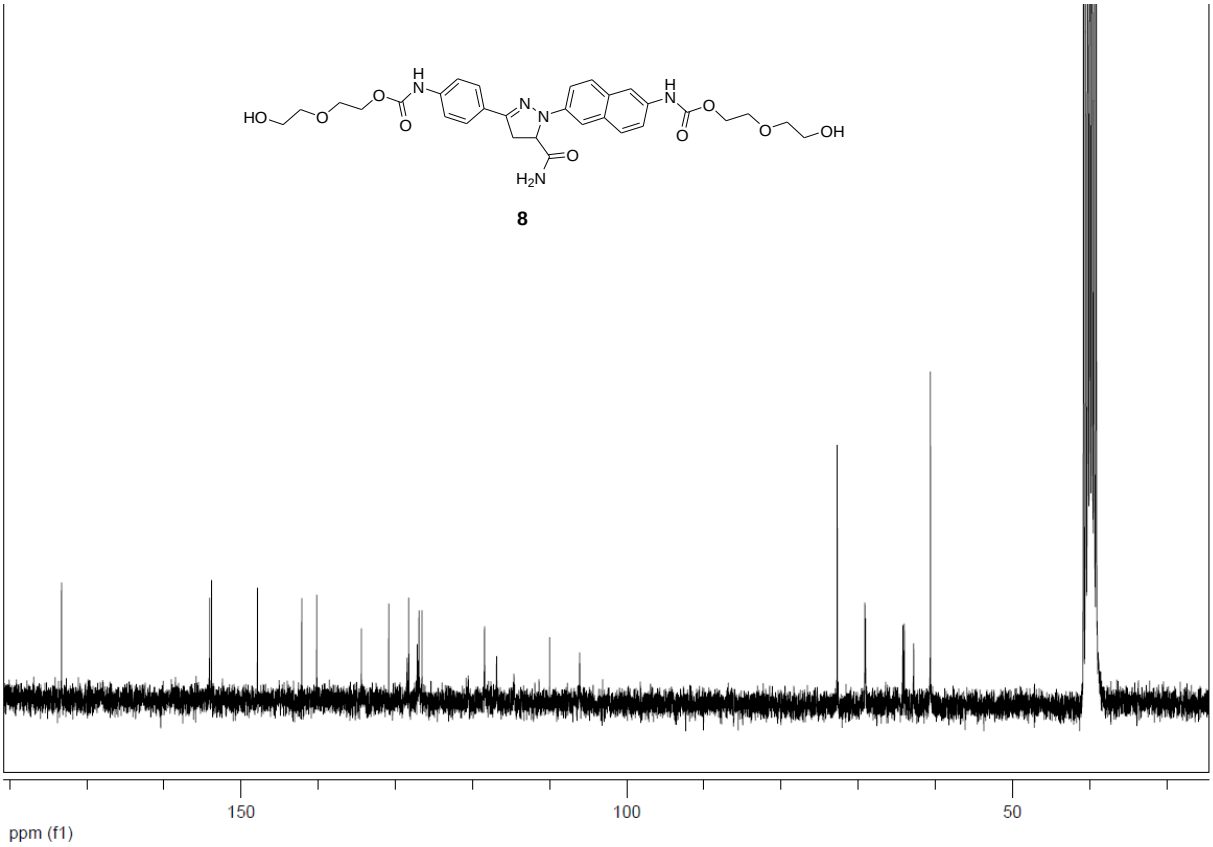
^{13}C


HO
O
O
H
N
O
N
N
Me
CO2Et
H
N
O
O
O
OH
6
150
100
50
0
ppm (f1)

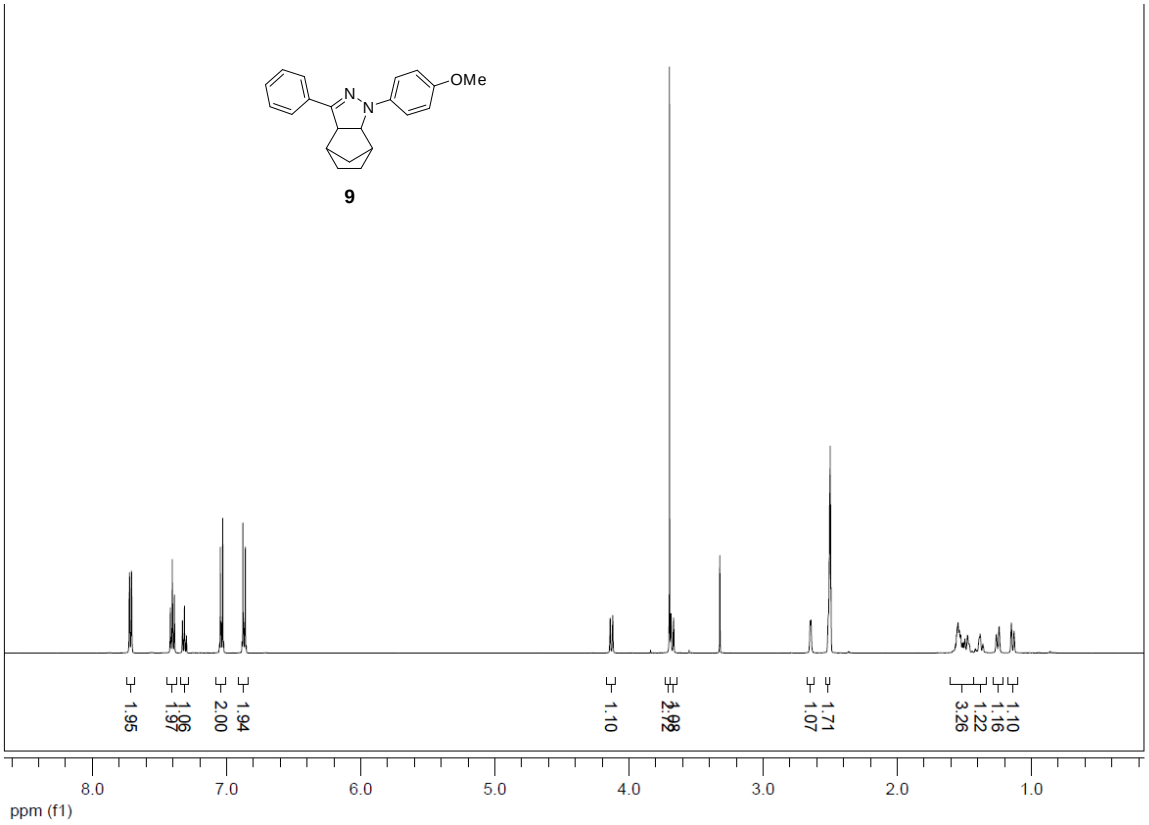

OMe
N
N
O
H2N
7
2.99
2.02
1.08
2.00
1.98
1.10
3.98
1.14
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)


OMe
N
N
O
H2N
7
150
100
50
ppm (t1)

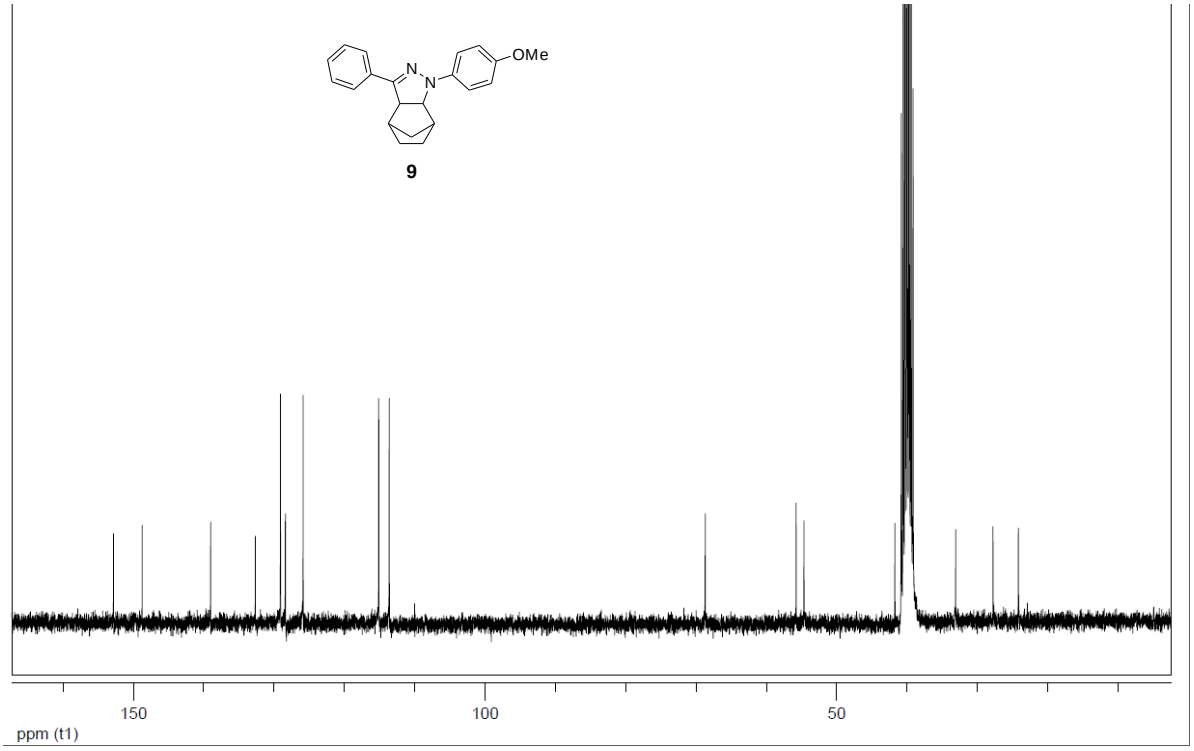

HO
O
O
H
N
O
N
N
O
H2N
H
N
O
O
O
OH
8
1.00
1.03
1.05
1.03
1.05
1.23
1.97
3.95
1.24
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
ppm (f1)


HO
O
O
O
H
N
O
N
N
O
H
N
O
O
O
OH
O
H2N
150
100
50
ppm (f1)

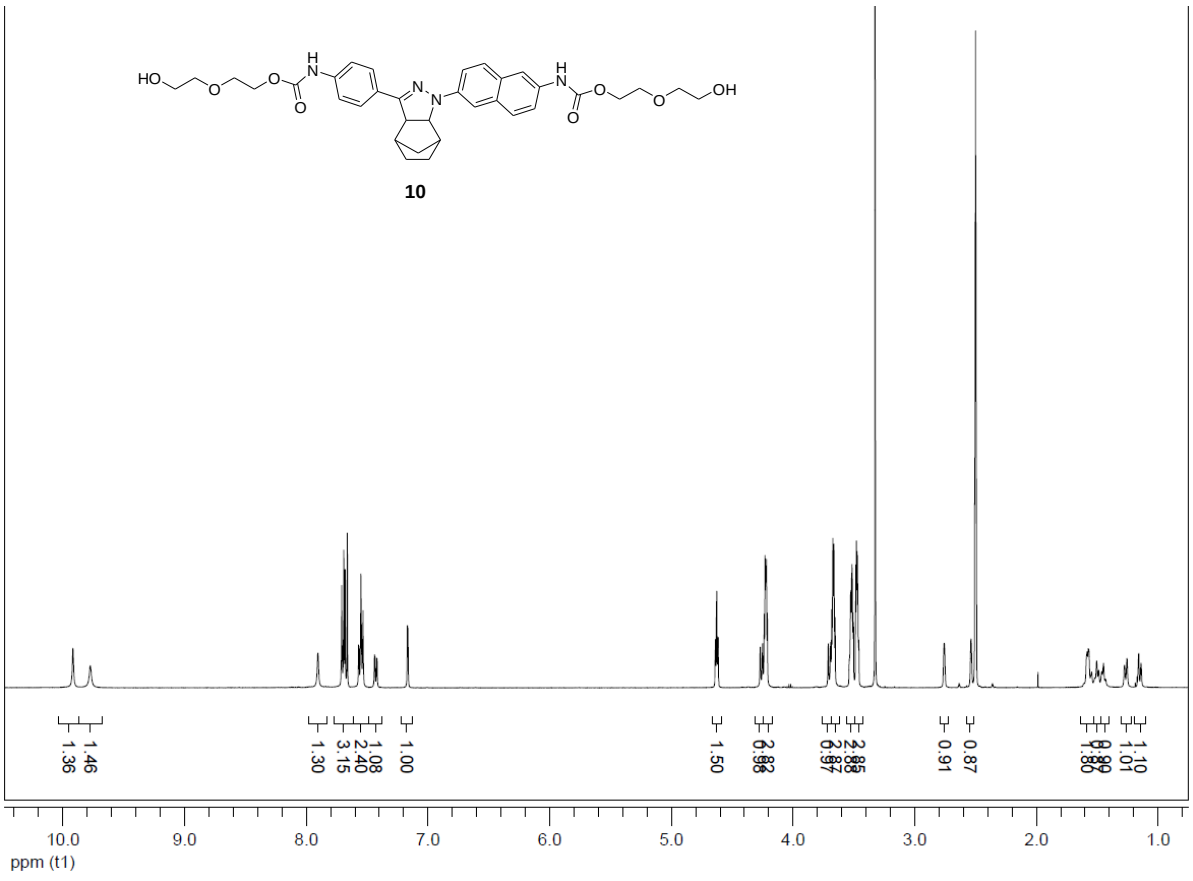
8


OMe
N
N
1.95
1.97
1.06
2.00
1.94
1.10
2.98
1.71
1.07
3.26
1.22
1.16
1.10
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

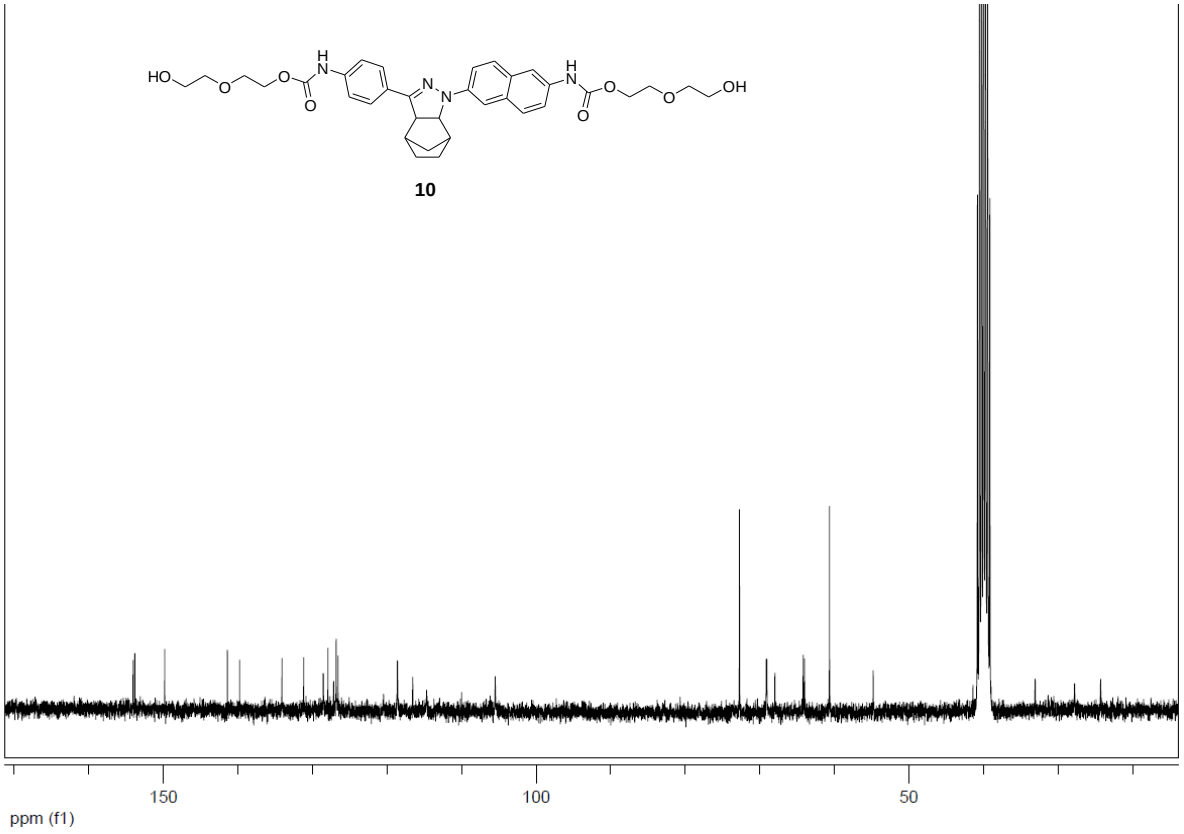
9


OMe
N
N
150
100
50
ppm (t1)

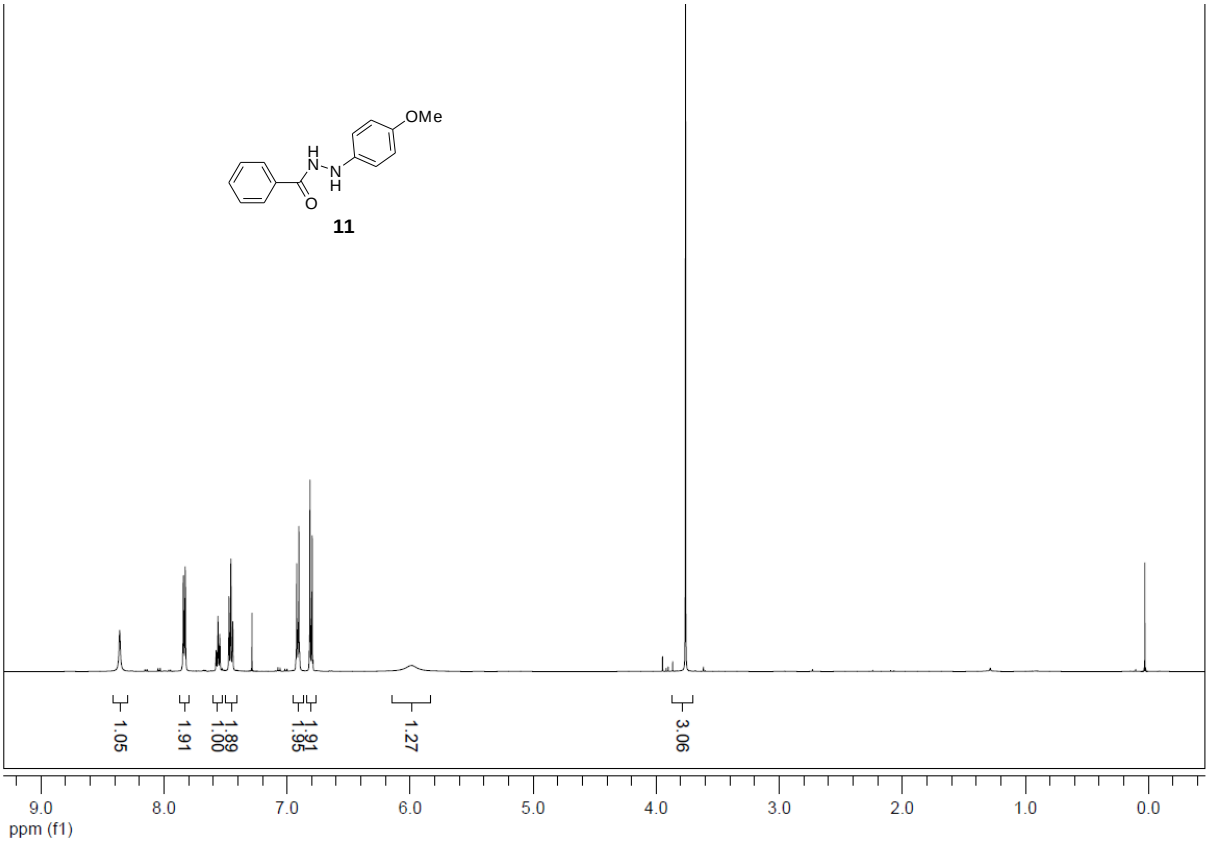
9


HO
O
O
H
N
O
N
N
H
N
O
O
O
O
OH
1.36
1.46
1.30
3.15
2.40
1.08
1.00
1.50
0.91
0.87
1.10
1.01
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)

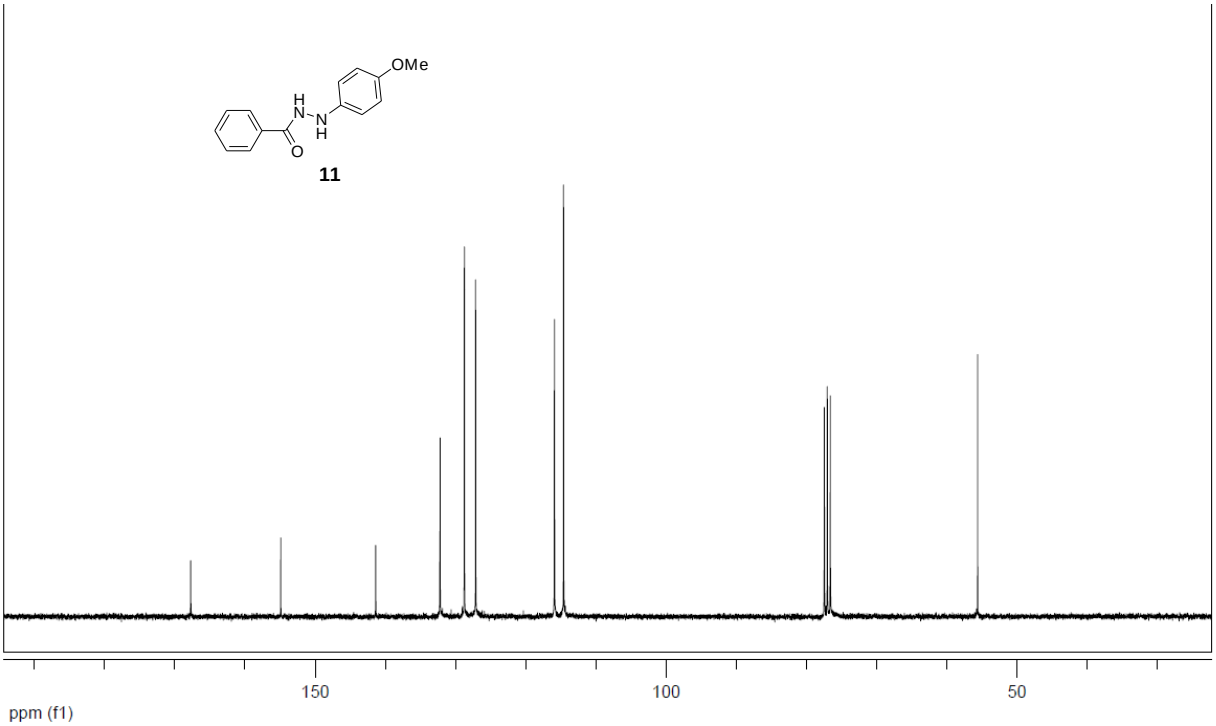
10

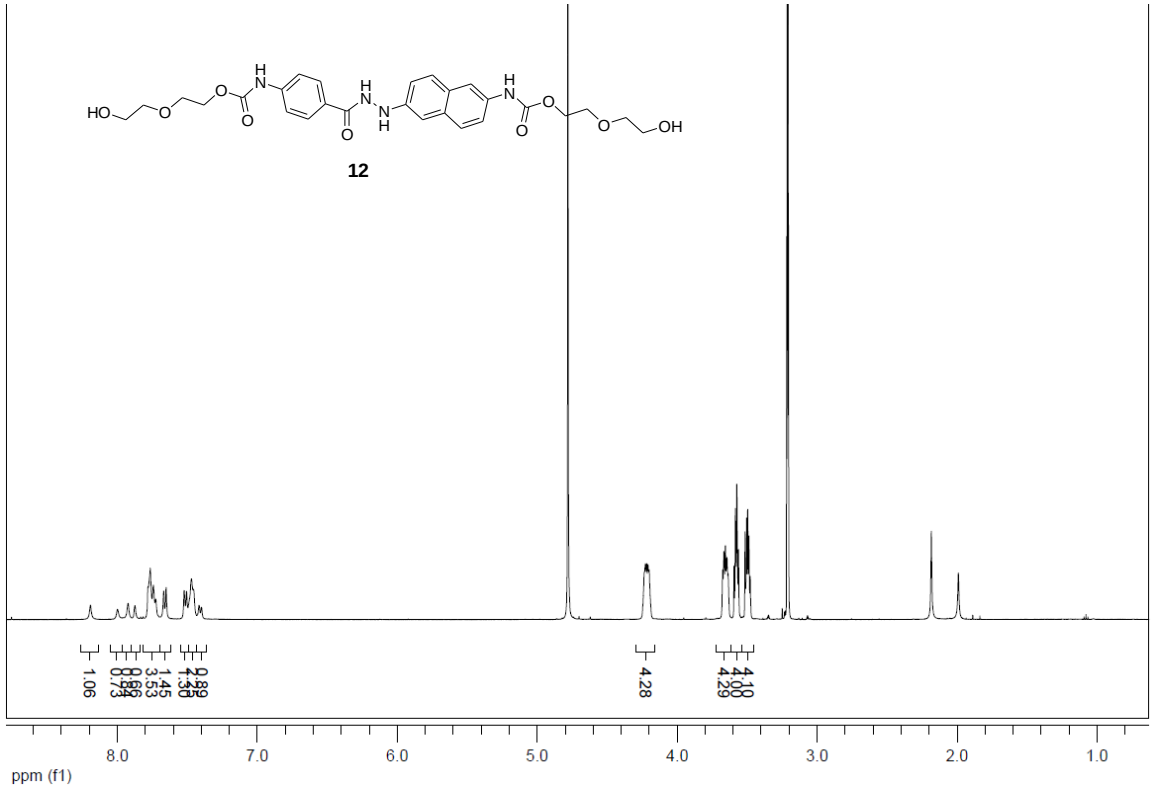

HO
O
O
H
N
O
N
N
H
N
O
O
O
OH
150
100
50
ppm (f1)

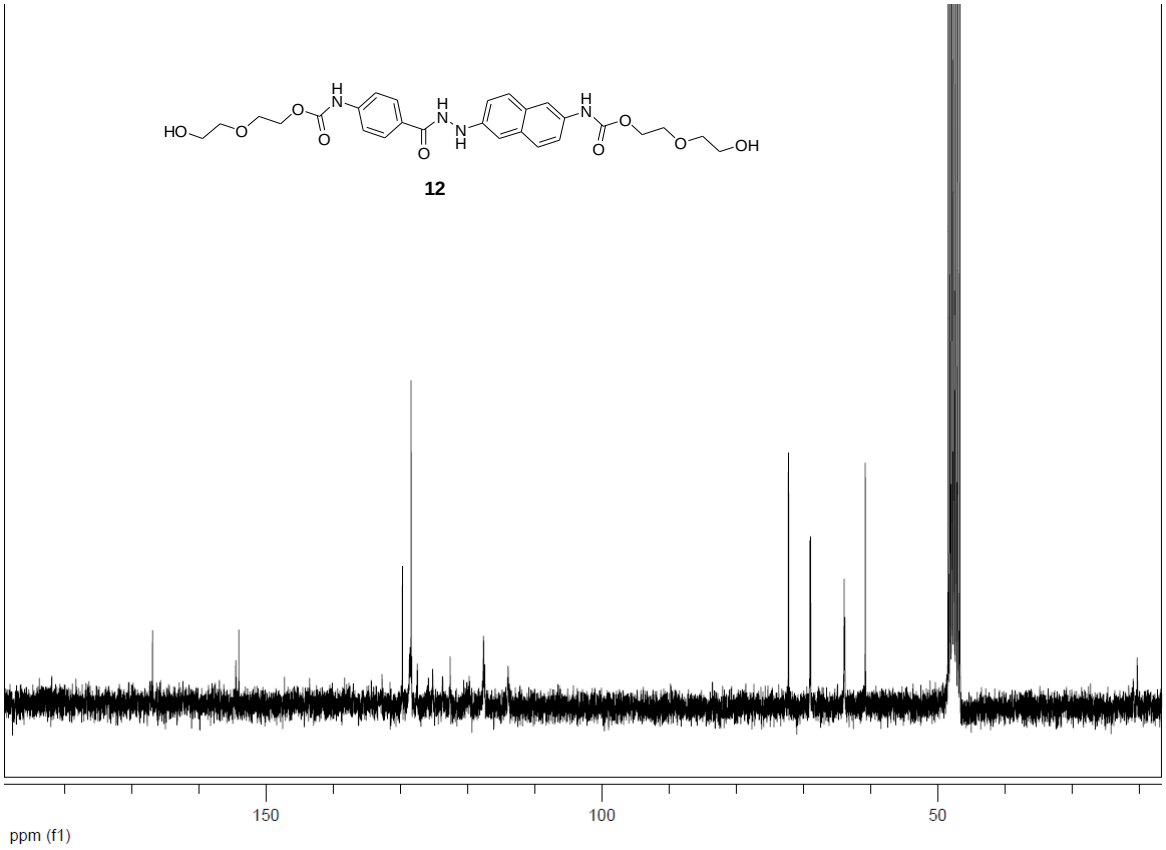
10


OMe
H
N
N
H
O
1.05
1.91
1.89
1.00
1.91
1.95
1.27
3.06
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

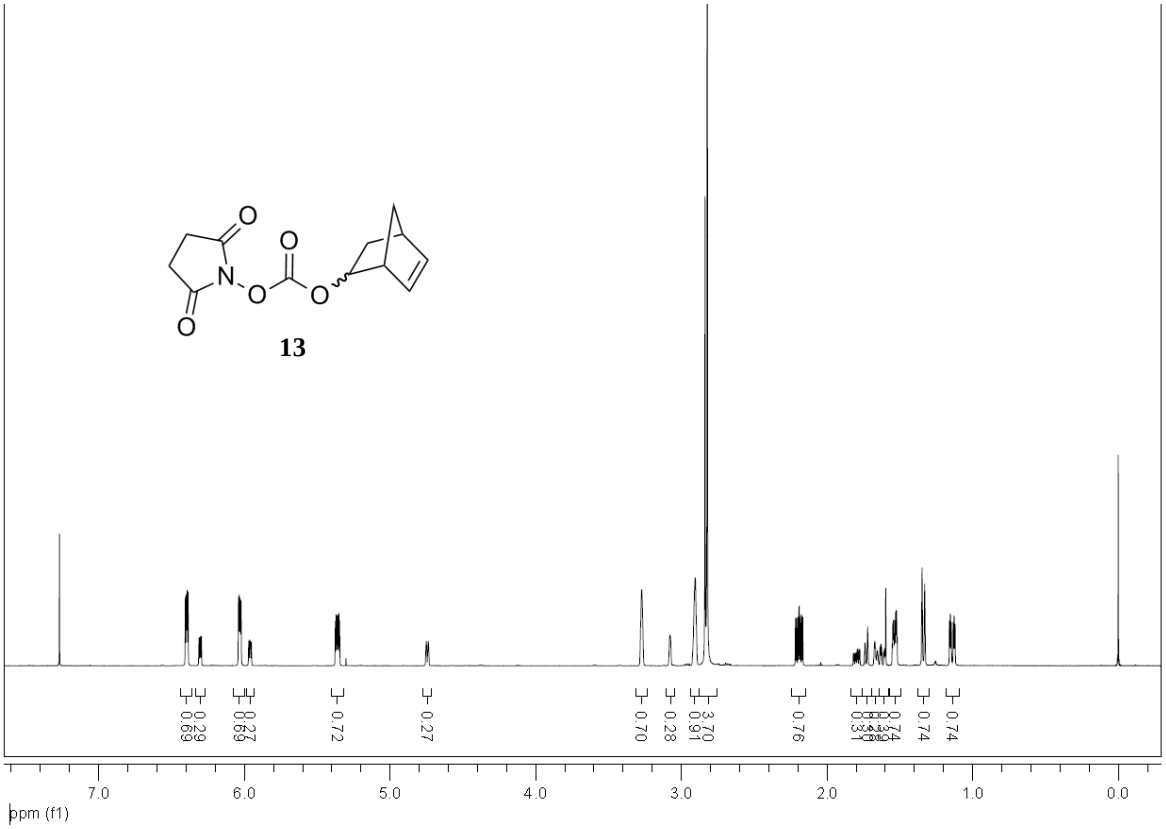
11


OMe
H
N
N
H
O
11
150
100
50
ppm (f1)

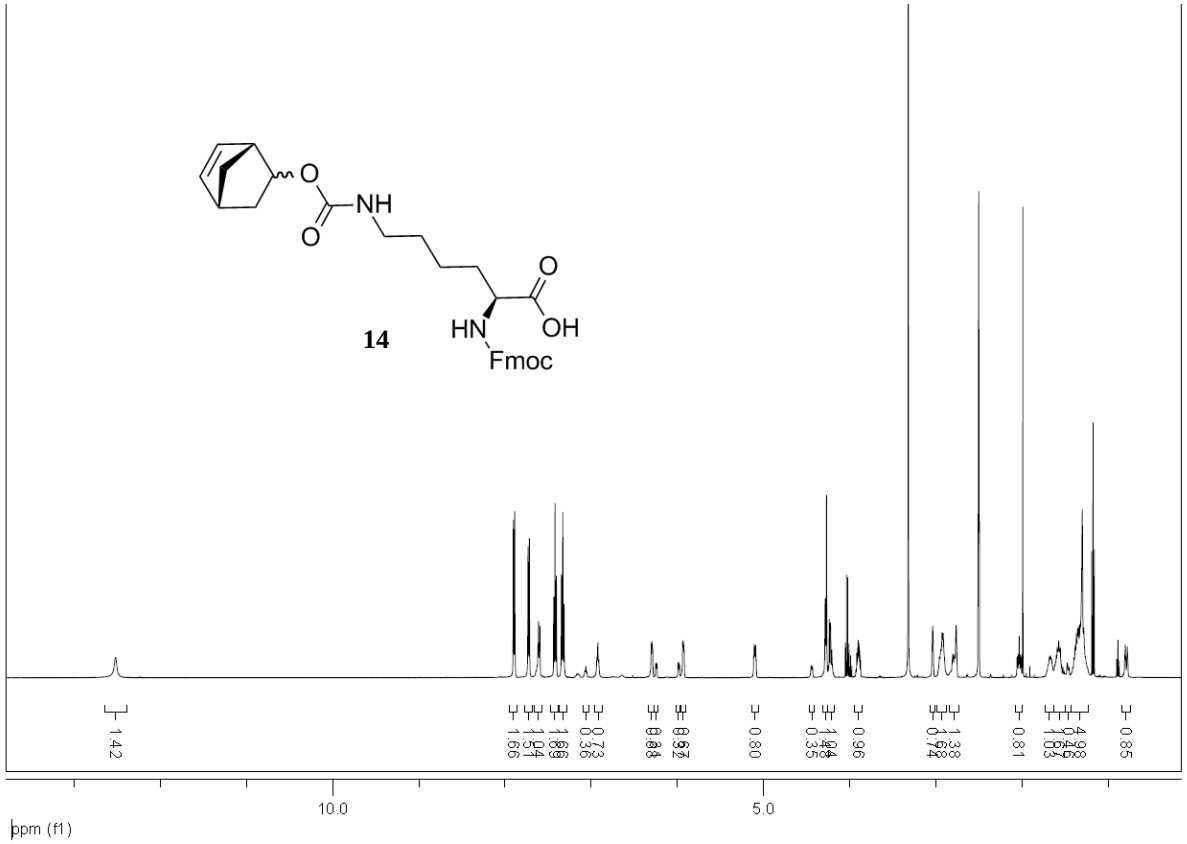

HO
O
O
H
N
O
H
N
N
O
H
N
O
O
O
OH
12
1.06
4.28
4.10
4.00
4.29
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)

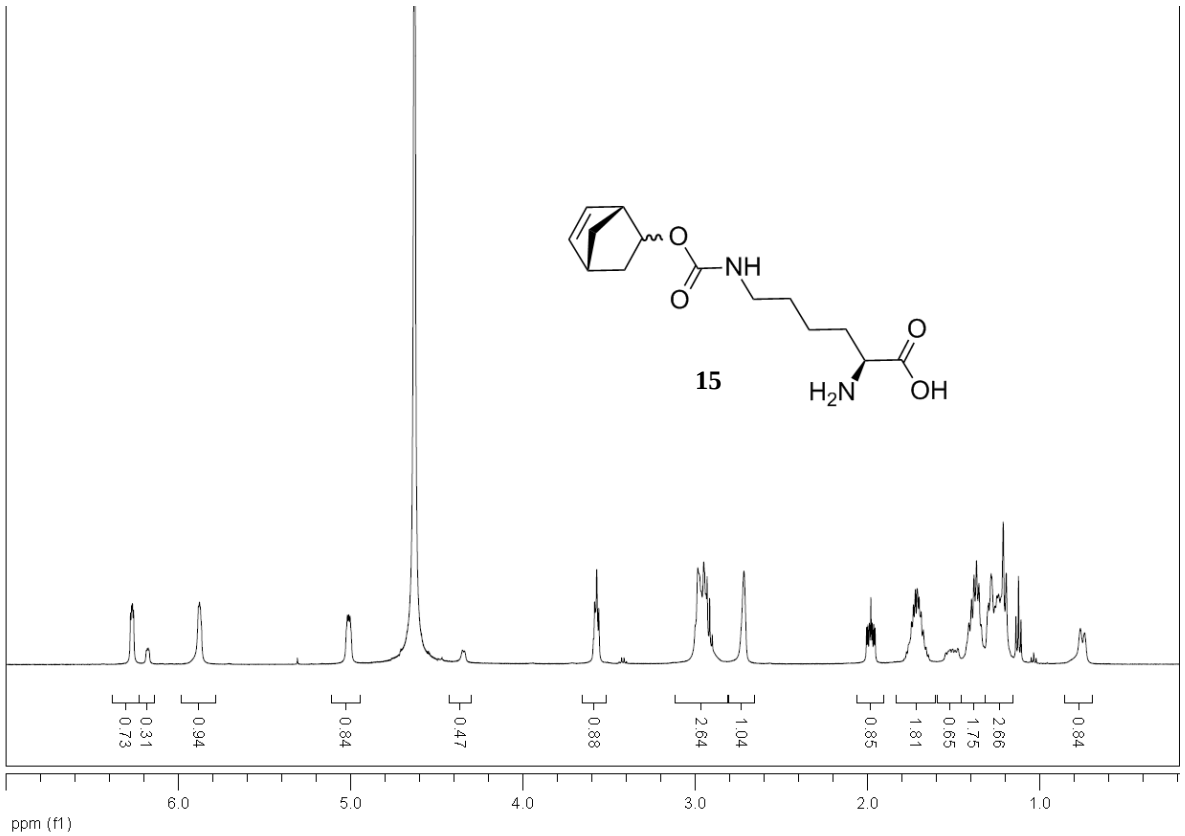

HO
O
O
O
H
N
O
H
N
H
N
O
H
N
O
O
O
OH
150
100
50
ppm (f1)

12


O
O
N
O
O
O
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (f1)

13


O
NH
O
O
HN
OH
Fmoc
14
1.42
0.73
0.36
0.80
0.96
0.81
0.85
10.0
5.0
ppm (f1)


O
NH
O
O
H2N
OH
15
0.73
0.31
0.94
0.84
0.47
0.88
2.64
1.04
0.85
1.81
0.65
1.75
2.66
0.84
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)