Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Exploring the versatility of pentafulvene-maleimide cycloaddition as a ligation strategy: buffer and pH effects

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1 Experimental procedures

1.0 Buffer preparation for stability studies

Table S1: Buffers for testing pentafulvene 1 and maleimide 2 stability in different buffer solutions after 1 and 24 h.

Buffer solution[a]	Buffer concentration (mM)	Buffering agent(s) (mg)	Final volume (mL)	pH[b]	pH*
Glycine HCl	100	Glycine (15.8)	2	3.7	3.5
MES	100	MES hydrate (37.8)	2	5.5	5.5
BTM	100	BTM (40.1)	2	6.4	6.5
BTP	100	BTP (58.5)	2	6.9	7.0
Imidazole HCl	100	Imidazole (13.7)	2	6.9	7.0
PIPES	100	PIPES (120)	2	6.9	7.0
HEPES	100	HEPES (47.7)	2	7.4	7.5
Trizma	100	Trizma HCl (26.1) Trizma base (5.0)	2	7.4	7.5
Tricine	100	Tricine (36.5)	2	8.0	8.1
Bicine	100	Bicine (33.5)	2	8.1	8.3
Glycine NaOH	100	Glycine (15.8)	2	8.7	8.9
CAPS	100	CAPS (43.2)	2	10.1	10.4

[a] All buffer solutions were prepared at 100 mM in D_2O and pH adjusted prior to addition of coupling partners. Abbreviations; MES: 2-(*N*-morpholino)ethanesulfonic acid, BTM: 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol, BTP: 2,2′-[propane-1,3-diylbis(azanediyl)]bis[2-(hydroxymethyl)propane-1,3-diol], PIPES: 2,2′-(piperazine-1,4-diyl)di(ethane-1-sulfonic acid),HEPES: 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid, Trizma: tris(hydroxymethyl)aminomethane, Tricine: *N*-(tri(hydroxymethyl)methyl)glycine, Bicine: *N*,*N*-bis(2-hydroxyethyl)glycine, CAPS: *N*-cyclohexyl-3-aminopropanesulfonic acid. [b] pH of buffer solutions calculated from pH* readings taken in D_2O.[1]

1.1 Buffer preparation for kinetics testing

Table S2: Buffers for determination of kinetics, half-lives and stereoselectivity for the Diels-Alder cycloaddition between pentafulvene 1 and maleimide 2 in different buffer solutions.

Buffer Solution	Buffer concentration (mM)	Buffering agents(s) (g)	Final volume (mL)	pH
glycine HCl	100	Glycine (0.380)	50	3.5
MES	100	MES (1.952)	100	5.5
BTM	100	BTM (1.035)	50	6.5
BTP	100	BTP (1.13)	50	7.0
Imidazole HCl	100	Imidazole (0.345)	50	7.0
PIPES	100	PIPES (3.01)	50	7.0
HEPES	100	HEPES (1.19)	100	7.5
Trizma	100	Trizma HCl (1.246) Trizma base (0.253)	100	7.5
Tricine	100	Tricine (1.79)	100	8.1
Bicine	100	Bicine (0.818)	50	8.3
Glycine NaOH	100	Glycine (0.38)	50	8.9
CAPS	100	CAPS (1.1)	50	10.4

1.2 Kinetics experiments

Table S3: The initial and final concentrations of pentafulvene 1 and maleimide 2 for determination of kinetics, half-lives and stereoselectivity for the Diels-Alder cycloaddition, including the quenching method used in different buffer solutions.

Buffer Solution	Fulvene 1 initial concentration (mmol)	Maleimide 2 initial concentration (mmol)	Final concentration 1 and 2 (mmol)	Quenching method[a]
glycine HCl	20	20	10	MCE
MES	25	25	12.5	MCE
BTM	25	25	12.5	MCE
BTP	20	20	10	MCE
Imidazole HCl	20	20	10	MeOH
PIPES	25	25	12.5	MeOH
HEPES	25	25	12.5	MeOH
Trizma	25	25	12.5	MCE
Tricine	25	25	12.5	MCE
Bicine	25	25	12.5	MCE
Glycine NaOH	20	20	10	MCE
CAPS	25	25	12.5	MeOH

[a] Quenched by the addition of mercaptoethanol (MCE) or methanol (MeOH)

Table S4: Comparison of rate constants and initial rates for the hydrolysis of maleimide 2 and the Diels-Alder cycloaddition.

Buffer[a]	$k_1^{obs}{}_{(Hyd)}$ ($\times 10^{-6}$ s^{-1})[b]	$k_{2,(DAC)}$ ($M^{-1}s^{-1}$)[c]	$K_{(Hyd)}$ ($\times 10^{-9}$ $M.s^{-1}$)[d]	$K_{(DAC)}$ ($\times 10^{-8}$ $M.s^{-1}$)[d]	($K_{(DAC)}/K_{(Hyd)}$)[d]	Conc. of 1 and 2 (mM)[e]
Imidazole	1.77	0.16	2.02	2.01	10	0.11
PIPES	4.69	0.14	1.55	1.55	10	0.33
HEPES	1.79	0.17	0.18	0.19	10	0.11
Tricine	2.58	0.25	0.27	0.27	10	0.10
Bicine	4.69	0.18	1.24	1.24	10	0.26

[a] Buffers in which maleimide hydrolysis was observed in the absence of pentafulvene (**Table 1**). [b] Pseudo first-order rate constant for hydrolysis of maleimide **2** in buffers in D_2O. [c] Second-order rate constant for Diels Alder cycloaddition between **1** and **2** (**Table 2**). [d] Initial rate of hydrolysis of maleimide **2** and Diels-Alder cycloaddition that provide a relative initial rate equal to 10. [e] Initial concentration of pentafulvene **1** and maleimide **2** when the $K_{(DAC)}/K_{(Hyd)}$ equals 10

2 Stability

2.1 Maleimide stability

2.1.1 Glycine HCl pH 3.7

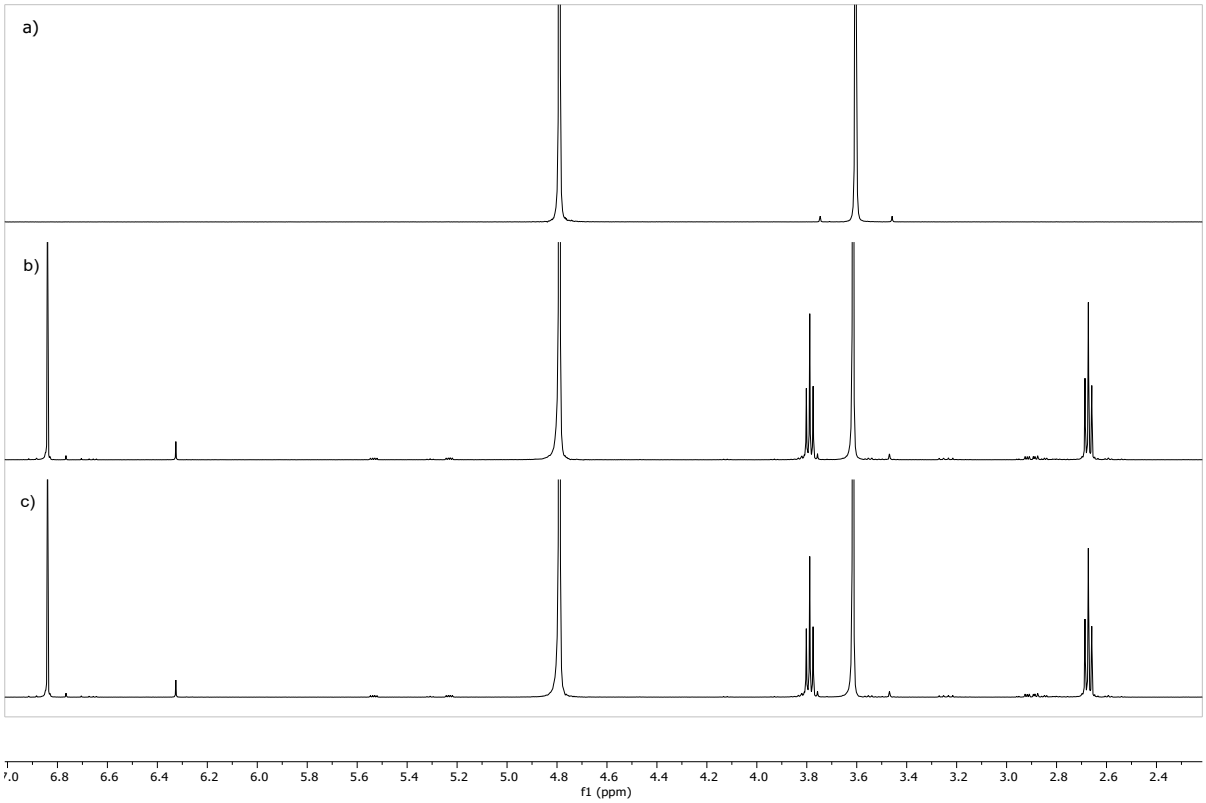

Figure S1: ^{1}H NMR spectra (500 MHz, D_2O) of (a) glycine HCl buffer (100 mM, pH 3.7) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.2 MES pH 5.5

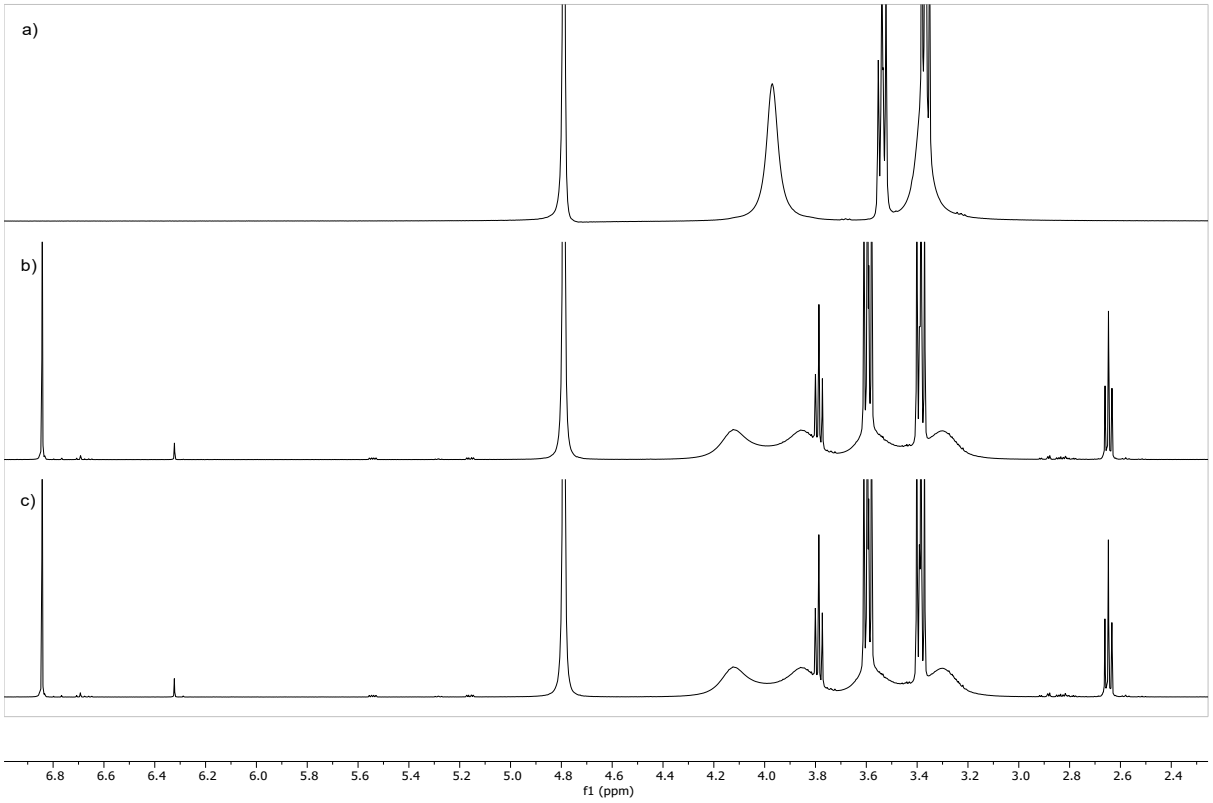

Figure S2: ^{1}H NMR spectra (500 MHz, D_2O) of (a) MES buffer (100 mM, pH 5.5) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.3 BTM pH 6.4

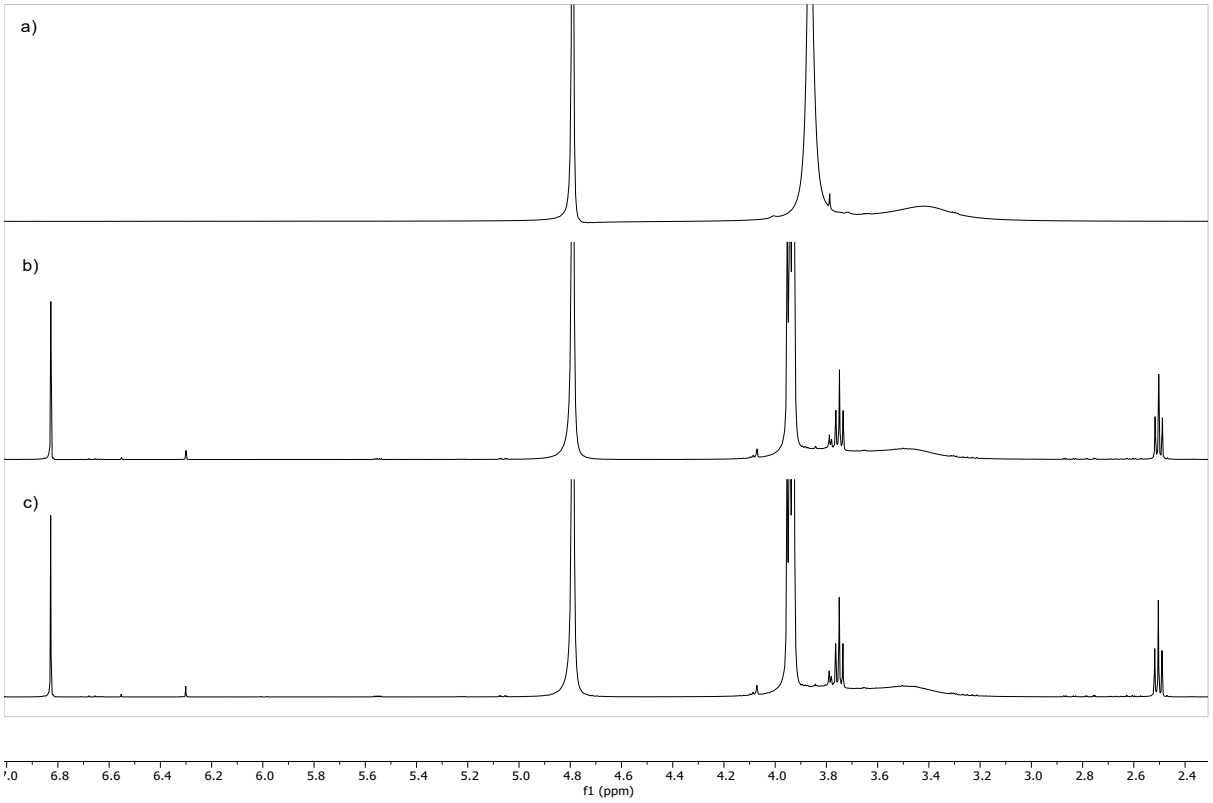

Figure S3: ^{1}H NMR spectra (500 MHz, D_2O) of (a) BTM buffer (100 mM, pH 6.4) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.4 BTP pH 6.9

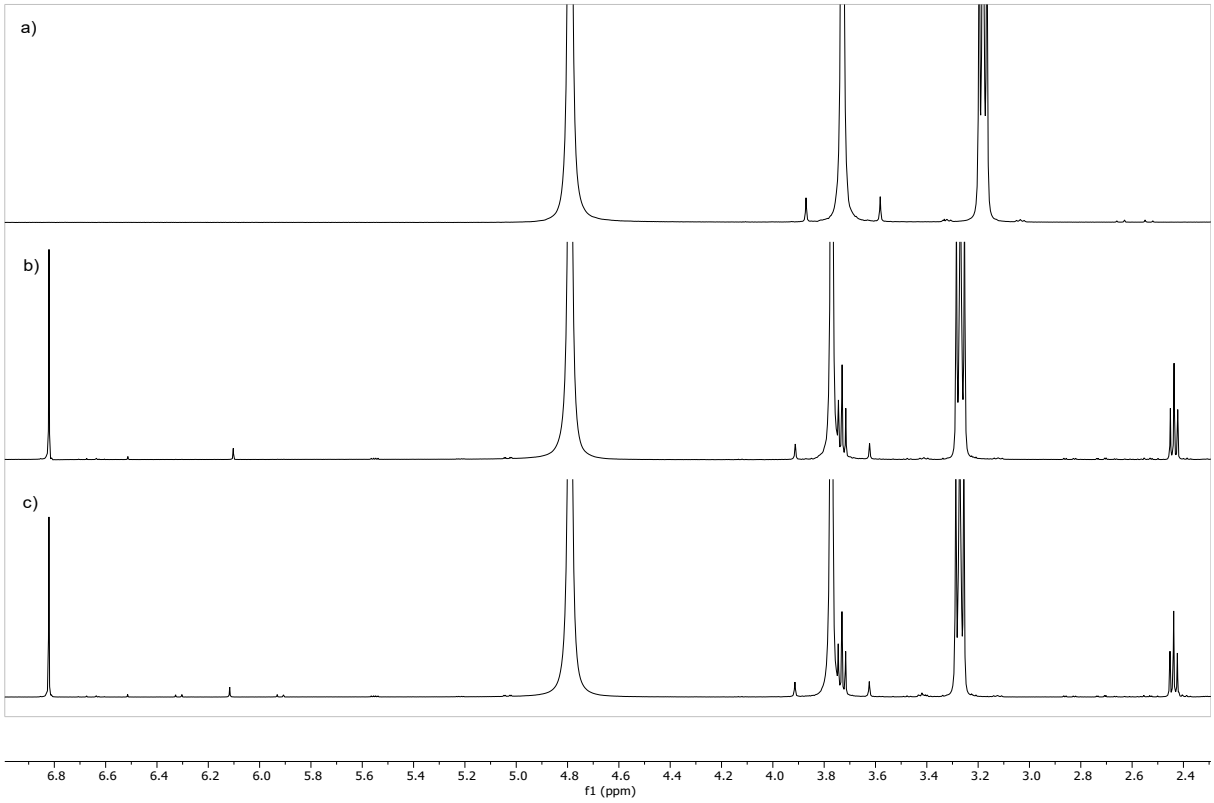

Figure S4: ^{1}H NMR spectra (500 MHz, D_2O) of (a) BTP buffer (100 mM, pH 6.9) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.5 Imidazole HCl pH 6.9

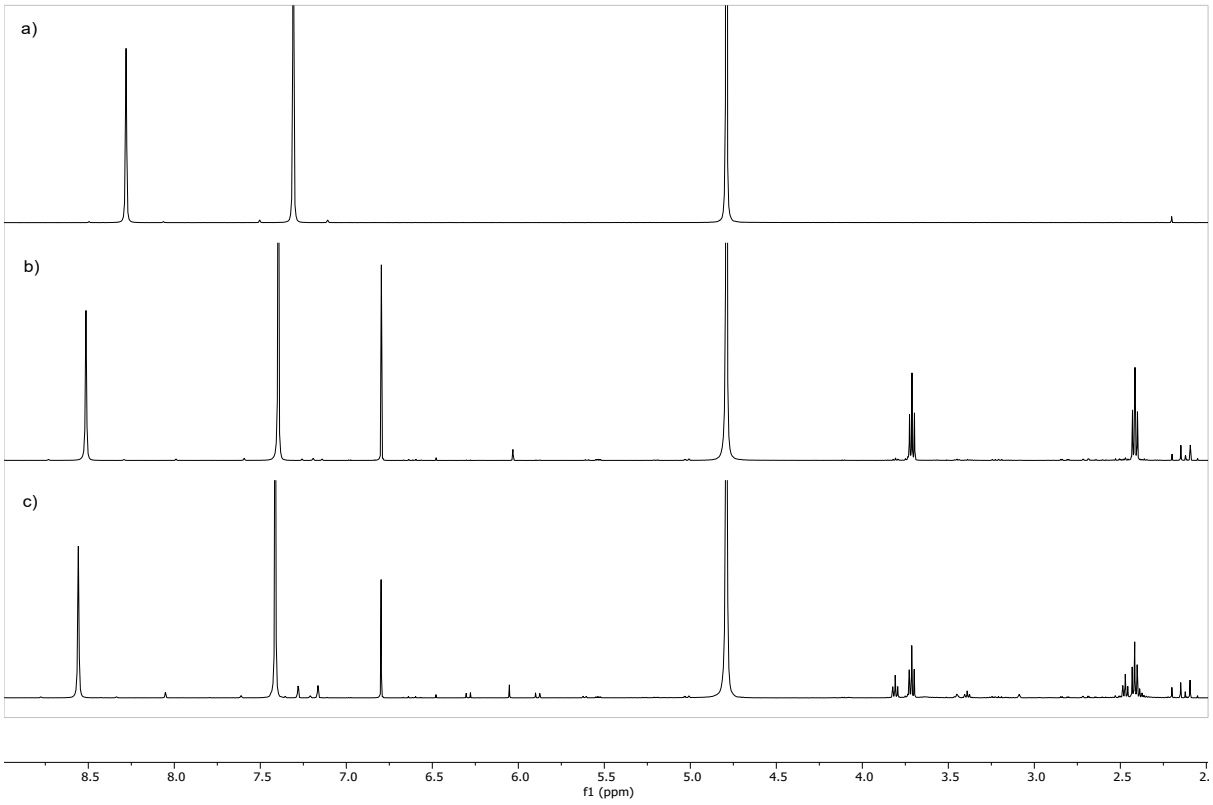

Figure S5: ^{1}H NMR spectra (500 MHz, D_2O) of (a) imidazole HCl buffer (100 mM, pH 6.9) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.6 PIPES pH 6.9

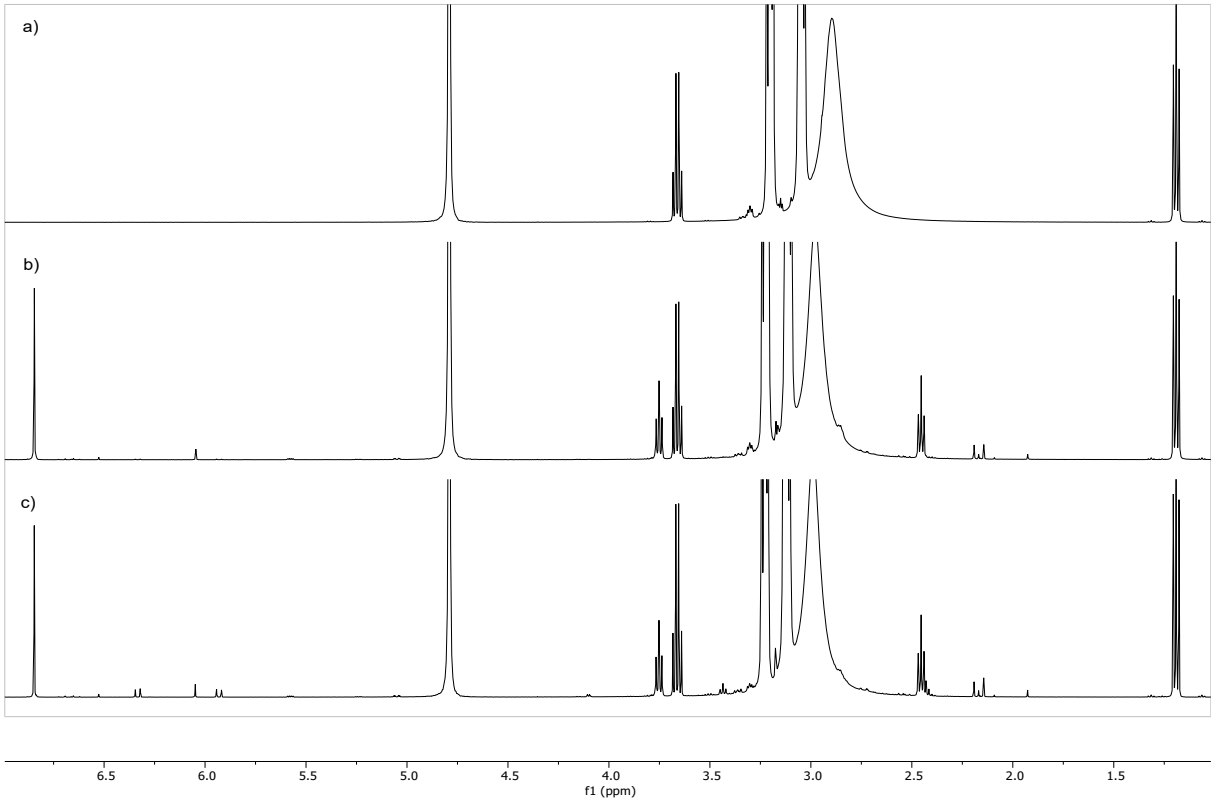

Figure S6: ^{1}H NMR spectra (500 MHz, D_2O) of (a) PIPES buffer (100 mM, pH 6.9) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.7 Deuterium oxide pH 7.1

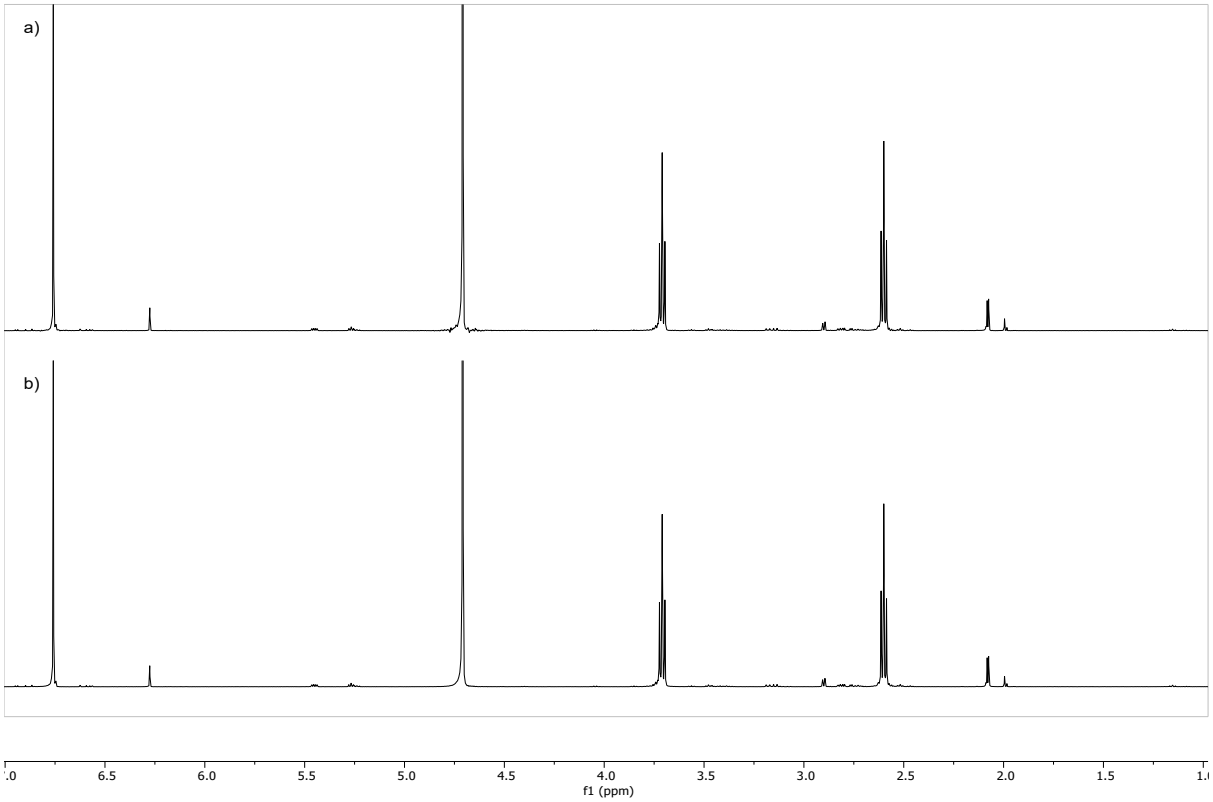

Figure S7: ^{1}H NMR spectra (500 MHz, D_2O) of maleimide **2** after (a) 1 and (b) 24 h in the D_2O (pH 7.1) at 21 ± 1 °C.

2.1.8 PBS pH 7.3

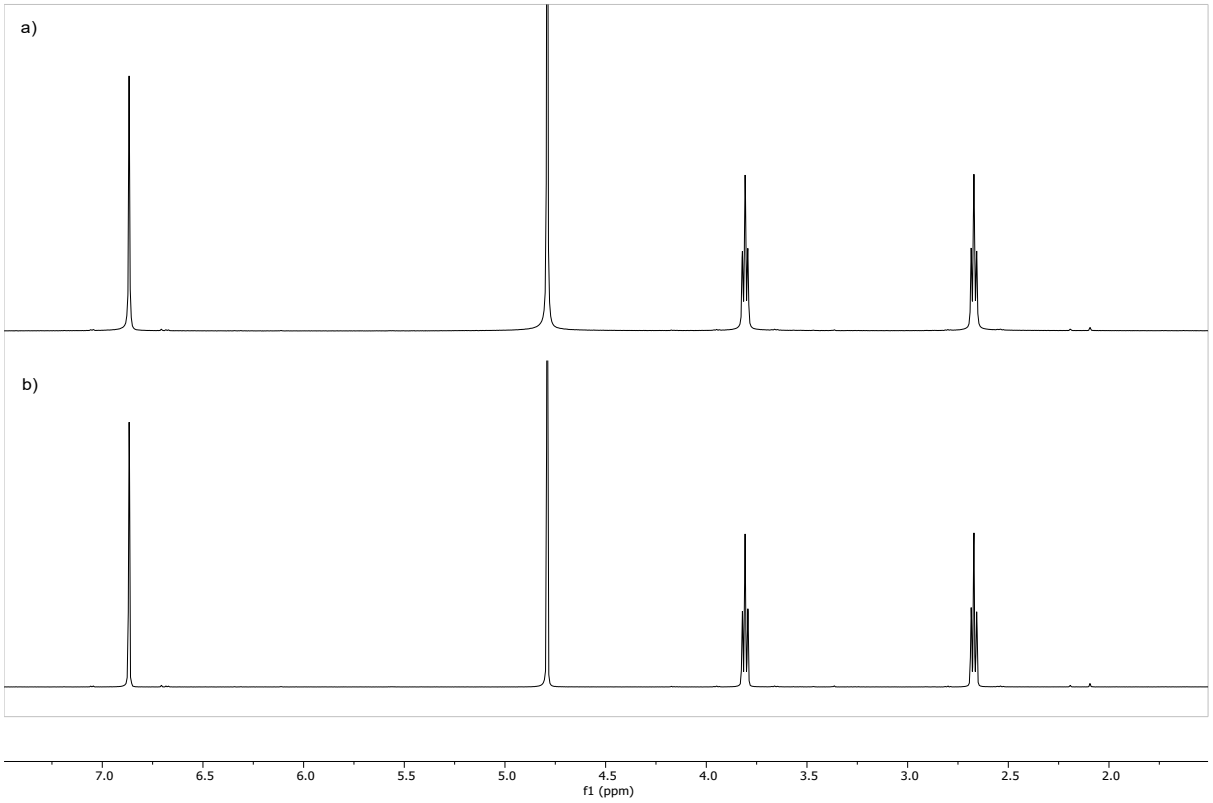

Figure S8: ^{1}H NMR spectra (500 MHz, D_2O) of (a) PBS buffer (100 mM, pH 7.3) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.9 HEPES pH 7.4

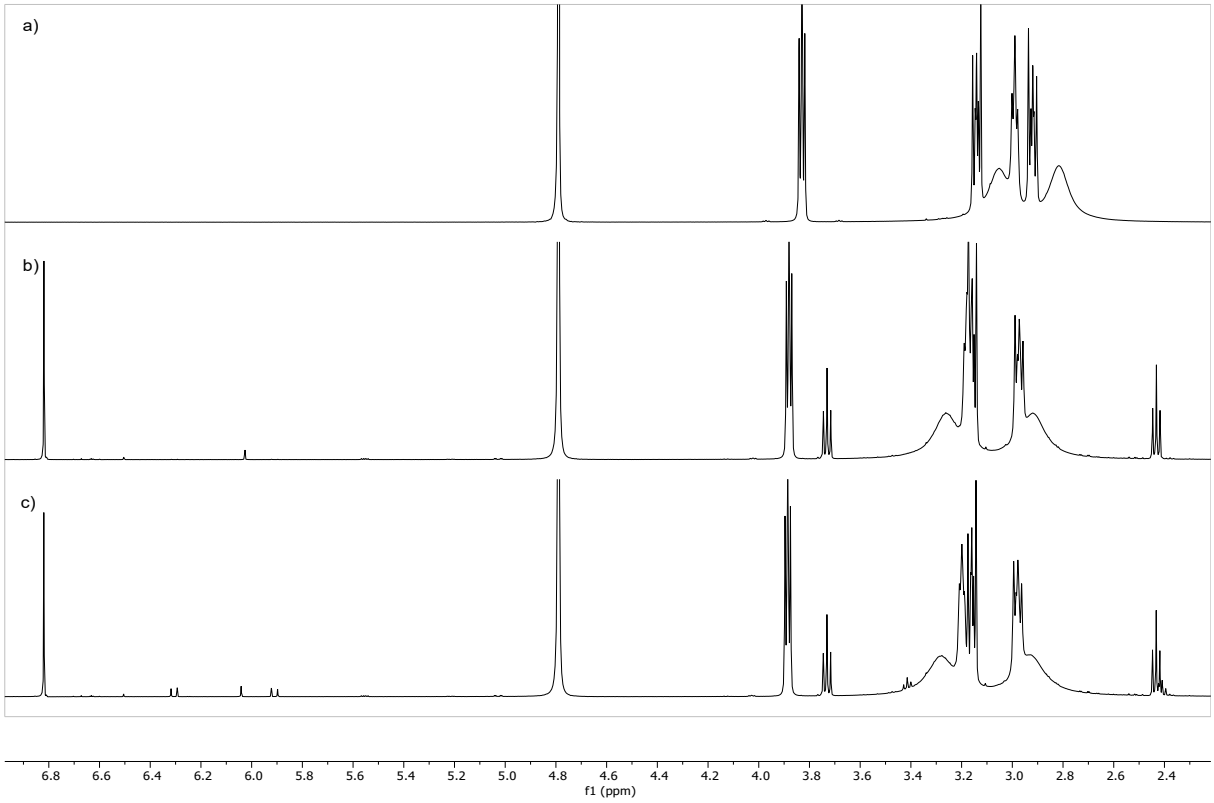

Figure S9: ^{1}H NMR spectra (500 MHz, D_2O) of (a) HEPES buffer (100 mM, pH 7.4) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.10 Trizma pH 7.4

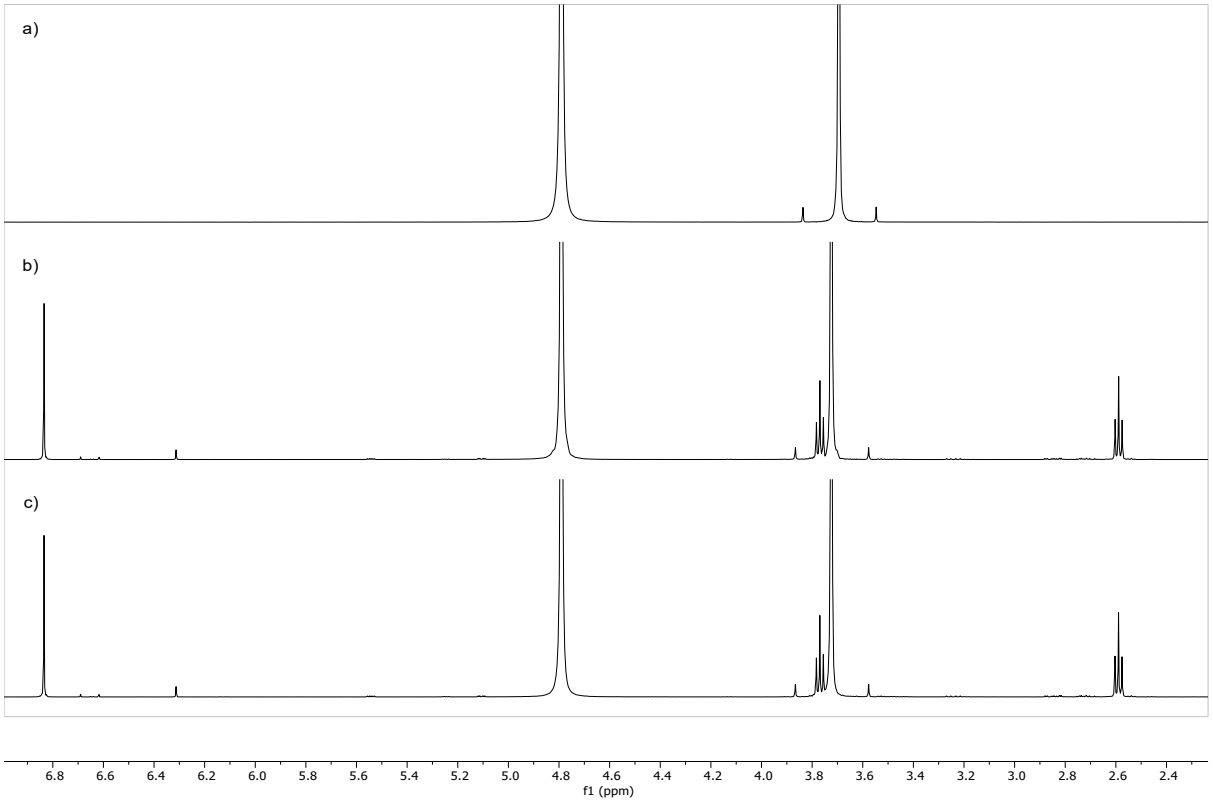

Figure S10: ^{1}H NMR spectra (500 MHz, D_2O) of (a) Trizma buffer (100 mM, pH 7.4) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.11 Tricine pH 8.0

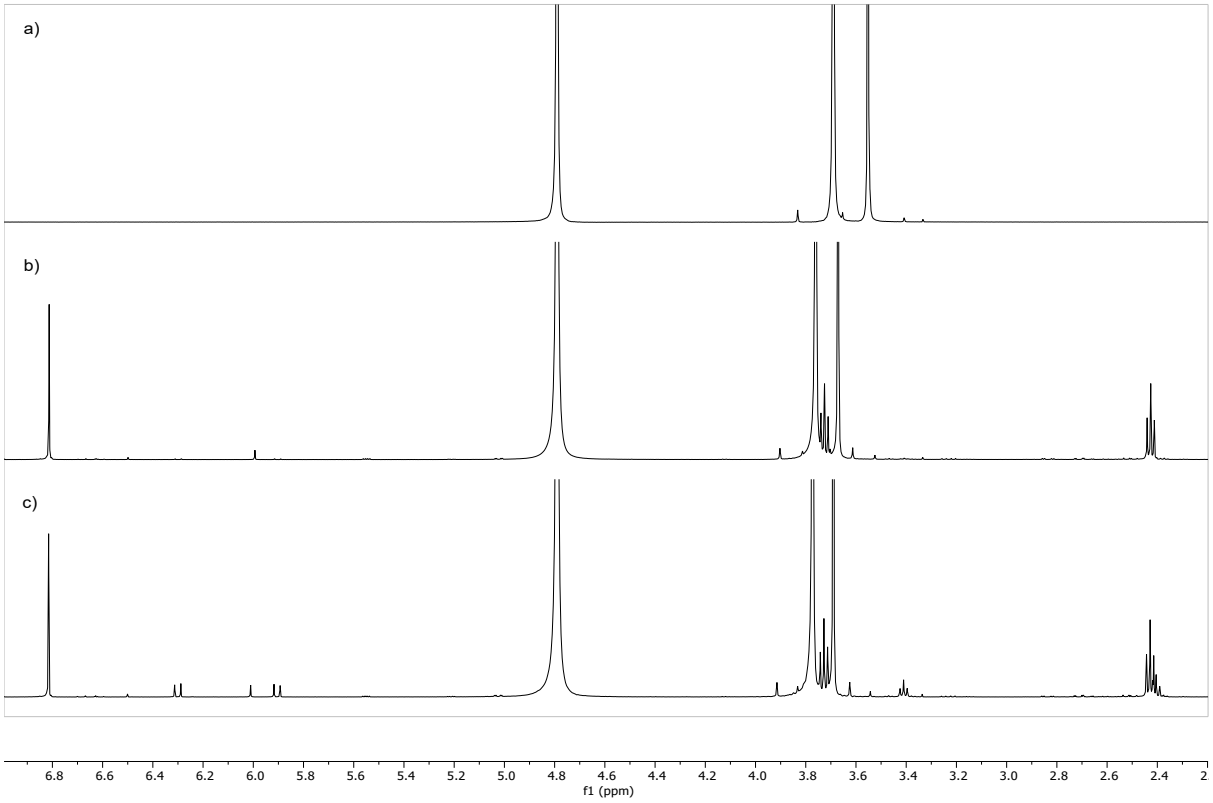

Figure S11: ^{1}H NMR spectra (500 MHz, D_2O) of (a) Tricine buffer (100 mM, pH 8.0) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.12 Bicine pH 8.1

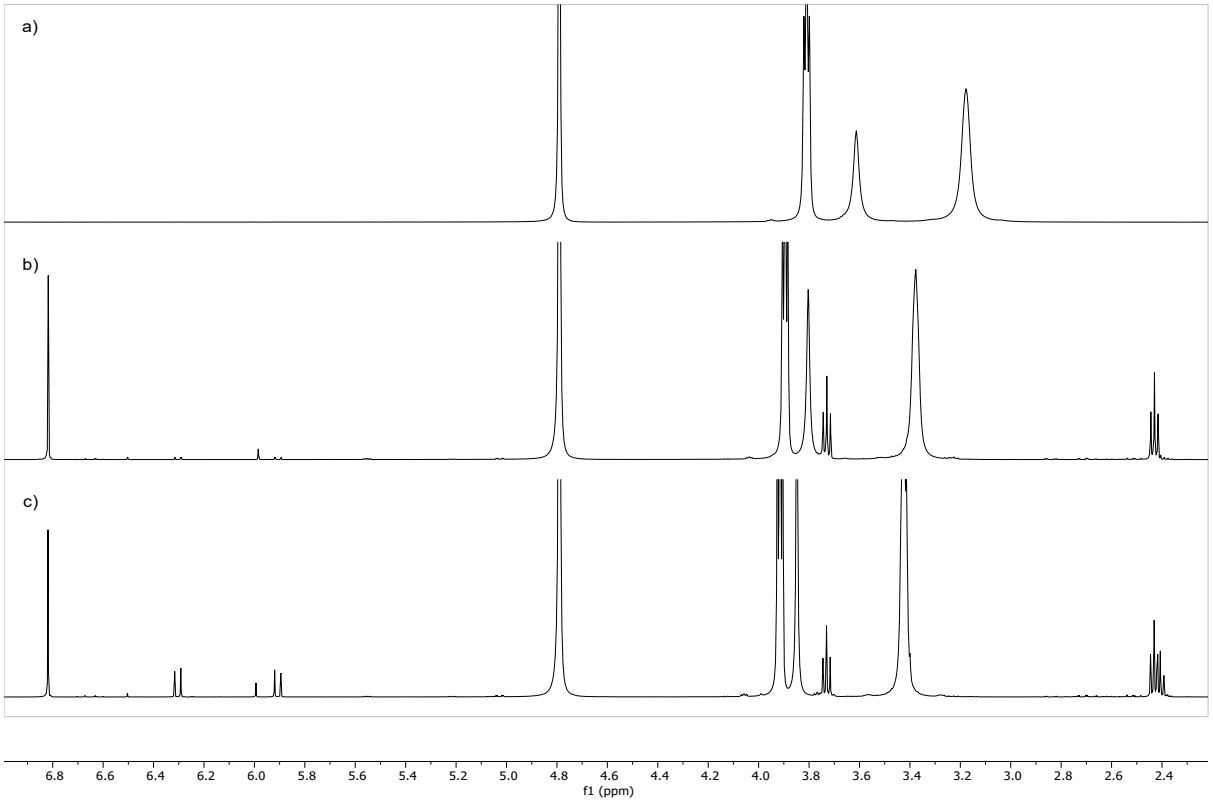

Figure S12: ^{1}H NMR spectra (500 MHz, D_2O) of (a) Bicine buffer (100 mM, pH 8.1) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.13 Glycine NaOH pH 9.2

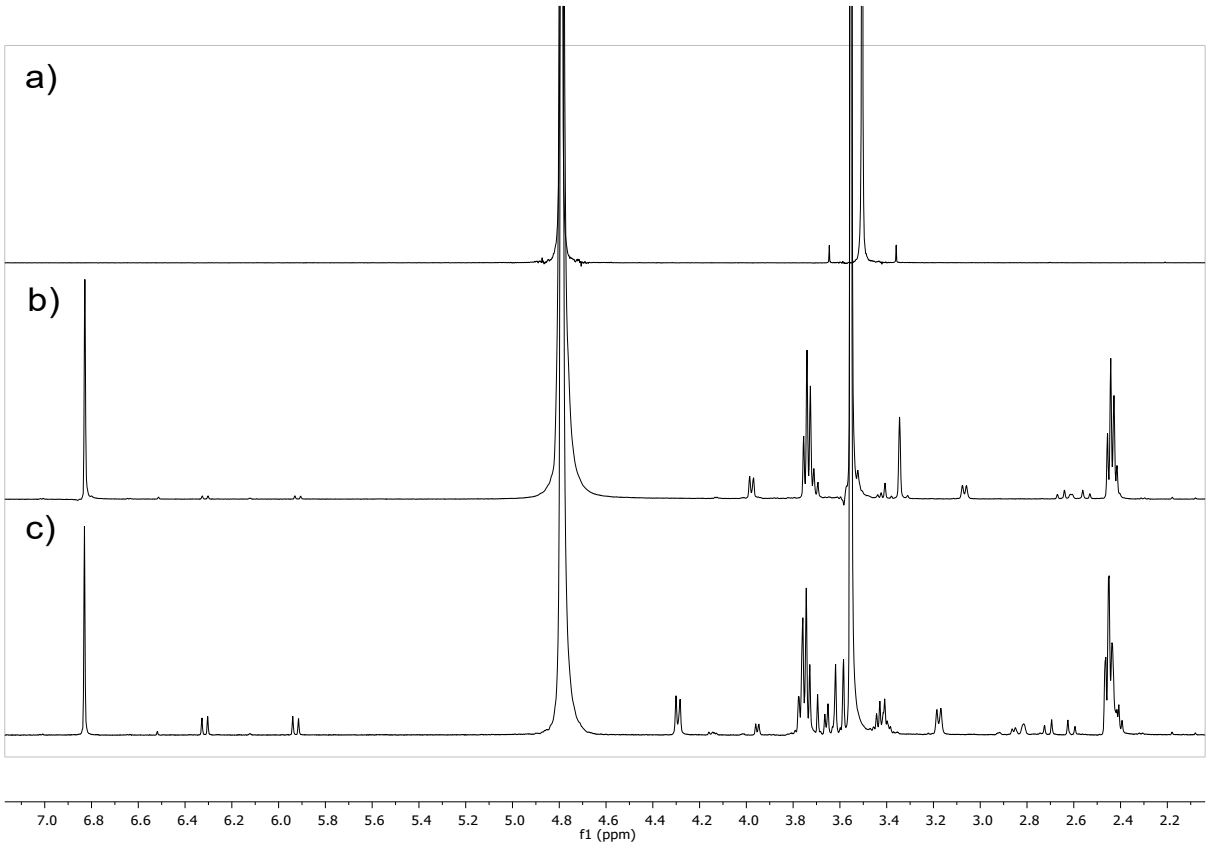

Figure S13: ^{1}H NMR spectra (500 MHz, D_2O) of (a) glycine NaOH buffer (100 mM, pH 8.7) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.1.14 CAPS pH 10.1

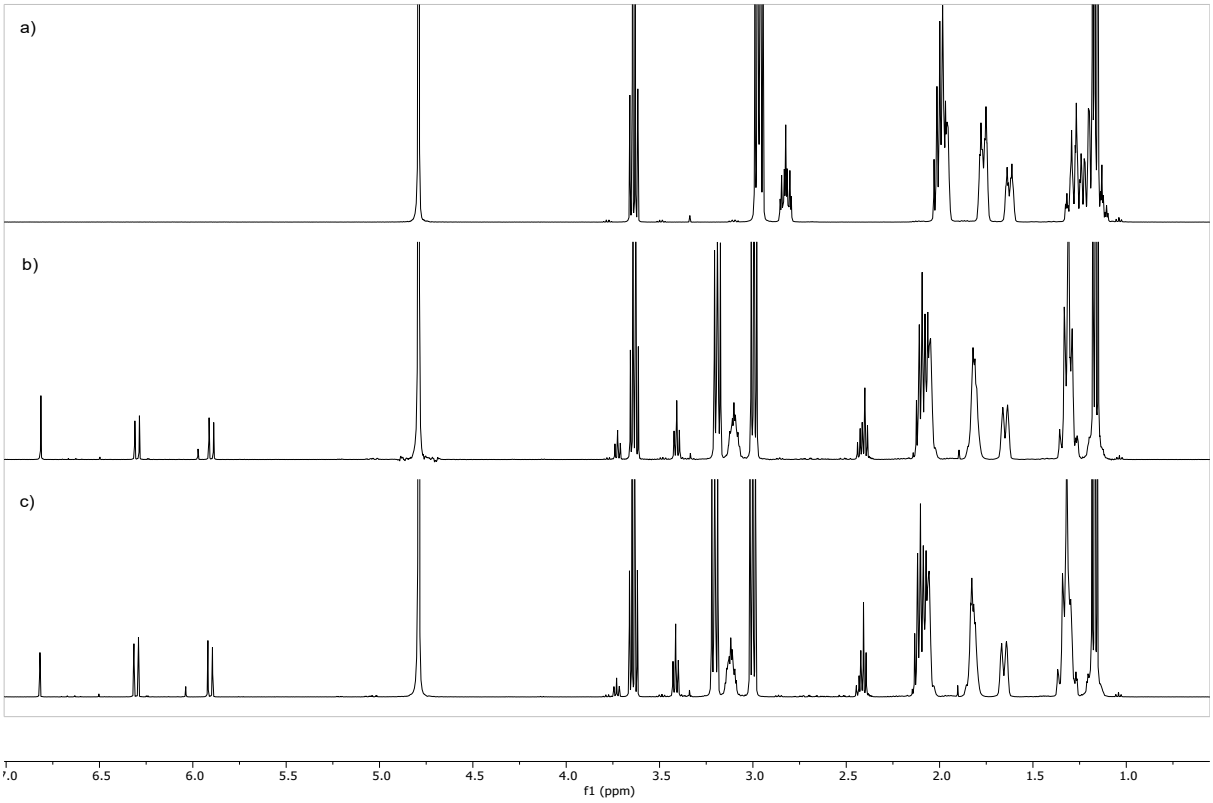

Figure S14: ^{1}H NMR spectra (500 MHz, D_2O) of (a) CAPS buffer (100 mM, pH 10.1) and maleimide **2** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2 Fulvene Stability

2.2.1 Glycine HCl pH 3.7

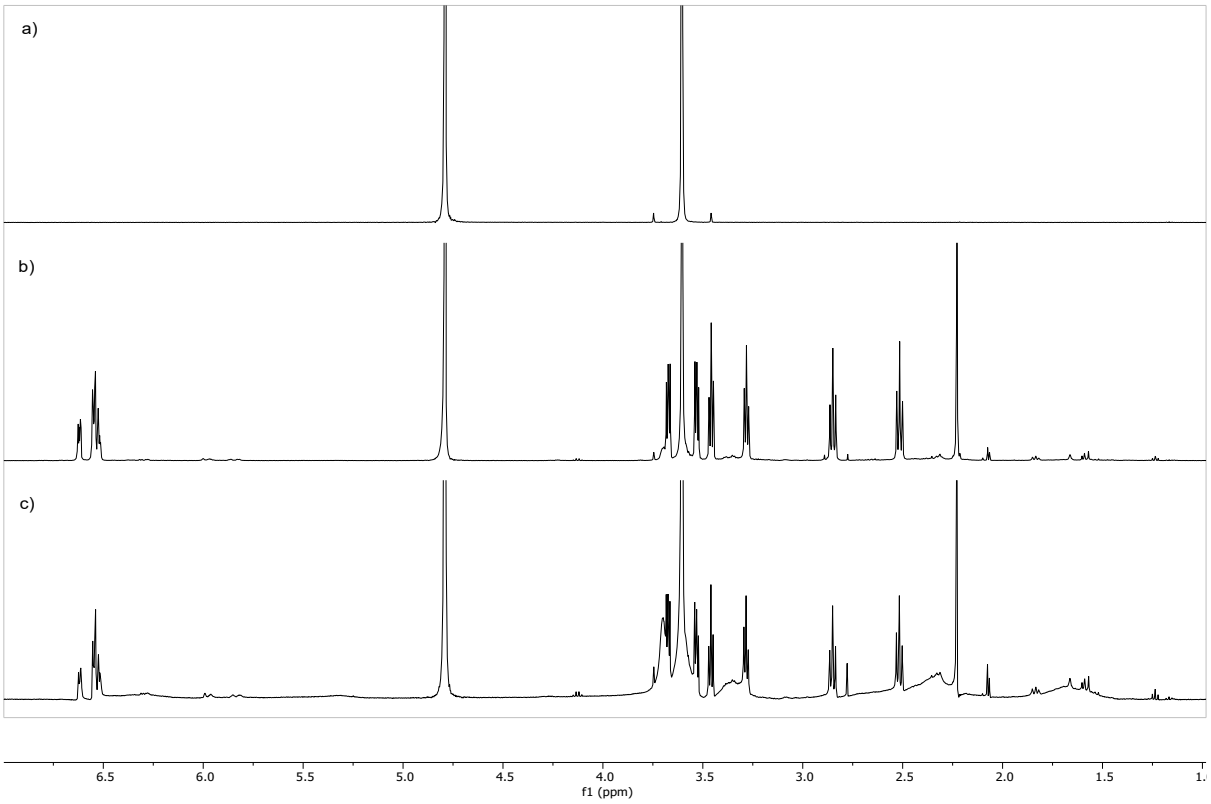

Figure S15: ^{1}H NMR spectra (500 MHz, D_2O) of (a) glycine HCl buffer (100 mM, pH 3.7) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.2 MES pH 5.5

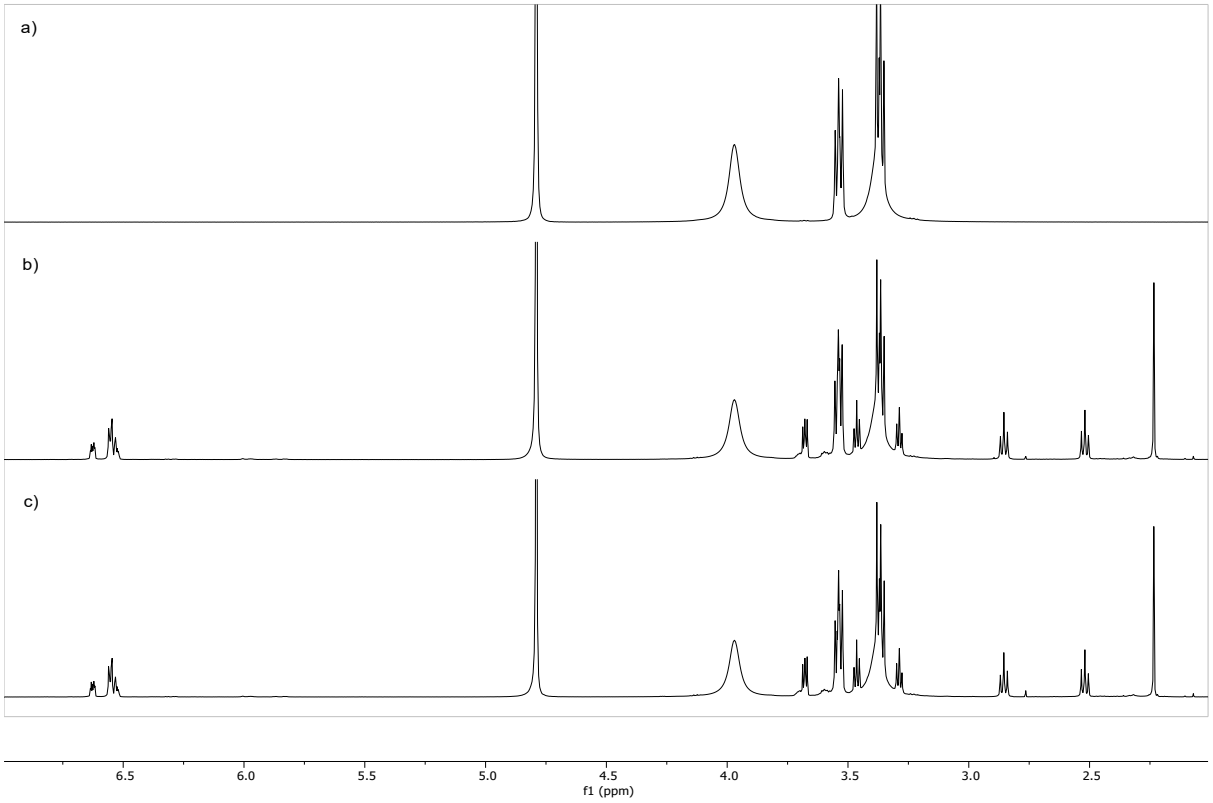

Figure S16: ^{1}H NMR spectra (500 MHz, D_2O) of (a) MES buffer (100 mM, pH 5.5) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.3 BTM pH 6.4

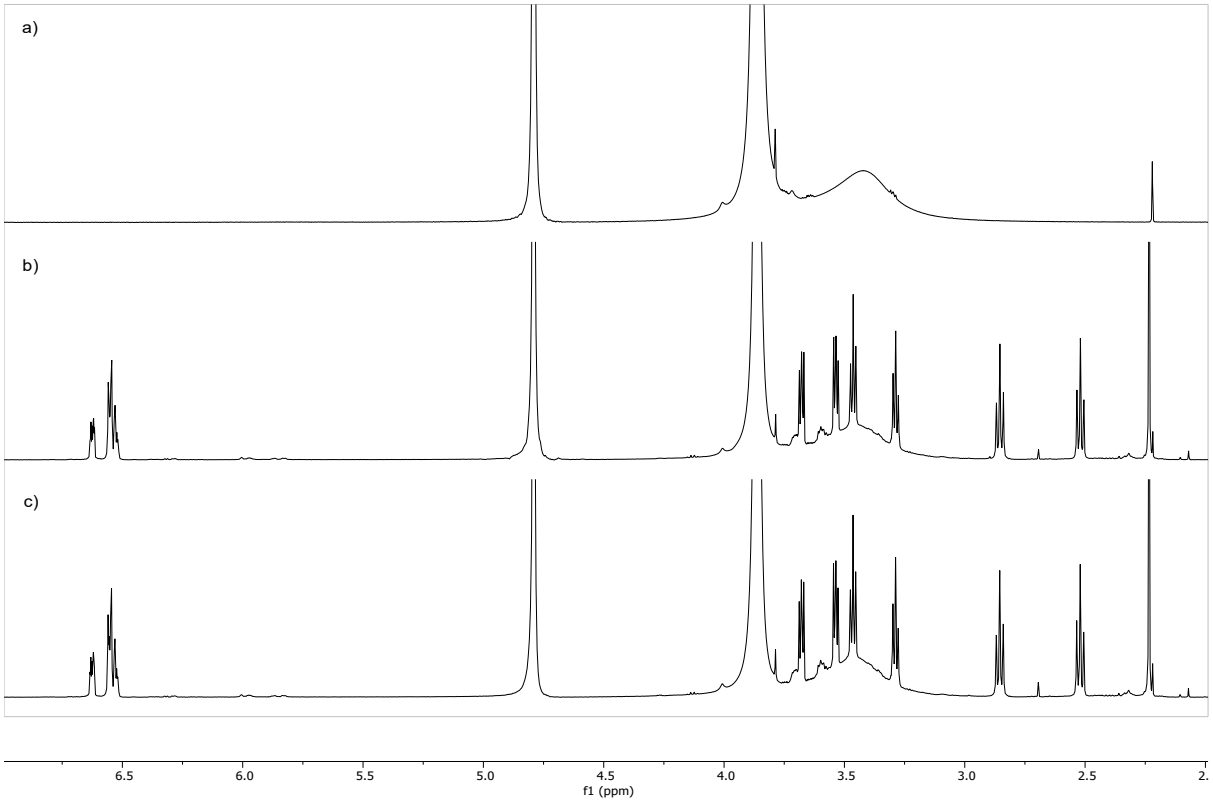

Figure S17: ^{1}H NMR spectra (500 MHz, D_2O) of (a) BTM buffer (100 mM, pH 6.4) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.4 BTP pH 6.9

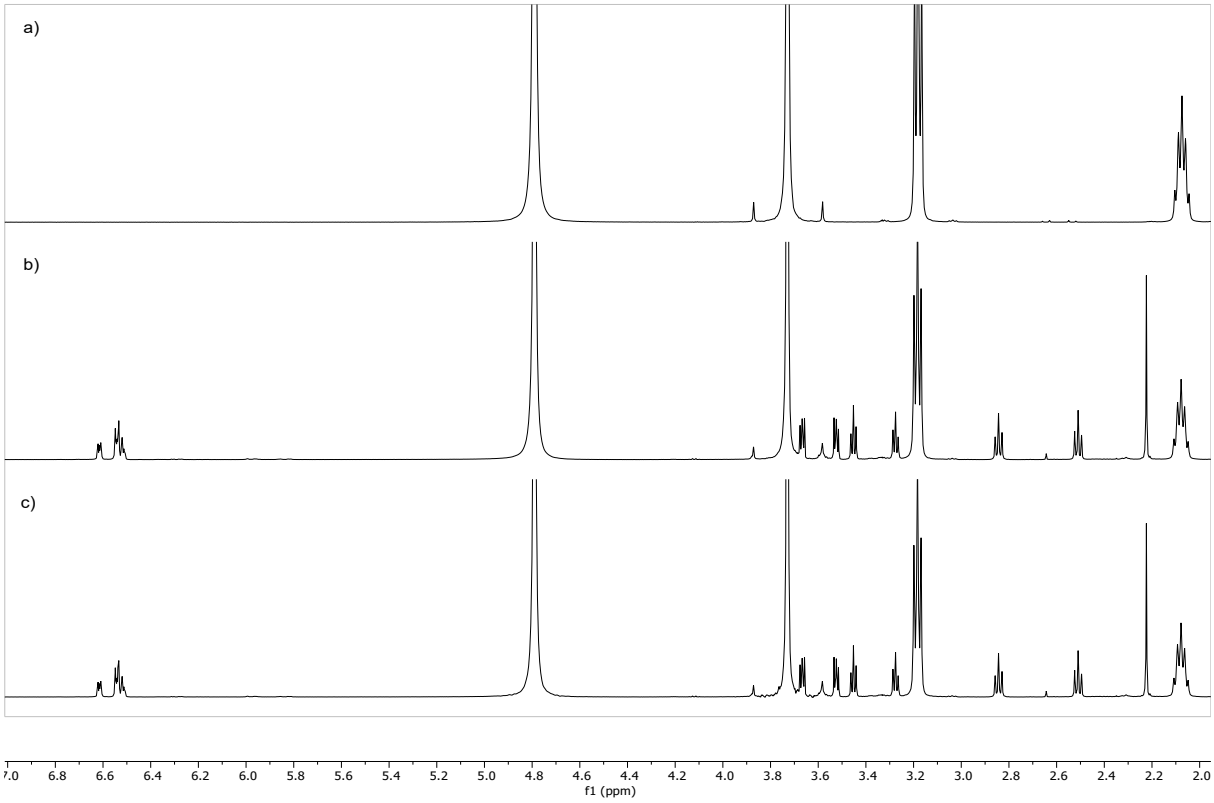

Figure S18: ^{1}H NMR spectra (500 MHz, D_2O) of (a) BTP buffer (100 mM, pH 6.9) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.5 Imidazole HCl pH 6.9

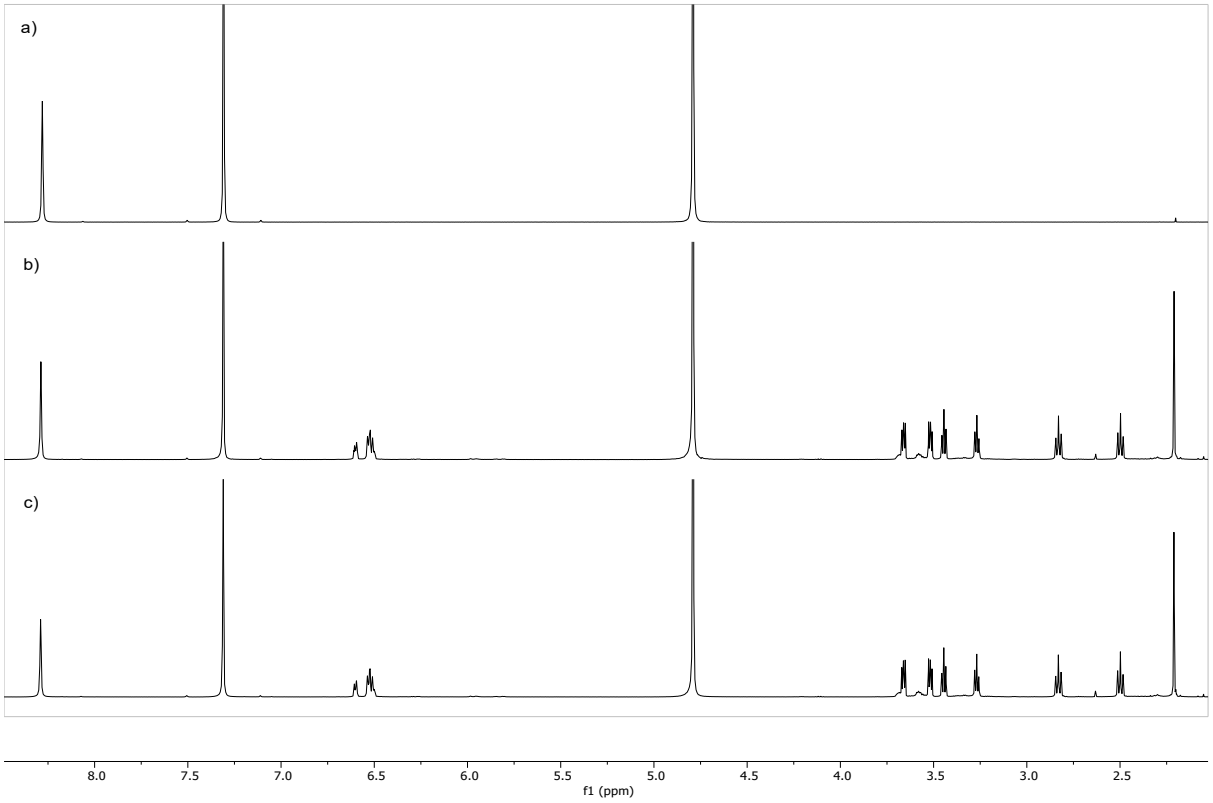

Figure S19: ^{1}H NMR spectra (500 MHz, D_2O) of (a) imidazole HCl buffer (100 mM, pH 6.9) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.6 PIPES pH 6.9

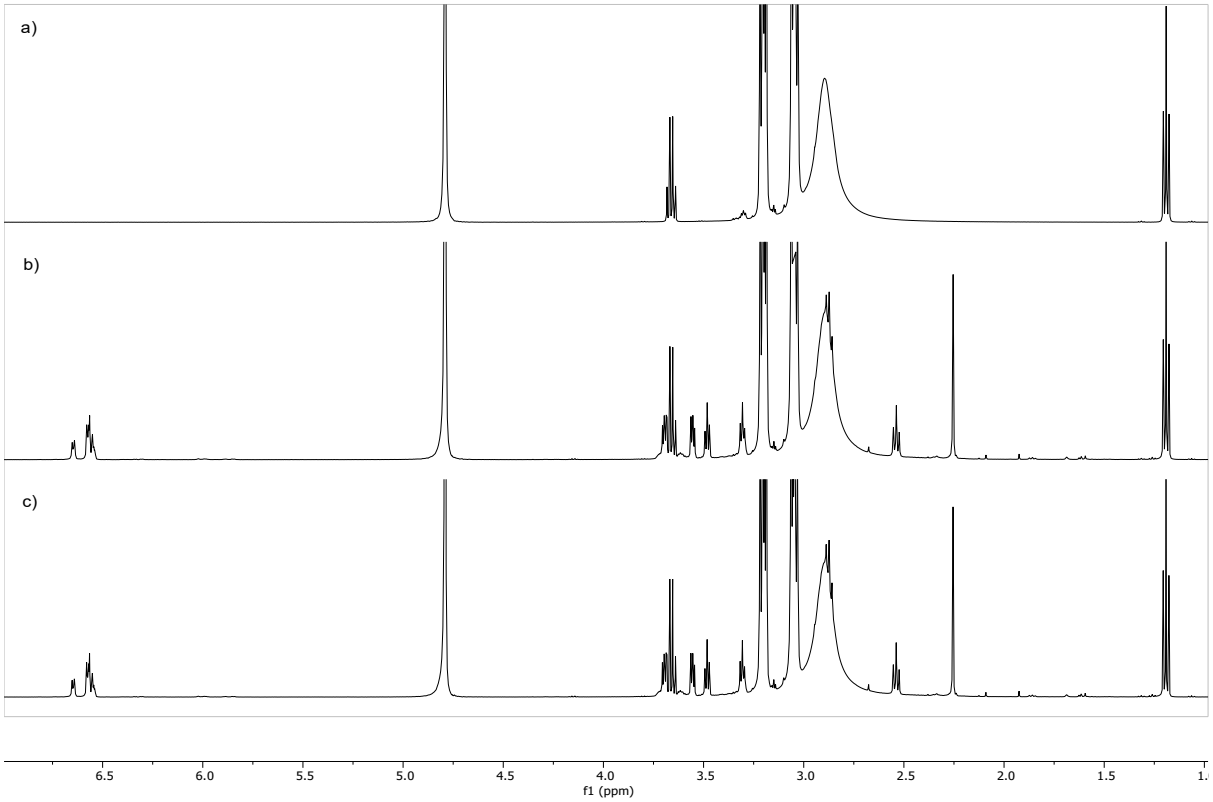

Figure S20: ^{1}H NMR spectra (500 MHz, D_2O) of (a) PIPES buffer (100 mM, pH 6.9) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.7 Deuterium oxide pH 7.1

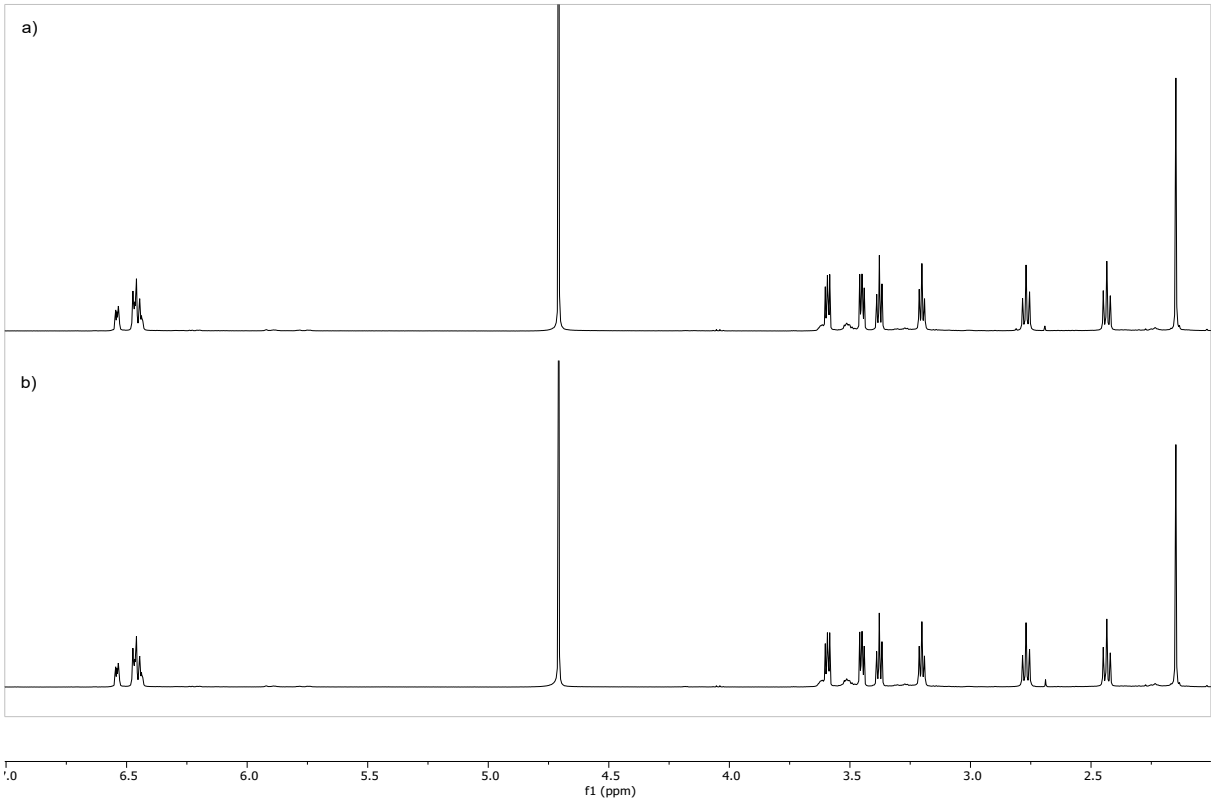

Figure S21: ^{1}H NMR spectra (500 MHz, D_2O) showing the stability of pentafulvene **1** in D_2O (pH 7.1) over (a) 1 and (b) 24 h at 21 ± 1 °C.

2.2.8 PBS pH 7.3

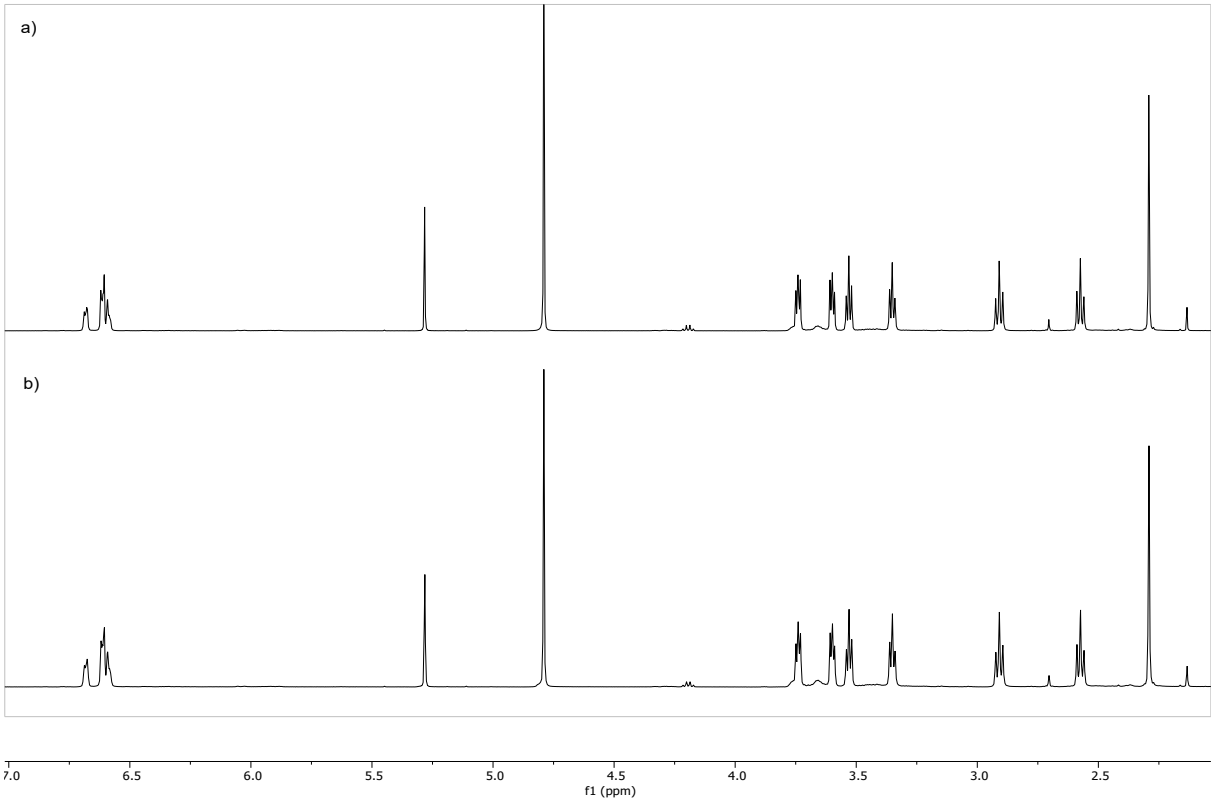

Figure S22: ^{1}H NMR spectra (500 MHz, D_2O) of (a) PBS buffer (100 mM, pH 7.3) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21± 1 °C.

2.2.9 HEPES pH 7.4

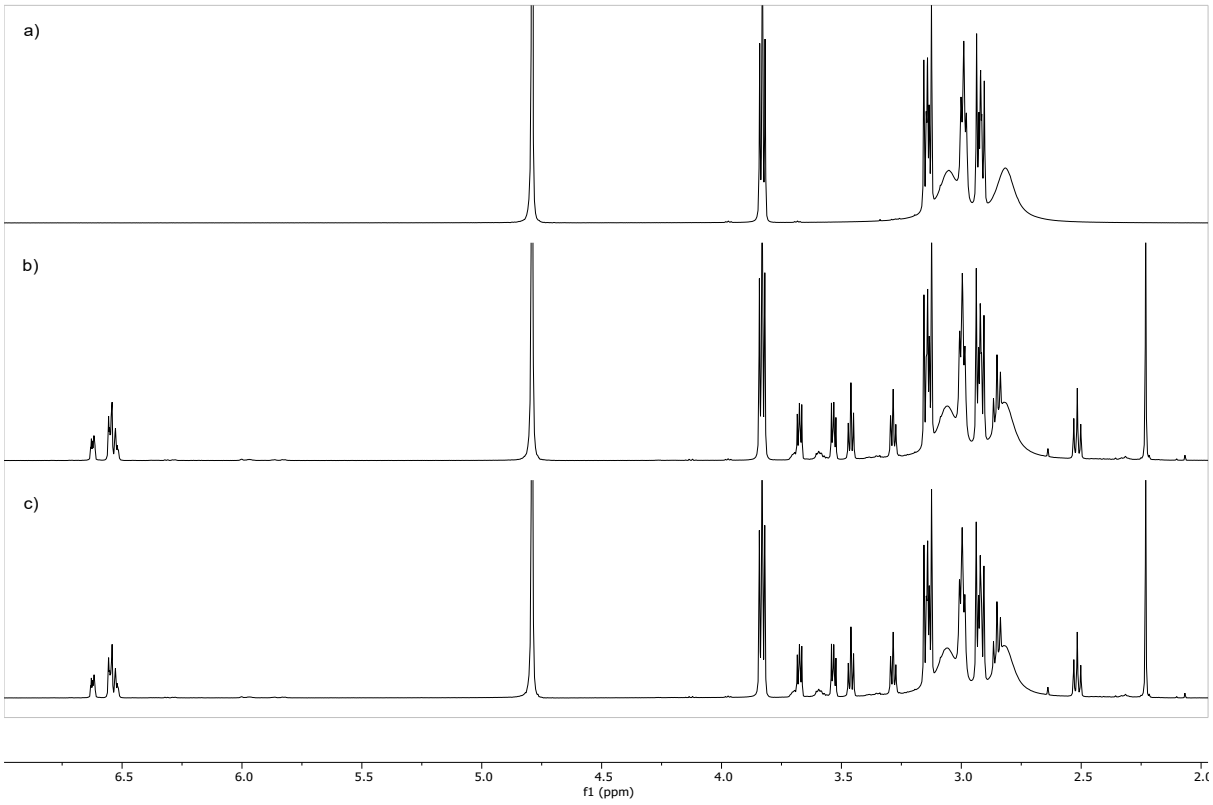

Figure S23: ^{1}H NMR spectra (500 MHz, D_2O) of (a) HEPES buffer (100 mM, pH 7.4) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.10 Trizma pH 7.4

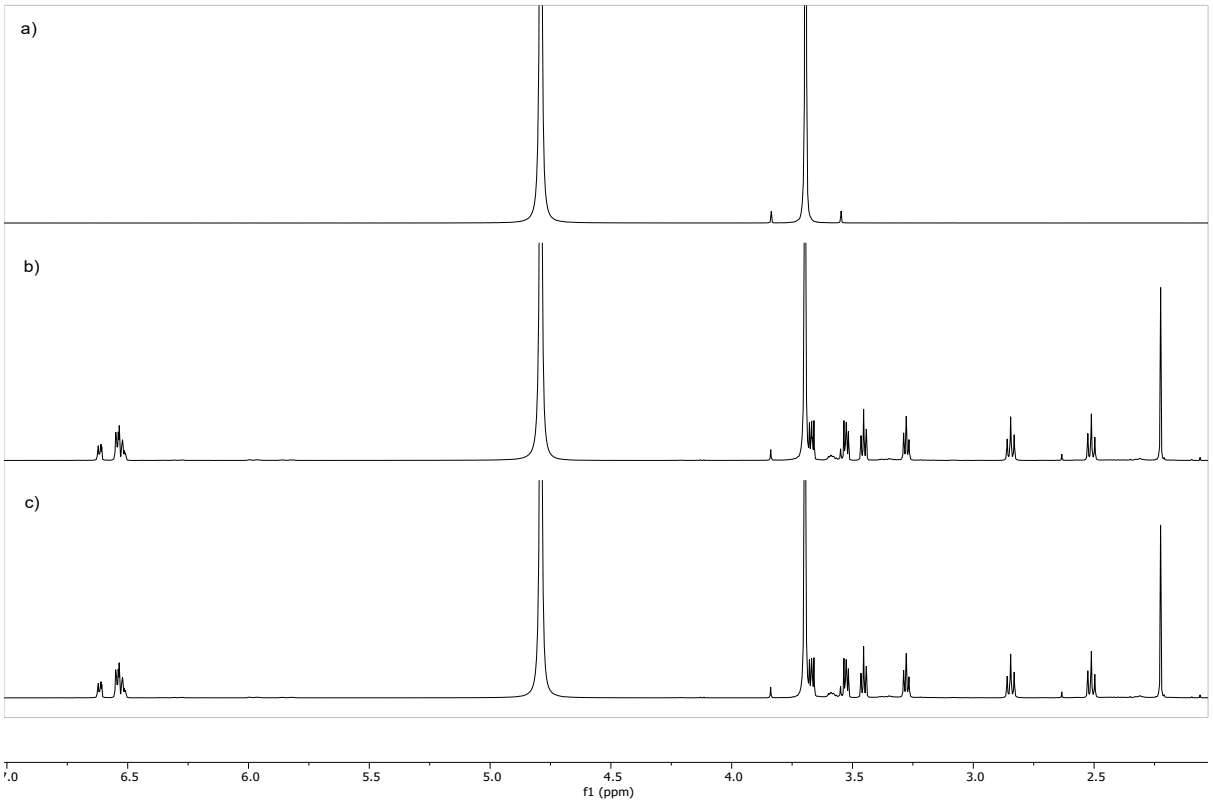

Figure S24: ^{1}H NMR spectra (500 MHz, D_2O) of (a) Trizma buffer (100 mM, pH 7.4) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.11 Tricine pH 8.0

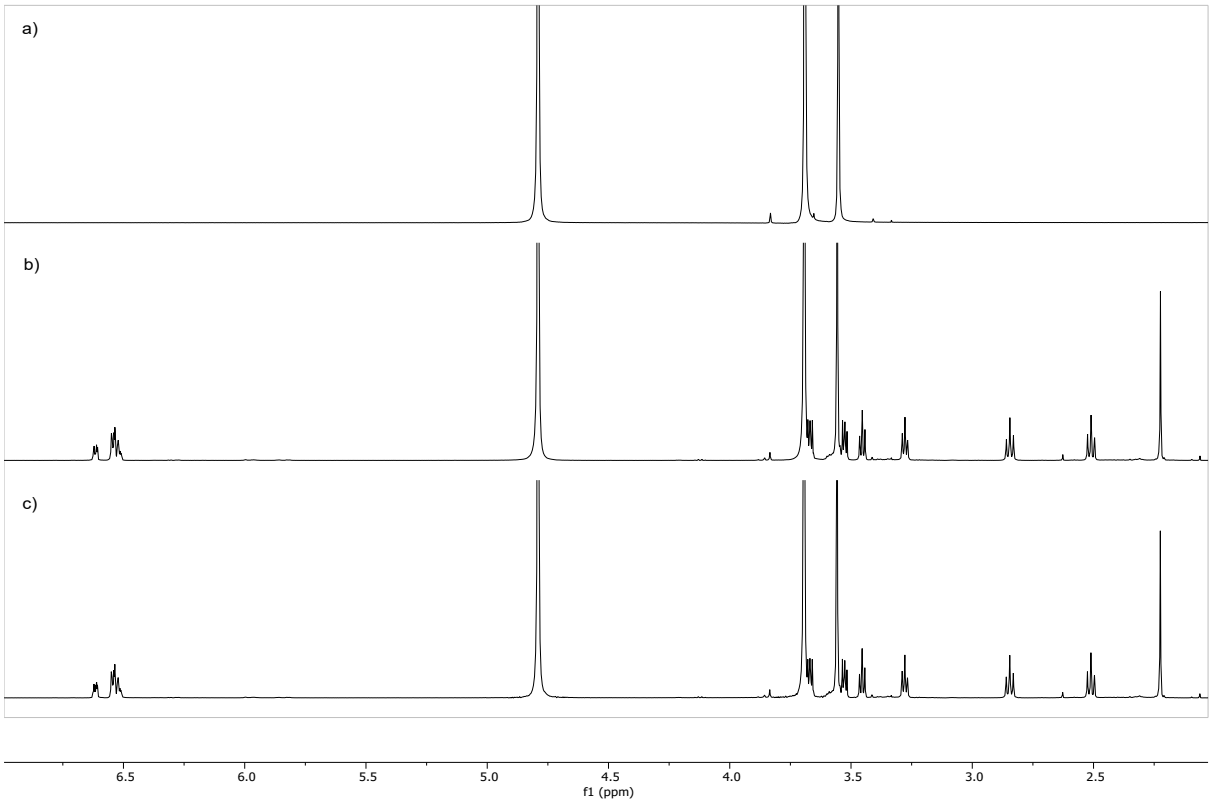

Figure S25: ^{1}H NMR spectra (500 MHz, D_2O) of (a) Tricine buffer (100 mM, pH 8.0) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.12 Bicine pH 8.1

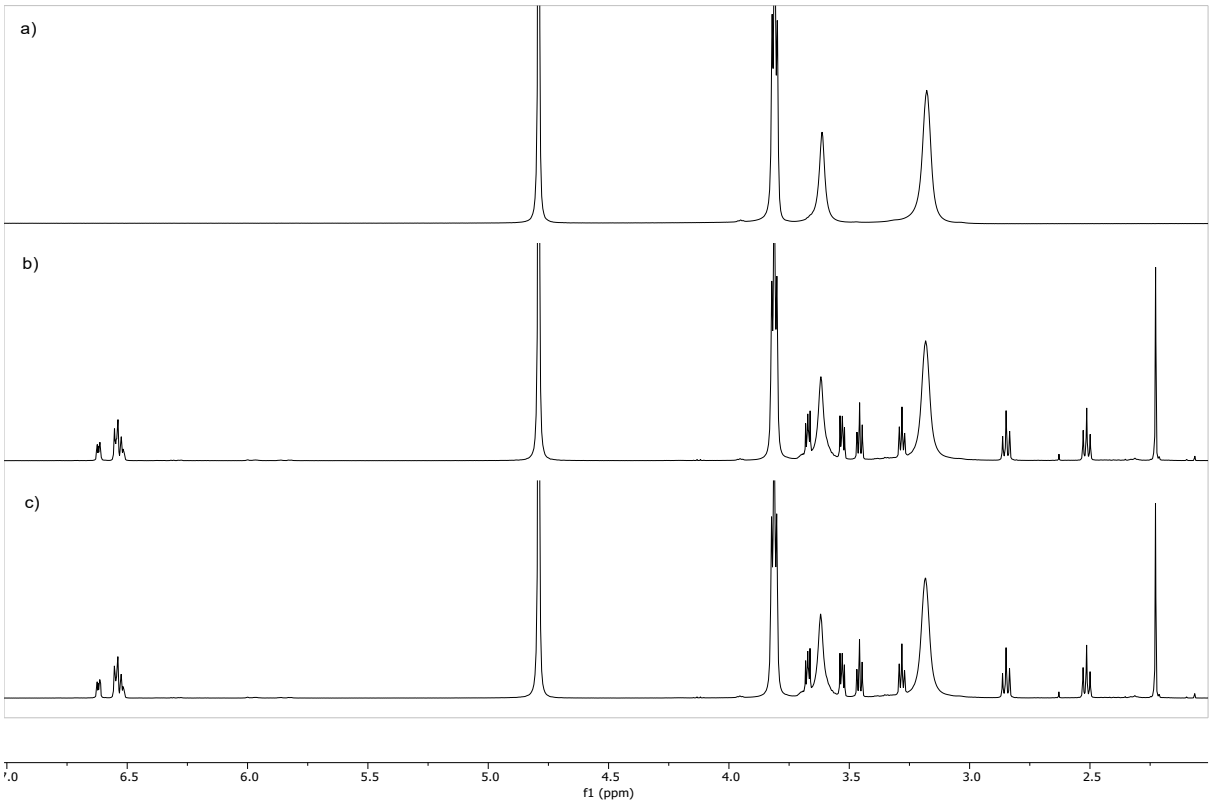

Figure S26: ^{1}H NMR spectra (500 MHz, D_2O) of (a) Bicine buffer (100 mM, pH 8.1) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.13 Glycine NaOH pH 8.7

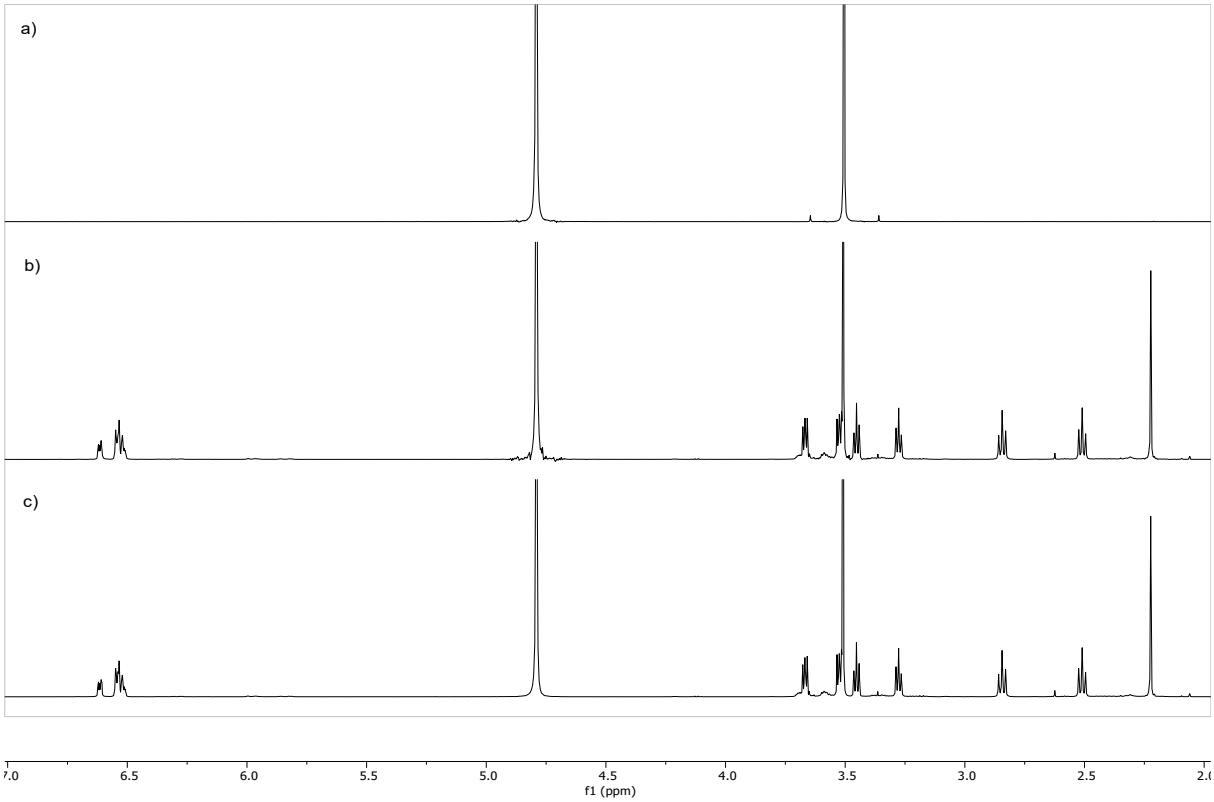

Figure S27: ^{1}H NMR spectra (500 MHz, D_2O) of (a) glycine NaOH buffer (100 mM, pH 8.7) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

2.2.14 CAPS pH 10.1

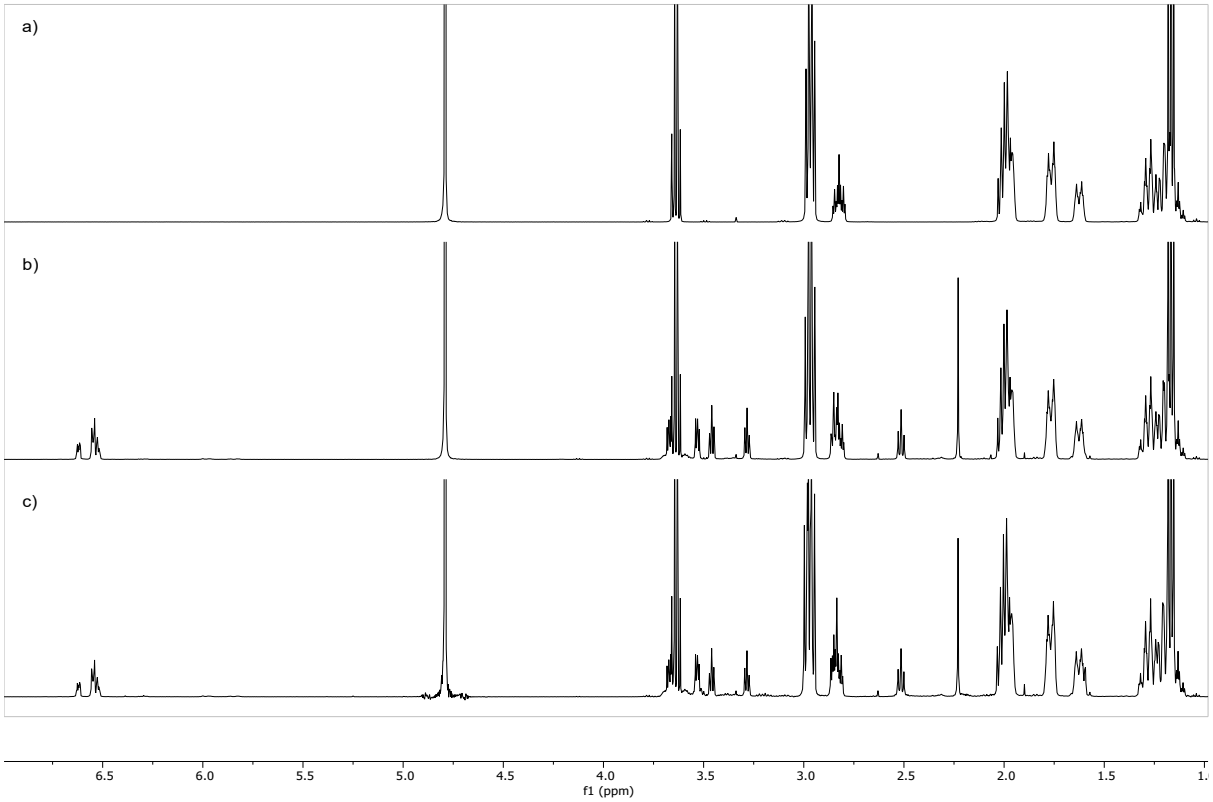

Figure S28: ^{1}H NMR spectra (500 MHz, D_2O) of (a) CAPS buffer (100 mM, pH 10.1) and pentafulvene **1** after (b) 1 and (c) 24 h in the buffer at 21 ± 1 °C.

3 Kinetic Studies

3.1 Glycine HCl pH 3.5

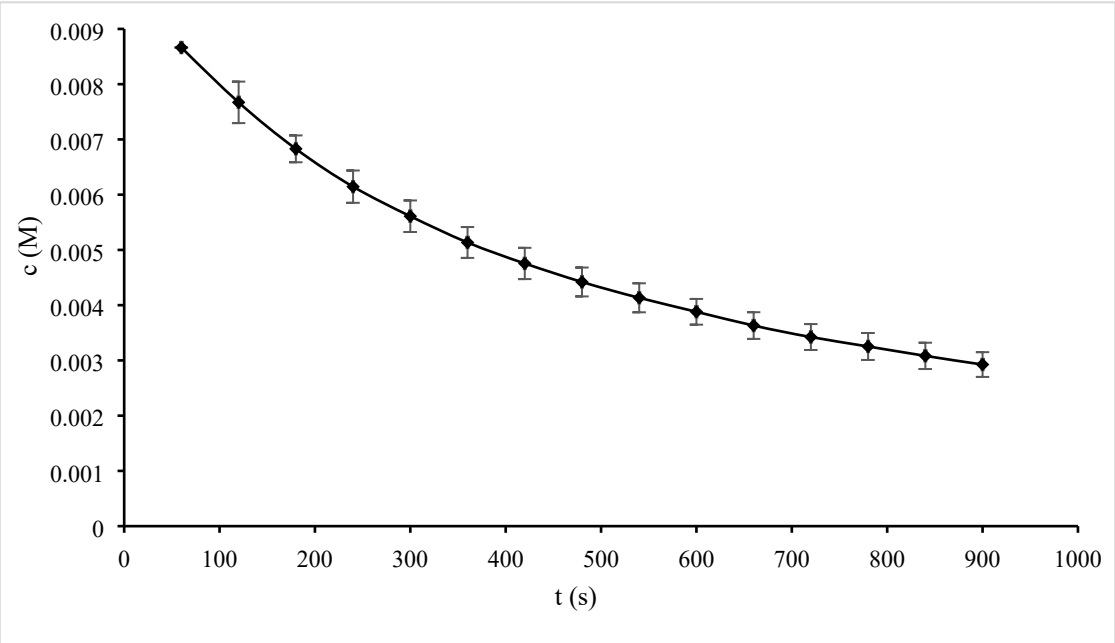

Figure S29: Concentration of pentafulvene **1** *versus* time for the reaction between **1** and **2** in glycine HCl buffer (100 mM, pH 3.5). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

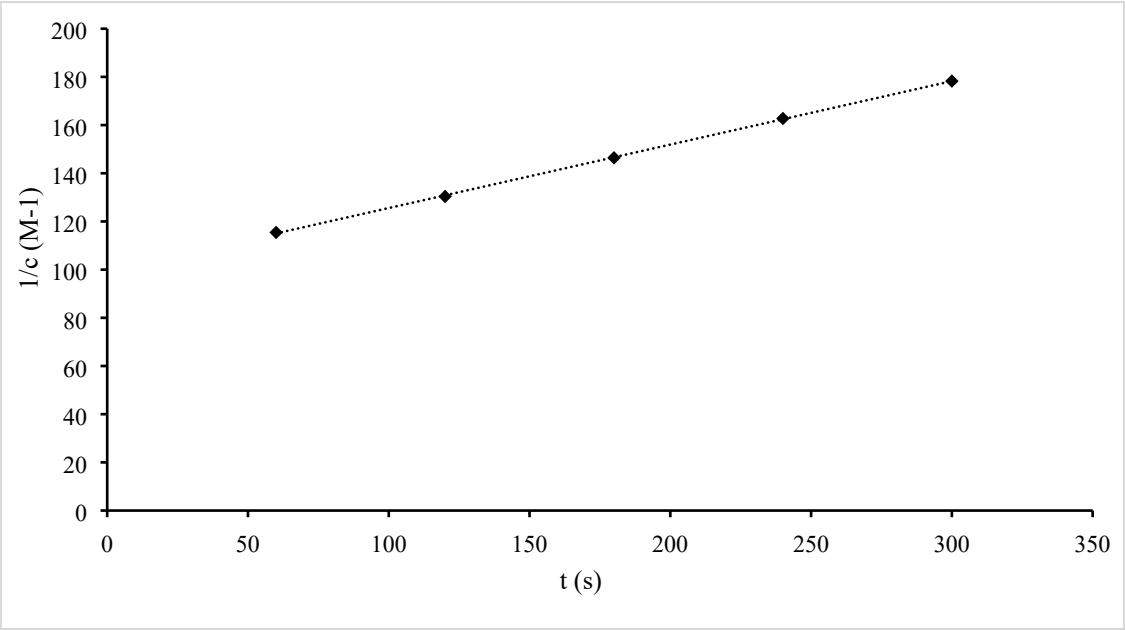

Figure S30: Second order kinetic plot for the reaction between **1** and **2** in glycine HCl buffer (100 mM, pH 3.5, 23 ± 1 °C); k_2 = 0.26 ± 0.02 $M^{-1}s^{-1}$.

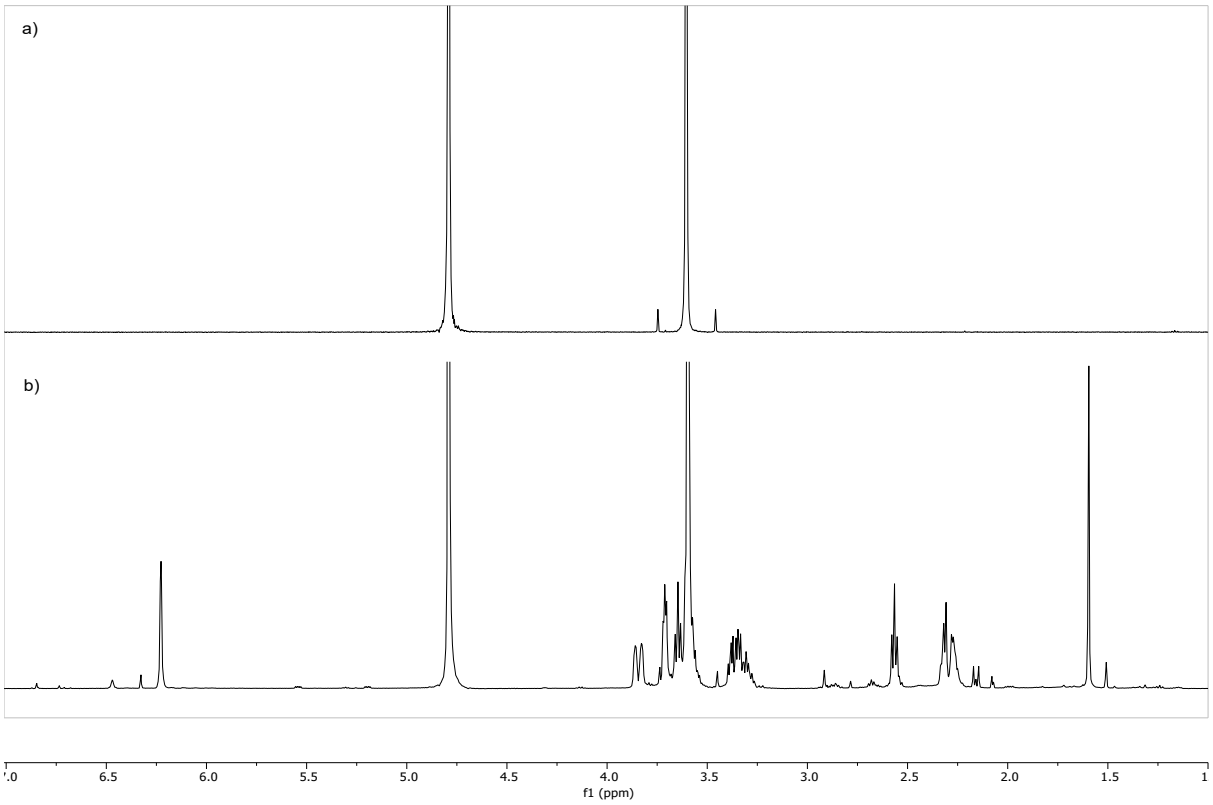

Figure S31: ^{1}H NMR spectra (500 MHz, D_2O) showing (a) glycine HCl buffer and (b) cycloadduct products from kinetic experiments.

3.2 MES pH 5.5

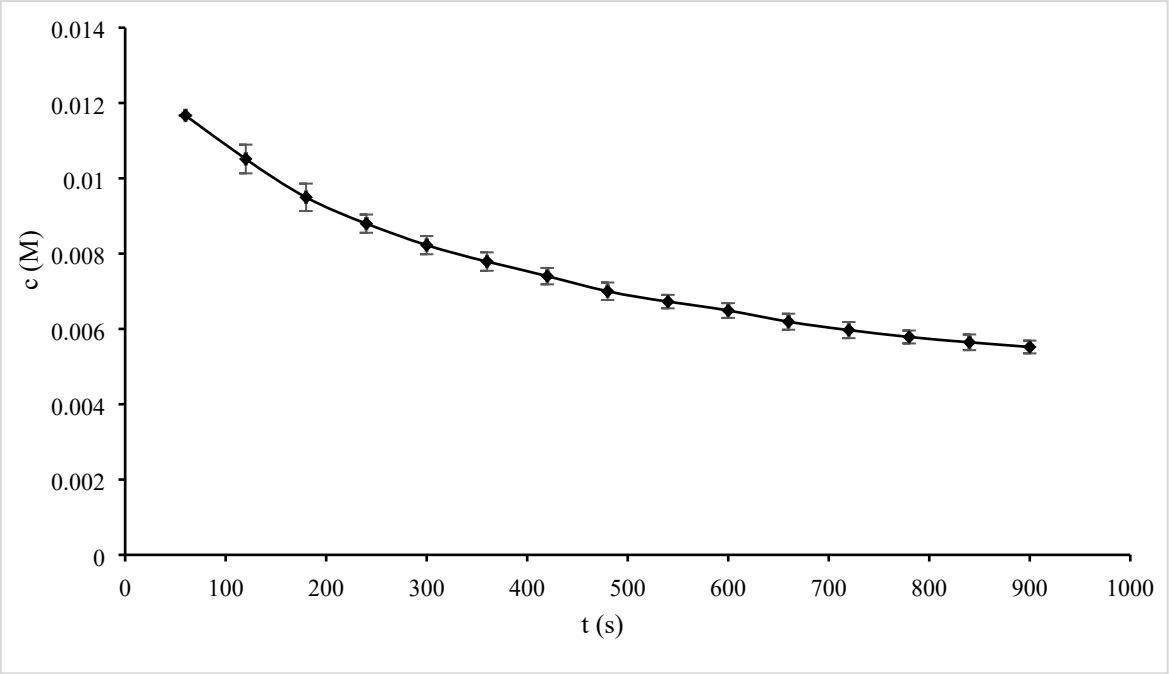

Figure S32: Concentration of pentafulvene **1** *versus* time for the reaction between **1** and **2** in MES buffer (100 mM, pH 5.5). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

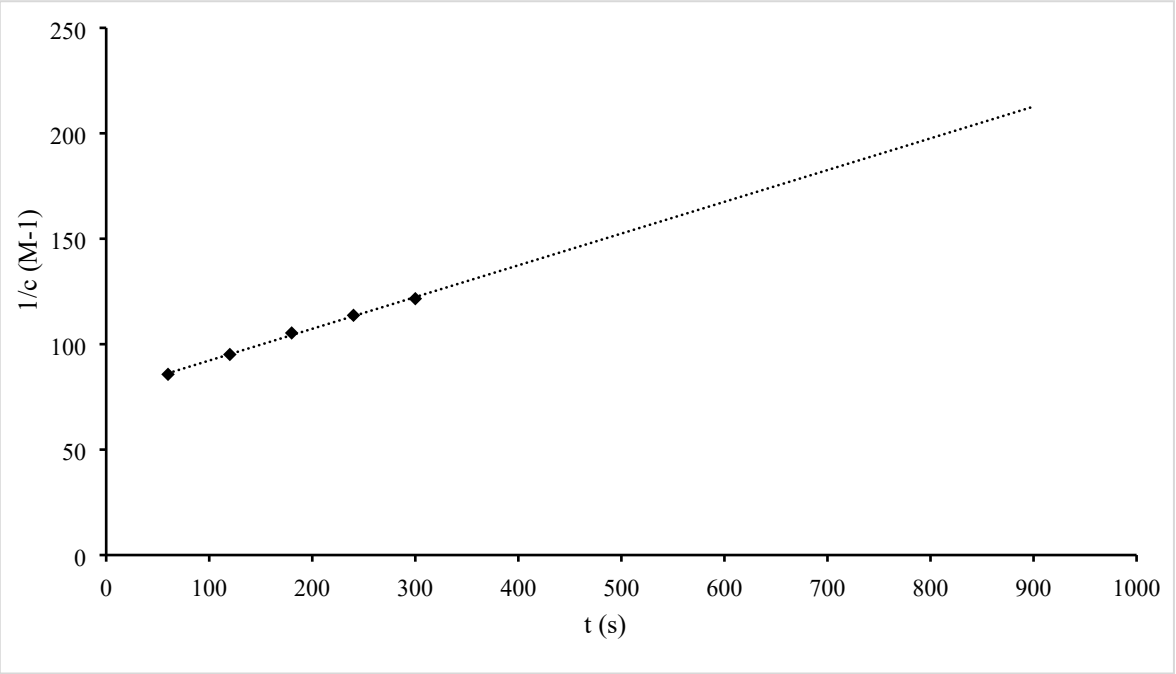

Figure S33: Second order kinetic plot for the reaction between **1** and **2** in MES buffer (100 mM, pH 5.5, 23 ± 1 °C); k_2 = 0.15 ± 0.01 $M^{-1}s^{-1}$.

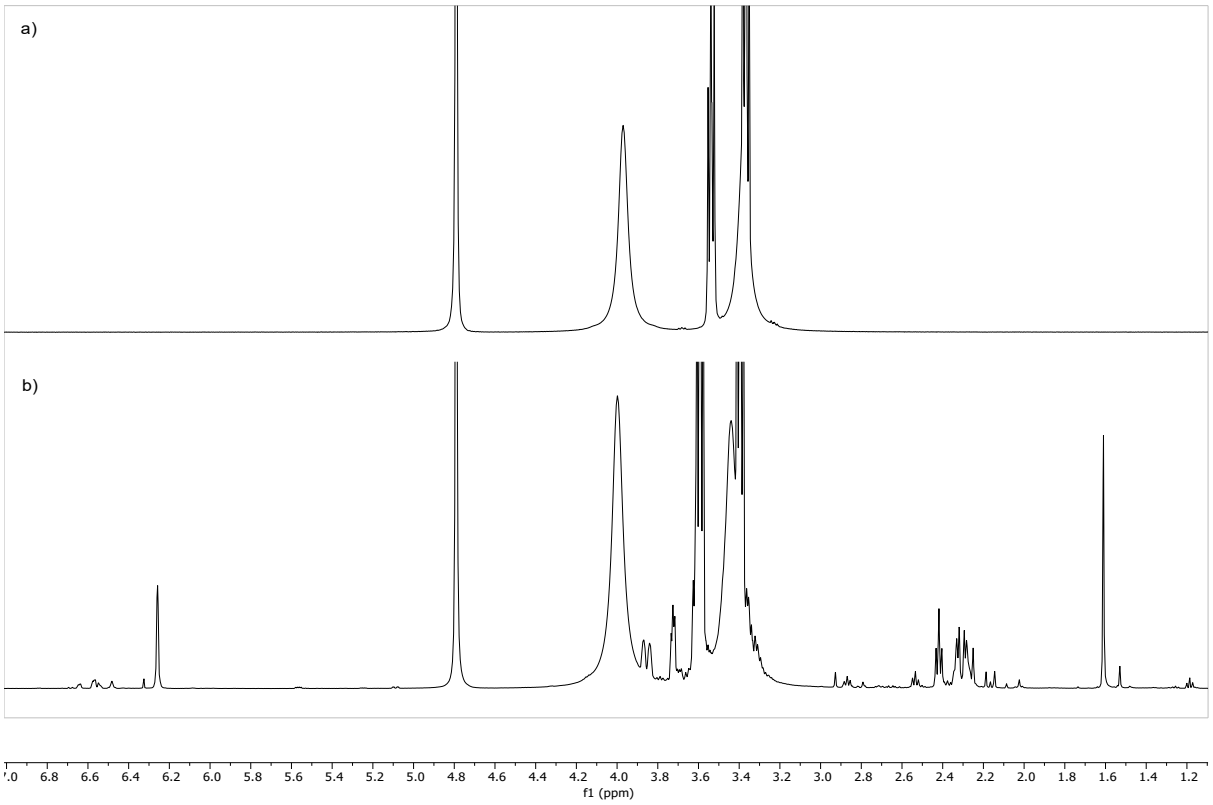

Figure S34: ^{1}H NMR spectra (500 MHz, D_2O) showing (a) MES buffer and (b) cycloadduct products from kinetic experiments.

3.3 BTM pH 6.5

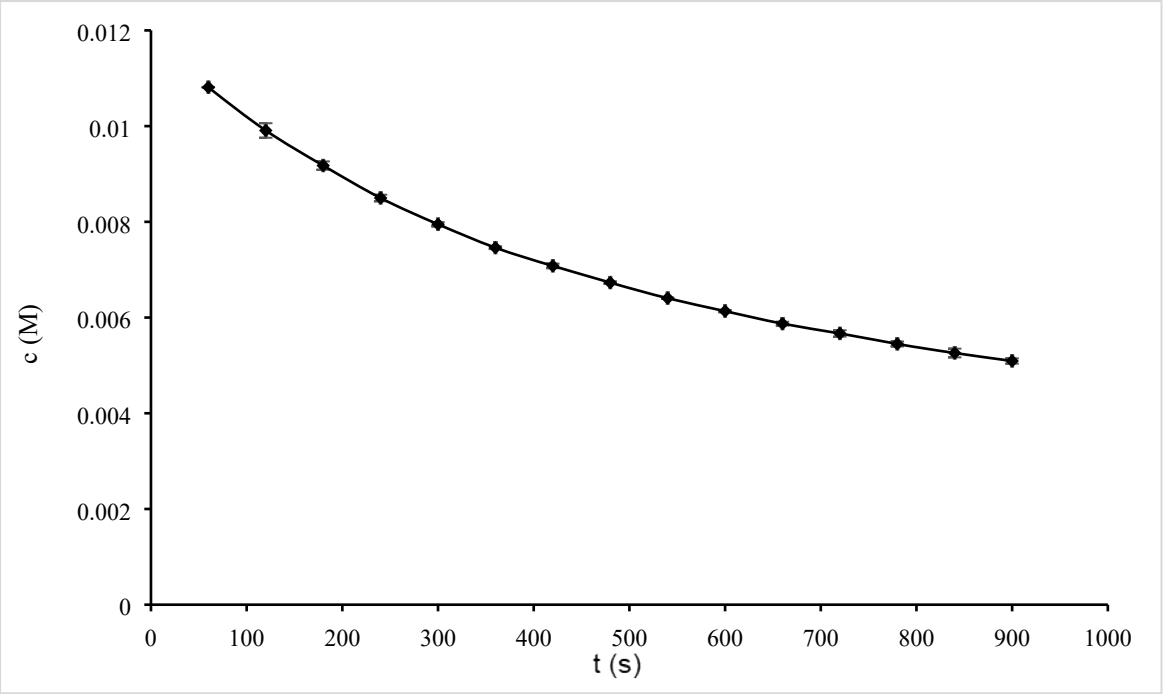

Figure S35: Concentration of pentafulvene **1** *versus* time for the reaction between **1** and **2** in BTM buffer (100 mM, pH 6.5). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

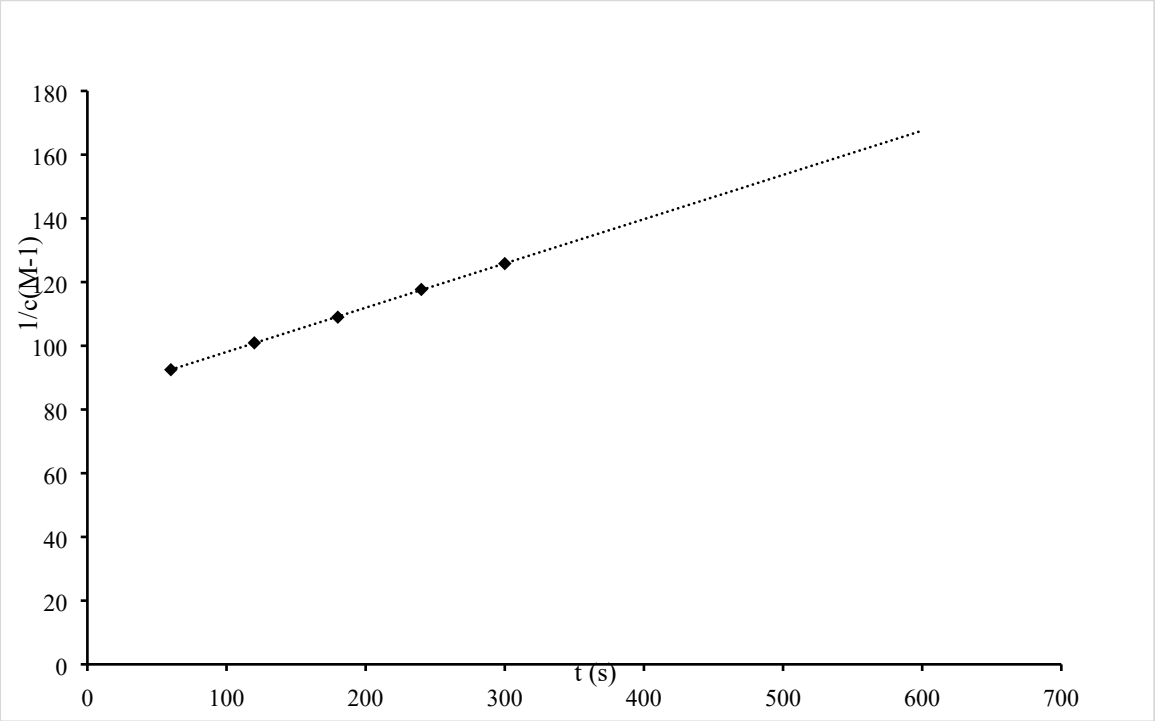

Figure S36: Second order kinetic plot for the reaction between **1** and **2** in BTM buffer (100 mM, pH 6.5, 23 ± 1 °C); k_2 = 0.14 ± 0.01 $M^{-1}s^{-1}$.

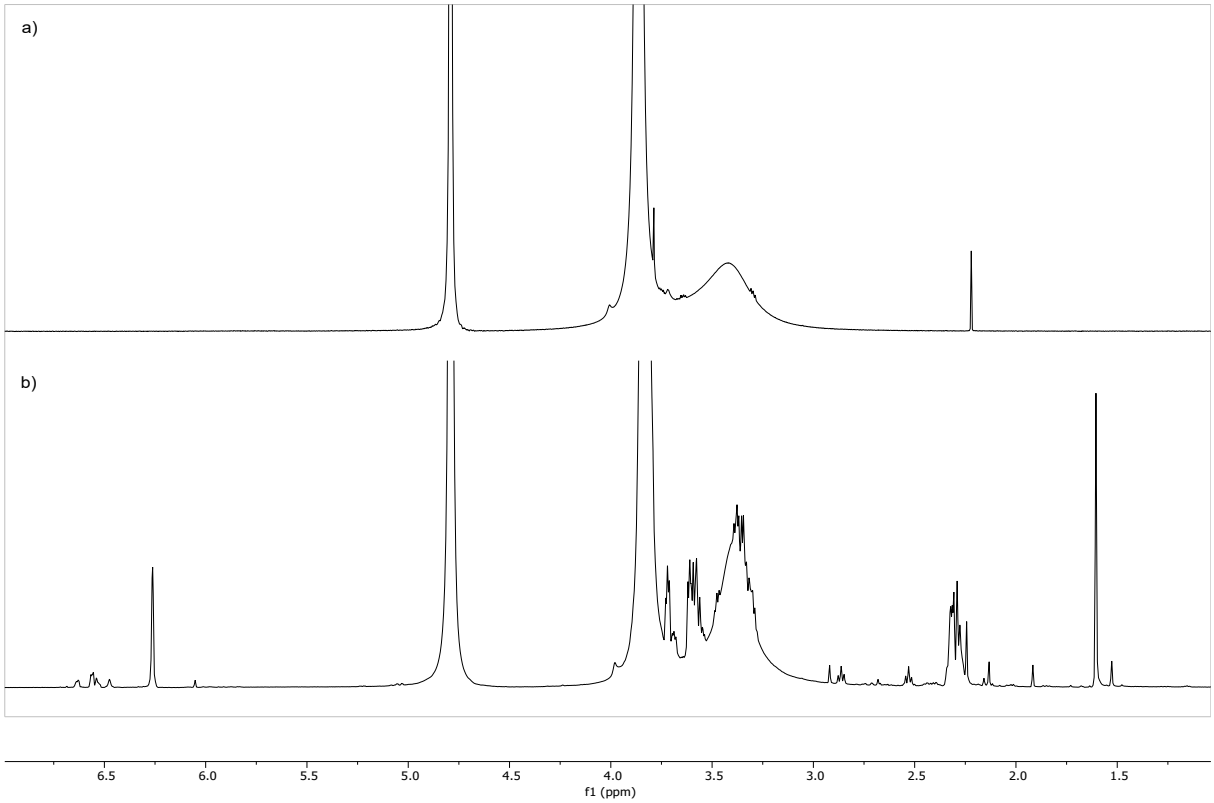

Figure S37: ^{1}H NMR spectra (500 MHz, D_2O) showing (a) BTM buffer and (b) cycloadduct products from kinetic experiments.

3.4 BTP pH 7.0

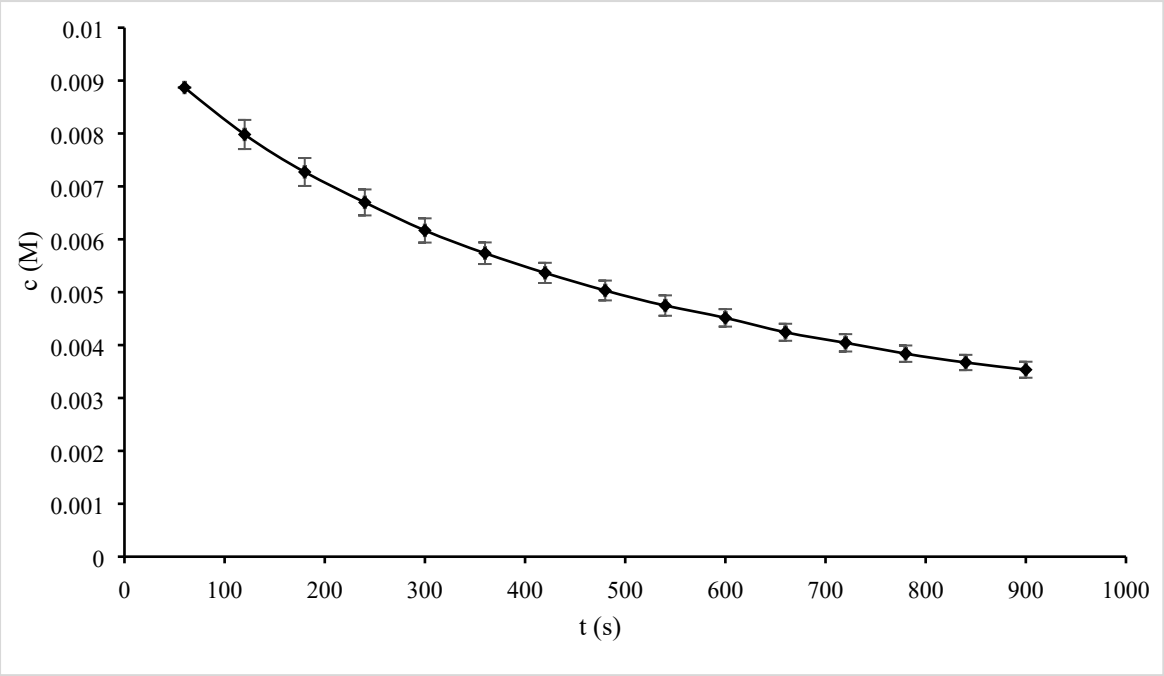

Figure S38: Concentration of pentafulvene **1** *versus* time for reaction between **1** and **2** in BTP buffer (100 mM, pH 7.0). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

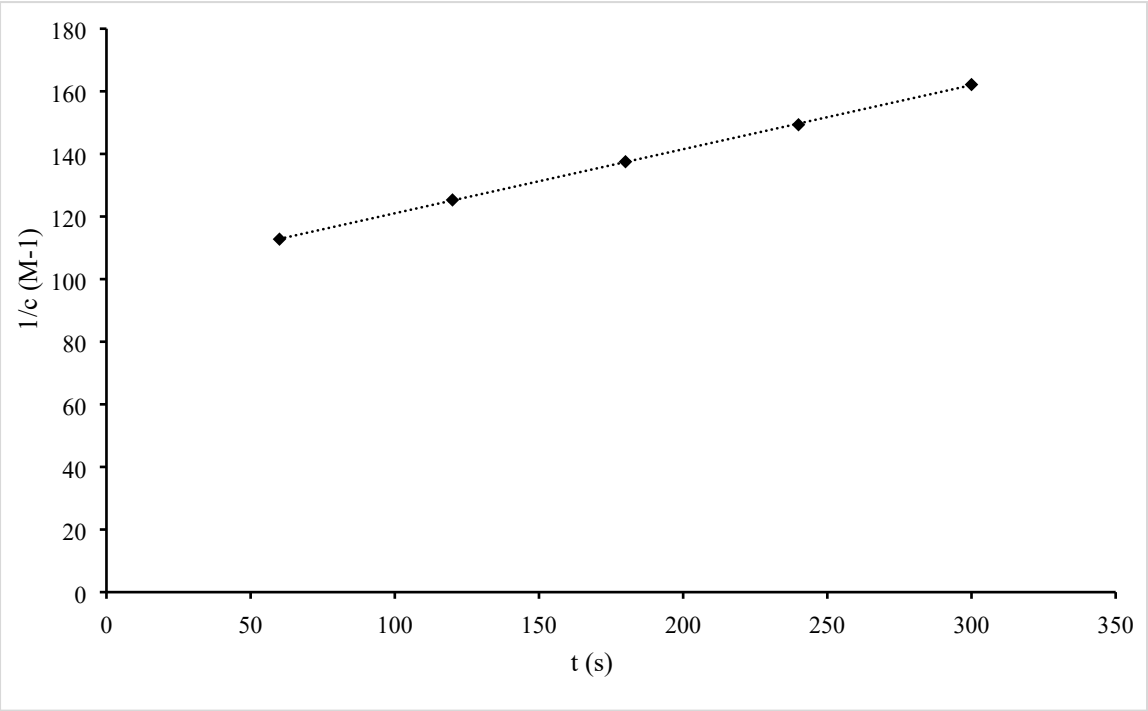

Figure S39: Second order kinetic plot for the reaction between **1** and **2** in BTP buffer (100 mM, pH 7.0, 23 ± 1 °C); k_2 = 0.21 ± 0.01 $M^{-1}s^{-1}$.

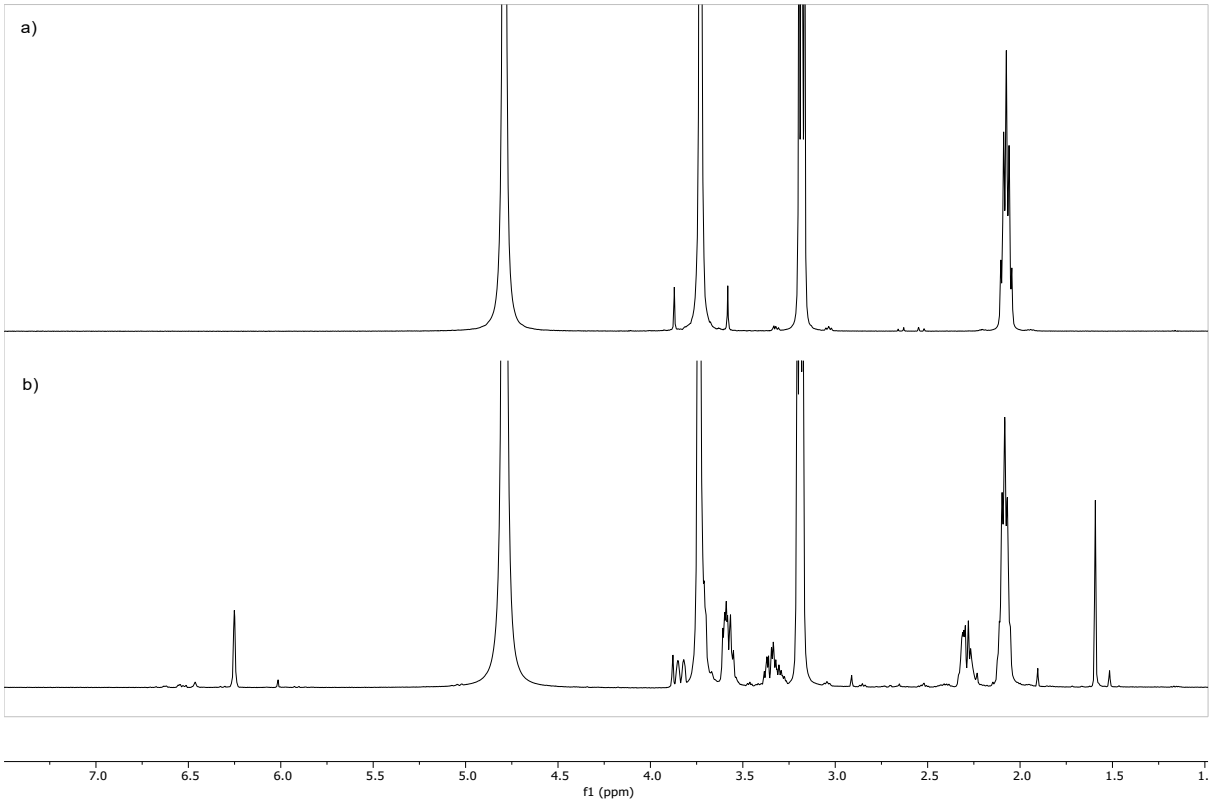

Figure S40: ^{1}H NMR spectra (500 MHz, D_2O) showing (a) BTP buffer and (b) cycloadduct products from kinetic experiments.

3.5 Imidazole HCl pH 7.0

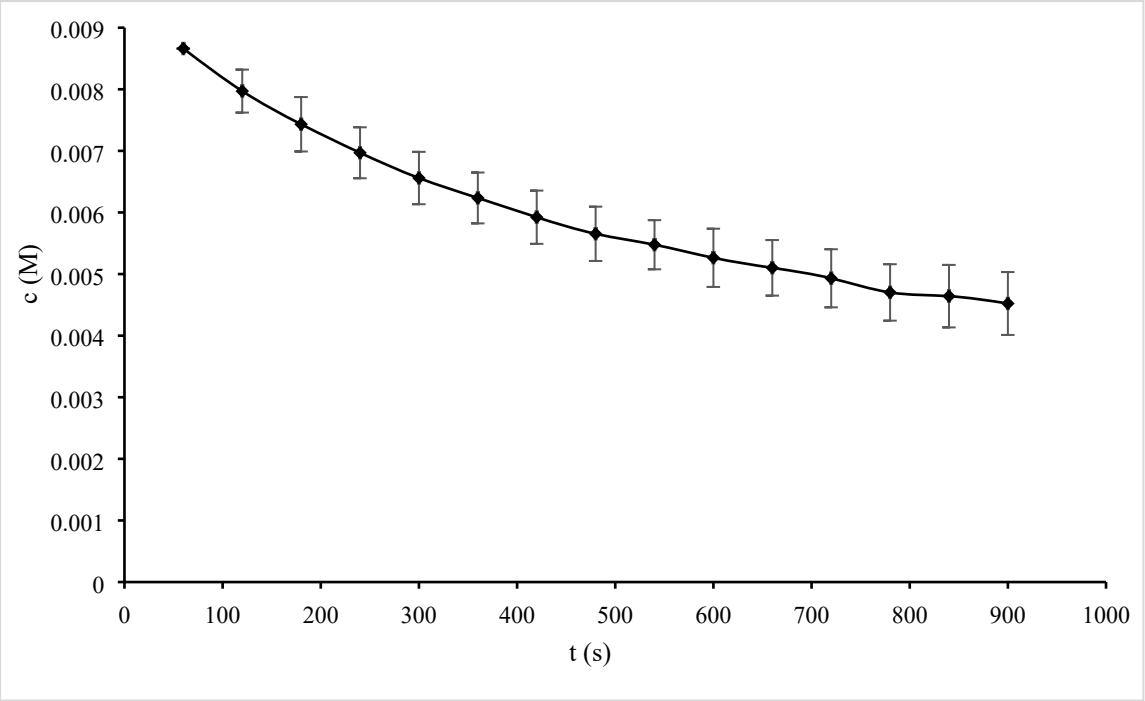

Figure S41: Concentration of pentafulvene **1** *versus* time for reaction between **1** and **2** in imidazole HCl buffer (100 mM, pH 7.0). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

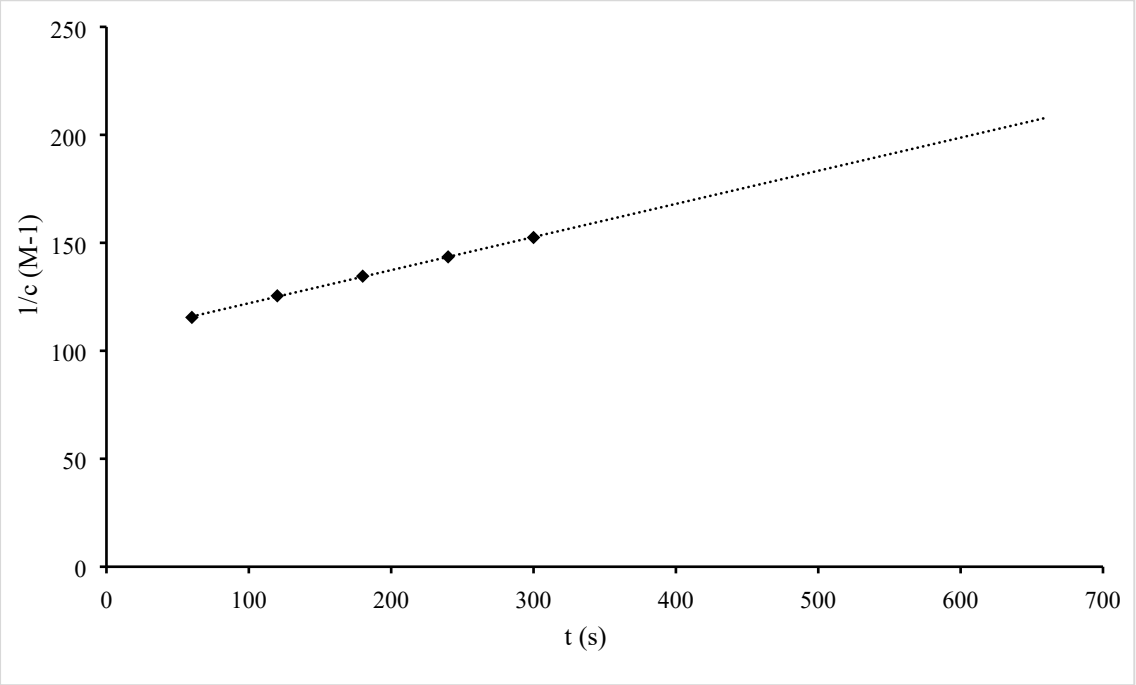

Figure S42: Second order kinetic plot for the reaction between **1** and **2** in imidazole HCl buffer (100 mM, pH 7.0, 23 ± 1 °C); k_2 = 0.16 ± 0.02 $M^{-1}s^{-1}$.

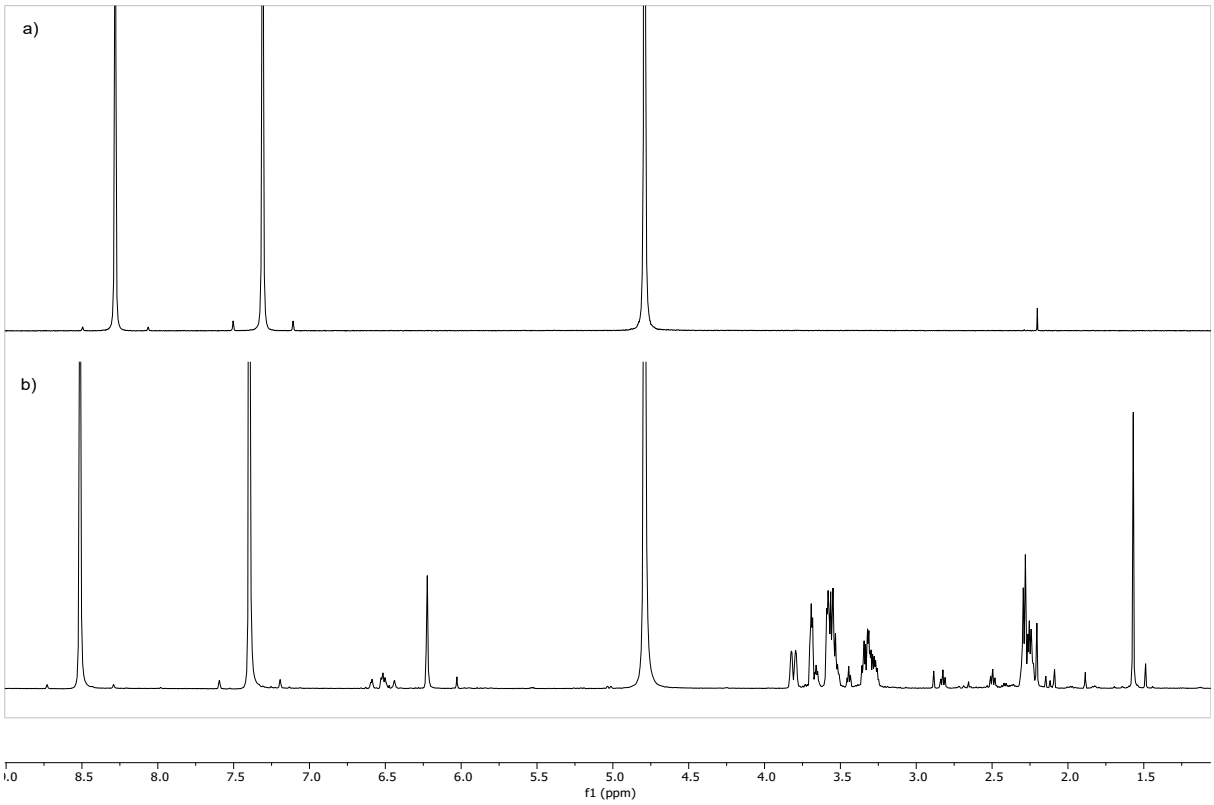

Figure S43: ^{1}H NMR spectra (500 MHz, D_2O) showing (a) imidazole HCl buffer and (b) cycloadduct products from kinetic experiments.

3.6 PIPES pH 7.0

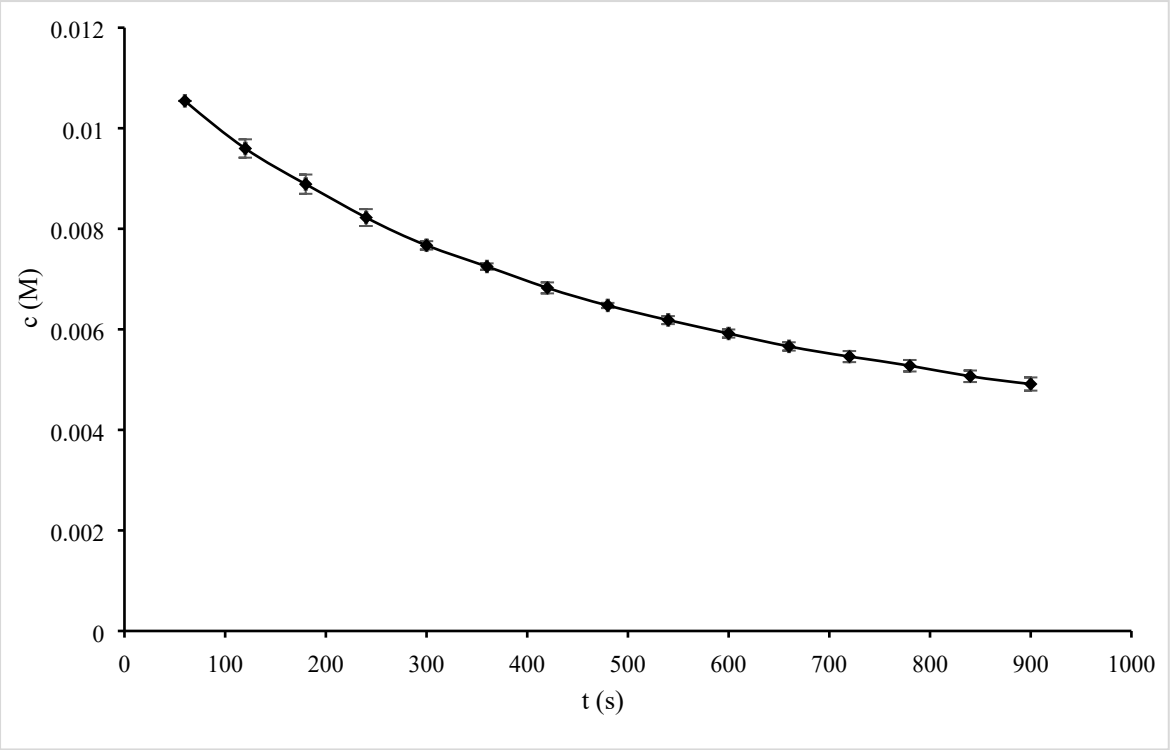

Figure S44: Concentration of pentafulvene **1** *versus* time for reaction between **1** and **2** in PIPES buffer (100 mM, pH 7.0). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

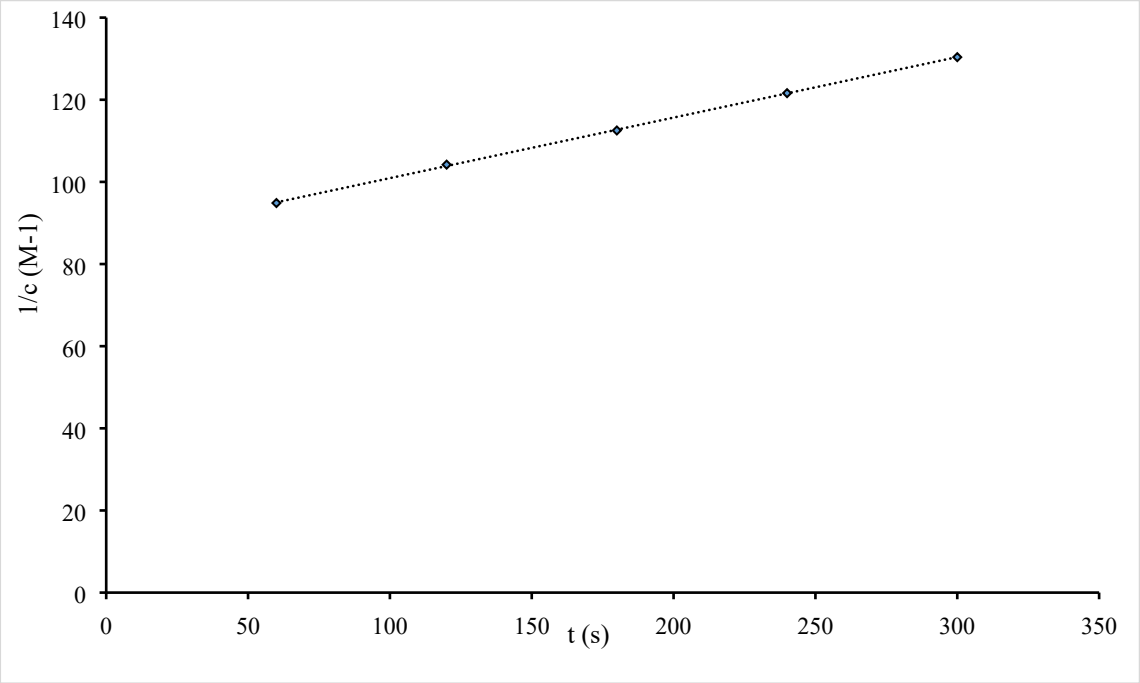

Figure S45: Second order kinetic plot for the reaction between **1** and **2** in PIPES buffer (100 mM, pH 7.0, 23 ± 1 °C); k_2 = 0.15 ± 0.01 $M^{-1}s^{-1}$.

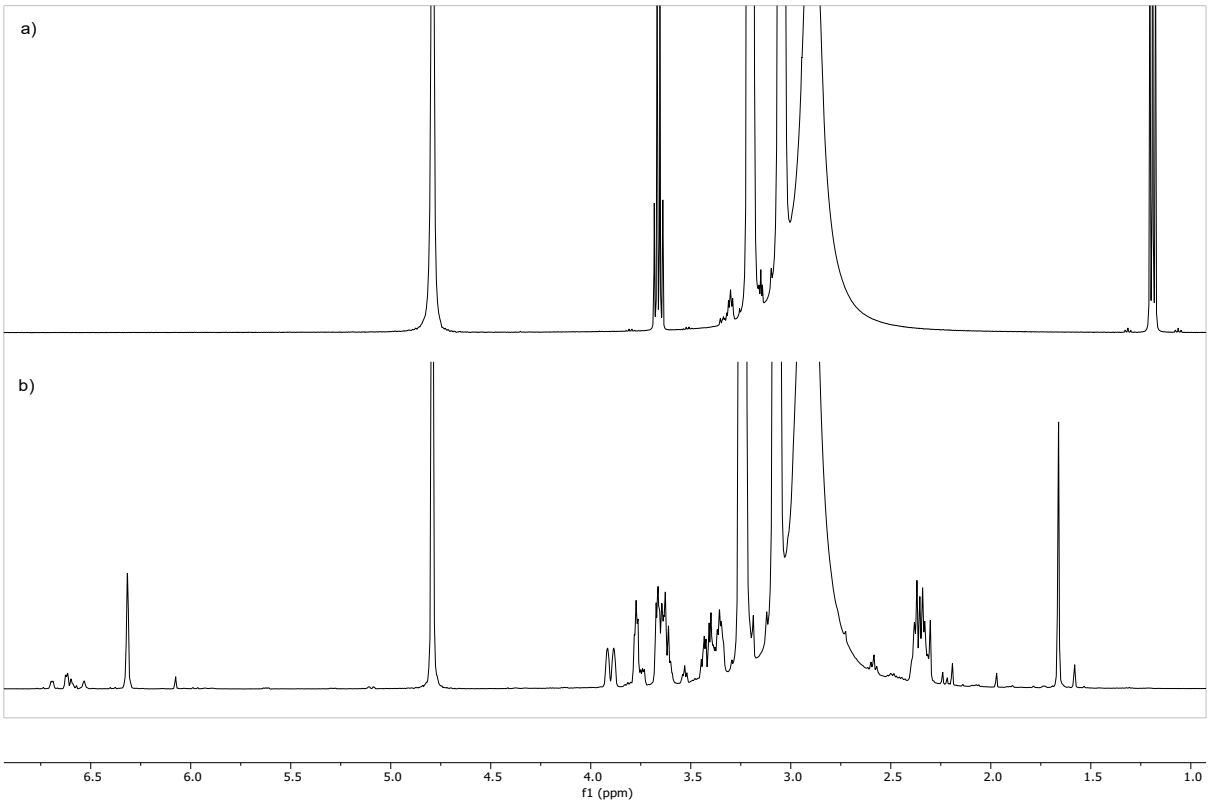

Figure S46: ^{1}H NMR spectra (500 MHz, D_2O) showing (a) PIPES buffer and (b) cycloadduct products from kinetic experiments.

3.7 Ultrapure Water pH 7.0

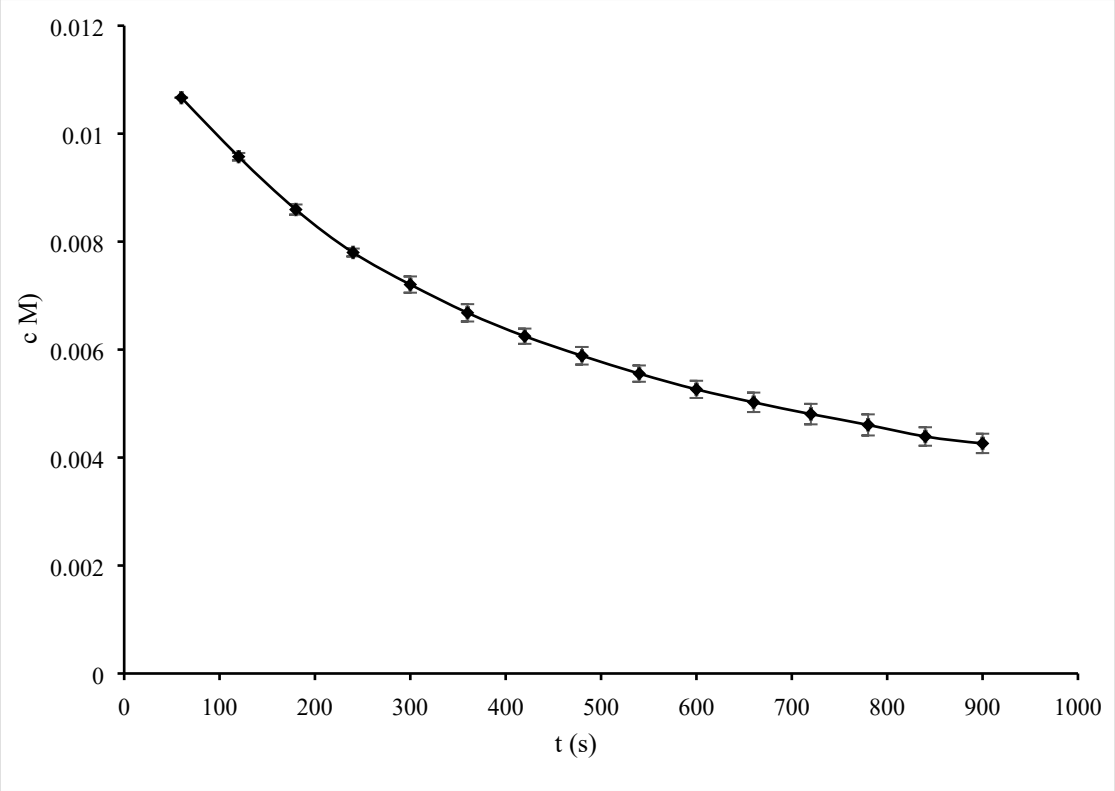

Figure S47: Concentration of pentafulvene **1** *versus* time for reaction between **1** and **2** in ultrapure water (pH 7.0). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

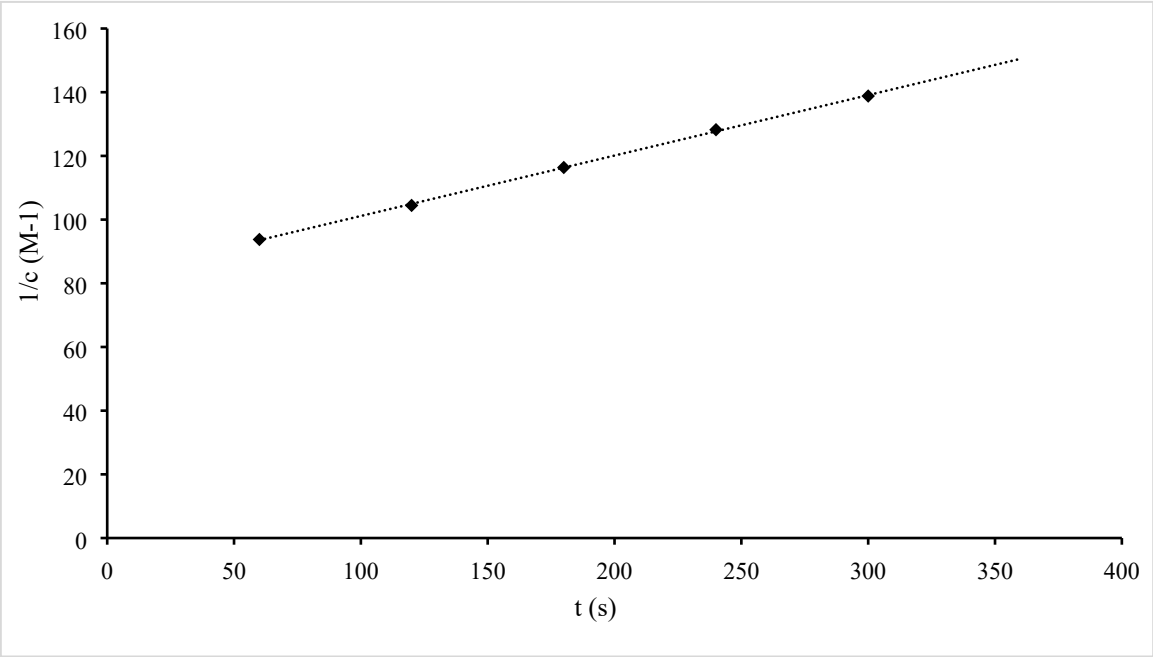

Figure S48: Second order kinetic plot for the reaction between **1** and **2** in ultrapure water (pH 7.0, 23 ± 1 °C); k_2 = 0.19 ± 0.01 $M^{-1}s^{-1}$.

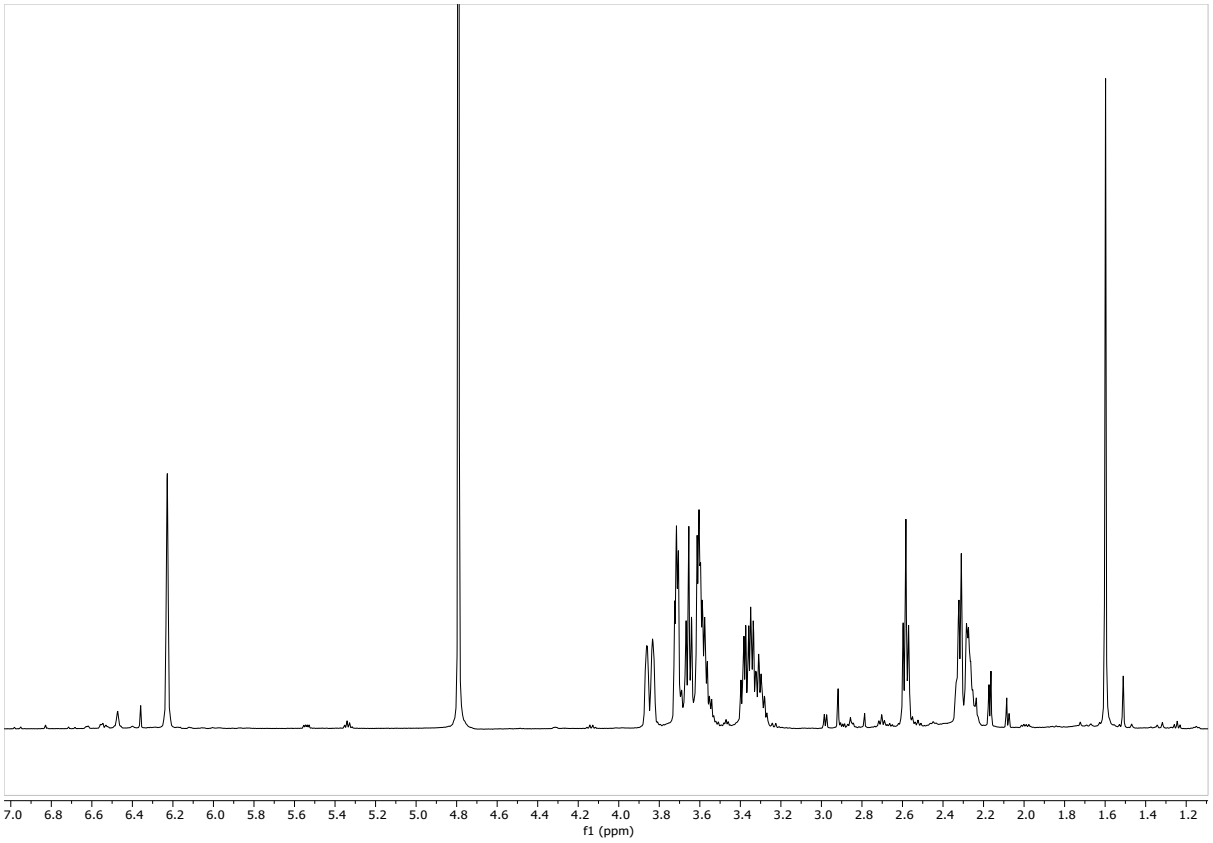

Figure S49: ^{1}H NMR spectrum (500 MHz, D_2O) showing cycloadduct products from kinetic experiments.

3.8 PBS pH 7.4

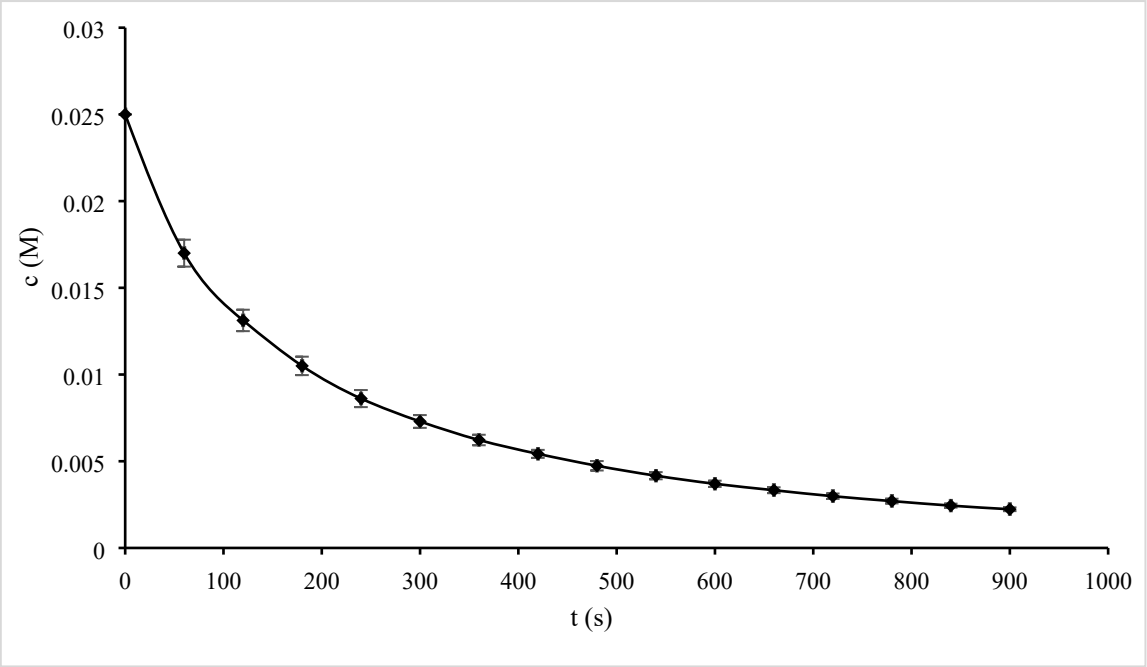

Figure S50: Concentration of pentafulvene **1** *versus* time for reaction between **1** and **2** in PBS buffer (100 mM, pH 7.4). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

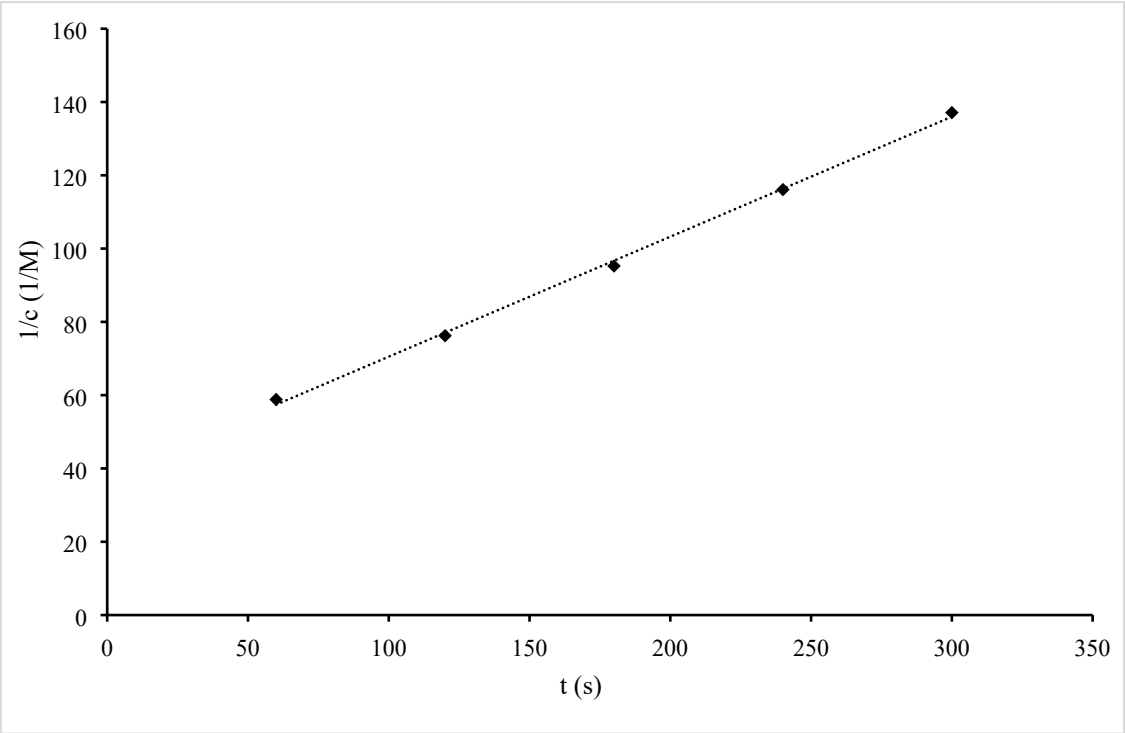

Figure S51: Second order kinetic plot for the reaction between **1** and **2** in PBS buffer (100 mM, pH 7.4, 23 ± 1 °C); k_2 = 0.33 ± 0.02 $M^{-1}s^{-1}$.

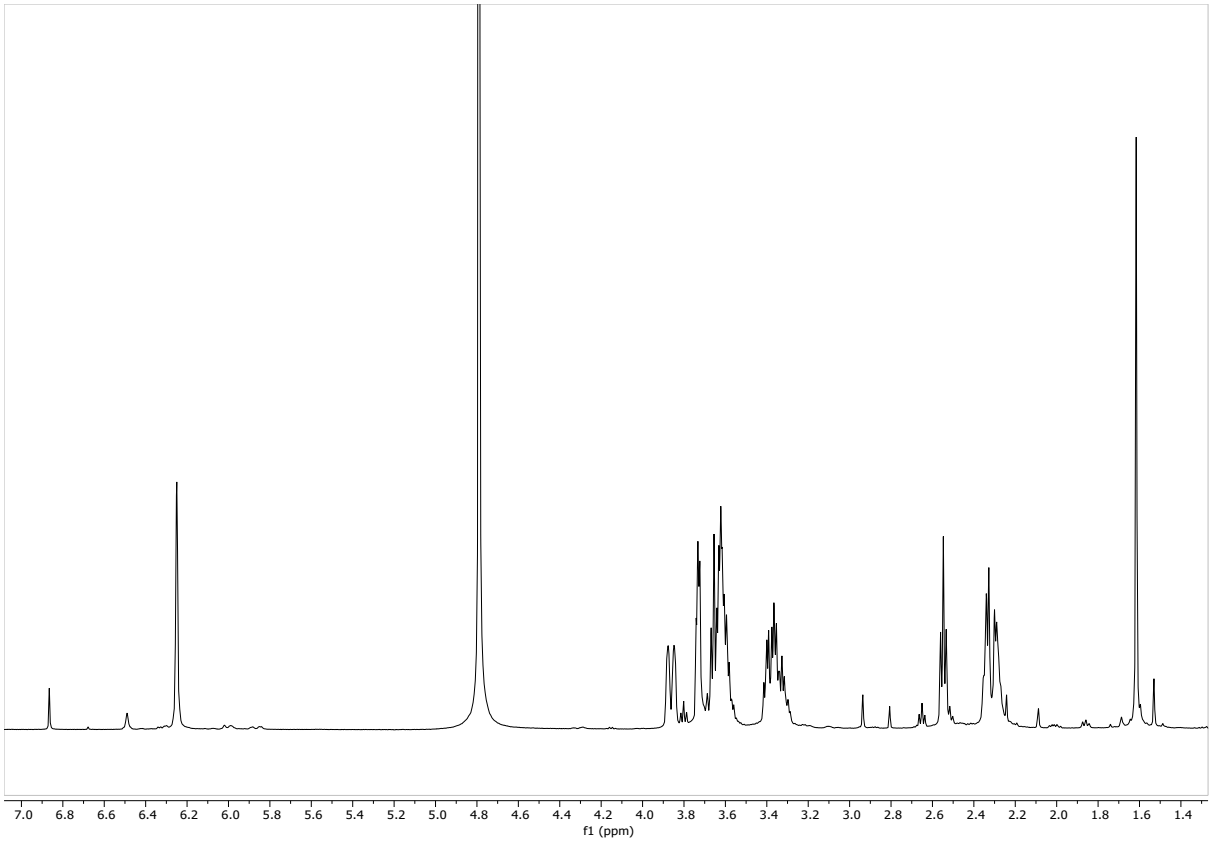

Figure S52: ^{1}H NMR spectrum (500 MHz, D_2O) showing cycloadduct products from kinetic experiments in PBS.

3.9 HEPES pH 7.5

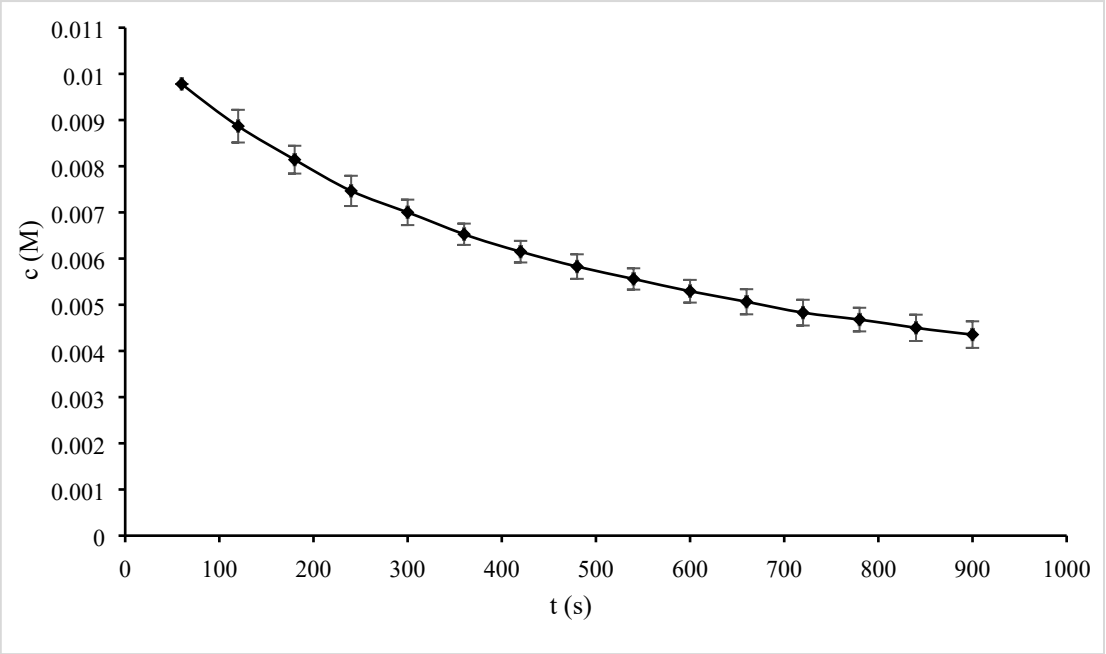

Figure S53: Concentration of pentafulvene **1** *versus* time for reaction between **1** and **2** in HEPES buffer (100 mM, pH 7.5). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

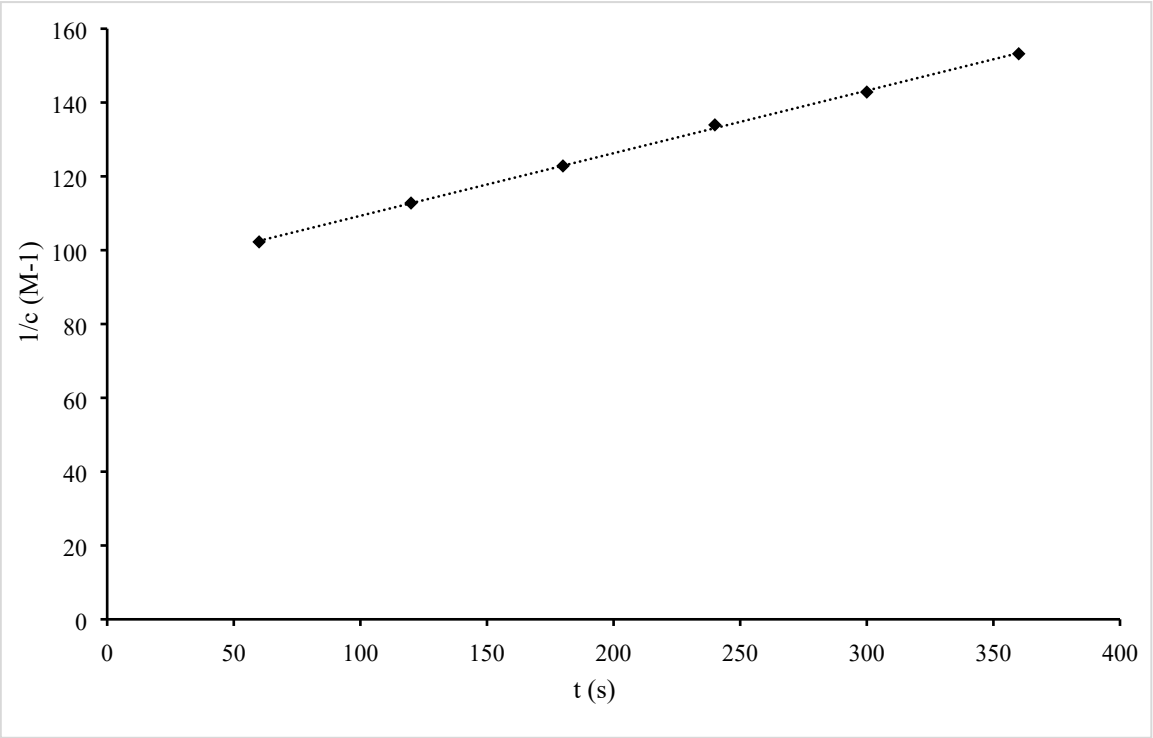

Figure S54: Second order kinetic plot for the reaction between **1** and **2** in HEPES buffer (100 mM, pH 7.5, 23 ± 1 °C); k_2 = 0.17 ± 0.01 $M^{-1}s^{-1}$.

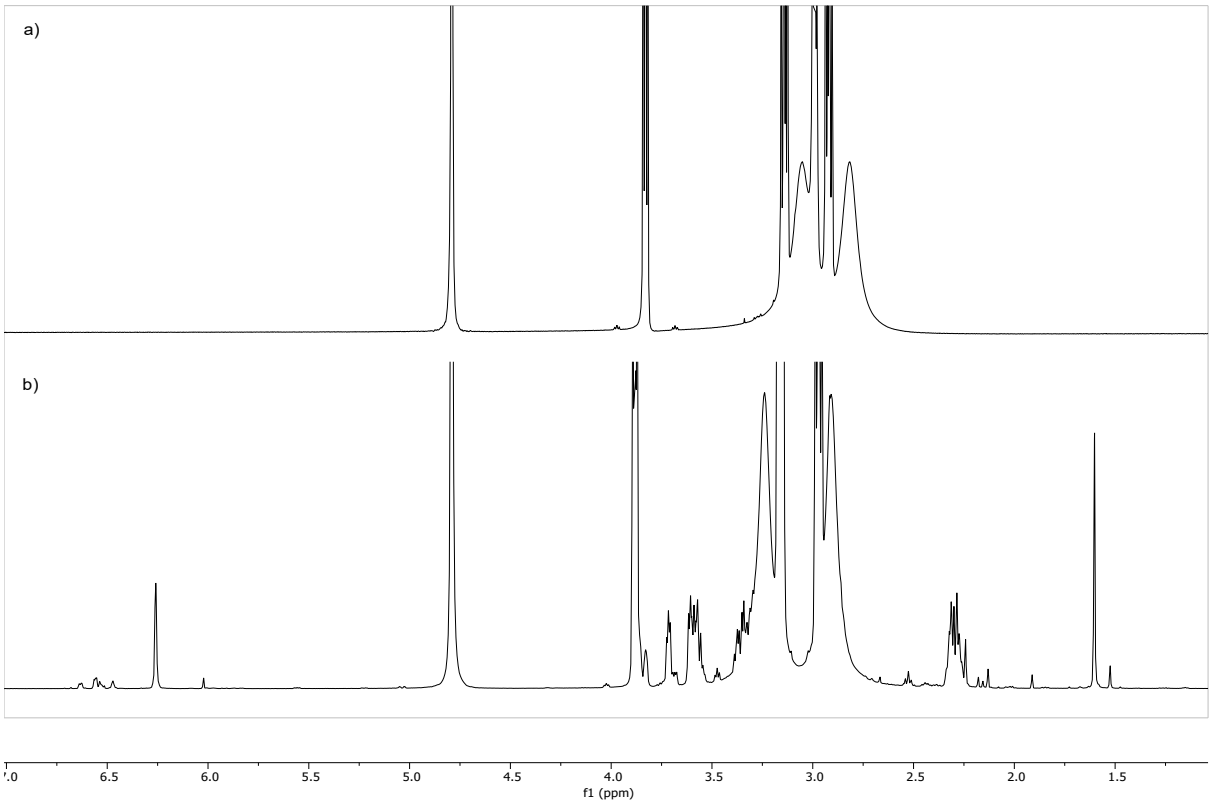

Figure S55: ^{1}H NMR spectra (500 MHz, D_2O) showing (a) HEPES buffer and (b) cycloadduct products from kinetic experiments.

3.10 Trizma pH 7.5

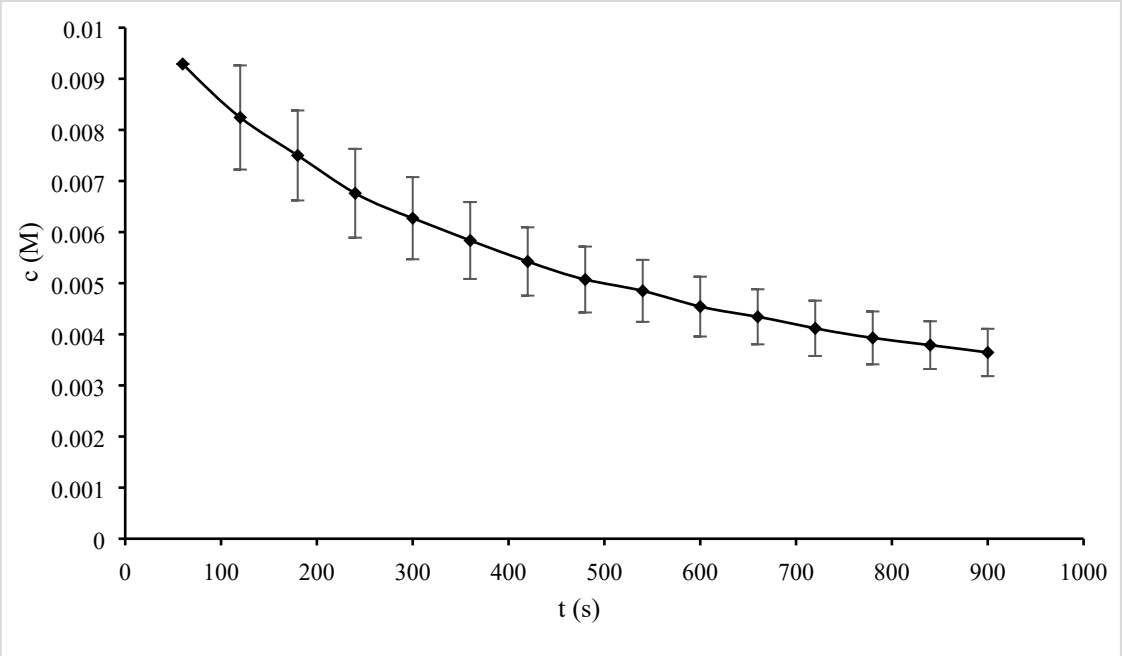

Figure S56: Concentration of pentafulvene **1** *versus* time for reaction between **1** and **2** in trizma buffer (100 mM, pH 7.5). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

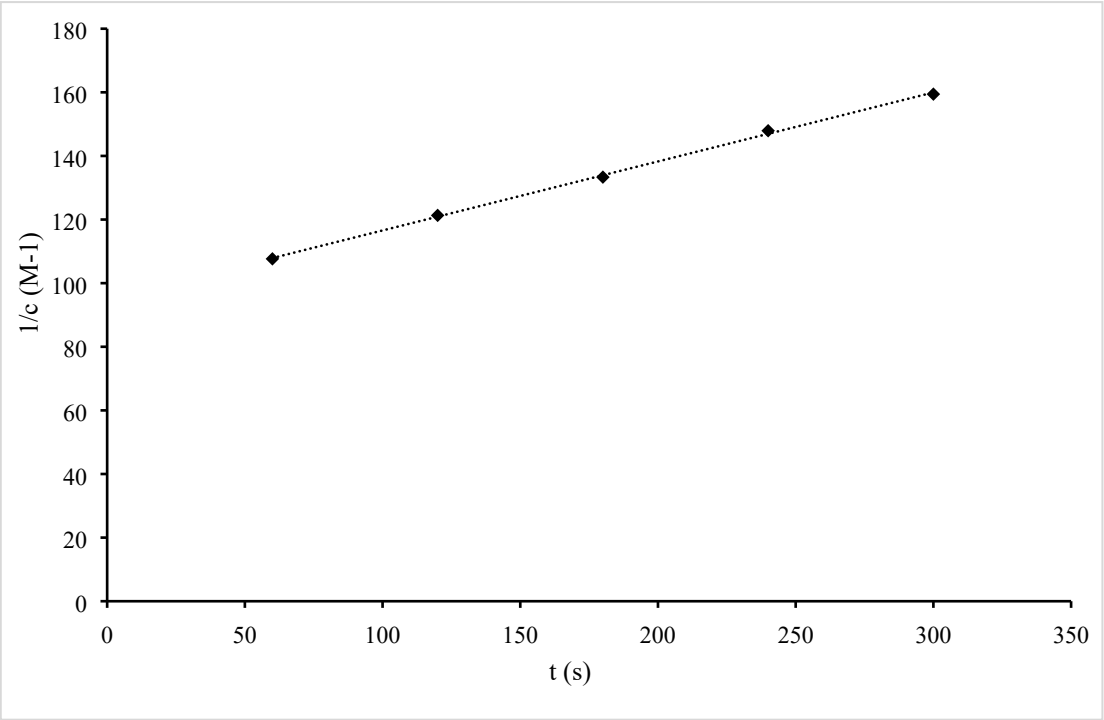

Figure S57: Second order kinetic plot for the reaction between **1** and **2** in trizma buffer (100 mM, pH 7.5, 23 ± 1 °C); k_2 = 0.22 ± 0.04 $M^{-1}s^{-1}$.

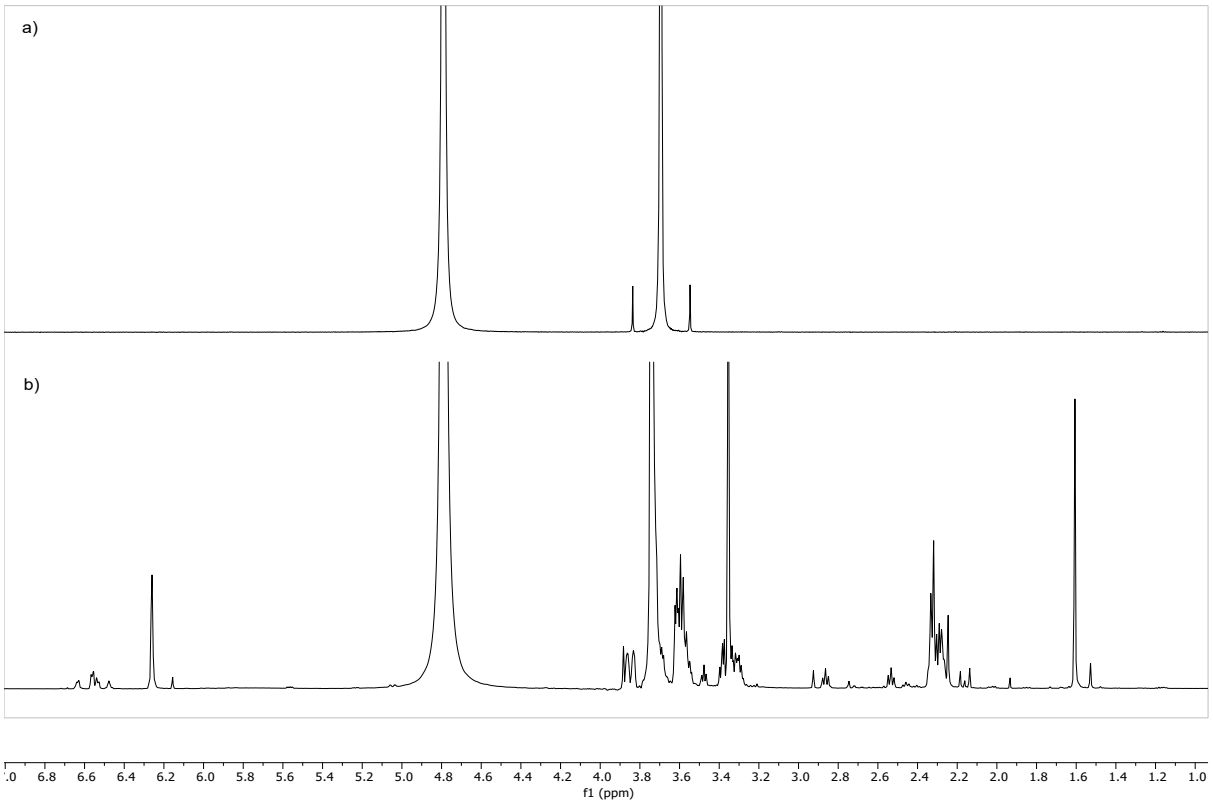

Figure S58: ^{1}H NMR spectra (500 MHz, D_2O) showing (a) trizma buffer and (b) cycloadduct products from kinetic experiments.

3.11 Tricine pH 8.1

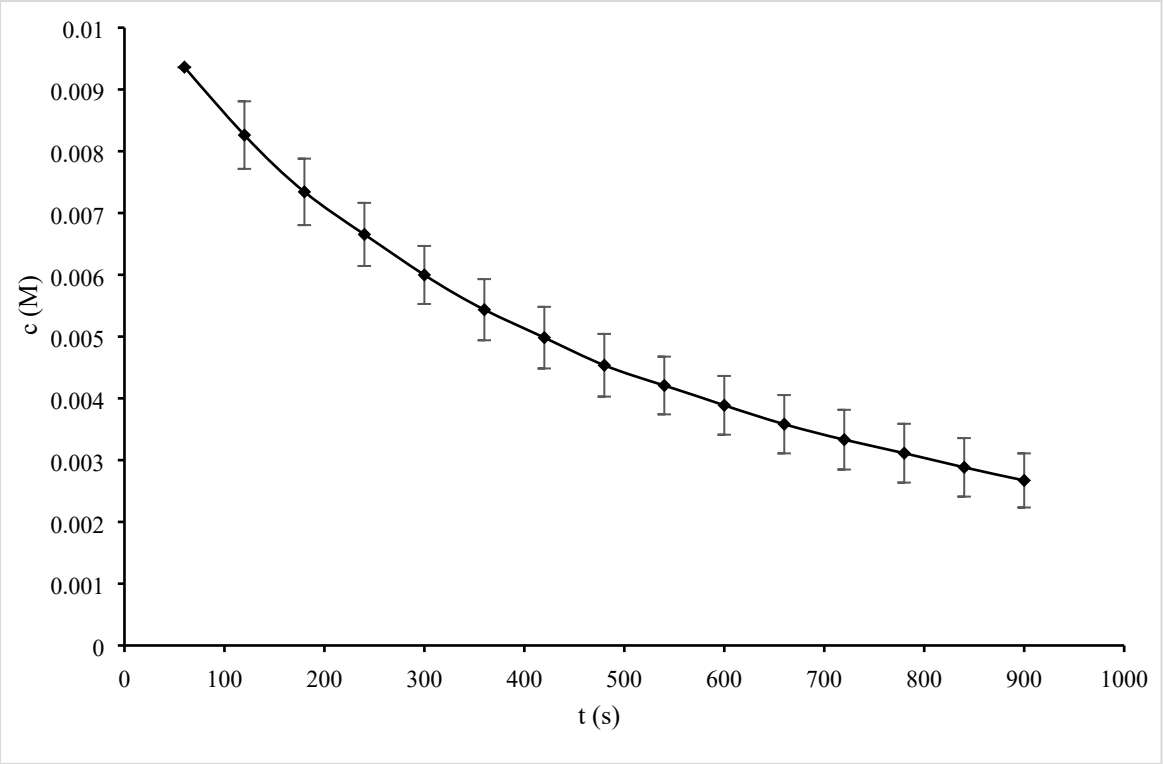

Figure S59: Concentration of pentafulvene **1** *versus* time for reaction between **1** and **2** in tricine buffer (100 mM, pH 8.1). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

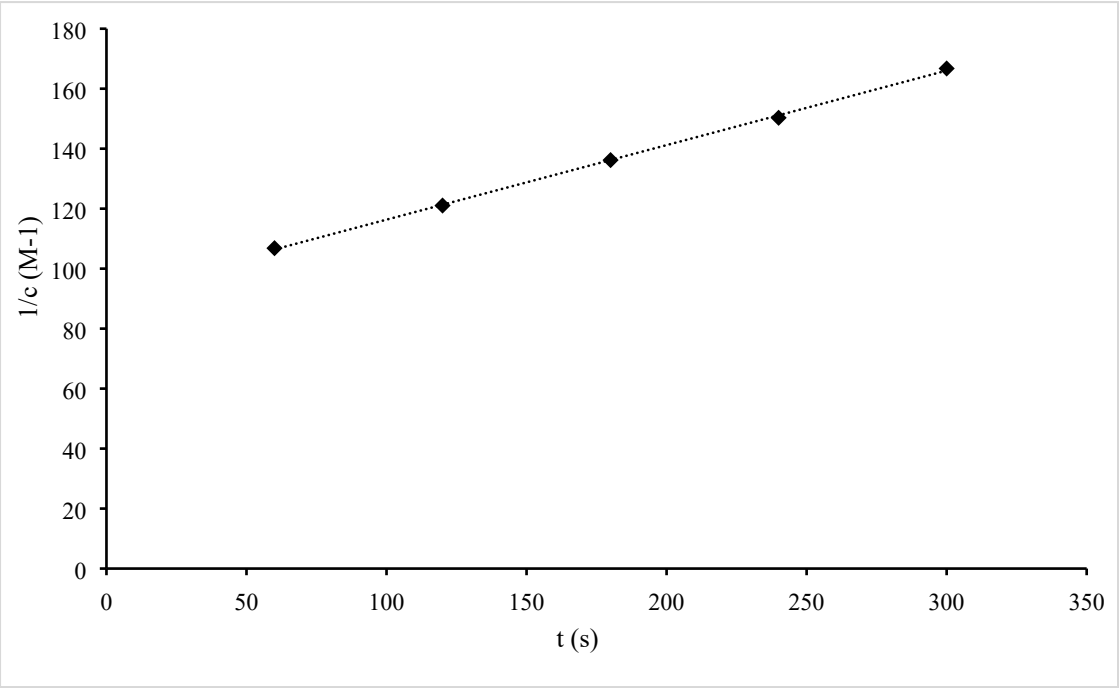

Figure S60: Second order kinetic plot for the reaction between **1** and **2** in tricine buffer (100 mM, pH 8.1, 23 ± 1 °C); k_2 = 0.25 ± 0.03 $M^{-1}s^{-1}$.

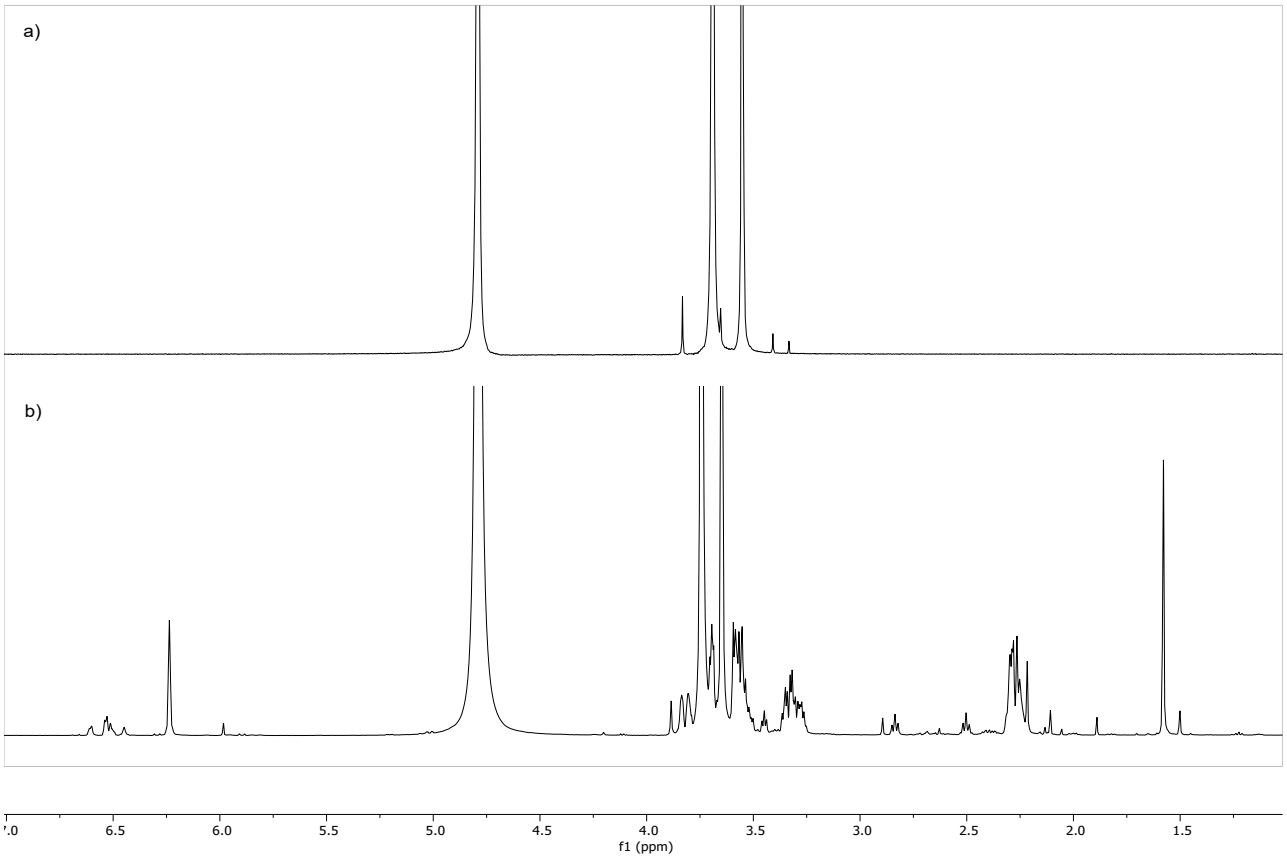

Figure S61: ^{1}H NMR spectrum (500 MHz, D_2O) showing (a) tricine buffer and (b) cycloadduct products from kinetic experiments.

3.12 Bicine pH 8.3

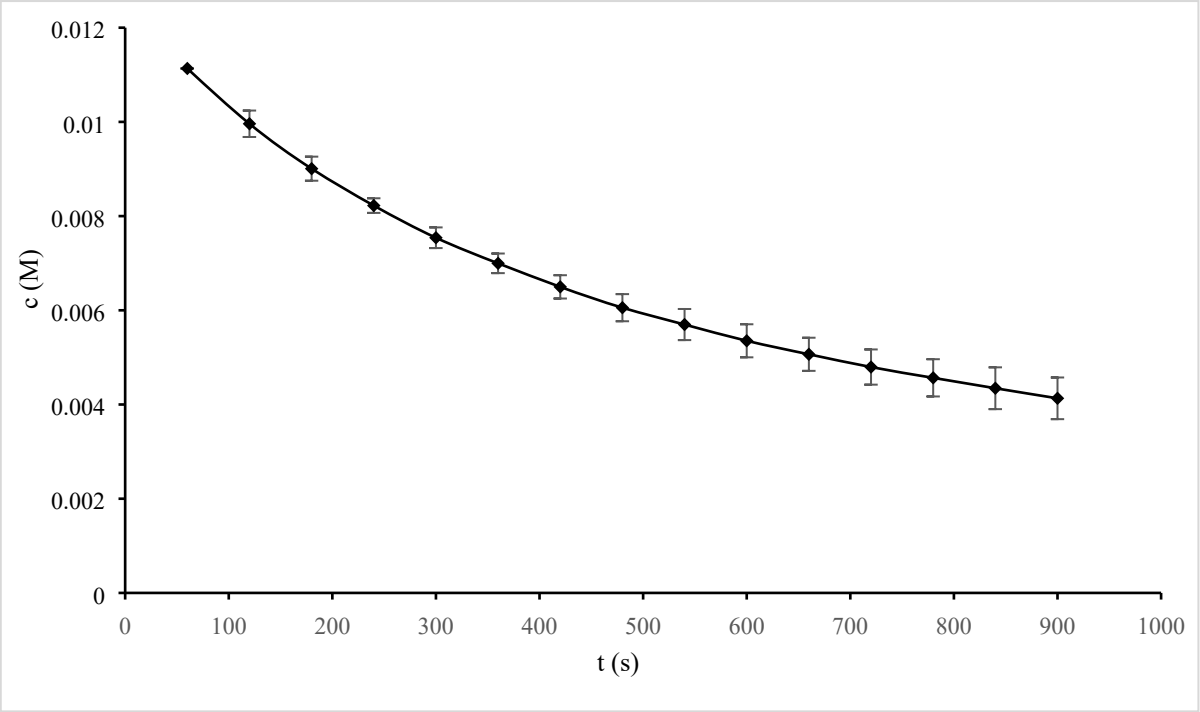

Figure S62: Concentration of pentafulvene **1** *versus* time for reaction between **1** and **2** in bicine buffer (100 mM, pH 8.3). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

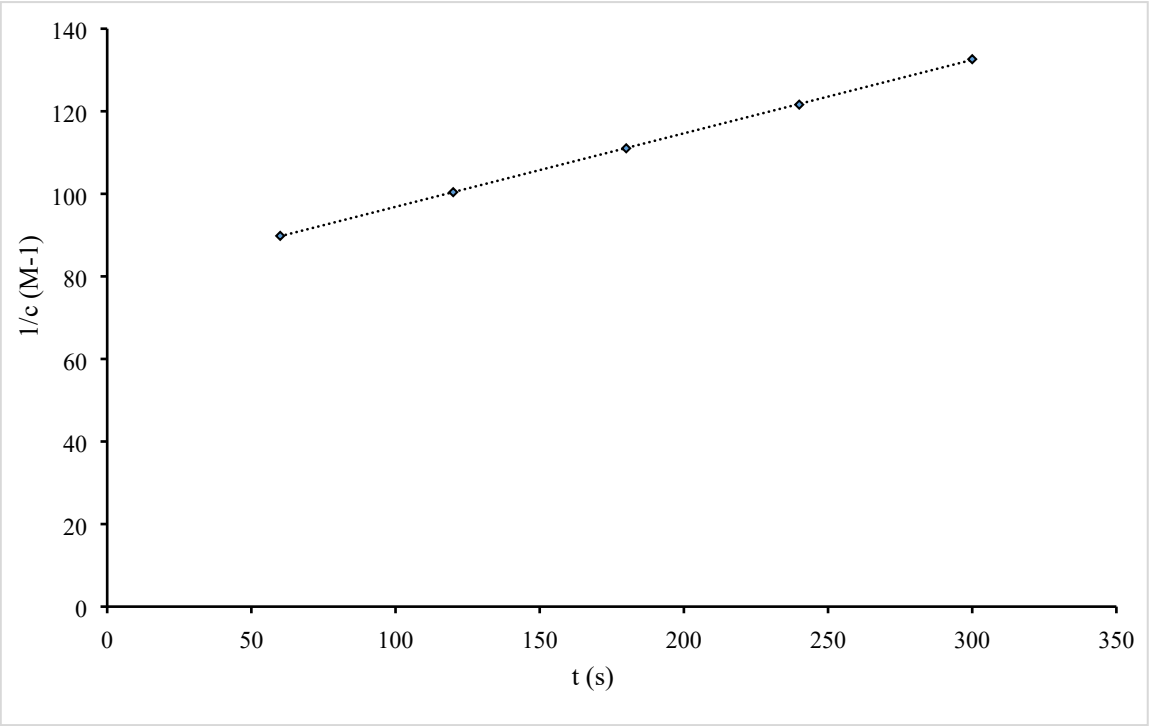

Figure S63: Second order kinetic plot for the reaction between **1** and **2** in bicine buffer (100 mM, pH 8.3, 23 ± 1 °C); k_2 = 0.18 ± 0.02 $M^{-1}s^{-1}$.

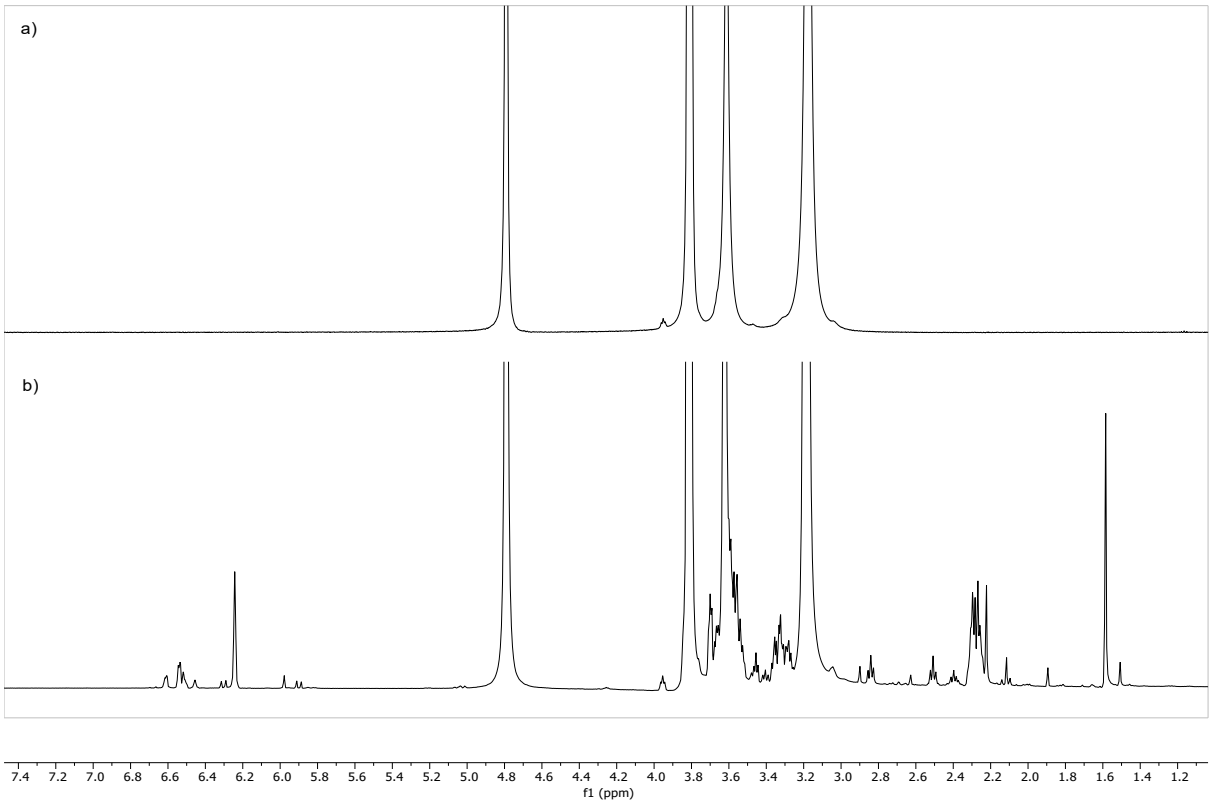

Figure S64: ^{1}H NMR spectrum (500 MHz, D_2O) showing (a) bicine buffer and (b) cycloadduct products from kinetic experiments.

3.13 Glycine NaOH pH 8.9

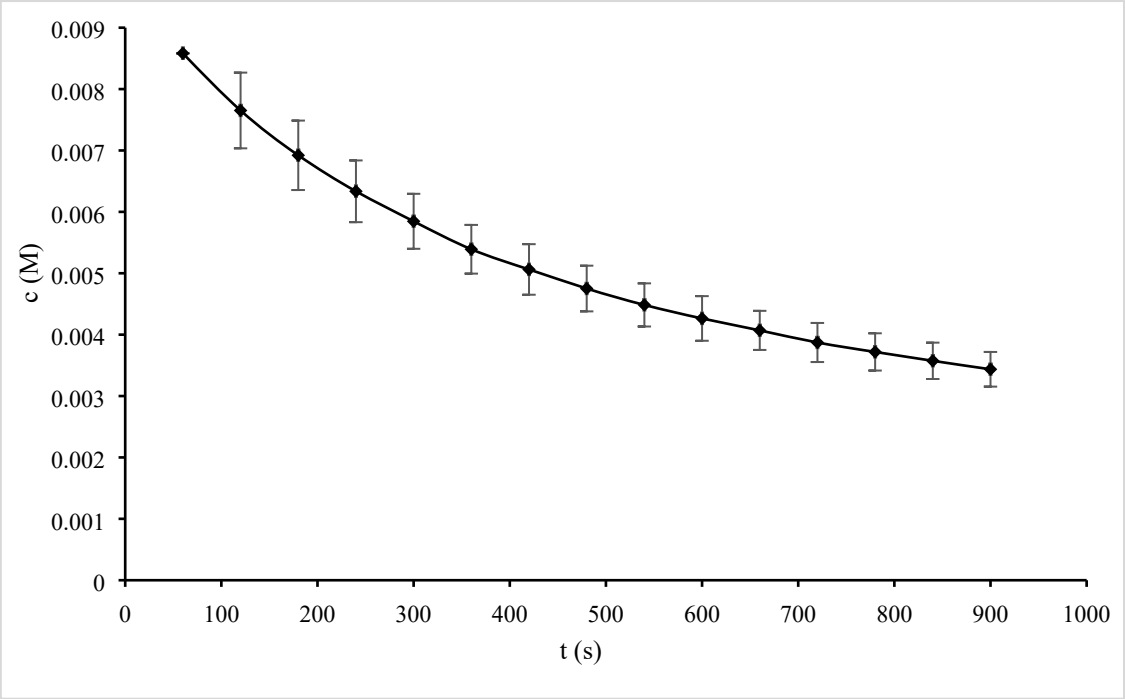

Figure S65: Concentration of pentafulvene **1** *versus* time for reaction between **1** and **2** in glycine NaOH buffer (100 mM, pH 8.9). Timepoints taken at 1 min intervals over a 15 min period as monitored *via* HPLC at 23 ± 1 °C (n = 3).

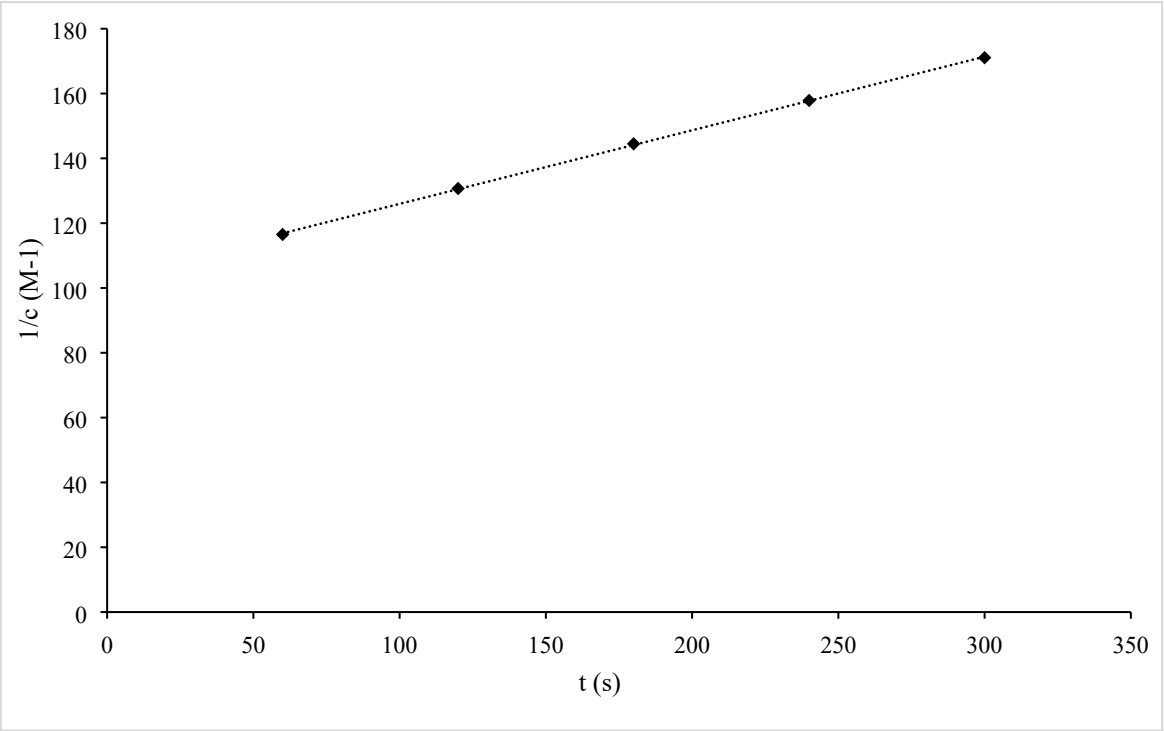

Figure S66: Second order kinetic plot for the reaction between **1** and **2** in glycine NaOH buffer (100 mM, pH 8.9, 23 ± 1 °C); k_2 = 0.23 ± 0.01 $M^{-1}s^{-1}$.

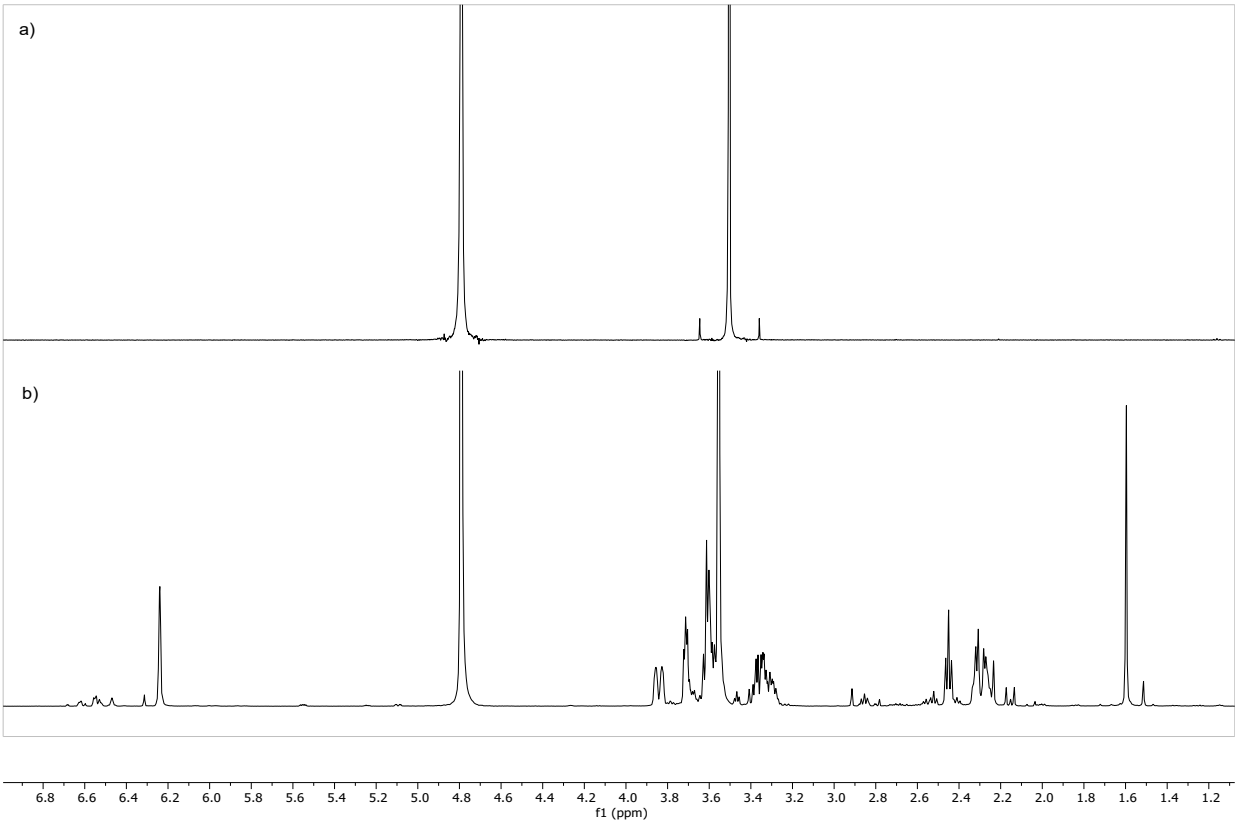

Figure S67: ^{1}H NMR spectra (500 MHz, D_2O) showing (a) glycine NaOH buffer and (b) cycloadduct products from kinetic experiments.

References

1. Krężel, A. and Bal, W. A formula for correlating pK_a values determined in D_2O and H_2O. *J. Inorg. Biochem*., **2004**, *98*, 161-166.