Electronic Supplementary Information

Indirect hard modeling of low resolution benchtop NMR data for the kinetic fitting of a complex organic reaction

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1 General Information

1.1 Materials and Methods

All materials were obtained from commercial suppliers and used without further purification. Percentages in brackets refer to purity reported by the manufacturer. 4-fluorothiophenol (**1**) (97.6%) was purchased from BLDpharm, *bis*-(4-fluorophenyl)-disulfide (**4**) (98%) from Thermo Fisher Scientific, *N*-chlorosuccinimide (**2**) (97%) from Thermo Fisher Scientific and diethylamine (>99.5%) from Sigma-Aldrich. The solvent, extra dry EtOAc (99.9%) over molecular sieves, from Thermo Fisher Scientific.

1.2 GC-MS

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using a Shimadzu GCMS-QP2010 SE, using an RTX-5MS column (30 m × 0.25 mm × 0.25 µm) and helium as carrier gas with a linear velocity of 40 cm/sec. The injector temperature was set to 280 °C. After 1 min at 50 °C, the oven temperature was increased by 25 °C/min to 300 °C and then kept at 300 °C for 3 min. The mass detector was a quadrupole with pre rods and electron impact ionization. The following settings were used in the detector: ion source temperature 200 °C, interface temperature 310 °C, solvent cut time 2 min 30 sec, acquisition mode scan, mass range m/z = 50 till m/z = 400.

1.3 GC-FID

GC analysis was performed on a Shimadzu GC FID 230 with a flame ionization detector (FID), using an RTX-5MS Cap. column (30 m × 0.25 mm ID × 0.25 µm) and helium as carrier gas (40 cm/sec-1 linear velocity). The injector temperature was set to 280 °C. After 1 min at 50 °C, the temperature was increased by 25 °C/min to 300 °C and kept constant at 300 °C for 4 min. FID was used for detection, and the detector gases used for flame ionization were hydrogen and synthetic air (5.0 quality).

1.4 High Field NMR

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 300 MHz instrument. ^{1}H and ^{13}C spectra were recorded at 300 MHz and 75 MHz, respectively. ^{19}F spectra were also recorded on the same instrument at 282 MHz. Chemical shifts (δ) are expressed in parts per million (ppm) relative to TMS. The samples were prepared in deuterated $CDCl_3$. The letters s, d, t and m are used to indicate singlet, doublet, triplet, and multiplet, respectively.

1.5 Flow Equipment

Pumps: In the single pass experiments, the feed solutions were pumped using Syrris Asia syringe pumps. Both pumps had internal pressure sensors and were equipped with check valves. The pressure limit was set to 15 bar, above which the pumps would stop automatically. During re-circulation a peristaltic pump from Innofluid was used. To ensure consistency between experiments the flow rates of both pumps were checked regularly and were always within ± 5% of the expected value.

Check valves (CVs): IDEX CVs (CV-3321) were attached at the outlet of the pumps.

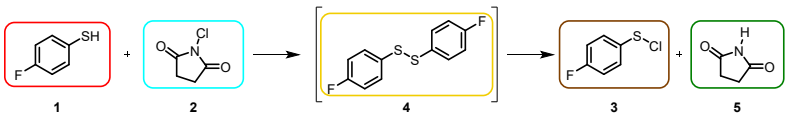
Tubing + mixer: PFA tubing with 1/16” o.d. and 0.8 mm i.d., were used as feedlines, connector tubings and coil reactors. Connectors and nuts made of polyether ether ketone (PEEK) or ethylene tetrafluoroethylene (ETFE) were used. A T-mixer made of PEEK with a thru-hole of 0.5 mm was used.

Back pressure regulator (BPR): The flow system was pressurized using a Zaiput BPR.

2 Initial GC-FID Screening

2.1 General Procedure

The desired amount of thiol **1** (30.8 mg, 0.240 mmol, 80 mM) was weighed into a glass vial (4 mL), followed by the addition of dry EtOAc (3 mL) and the desired amount of water. The glass vial was mounted in a metal heating block, which was placed on a magnetic stirrer and subsequently mixed and heated to 25 °C. An aliquot of the reaction mixture (50 µL) was taken and directly quenched by adding the aliquot to an HPLC vial containing a preprepared mixture of diethyl amine in acetonitrile (275 mM). After addition the HPLC vial was sealed and vigorously shaken. Under mixing the desired amount of NCS (**2**) was added to the reaction mixture. Aliquots were taken and quenched as described above after 30 s, 60 s, 120 s, and thereafter every 120 s for 20 min. All aliquots were then measured on the GC-FID and the relative peak areas used to create reaction profiles.



Scheme S1. Simplified reaction scheme. Disulfide (**4**) observed as an intermediate during chlorination, likely due to both condensation during reaction and re-oxidation during sample preparation or analysis.

While diethylamine successfully stabilized product **3** as the corresponding sulfenamide **3a**, the quench also resulted in the dimerization of the starting material **1** in the absence of NCS (**2**).[1] This was confirmed by GC-MS analysis of an aliquot before the addition of **2** with and without diethylamine quench (**Figure S1**). Performing the chlorination in the absence of water led to **4** and **3a** as the only observable products. However, if the reaction was carried out with an excess of NCS (**2**) and water further oxidation products were observed (**Figure S2**).

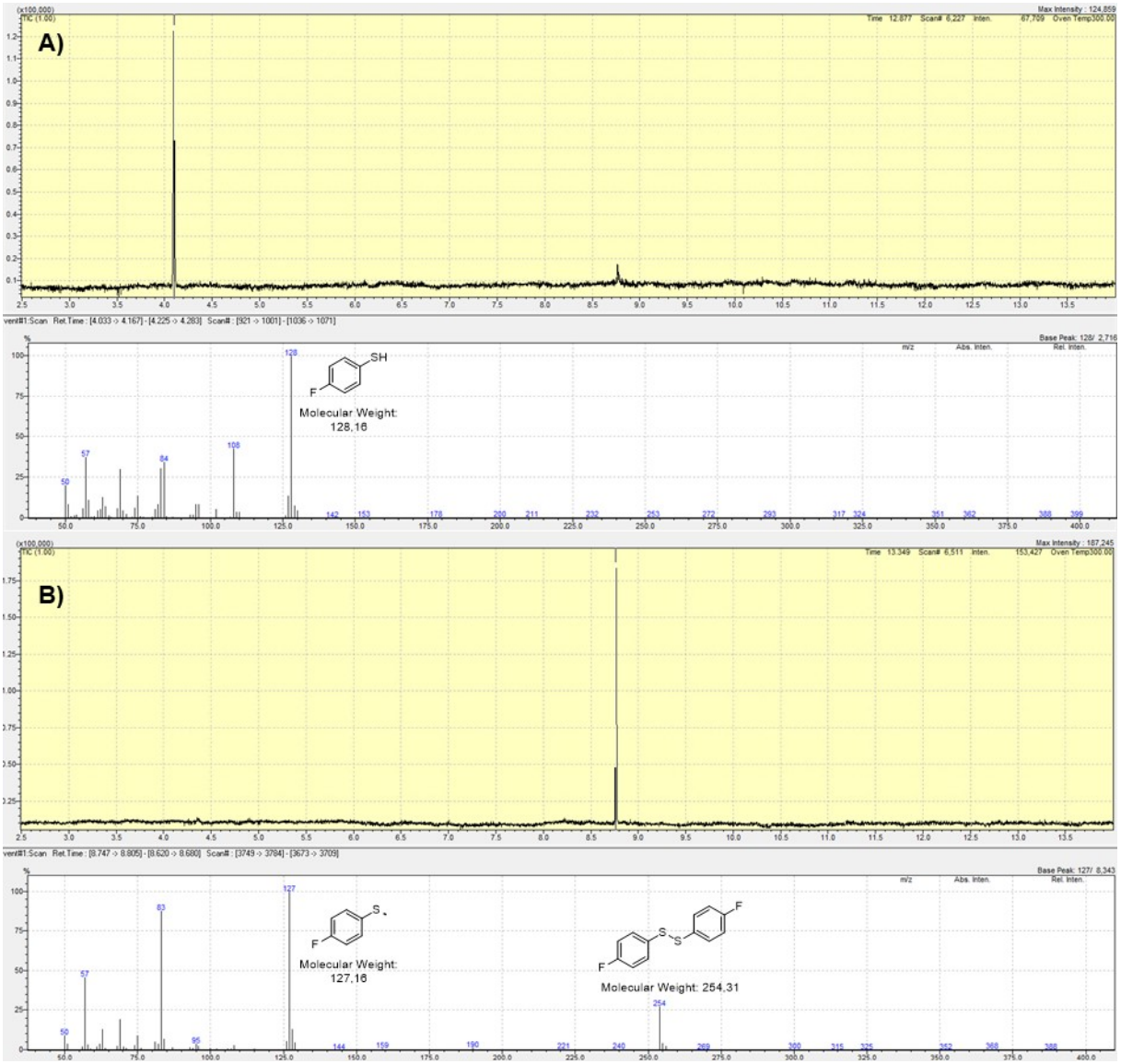


Figure S1. GC-MS spectra of **1** in dry EtOAc (80 mM) before the addition of NCS (**2**) as described in the general procedure above. **A**) The aliquot (50 µL) was added to pure acetonitrile (950 µL) and then measured. 4-fluorothiophenol (**1**), the starting material, is the main compound and only minor amounts of the corresponding disulfide (**4**) are present. **B**) The aliquot (50 µL) was added to the quenching solution, diethylamine in acetonitrile (950 µL) and then measured. *Bis*-(4-fluorophenyl)-disulfide (**4**) is the only detected compound.

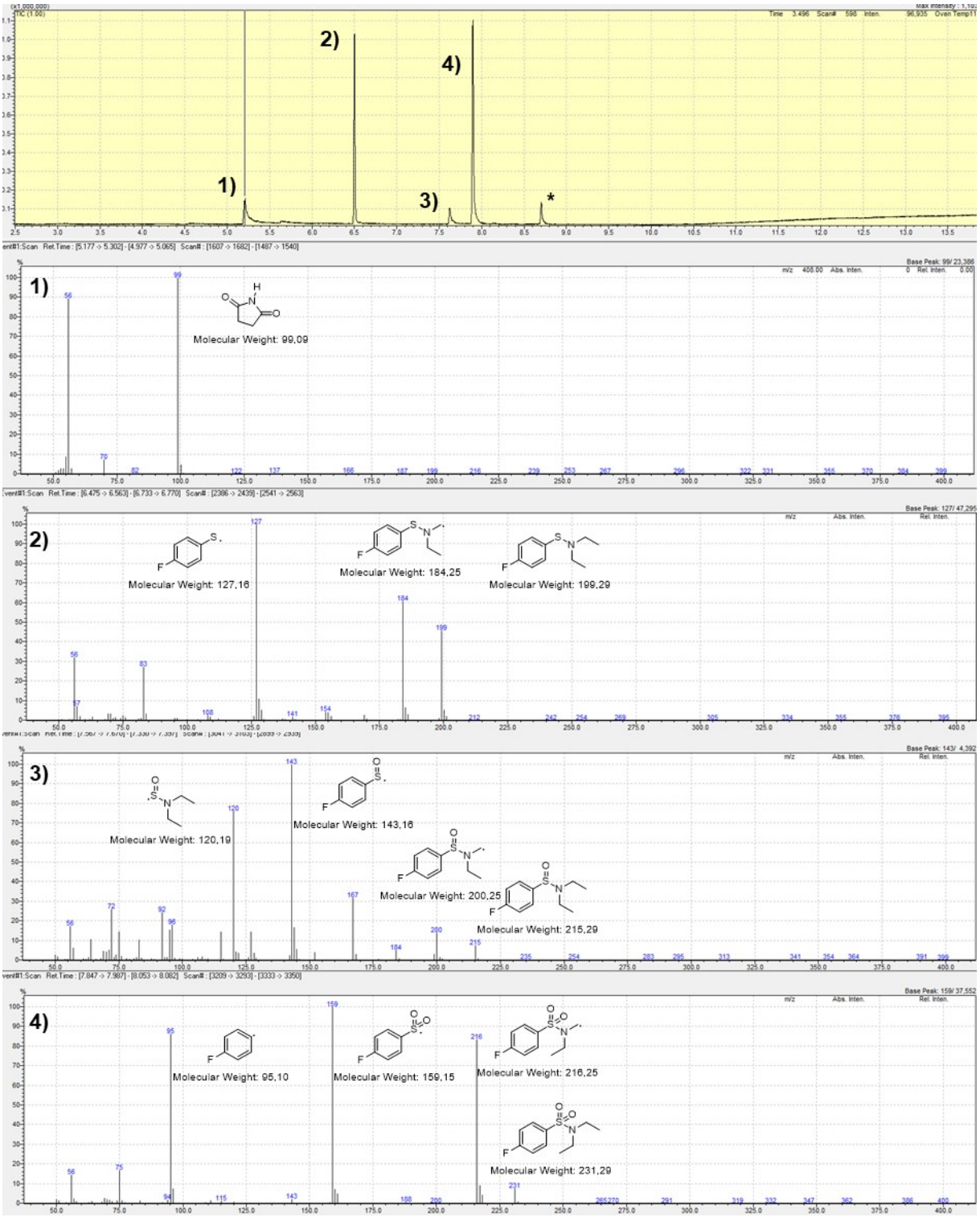


Figure S2. GC-MS chromatogram of the chlorination reaction after 8 minutes, using the following initial concentrations: 100 mM thiol **1**, 200 mM NCS (**2**), 150 mM H_2O. *Last peak corresponds to disulfide **4** as identified in **Figure S1**.

3 Chemometrics

3.1 Reaction Monitoring

Reaction monitoring was accomplished using a low field benchtop NMR spectrometer (Magritek, Spinsolve Ultra 43 MHz). Measurements were carried out using either a borosilicate glass NMR tube or a glass flow through cell (internal volume = 800 µL, length = 500 µL). In case of the flow cell, the reaction mixture would enter at the bottom and exit at top of the instrument. Before entry to the NMR, a six-port valve was installed, enabling the reaction mixture to by-pass the NMR and allow for shimming without the need to stop the process stream. Additionally, a T-piece was installed before the six-port valve, connecting the process stream to the waste. Along with this connection a cartridge back pressure regulator (2.8 bar) was installed, to avoid damage to the NMR device in the case of a blockage within the flow cell.

Shims were performed with either an NMR tube or the flow cell filled with dry EtOAc and referenced to the methyl singlet at 2.1 ppm. Typically, a “QUICKSHIM: ALL” was performed in the Spinsolve software (Magritek) and shim values were below 0.45 Hz linewidth at 50%, below 7.5 Hz linewidth at 0.55% and a signal to noise ratio above 20000.

Spectra were recorded in the reaction monitoring mode with a pulse angle of 90°. ^{1}H spectra were recorded with an acquisition time of 6.4 s, repetition time of 10 s, four scans and a delay of two s between spectra recording. ^{19}F spectra were recorded with an acquisition time of 6.4 s, a repetition time of 15 s, four scans and a delay of two s between spectra recording. The recorded spectra were processed with an indirect hard model in the PEAXACT software.

3.2 Indirect Hard Modeling

3.2.1 Pretreatment

All ^{1}H spectra were processed with the same pretreatment conditions. Exponential NMR apodization (0.3 Hz), zero filling (131072 (128 k)), phase correction (auto, negative peak penalization). Fixed baseline (Intercept and slope fixed at 0) and baseline correction (rubber band subtraction). Global range was set from 1.8 to 10 ppm and all spectra referenced to the highest peak, methyl group of EtOAc, at 2.1 ppm.

All ^{19}F spectra were processed with the same pretreatment conditions. Exponential NMR apodization (0.3 Hz), zero filling (131072, 128k), phase correction (auto, negative peak penalization) and baseline correction by rubber band subtraction (Nodes: −120 ppm, −118.3 ppm, −116 ppm, −115.5 ppm, −110.7 ppm, −109 ppm, −63 ppm). The global

range was set from −125 to −50 ppm, excluding the range from −97 to −65 ppm and all spectra referenced to the highest peak (trifluoro toluene) at −63.72 ppm.

3.2.2 Model Creation

An NMR tube containing only EtOAc was measured with the benchtop NMR. The resulting ^{1}H NMR spectrum was used to create a hard model of the solvent and its more prominent ^{13}C satellites by stepwise addition of peaks, minimizing the residuals. A total of eleven peaks were used and the fitting mode was set to maximal interaction, maximizing flexibility of peak position and shape. Thereafter, 4-fluorothiophenol (**1**) was dissolved in an NMR tube in dry EtOAc, a spectrum measured, and a hard model created, as described above, using twelve peaks. This process was separately repeated for *bis*-(4-fluorophenyl)-disulfide (**4**, thirteen peaks), NCS (**2**, 1 peak), NHS (**5**, 1 peak) and trifluorotoluene (internal standard, 2 peaks). As product **3** is very reactive, it was created in situ by reaction of **1** with an excess of **2** in dry EtOAc directly in the NMR tube. The reaction was constantly monitored via ^{1}H and ^{19}F NMR, until no more change in the concentration of **2** and **5,** as well as full consumption of **1** and **4** was observed, indicating quantitative conversion. The product was identified as **3**, based on the observed shift in the ^{19}F NMR spectrum (−109.8 ppm). A ^{1}H NMR spectrum recorded after completion of the reaction was then used to create a hard model for **3** using 22 peaks. These 7 hard models were then combined in a mixture model intended of spectral deconvolution during reaction monitoring.

Evaluating NMR spectra with the previously established spectral hard model in PEAXACT directly returns the peak areas of each component. Quantification of the individual species was done directly by comparison of an analyte's peak area ($A_{analyte}$) with the area of the internal standard (TFT) of known concentration (100 mM). Additionally, the protons that make up each peak had to be taken into consideration. Quantification was implemented in the PEAXACT software via the "custom function" functionality, directly returning concentrations ($c_{analyte}$) after spectral analysis. Model validation showed strong overprediction of high concentration values for **4** and **5**, resulting in the addition of a correction factor (0.95) to the equations of these two compounds. The five custom functions are stated below (Equations S1-S5).

$$c_{thiol} = \frac{\frac{A_{thiol}}{5}}{\frac{A_{TFT}}{4}} * c_{TFT} \quad \#S1$$

$$c_{disulfide} = \frac{\frac{A_{disulfide}}{5}}{\frac{A_{TFT}}{8}} * c_{TFT} * 0.95 \quad \#S2$$

$$c_{sulfenyl\ chloride} = \frac{\frac{A_{sulfenyl\ chloride}}{5}}{\frac{A_{TFT}}{4}} * c_{TFT}\ \#S3$$

$$c_{NCS} = \frac{\frac{A_{NCS}}{5}}{\frac{A_{TFT}}{4}} * c_{TFT}\ \#S4$$

$$c_{NHS} = \frac{\frac{A_{NHS}}{5}}{\frac{A_{TFT}}{4}} * c_{TFT} * 0.95\ \#S5$$

3.2.3 Model Validation

To evaluate the performance of the model, two sets of validation experiments were carried out, consisting of five samples of known concentration each. The first series contained NCS (**2**) and NHS (**5**) in concentrations between 0 mM and 150 mM, the second one thiol **1** and disulfide **4** in concentrations of up to 150 mM and 75 mM respectively (product **3** could not be added for validation, as it would react with the thiol **1**). The internal standard (TFT) was present in all samples at a concentration of 100 mM.

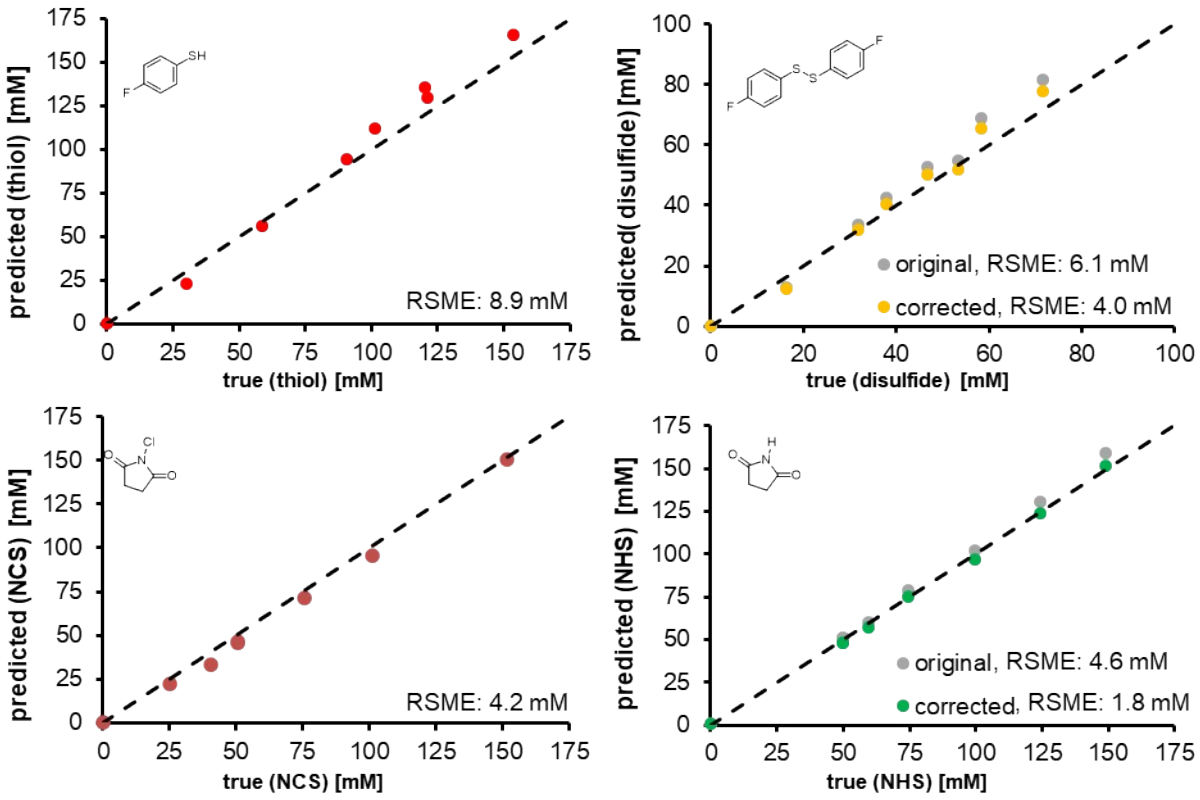


Figure S3. Predicted vs true plots for concentrations of **1**, **4**, **2** and **5** derived from the spectral hard modeling and the custom functions (equations S1, S2, S4 and S5), including the target line (y = x).

4 Calorimetry

4.1.1 Temperature Monitoring

To investigate the exothermicity of the chlorination reaction the internal temperature was monitored over the course of the reaction. NCS (**2**, 100.1 mg, 750 µmol, 150 mM) was added to a two neck round bottom flask (10 mL) and dissolved in dry EtOAc (5 mL). The round bottom flask was mounted above a magnetic stirrer and equipped with a stopper and a perforated septum, through which a temperature sensor was put into the solution. **1** (96.1 mg, 750 µmol, 150 mM) was added to the solution under stirring and the measured temperature noted every 30 s. This experiment was performed twice, showing a small temperature increase (0.3-0.4 °C) upon the addition of **1** and an overall increase in temperature of about 2 °C over the course of 14-16 minutes. The recorded temperature profiles are shown below.

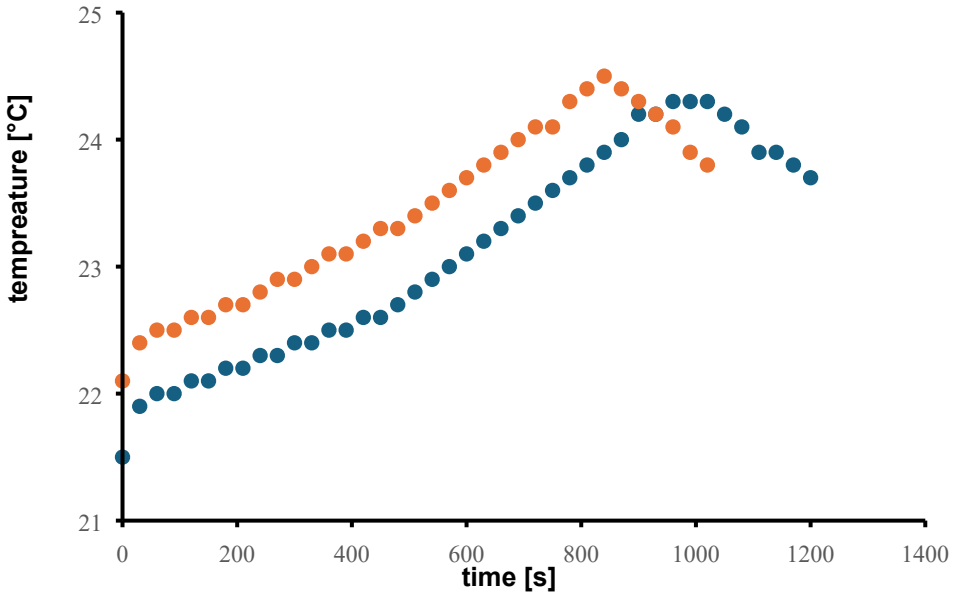


Figure S4. Observed temperature evolution over the course of the reaction of **1** and **2** at a concentration of 150 mM each in dry EtOAc. Experiment conducted in double determination. Blue dots = first run, orange dots = repeat experiment.

4.1.2 Calorimetric Titration

Calorimetric titration experiments were conducted using a Thermal Hazard Technology (THT) µRC Microreaction Calorimeter. A standard vial was prepared with a preprepared solution of **2** (300 mM) in dry EtOAC (500 µL) and a 250 µL syringe was loaded with a preprepared solution of **1** (600 mM) in dry EtOAc (250 µL). The solution in the syringe was then stepwise added over ten steps of 25 µL to the standard vial, under mixing. A 300 s interval between injections was chosen to allow for stabilization in between injections. Figure S5 depicts the observed power response to these injections in mW. As the tenth injection gave an error message, the heat of reaction was calculated as the mean value of the remaining nine injections, resulting in 121 ± 6 KJ/mol. All integration values are shown below in table S1.

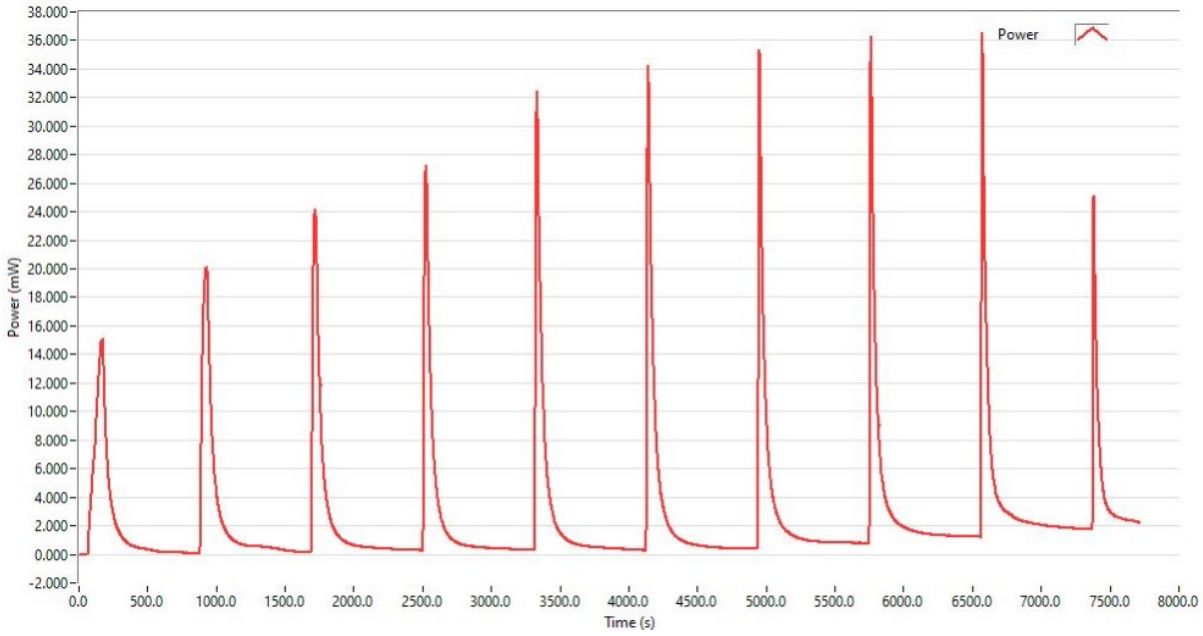


Figure S5. Power response observed over the calorimetric titration experiment. Heat of reaction, derived form integration, noted below in table S1. The tenth and last injection was not considered in the analysis.

Table S1. Heat of reaction measured during calorimetric titration experiments.

Injection	Heat of reaction [kJ/mol]	Injection	Heat of reaction [kJ/mol]
1	112.280	7	125.731
2	122.760	8	125.206
3	114.540	9	123.258
4	111.309	10	NaN (Error)
5	123.664	Average	121 +/- 6
6	127.050		

Subsequently the heat of reaction was used to calculate the adiabatic temperature rise ($\Delta T_{adiabatic}$) as described in equation S6. We only considered the heat capacity of the solvent in this calculation,[2] resulting in a $\Delta T_{adiabatic}$ of 10.5 K. This value is a lot higher than the temperature rise of ~2 °C we observed, which is likely to be lower due to the heat exchange with the surrounding air, as can also be seen in the temperature drop after reaction completion in figure S4.

$$\Delta T_{adiabatic} = -\frac{\Delta H_{reaction} \times n_{Thiol}}{c_{p,\,EtOAc} \times n_{EtOAc}} = -\frac{121\frac{kJ}{mol} * 1\ mmol}{169.06\frac{J}{mol\ K} * 68.3\ mmol} = 10.5\ K\ \#S6$$

5 NMR Tube Reactions

5.1 Experimental procedure

A solution of trifluorotoluene (100 mM) in dry ethyl acetate was prepared in a volumetric flask (20 mL). Two separate solutions of the starting materials were prepared by weighing **1** and **2** at the desired amount into two different volumetric flasks (5 mL). The starting materials were then dissolved in the preprepared solution of trifluorotoluene (100 mM) in dry EtOAc and the volumetric flasks subsequently filled to the mark. Both solutions were ultrasonicated until **2** was completely dissolved. Reactions were carried out by combining these three different solutions in varying volumes, resulting in a combined total volume of 1 mL in an NMR tube. An overview of the different initial concentrations is given in table S2. The tube was then vigorously shaken for 10 seconds and subsequently placed in the benchtop NMR and the reaction monitoring started. The raw spectra were imported into PEAXACT to obtain concentrations for the different species The reported concentration values were calculated using a moving average of three, applied for smoothing. These data were then used to fit rate constants for the five-step reaction network described in table S3. As a comparison, each individual profile experiment was also fitted separately and the fits from this are shown in figures S16-S20.

Table S2. Initial concentrations of NCS (**2**) and starting material **1** for the NMR tube experiments performed under ambient conditions. Reaction profiles of these experiments are depicted in figures S6-S10.

Experiment	NCS conc. [mM]	Thiol conc. [mM]
1	60	60
2	100	75
3	100	150
4	150	120
5	150	150

Table S3. Fitted rate constants for the five-step reaction network, derived from the simultaneous fitting of the five experiments shown in table S2. Values stated without deviation were fitted with high uncertainty. Reaction profiles with the corresponding model fit are depicted in figures S13-S17. Standard error based on 95% confidence limit.

Reaction Equations	K ± SE [M^{-1} min^{-1}]
thiol + NCS → sulfenyl chloride + NHS	0.51 ± 0.04
thiol + sulfenyl chloride → disulfide + HC	92 ± 28
NCS + HCl → Cl_2 + NHS	1.85 ± 0.06
thiol + Cl_2 → sulfenyl chloride + HCl	1E-8

disulfide + Cl_2 → 2 sulfenyl chloride 4.35E5

5.2 Experimental Data

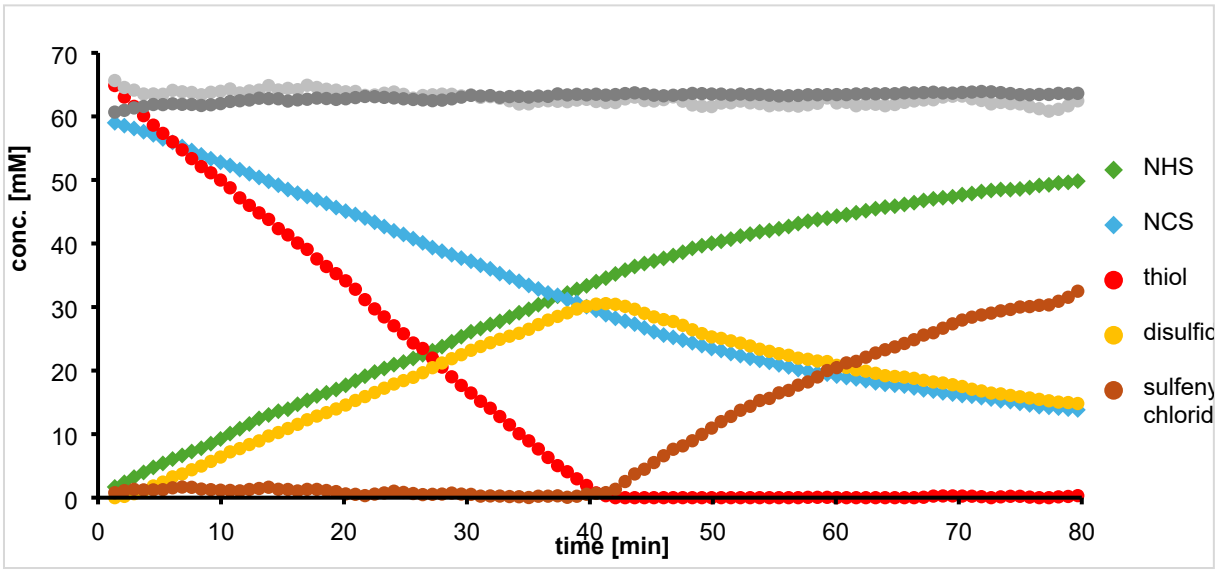


Figure S6. Reaction profile of the reaction of **1** and **2** in an NMR tube at ambient conditions. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

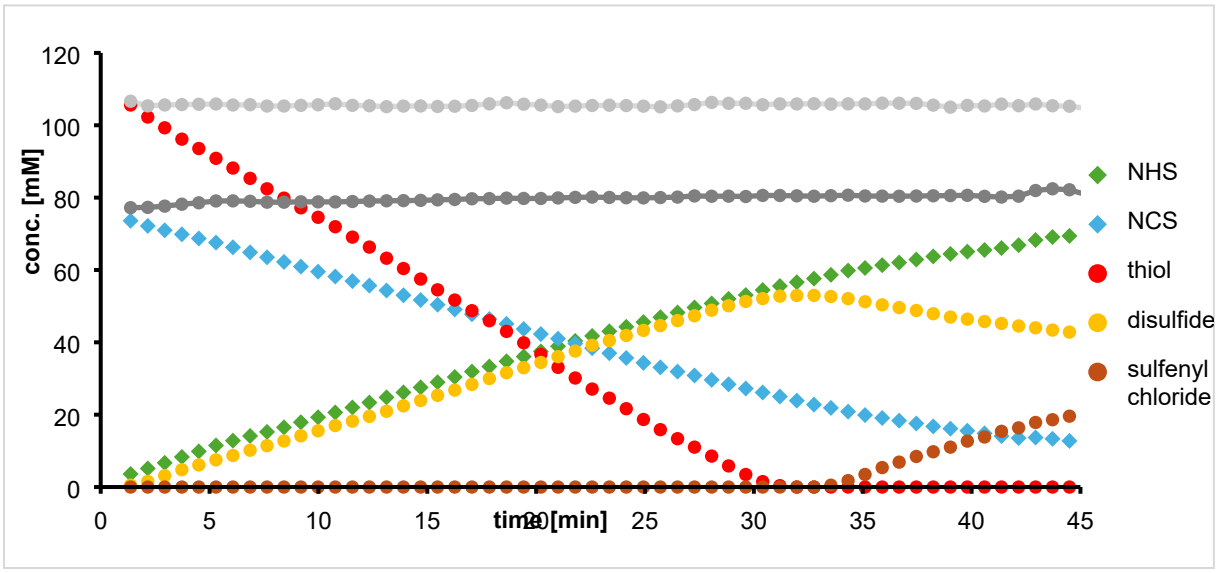


Figure S7. Reaction profile of the reaction of **1** and **2** in an NMR tube at ambient conditions. Initial concentrations: 75 mM NCS (**2**), 100 mM thiol **1**. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

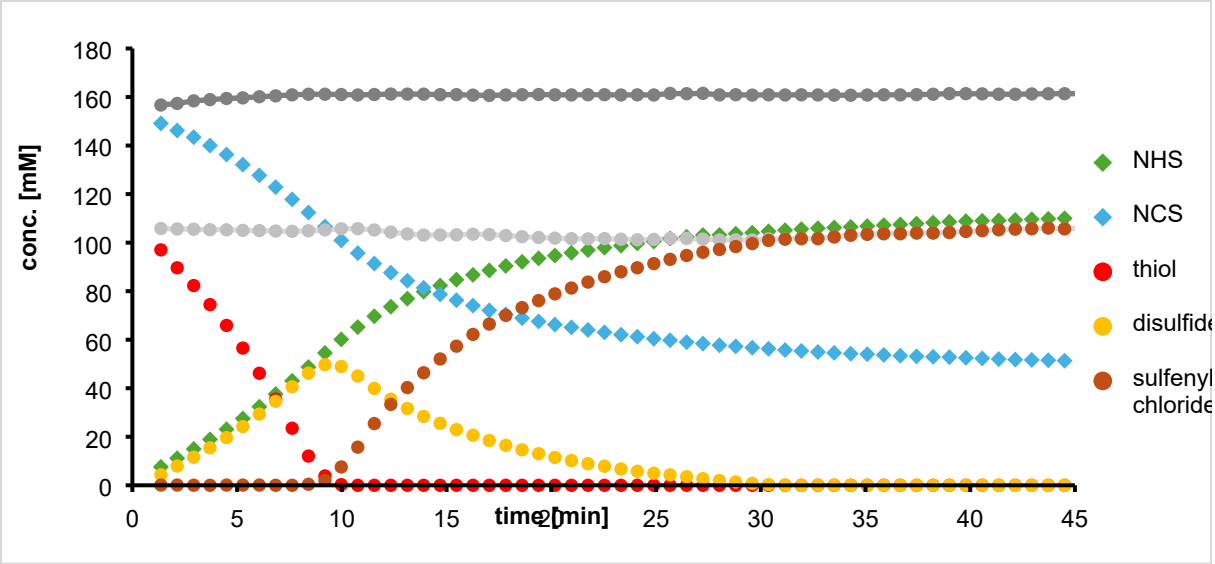


Figure S8. Reaction profile of the reaction of **1** and **2** in an NMR tube at ambient conditions. Initial concentrations: 150 mM NCS (**2**), 100 mM thiol **1**. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

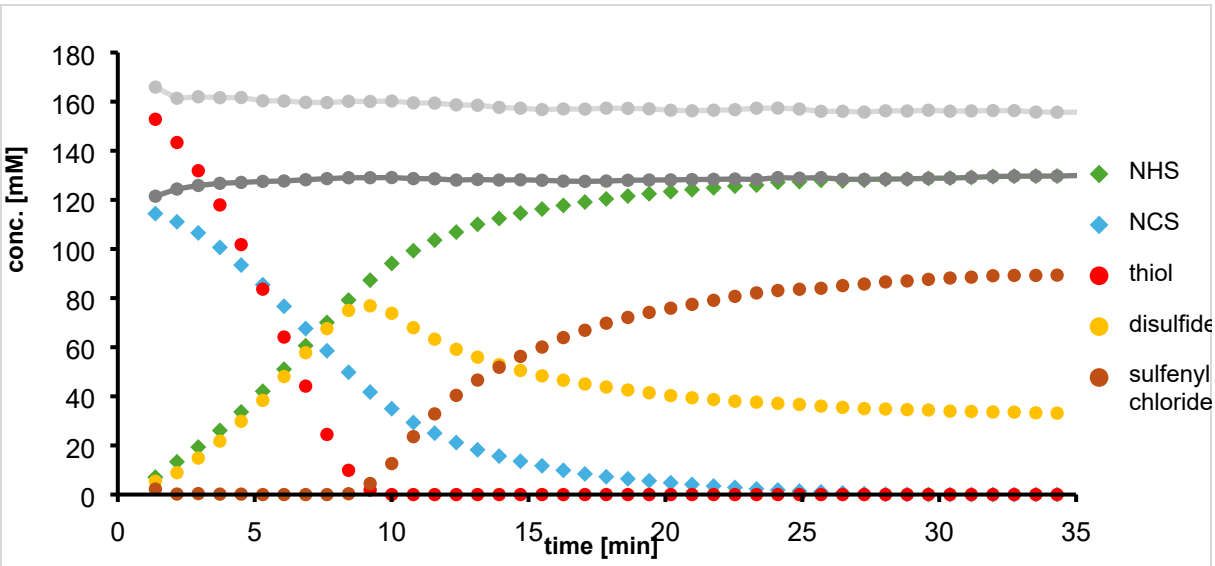


Figure S9. Reaction profile of the reaction of **1** and **2** in an NMR tube at ambient conditions. Initial concentrations: 120 mM NCS (**2**), 150 mM thiol **1**. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

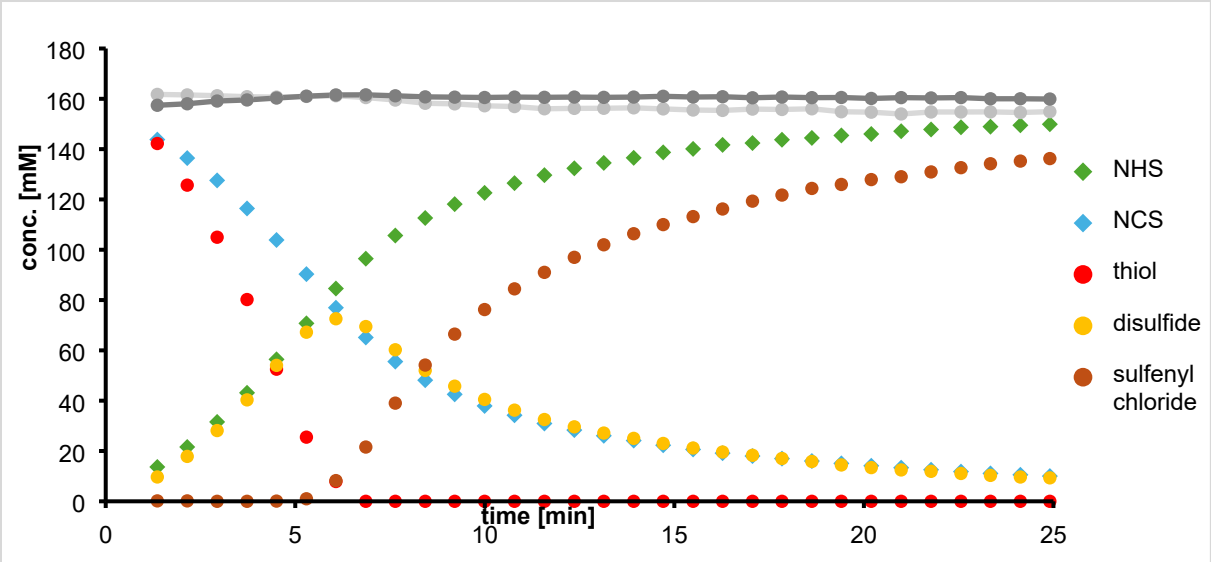


Figure S10. Reaction profile of the reaction of **1** and **2** in an NMR tube at ambient conditions. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

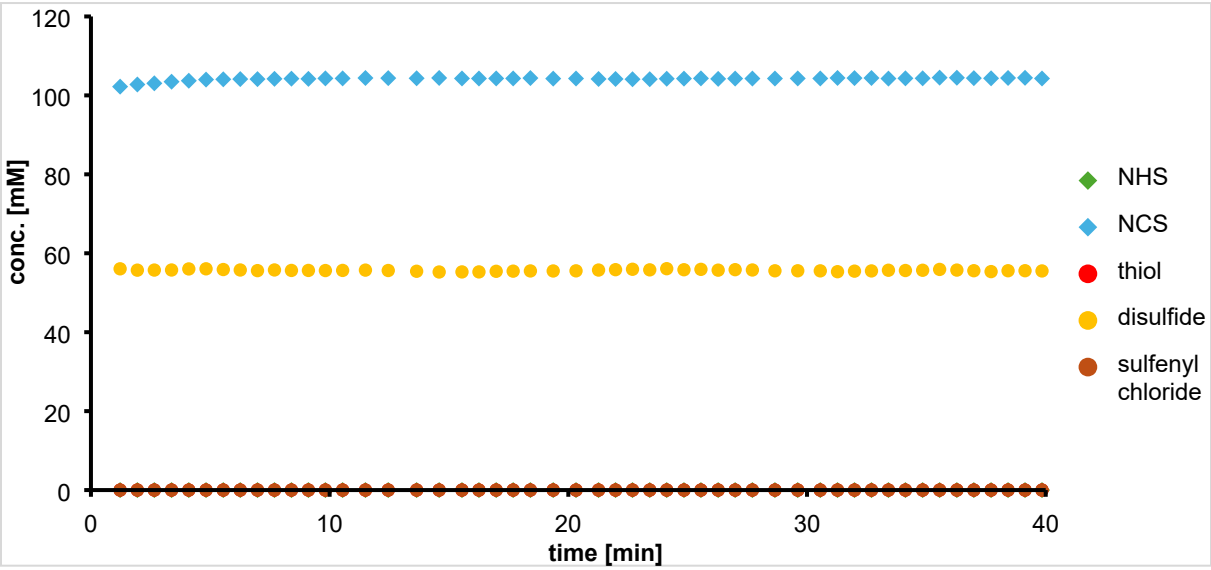


Figure S11. Reaction profile of the attempted chlorination of **4** with **2** at ambient conditions. Both reagent concentrations stayed constant over the course of 40 minutes and no chlorinated product **3** was observed. Initial concentrations: 100 mM NCS (**2**), 50 mM disulfide **4**.

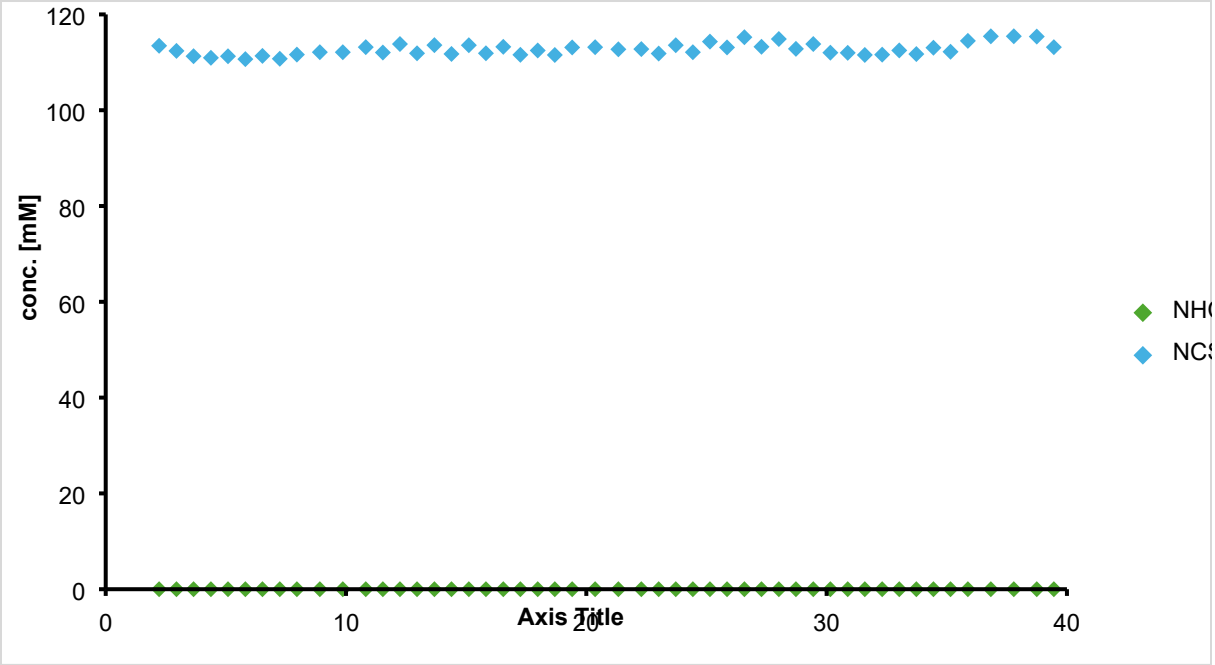


Figure 12. Reaction profile of the attempted hydrolysis of **2** at ambient conditions. No hydrolysis product **5** was observed and the NCS (**2**) concentration was constant over the course of the 40 minutes experiment. Initial concentrations: 100 mM NCS (**2**), 20 mM H_2O.

5.3 Kinetic Fit

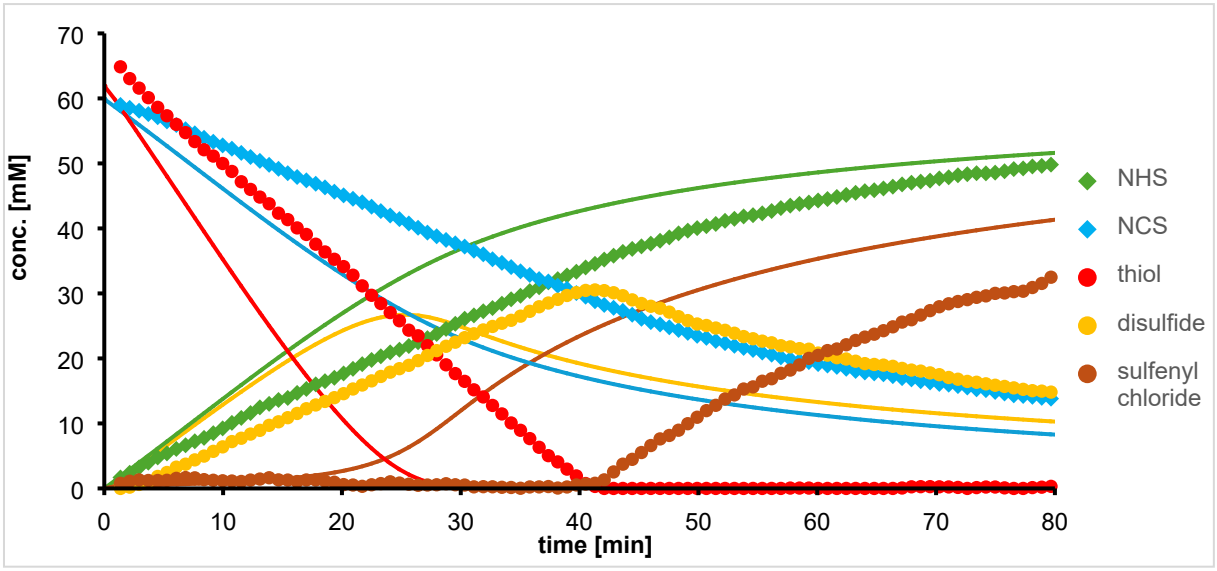


Figure S13. Experimental data and fit for experiment in NMR tube at ambient temperature. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

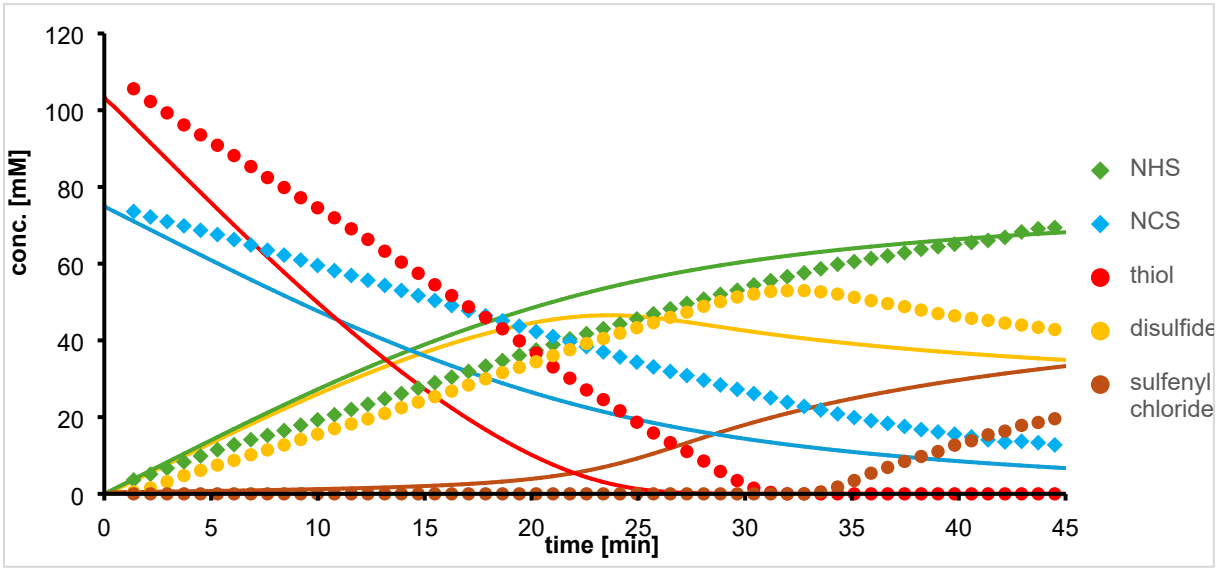


Figure S14. Experimental data and fit for experiment in NMR tube at ambient temperature. Initial concentrations: 75 mM NCS (**2**), 100 mM thiol **1**. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

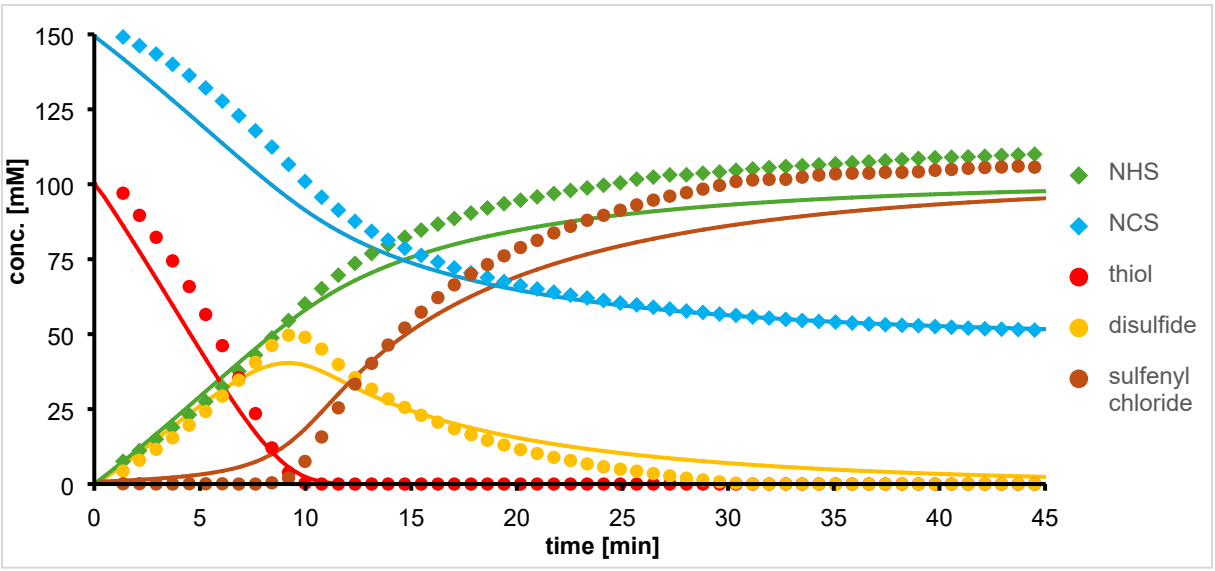


Figure S15. Experimental data and fit for experiment in NMR tube at ambient temperature. Initial concentrations: 150 mM NCS (**2**), 100 mM thiol **1**. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

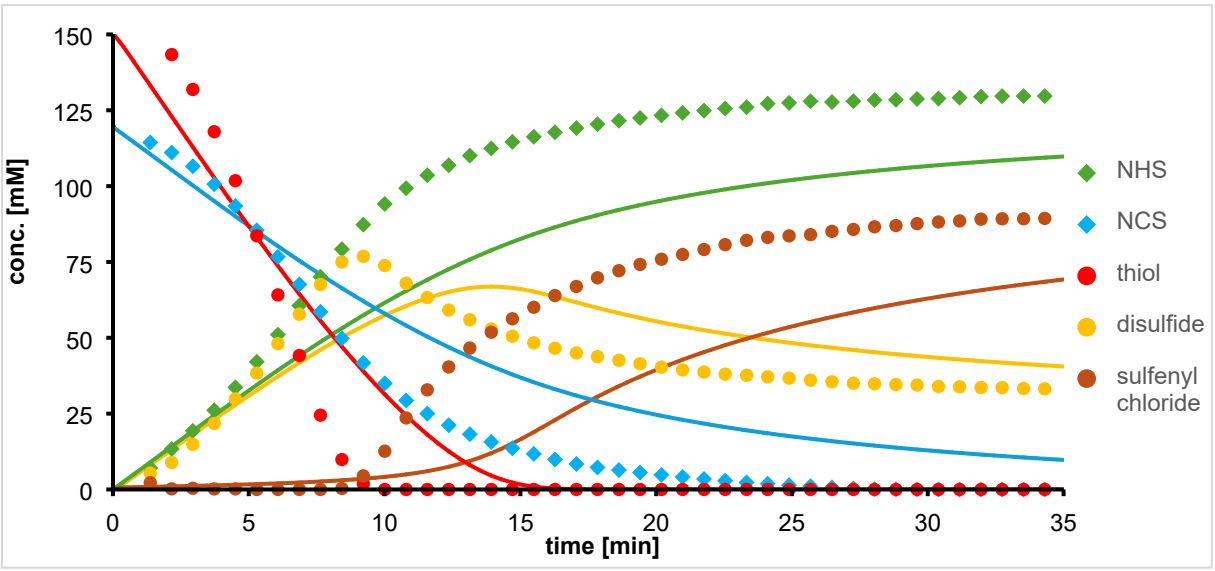


Figure S16. Experimental data and fit for experiment in NMR tube at ambient temperature. Initial concentrations: 120 mM NCS (**2**), 150 mM thiol **1**. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

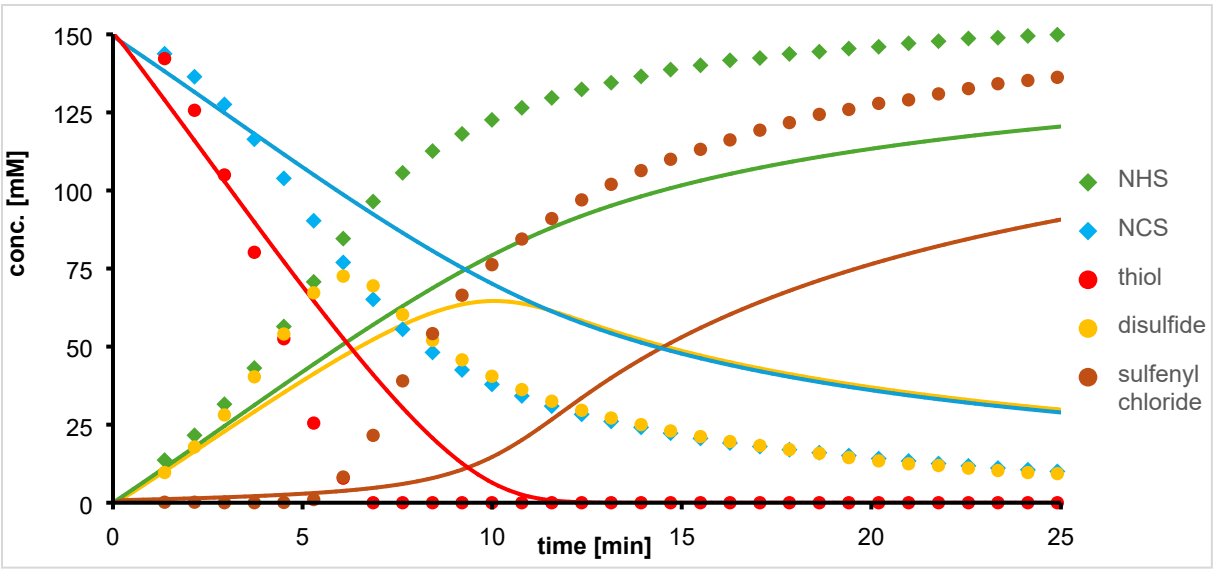


Figure S17. Experimental data and fit for experiment in NMR tube at ambient temperature. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

5.4 Individual Kinetic Fits

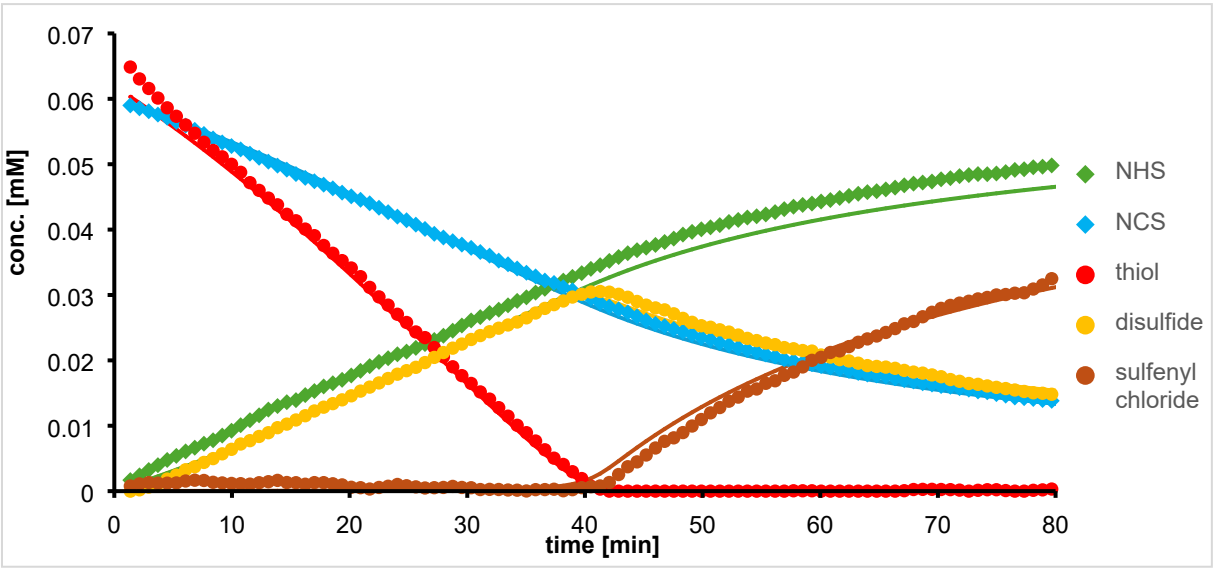


Figure S18. Experimental data and individual fit for this experiment. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

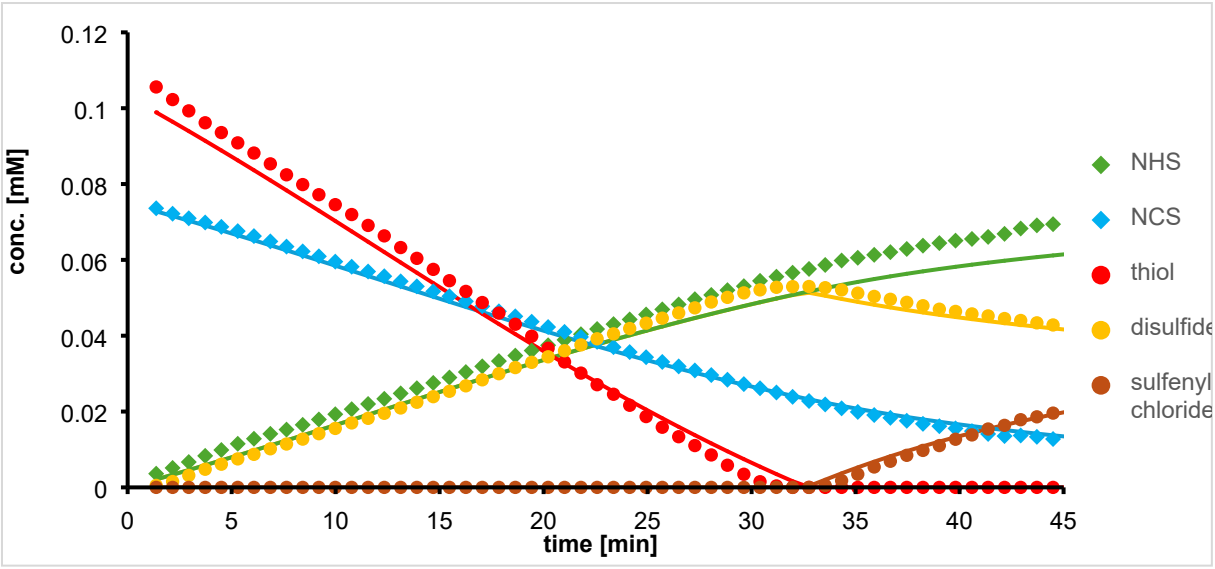


Figure S19. Experimental data and individual fit for this experiment. Initial concentrations: 75 mM NCS (**2**), 100 mM thiol **1**. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

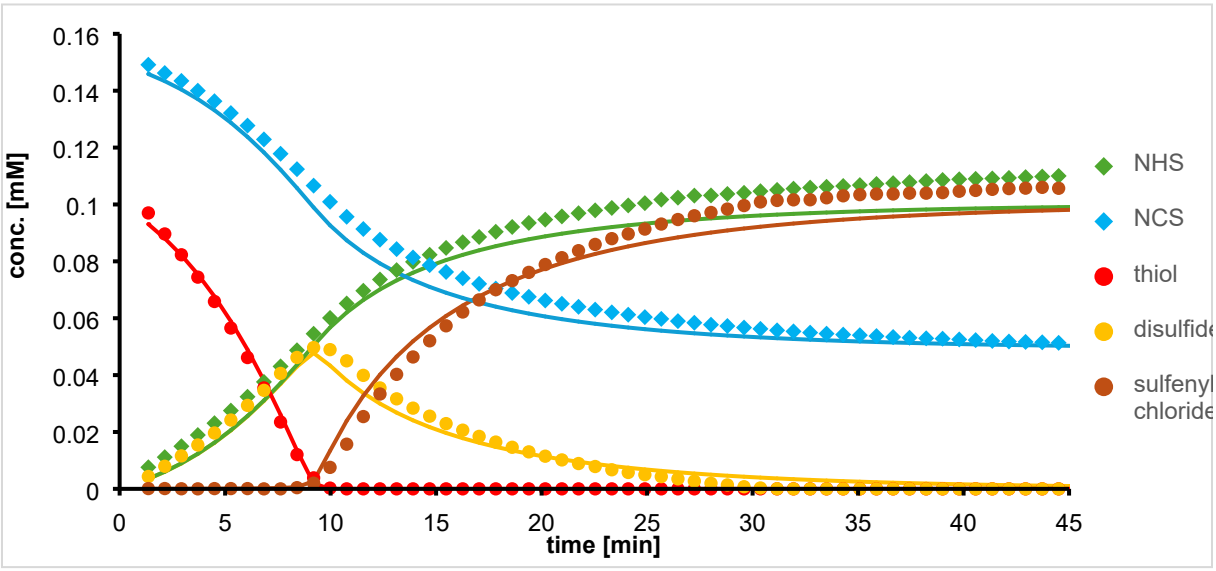


Figure S20. Experimental data and individual fit for this experiment. Initial concentrations: 150 mM NCS (**2**), 100 mM thiol **1**. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

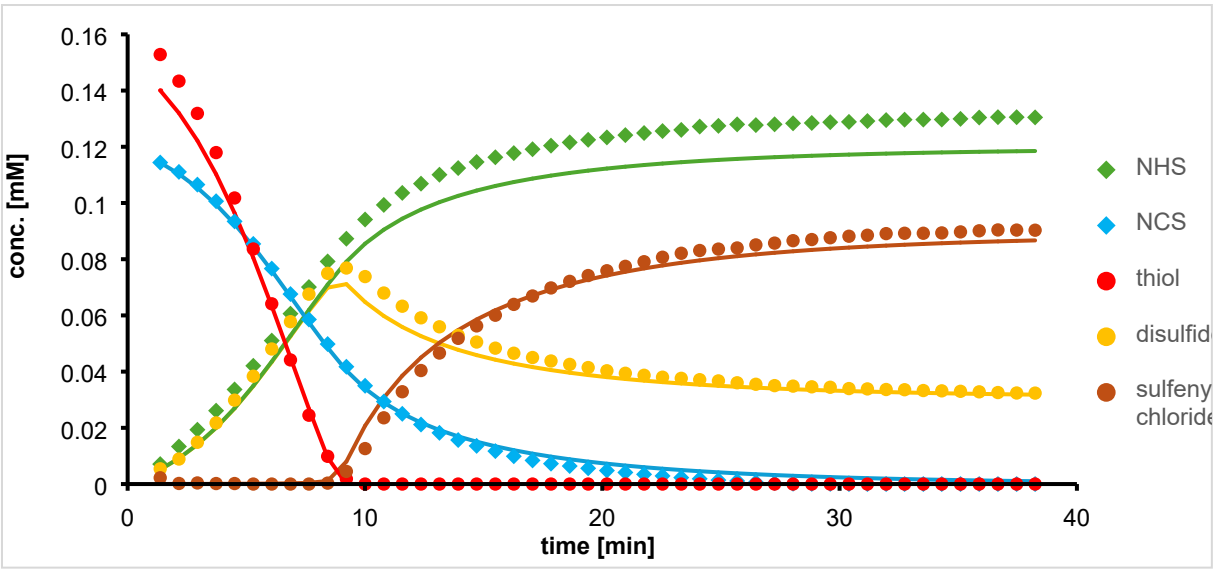


Figure S21. Experimental data and individual fit for this experiment. Initial concentrations: 120 mM NCS (**2**), 150 mM thiol **1**. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

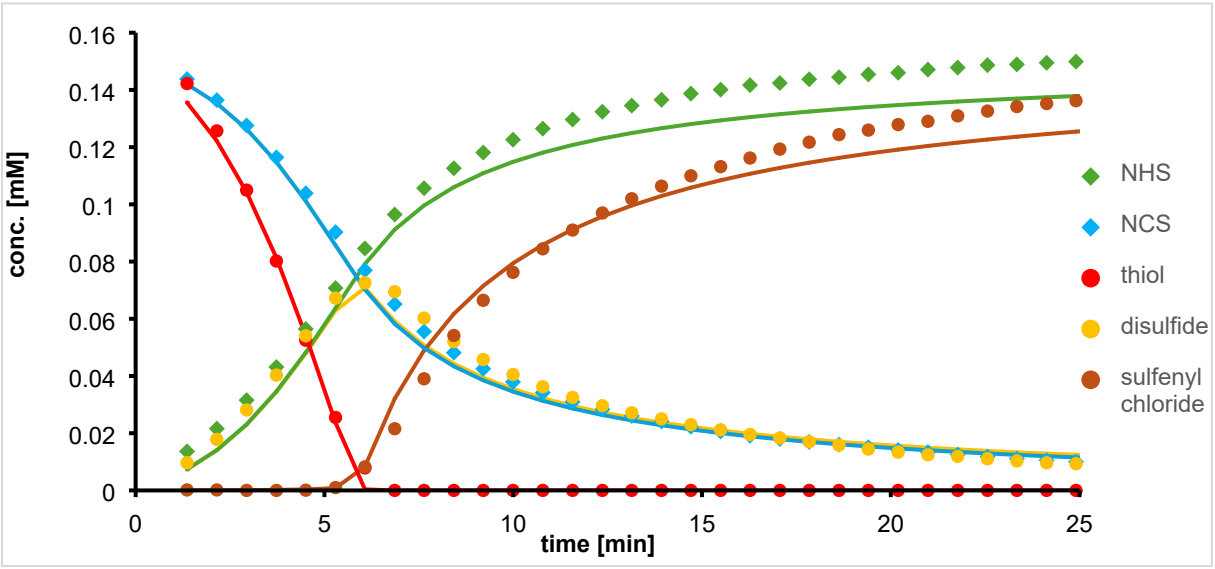


Figure S22. Experimental data and individual fit for this experiment. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

6 Re-Circulation

6.1 General Procedure

NCS (**2**) and a magnetic stir bar were added to a 50 mL two necked round bottom flask, that was equipped with a perforated septa and a stopper. The flask was placed in a water bath, which was heated to the desired temperature with a magnetic stirrer, before 25 mL of dry EtOAc and TFT (304 µL, 365 mg, 2.50 mmol, 100 mM) were added and the NCS (**2**) was dissolved through vigorous stirring (600 rpm). The end of the tubing from which liquid was pumped into the NMR-cell was placed into the reaction mixture through the perforated septum. Thiol **1** was added to the reaction mixture and after ten seconds the stirring was stopped (as the magnetic stirrer interferes with the bench-top NMR) and the peristaltic pump started at 2 mL/min. For the first five seconds the reaction mixture was discarded and afterwards fed back into the flask in recirculation. Reaction monitoring started 70 seconds after the thiol addition and as the first scan takes 40 seconds, 90 seconds was selected as the first data point. were imported into PEAXACT to obtain the concentrations for the different species. A moving average of 3 was applied for smoothing.

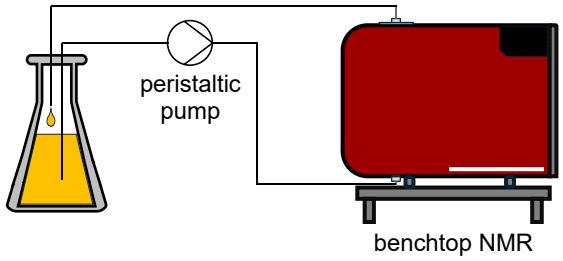


Figure S23. Schematic depiction of the experimental setup used during re-circulation experiments.

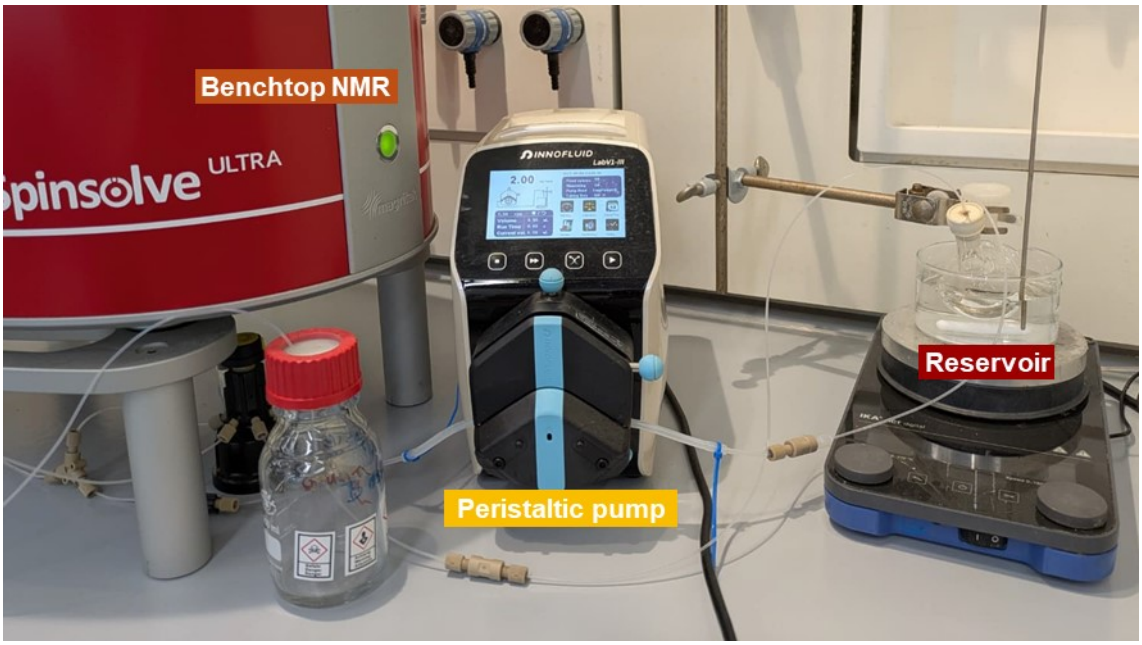


Figure S24. Labeled image of the experimental setup used during re-circulation experiments.

Table S4. Initial concentrations of NCS (**2**), starting material **1**, H_2O and water bath temperature for re-circulation experiments. Reaction profiles of these experiments are depicted in figures S25-36.

Experiments (fitting)	NCS conc. [mM]	Thiol conc. [mM]	H_2O conc. [mM]	T. [°C]
1	60	60	-	25
2	100	75	-	25
3	100	100	-	25
4	100	150	-	25
5	150	150	-	25
6	60	60	-	35
7	100	75	-	35
8	60	60	6	25
9	60	60	15	25
10	100	75	6	25
Experiments (simulation)				
11	150	120	-	25
12	100	100	10	25

Table S5. Fitted rate constants and activation energies for the six-step reaction network, using ten re-circulation experiments (table S4, entries 1-10). Values stated without deviation were fitted with high uncertainty. Reaction profiles with the corresponding model fit are depicted in figures S37-S48. Standard error based on 95% confidence limit. [1] Unit of rate constant for sixth reaction step: [M^{-2} min^{-1}]. [2] Model fitting showed low temperature sensitivity for second reaction step.

Reaction Equations	k ± SE [M^{-1} min^{-1}]	E_a ± SE [kJ/mol]

thiol + NCS → sulfenyl chloride + NHS	0.10 ± 0.01	58.8 ± 2.7
thiol + sulfenyl chloride → disulfide + HC	564	-[2]
NCS + HCl → Cl_2 + NHS	1.69 ± 0.03	35.8 ± 1.5
thiol + Cl_2 → sulfenyl chloride + HCl	192 ± 49	132
disulfide + Cl_2 → 2 sulfenyl chloride	18.3 ± 1.2	68 ± 22
NCS + HCl + H_2O → Cl_2 + NHS + H_2(	293 ± 6[1]	-

6.2 Experimental Data

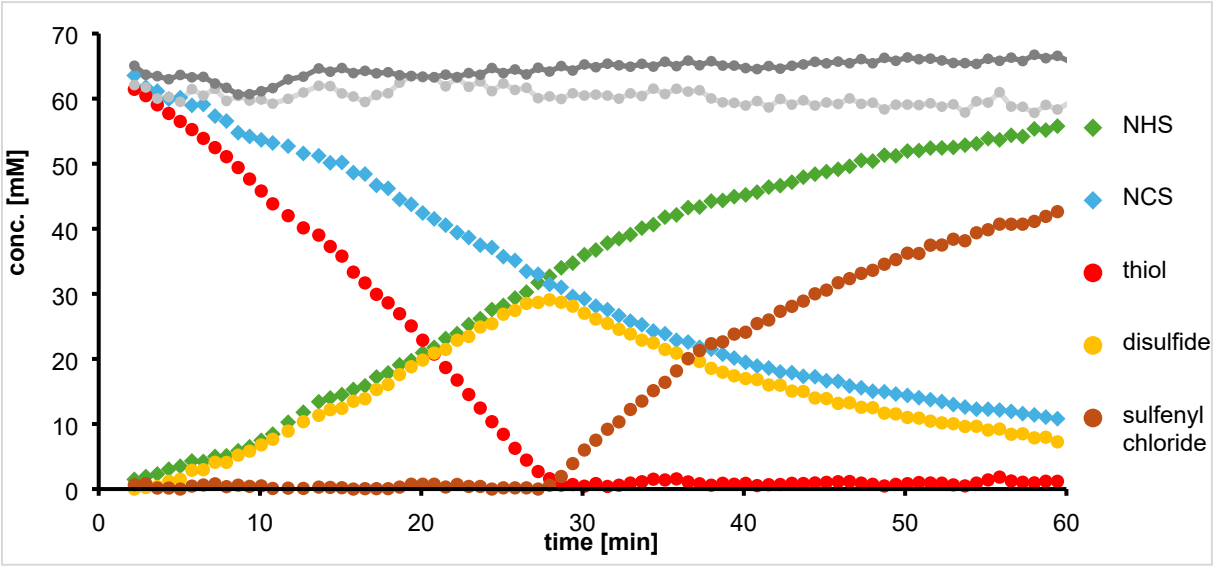


Figure S25. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

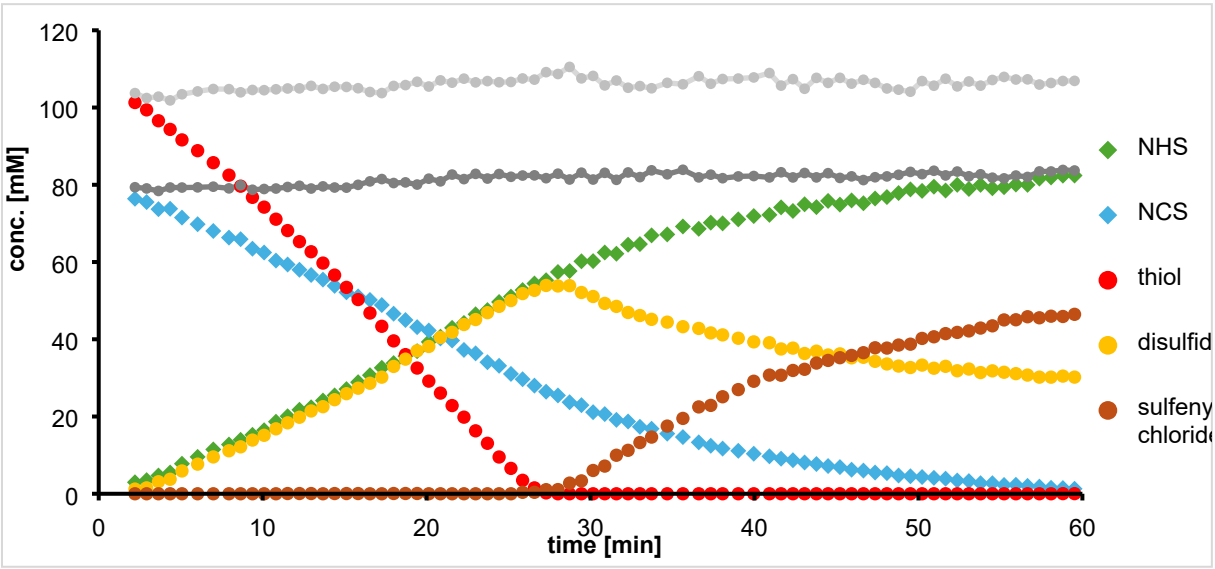


Figure S26. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 75 mM NCS (**2**), 100 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

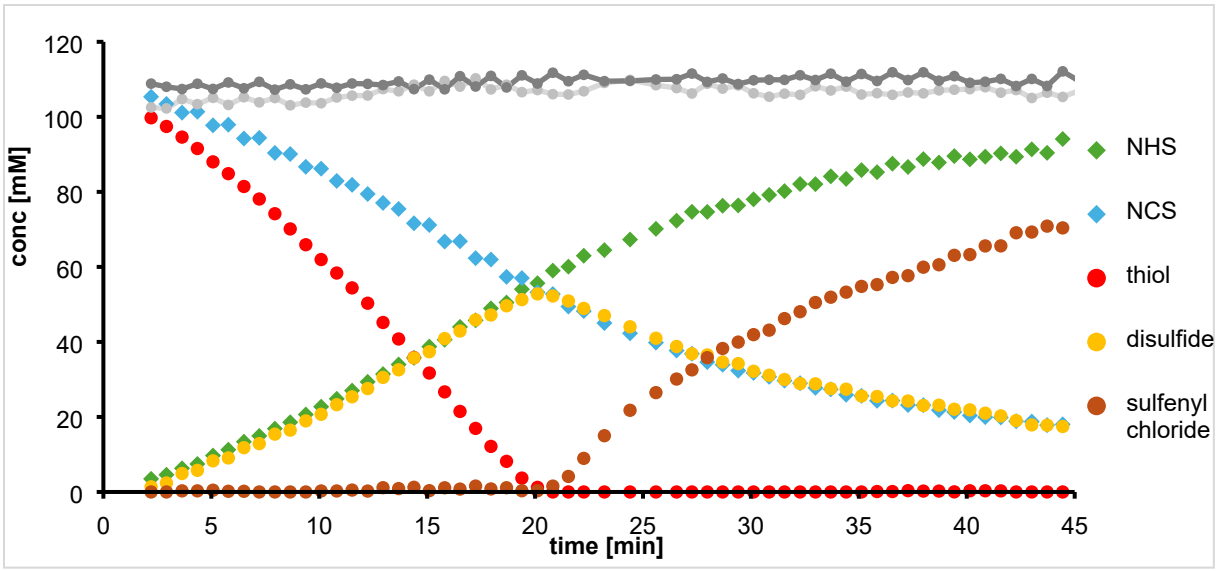


Figure S27. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 100 mM NCS (**2**), 100 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

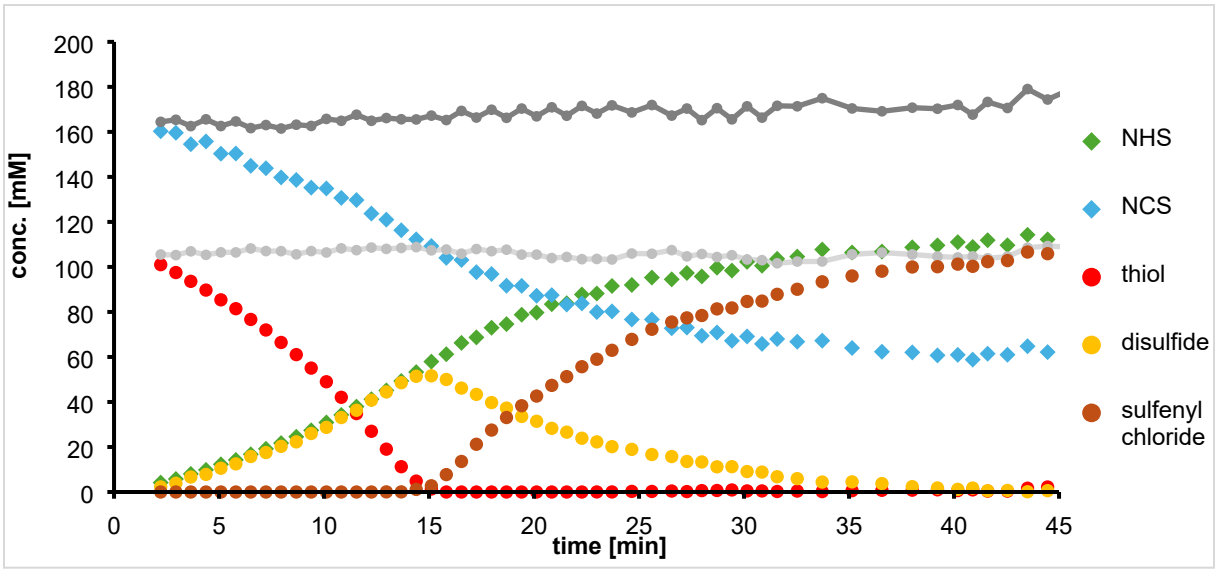


Figure S28. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 100 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

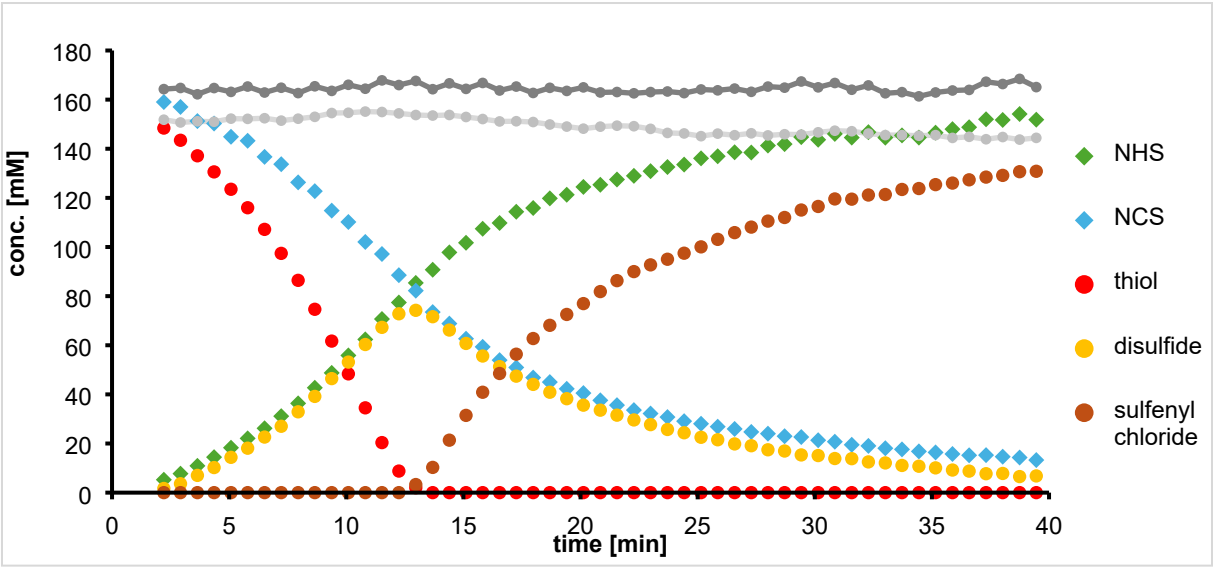


Figure S29. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

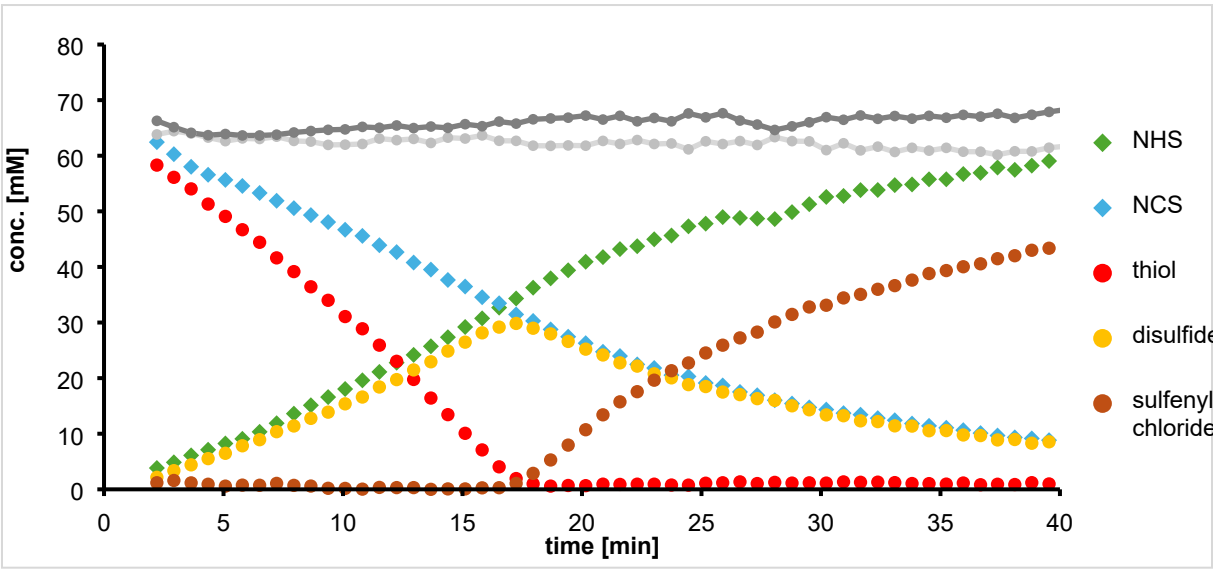


Figure S30. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 0 mM H_2O, temperature = 35 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

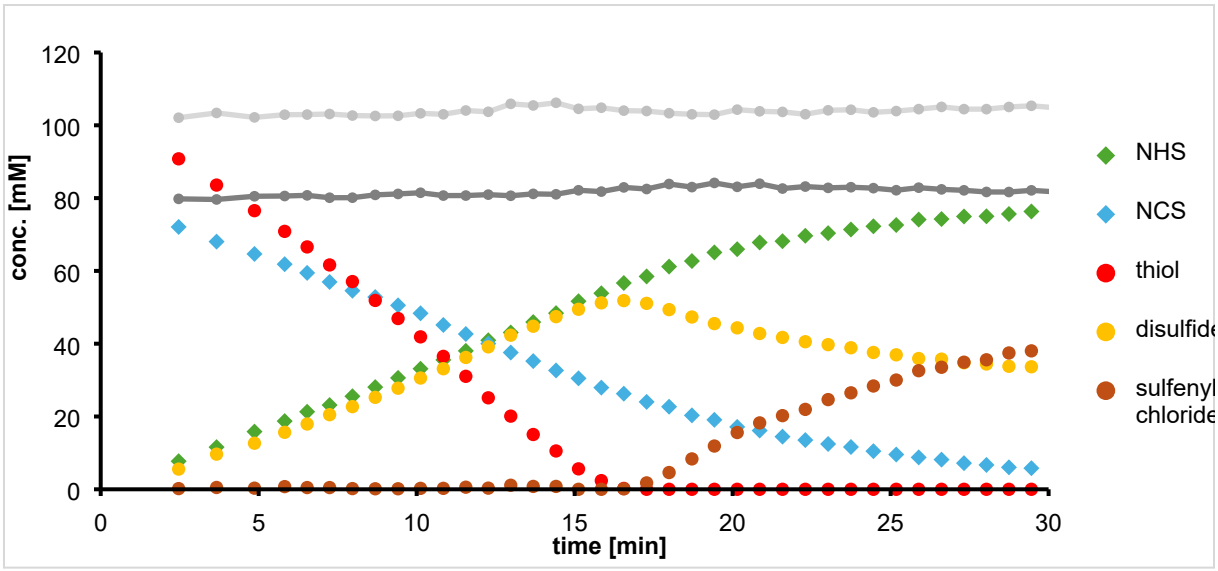


Figure S31. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 75 mM NCS (**2**), 100 mM thiol **1**, 0 mM H_2O, temperature = 35 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

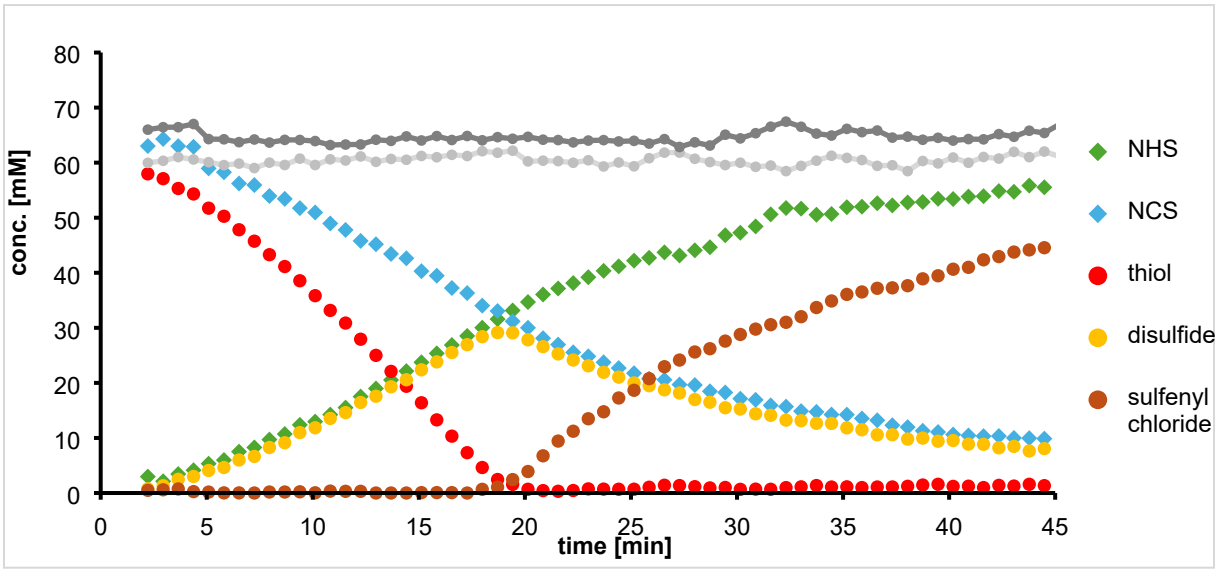


Figure S32. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 6 mM H_2O, temperature = 25 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

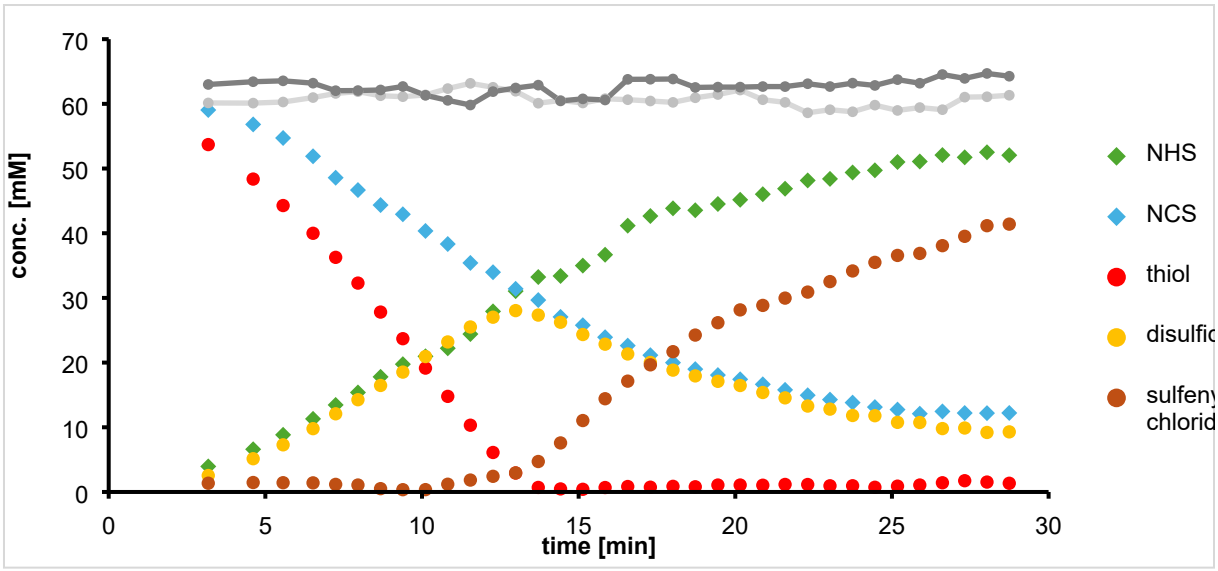


Figure S33. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 15 mM H_2O, temperature = 25 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

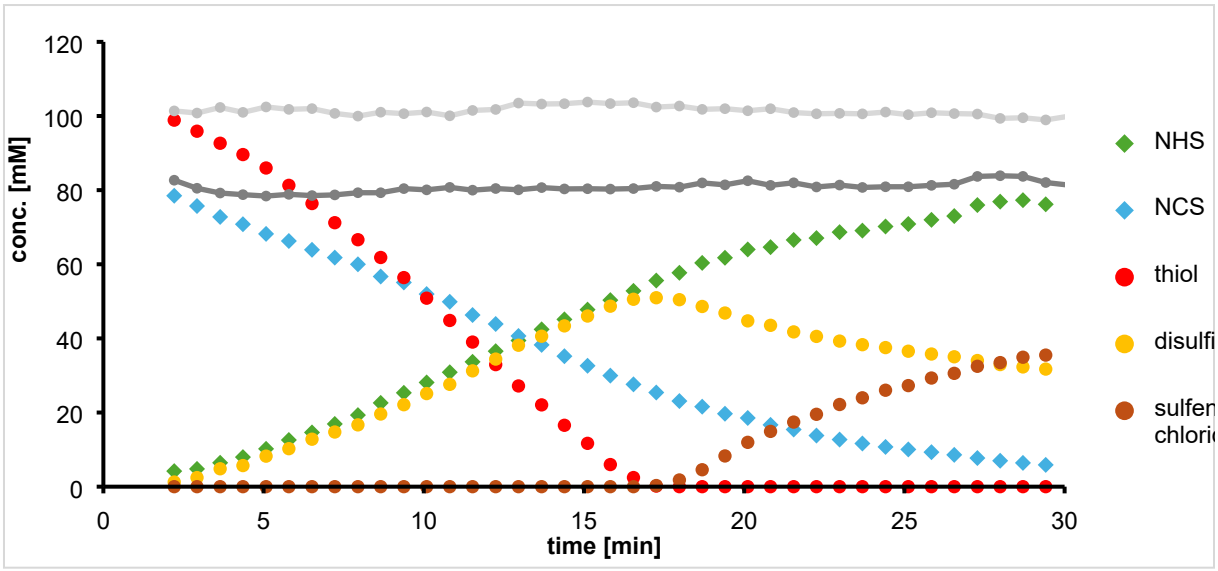


Figure S34. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 75 mM NCS (**2**), 100 mM thiol **1**, 6 mM H_2O, temperature = 25 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

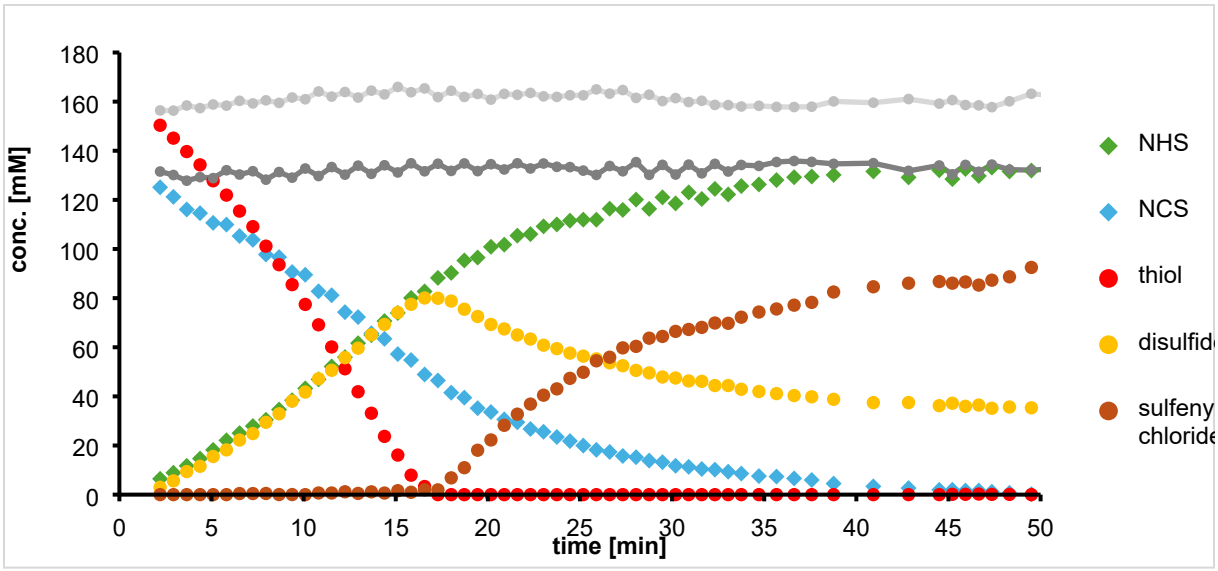


Figure S35. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 120 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

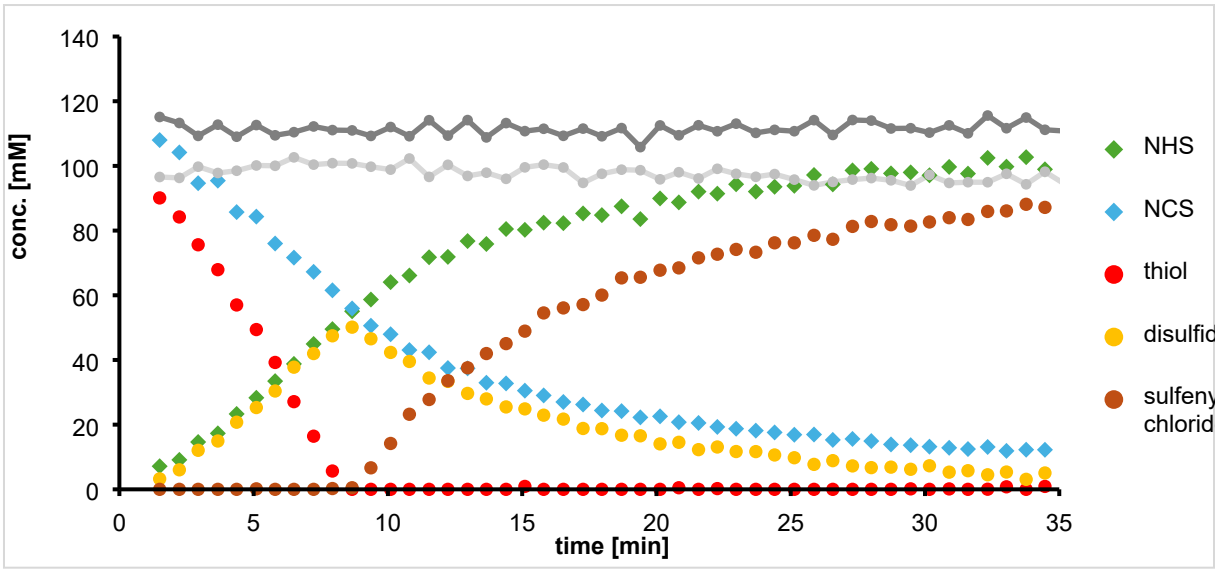


Figure S36. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 100 mM NCS (**2**), 100 mM thiol **1**, 10 mM H_2O, temperature = 25 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

6.3 Kinetic Fit

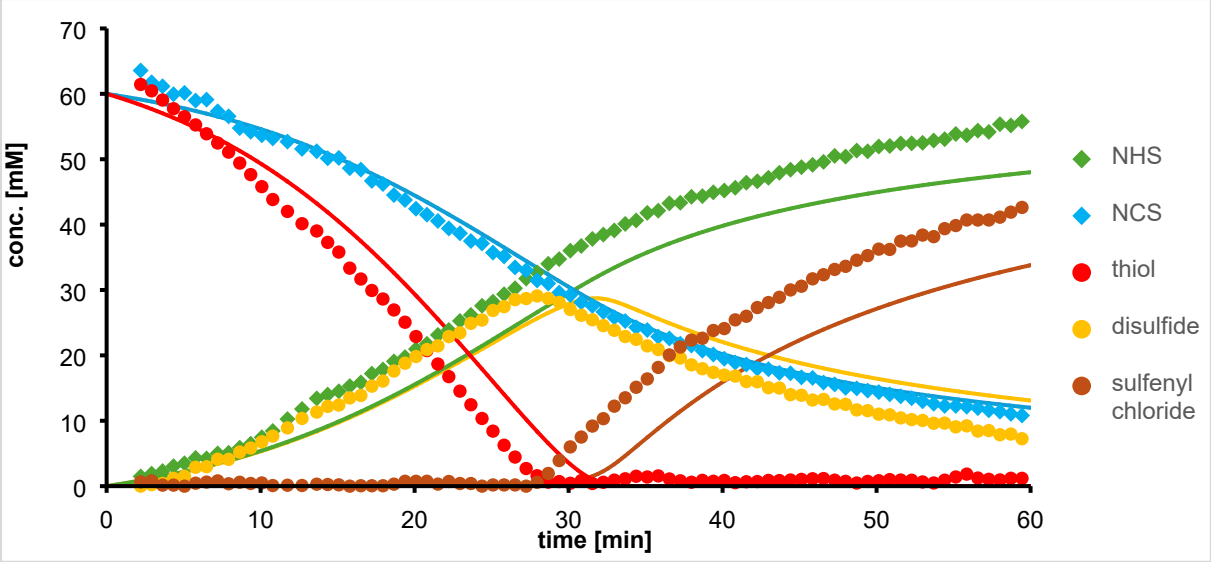


Figure S37. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Experiment was used for fitting, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

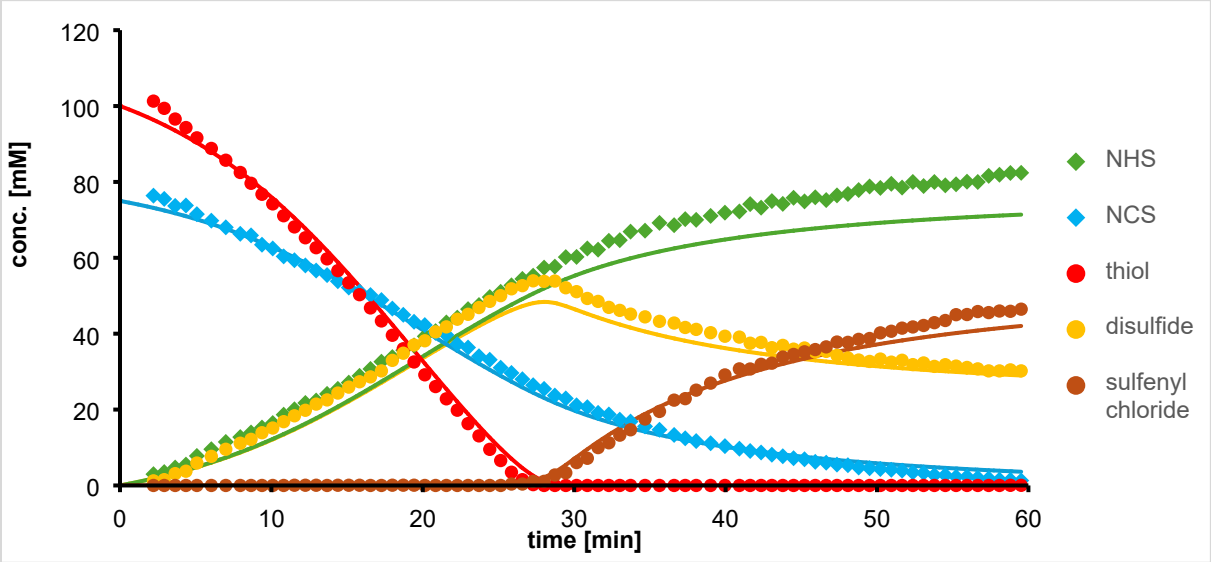


Figure S38. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 75 mM NCS (**2**), 100 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Experiment was used for fitting, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

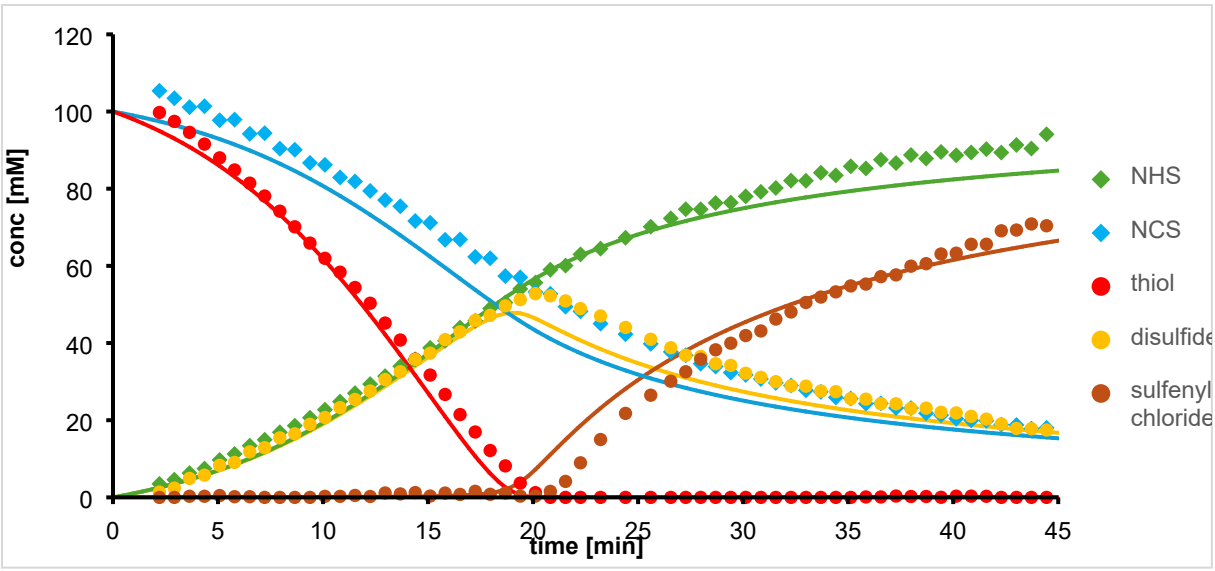


Figure S39. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 100 mM NCS (**2**), 100 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Experiment was used for fitting, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

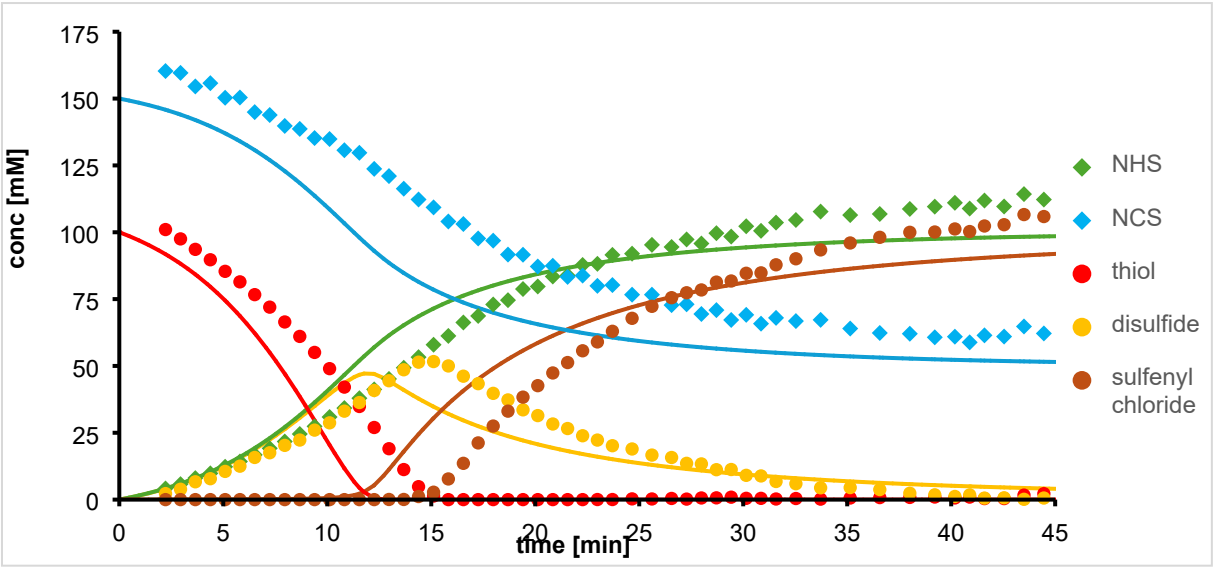


Figure S40. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 150 mM NCS (**2**), 100 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Experiment was used for fitting, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

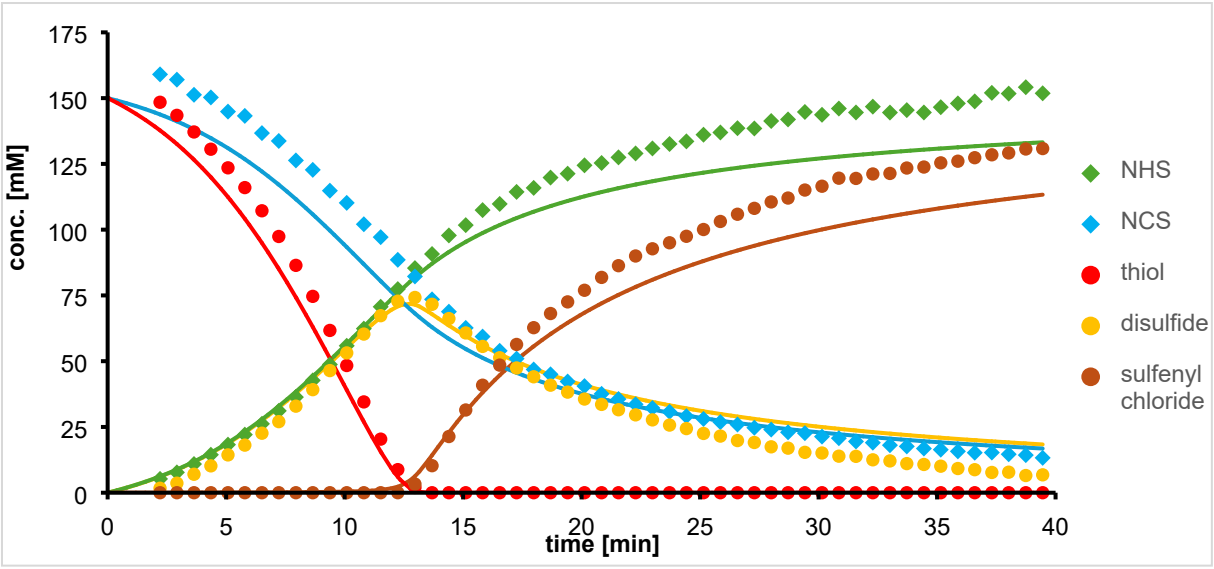


Figure S41. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Experiment was used for fitting, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

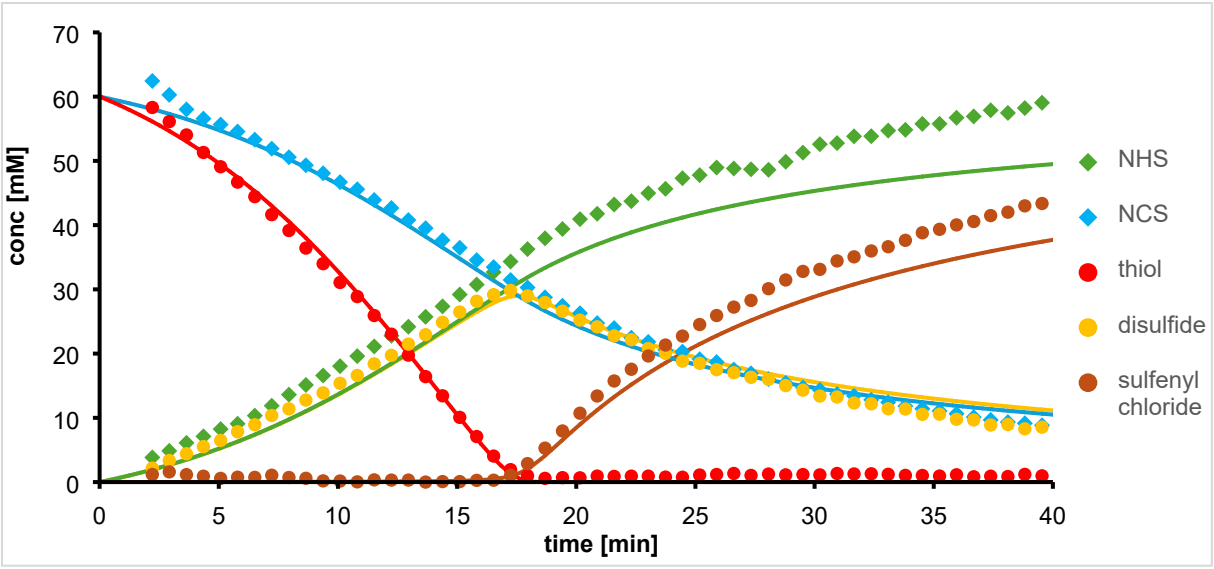


Figure S42. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 0 mM H_2O, temperature = 35 °C. Experiment was used for fitting, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

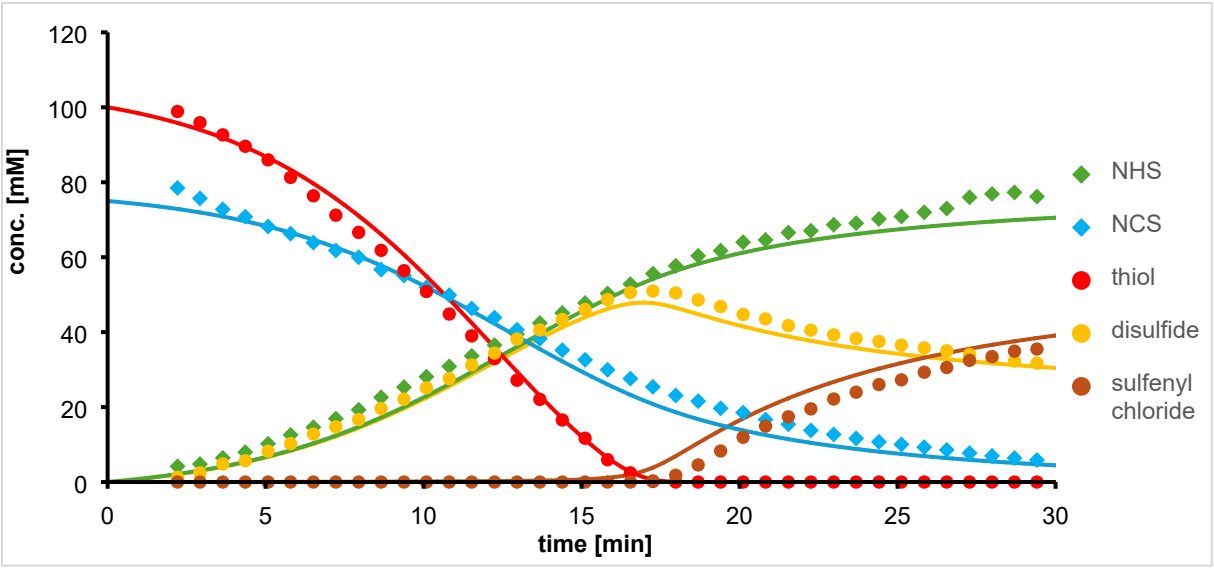


Figure S43. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 75 mM NCS (**2**), 100 mM thiol **1**, 0 mM H_2O, temperature = 35 °C. Experiment was used for fitting, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

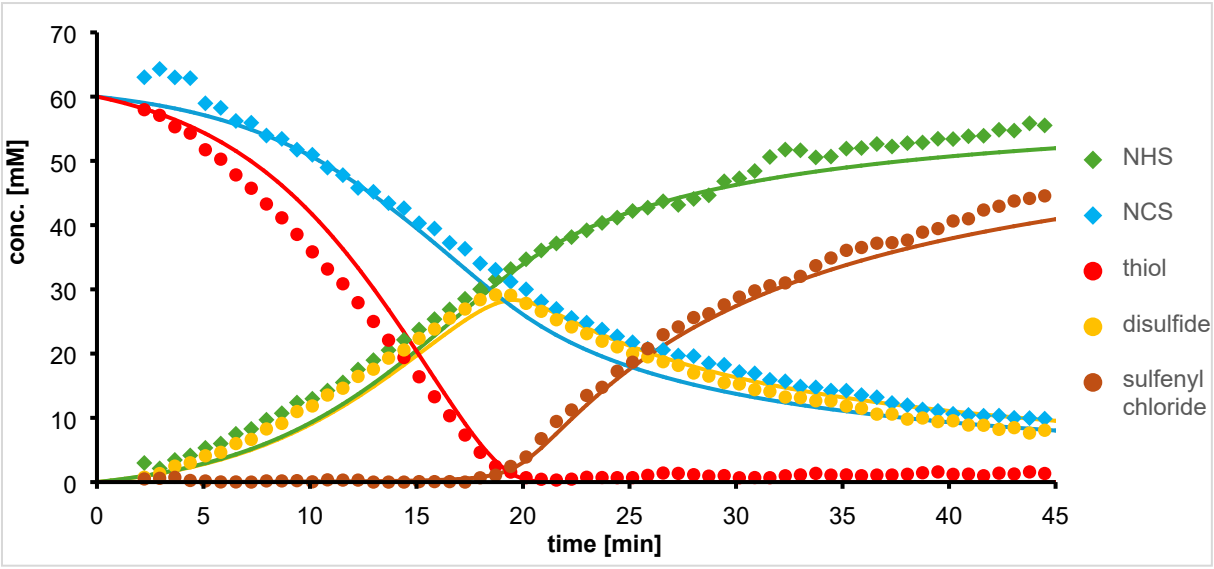


Figure S44. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 6 mM H_2O, temperature = 25 °C. Experiment was used for fitting, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

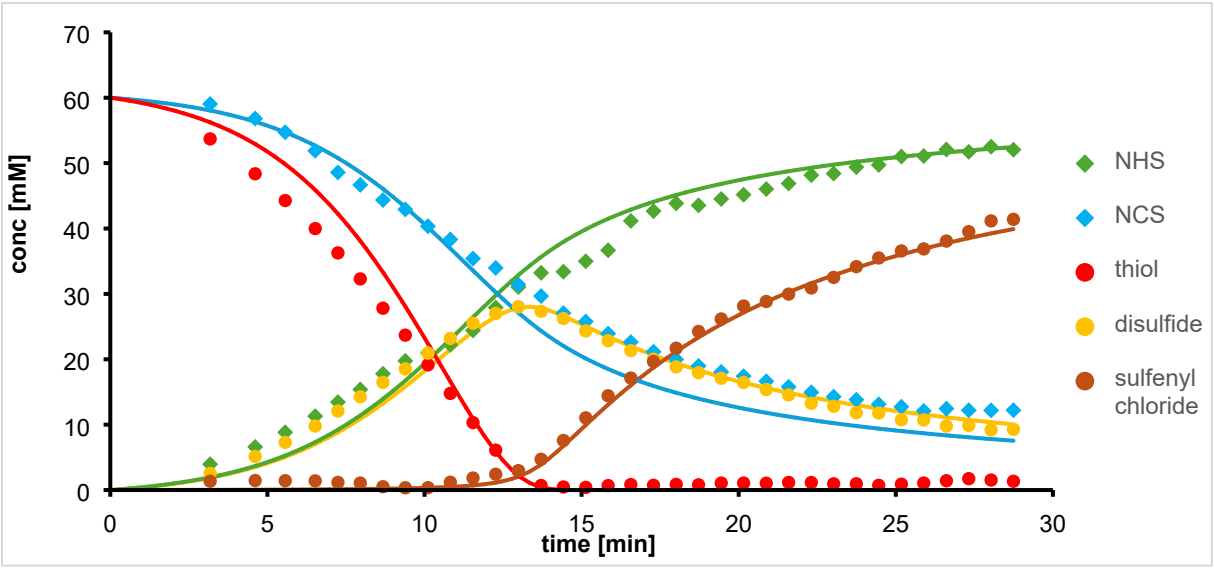


Figure S45. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 15 mM H_2O, temperature = 25 °C. Experiment was used for fitting, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

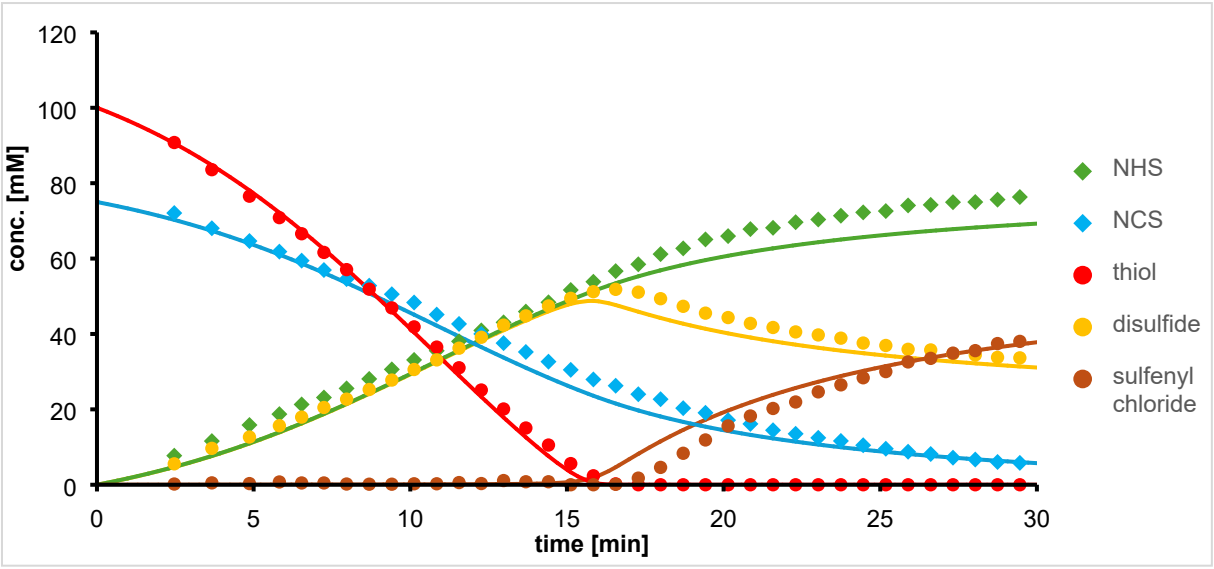


Figure S46. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 75 mM NCS (**2**), 100 mM thiol **1**, 6 mM H_2O, temperature = 25 °C. Experiment was used for fitting, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

6.4 Reaction Predictions

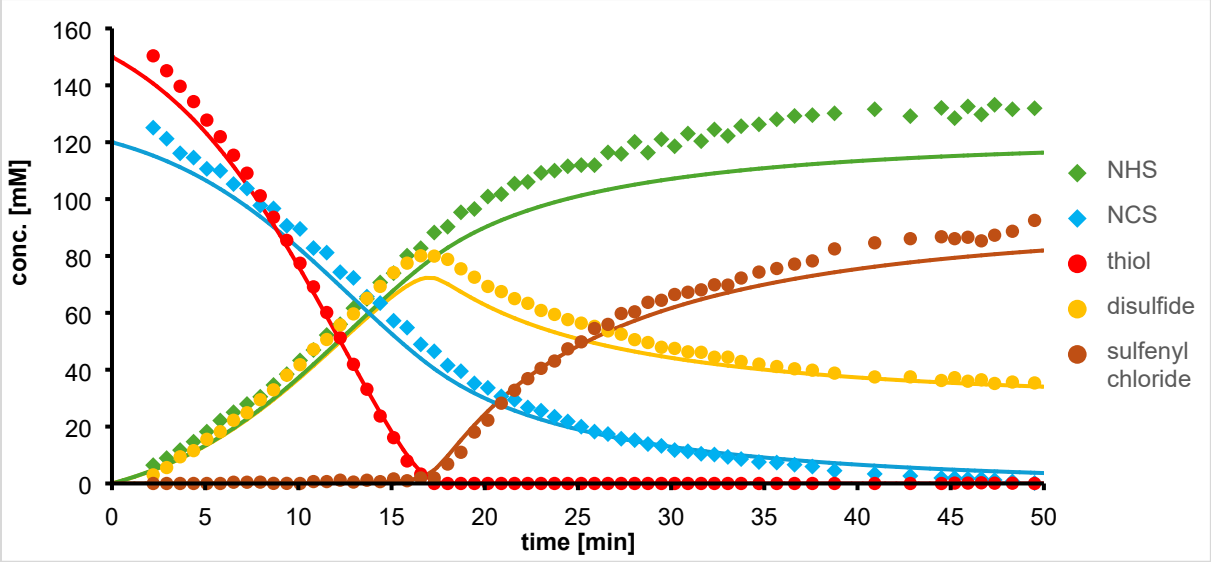


Figure S47. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 120 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 25 °C. Experiment was used for validation, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

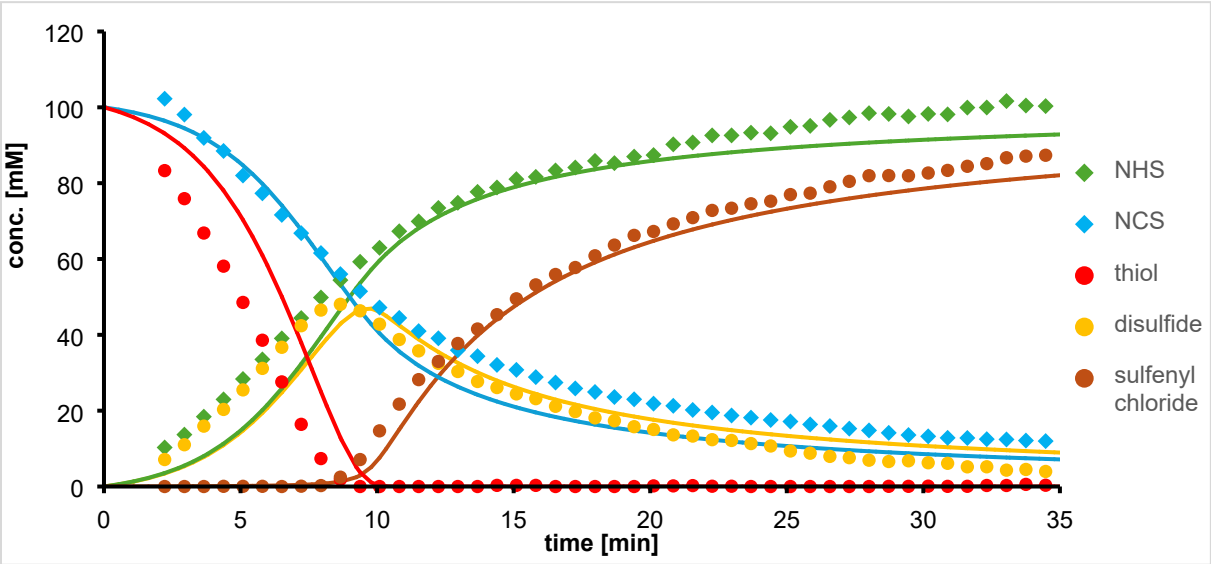


Figure S48. Reaction profile of the reaction of **1** and **2** performed in re-circulation. Initial concentrations: 100 mM NCS (**2**), 100 mM thiol **1**, 10 mM H_2O, temperature = 25 °C. Experiment was used for validation, the experimental data depicted is as dots and diamonds, model predicted fit depicted as lines of the same color.

7 Single pass Reactions

7.1 General Procedure

All single-pass flow experiments were conducted following the same general procedure. The different initial regent concentrations and temperatures of individual experiments are summarized in table S6. Two feed solutions were prepared by weighing the desired amount of the starting material **1** and NCS (**2**) reagent into a volumetric flask (50 mL). Trifluorotoluene (0.73 g) was weighed into both flasks, before the solids were dissolved in dry EtOAc and the volumetric flasks subsequently filled to the mark with dry EtOAc. Both solutions were then ultrasonicated until **2** was completely dissolved. After the water bath was heated to the desired temperature, the reactor system was flushed with dry EtOAc at a flow rate of 1 mL/min for five minutes. Thereafter, the pumps were emptied, the EtOAc exchanged for the preprepared feed solutions, and the pumps restarted at 1 mL/min each (total flow = 2 mL/min; residence time (t_{res}) = 1.25 min). The reactor was operated for five minutes (four reactor volumes) and then three spectra collected, before both flow rates were changed. After each change to the flow rates two reactor volumes were allowed to pass through, enabling the reactor system to reach steady state before spectra collection was started. A total of 12 different total flow rates (2, 1.6, 1.2, 1, 0.88, 0.75, 0.66, 0.6, 0.55, 0.5, 0.45 and 0.4 mL/min) and residence times (1.25, 1.56, 2, 2.5, 2.84, 3.33, 3.79, 4.17, 4.55, 5, 5.56 and 6.25 min) were investigated. The raw spectra were imported into PEAXACT to obtain the concentrations for the different species. The reported concentration values were calculated as a mean value of three steady state spectra and used to fit rate constants and activation energies for the six-step reaction model described in table S7.

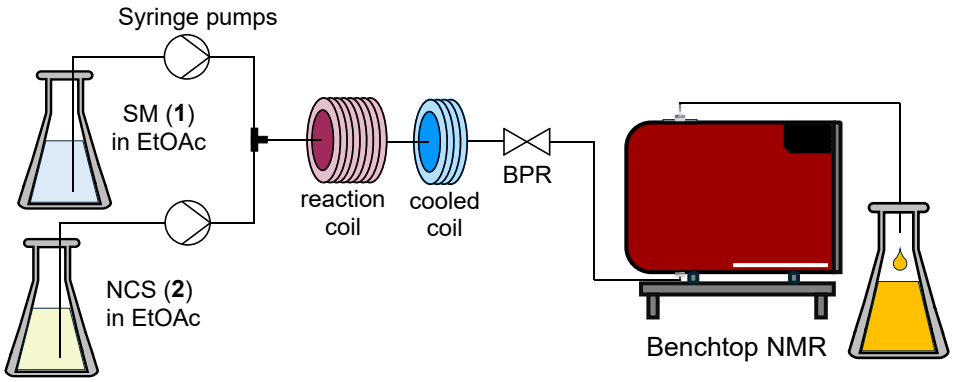


Figure S49. Schematic depiction of the flow setup used for the single-pass experiments.

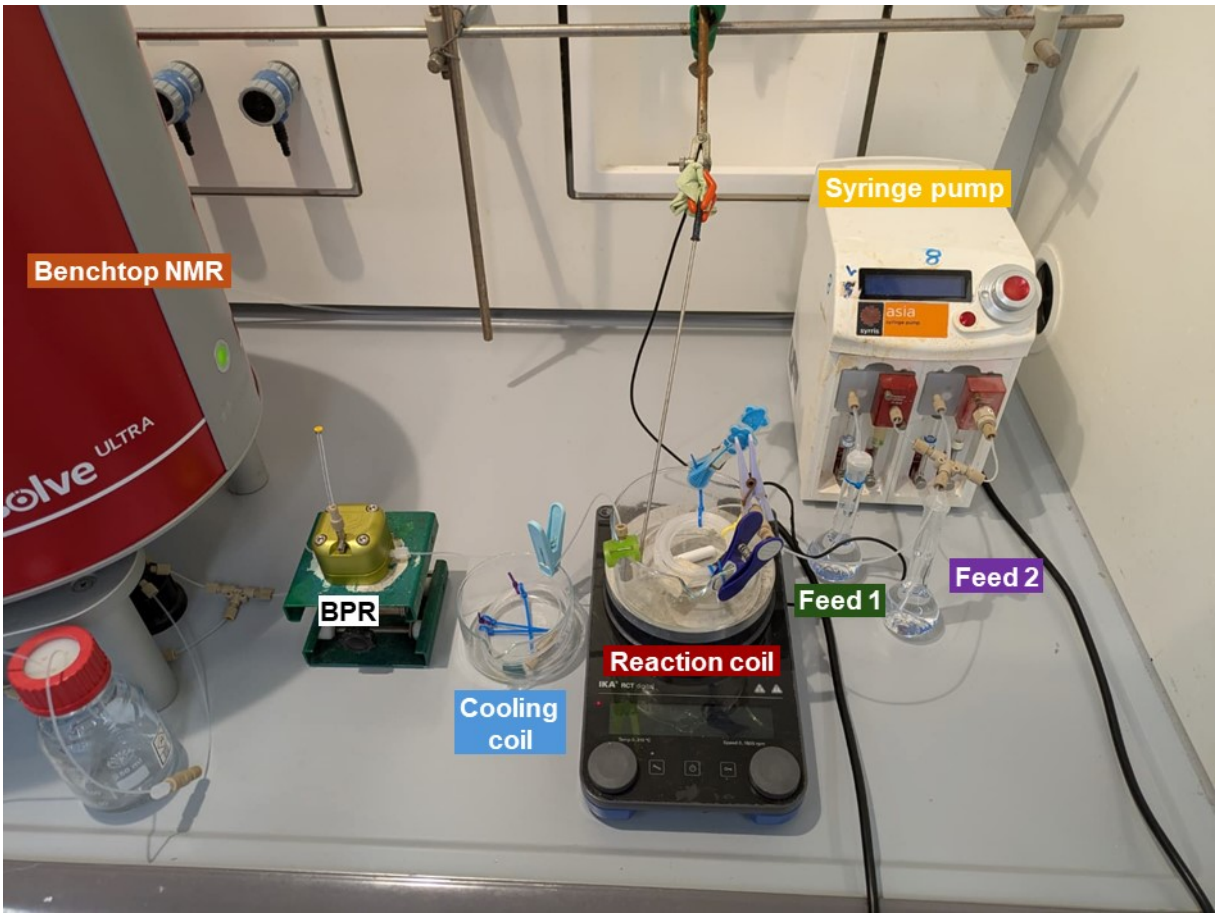


Figure S50. Labeled image of the flow setup used for the single pass experiments.

Table S6. Initial concentrations of NCS (**2**), starting material **1**, H_2O and water bath temperature for single pass flow experiments. Reaction profiles of these experiments are depicted in figures S51-S57.

Experiment	NCS conc. [mM]	Thiol conc. [mM]	H_2O conc. [mM]	T. [°C]
1	60	60	-	80
2	150	150	-	80
3	150	150	-	70
4	150	150	-	50
5	60	60	6	70
6	60	60	12	70
7	60	60	12	80

Table S7. Fitted rate constants and activation energies for the six-step reaction network, derived from the seven experiments described in table S6. Values stated without deviation were fitted with high uncertainty. Reaction profiles with the corresponding model fit are depicted in figures S58-S64. Standard error based on 95% confidence limit. [1] Unit of rate constant for sixth reaction step: [M^{-2} min^{-1}]. [2] Model fitting showed low temperature sensitivity for second reaction step.

Reaction Equations	k ± SE [M^{-1} min^{-1}]	E_a ± SE [kJ/mol]
thiol + NCS → sulfenyl chloride + NHS	0.24 ± 0.02	25.5 ± 0.4
thiol + sulfenyl chloride → disulfide + HC	606	-[2]
NCS + HCl → Cl_2 + NHS	0.07 ± 0.01	79.4 ± 0.3
thiol + Cl_2 → sulfenyl chloride + HCl	13.2 ± 3.7	13.7 ± 0.2
disulfide + Cl_2 → 2 sulfenyl chloride	24.7 ± 3.7	7.9 ± 0.2
NCS + HCl + H_2O → Cl_2 + NHS + H_2(	32.2 ± 1.9[1]	65 ± 0.6

7.2 Experimental Data

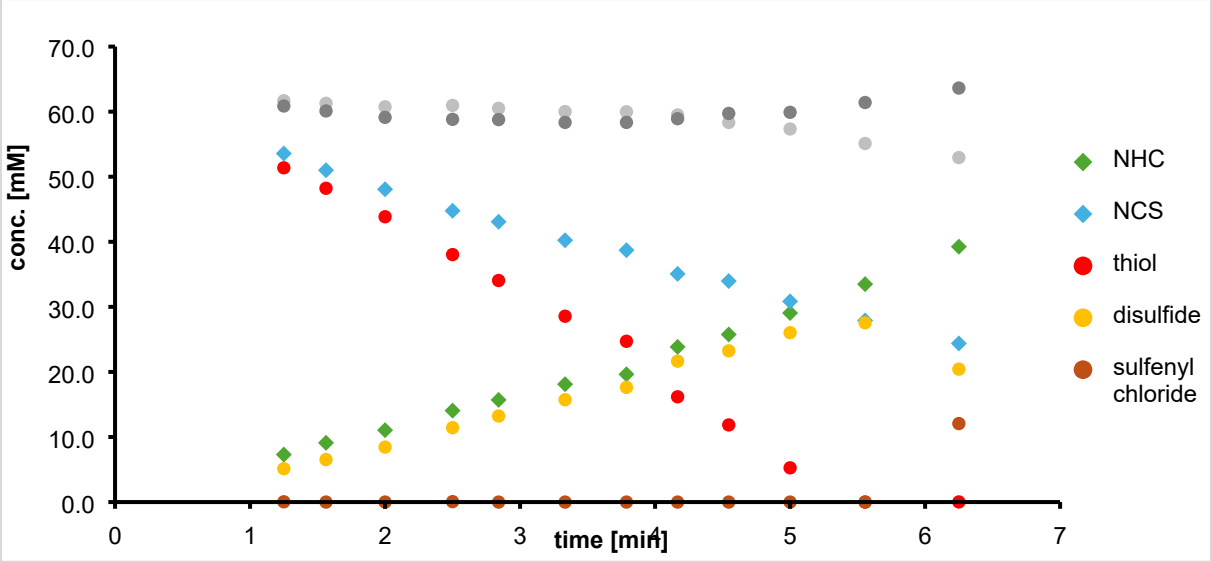


Figure S51. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 0 mM H_2O, temperature = 80 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

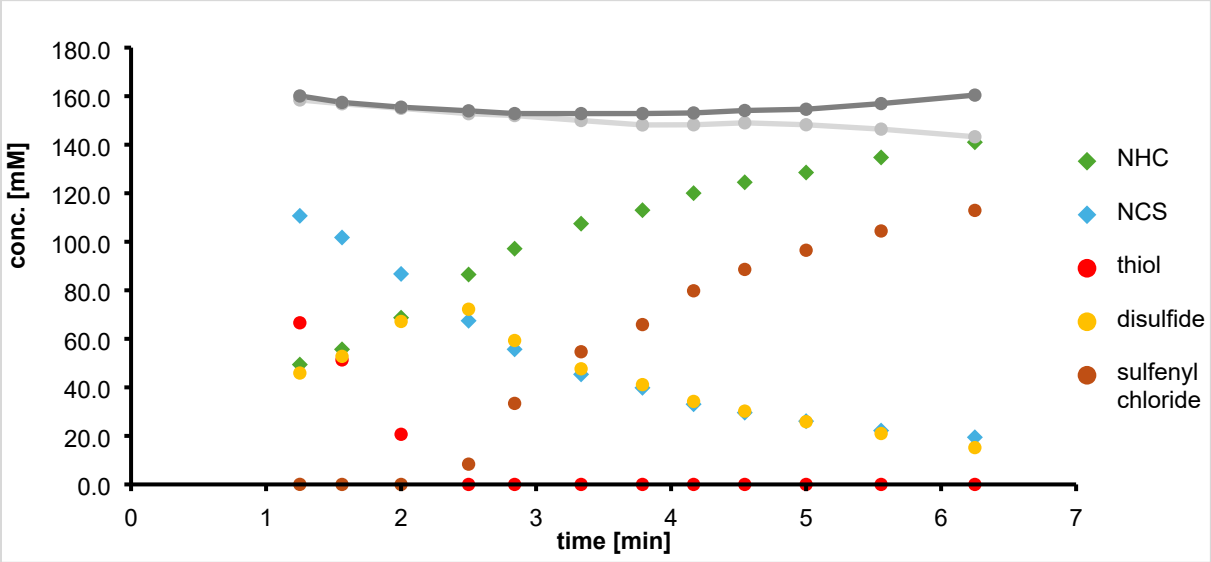


Figure S52. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 80 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

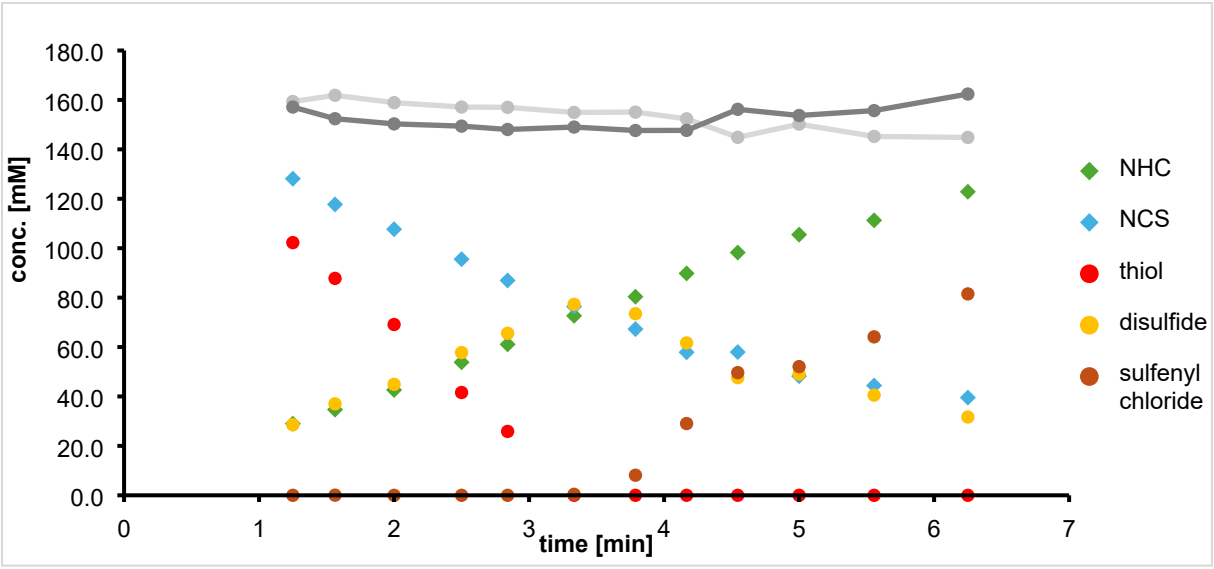


Figure S53. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 70 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

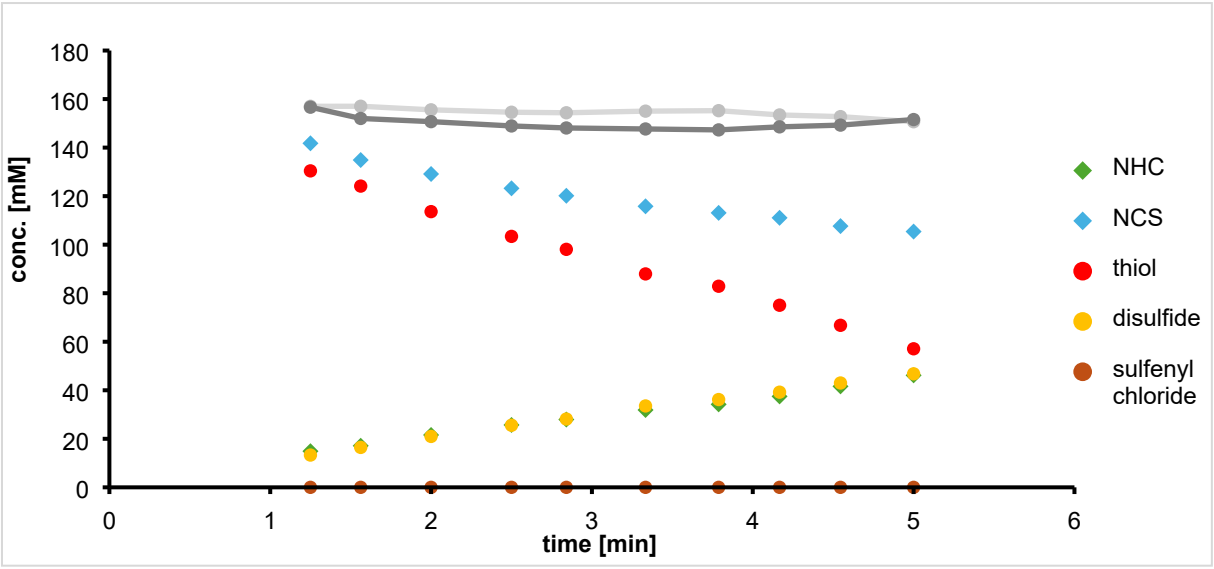


Figure S54. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 50 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey. Only ten instead of the usual 12 data points were obtained in this run.

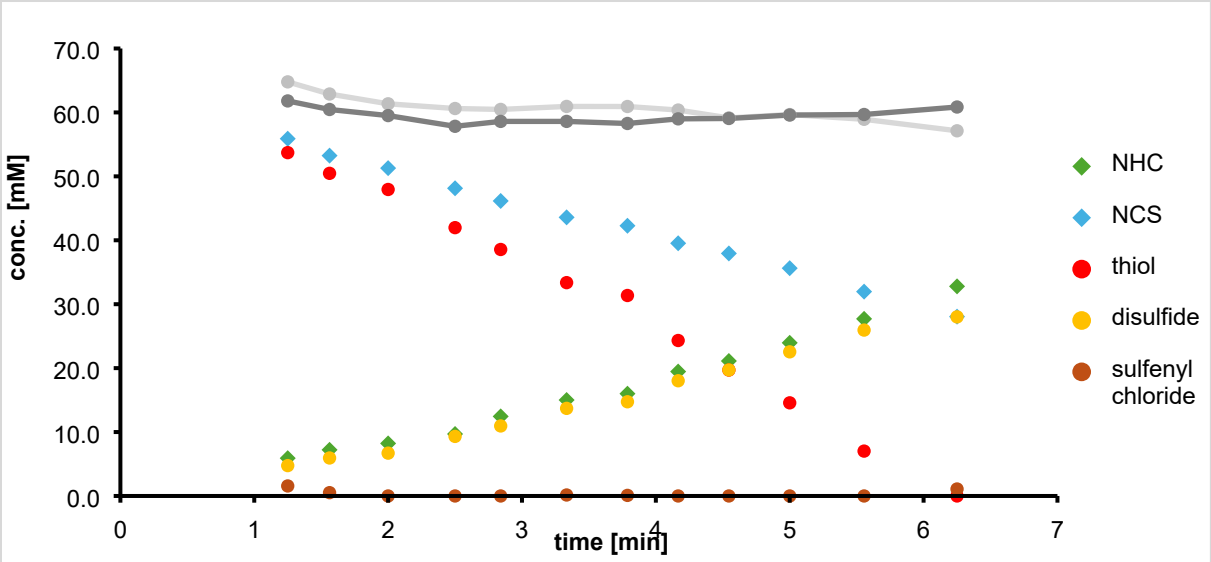


Figure S55. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 6 mM H_2O, temperature = 70 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

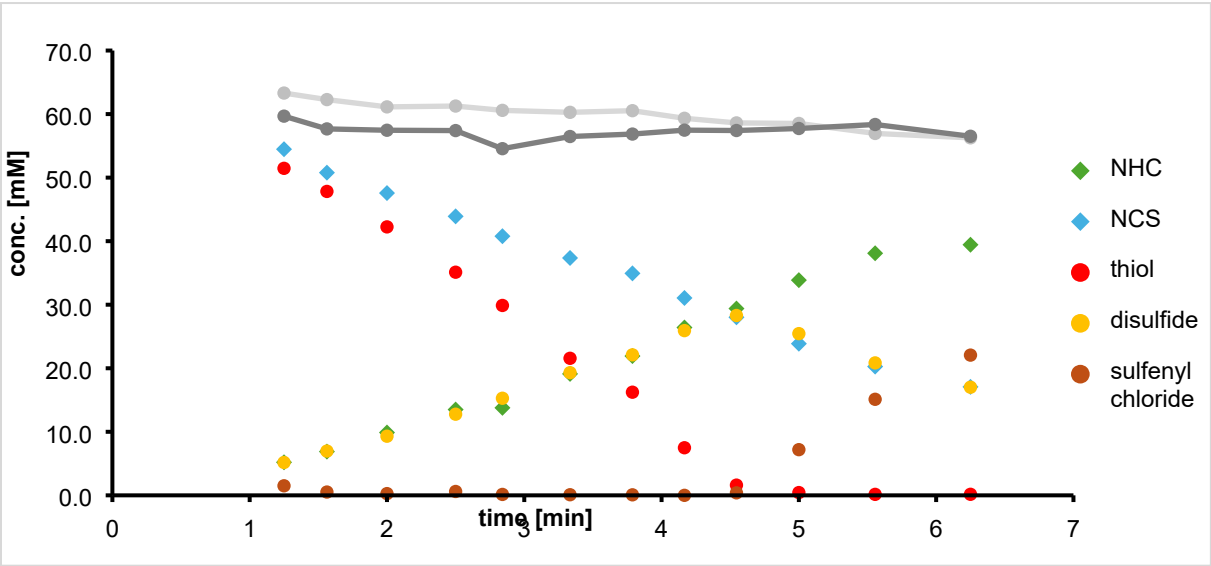


Figure S56. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 12 mM H_2O, temperature = 70 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

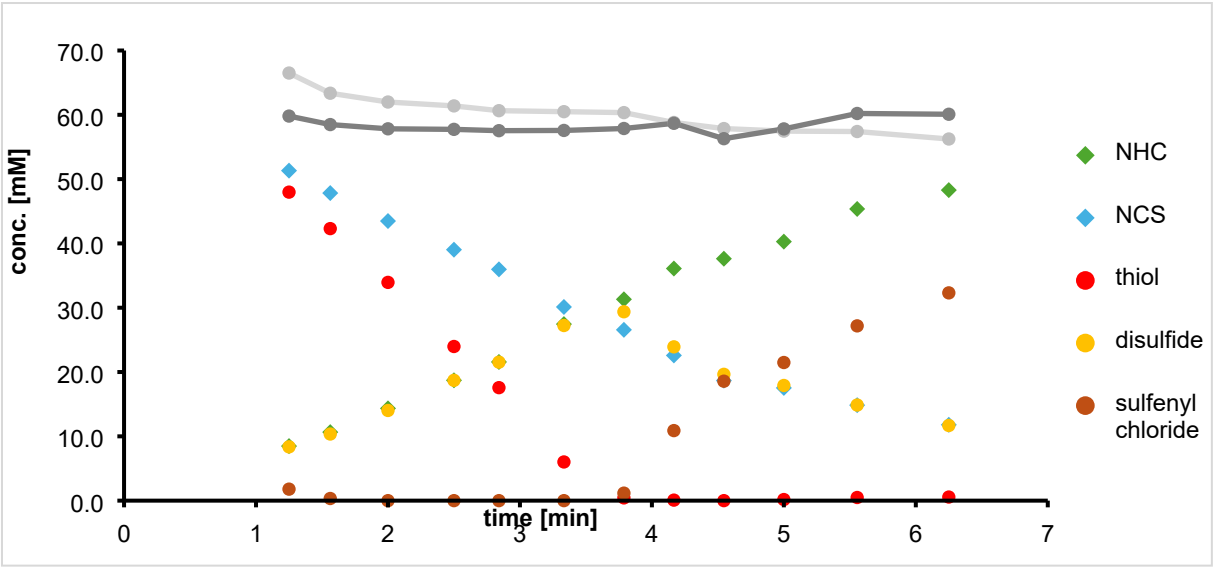


Figure S57. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 12 mM H_2O, temperature = 80 °C. Mass balance of sulfur species (thiol **1** + disulfide **4** + sulfenyl chloride **3**) depicted in light grey. Mass balance of NCS (**2**) and NHS (**5**) depicted in dark grey.

7.3 Kinetic Fit

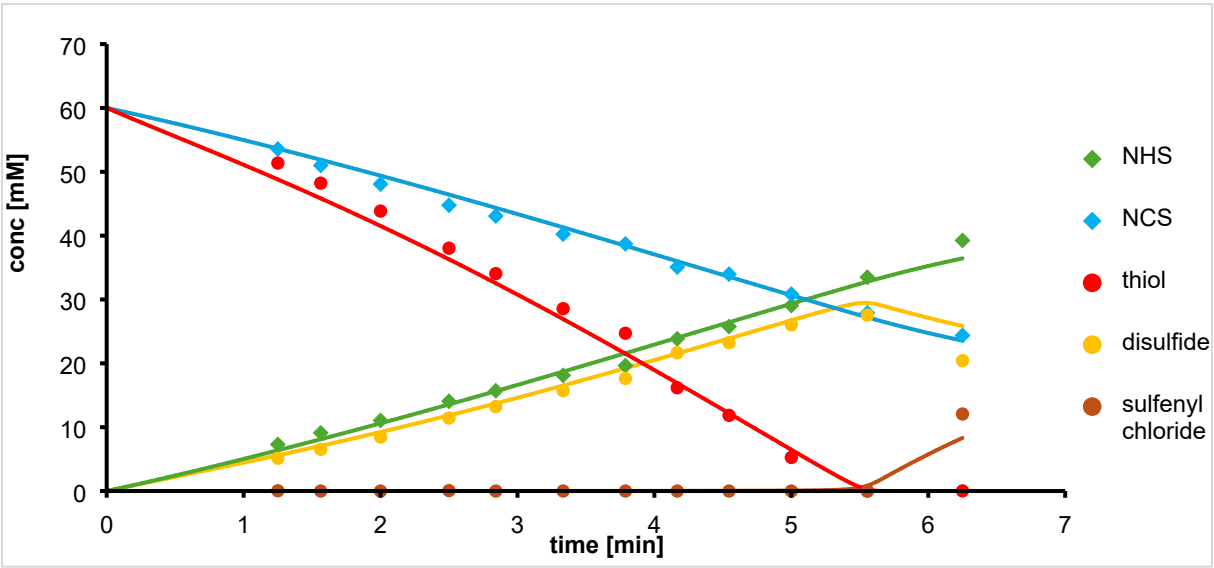


Figure S58. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 0 mM H_2O, temperature = 80 °C. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

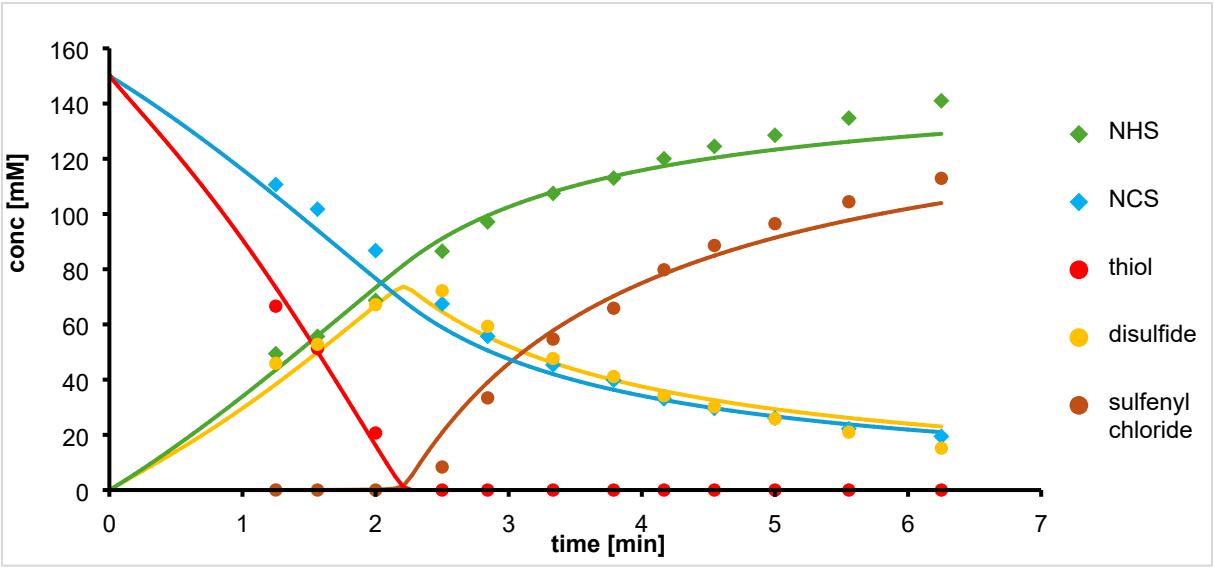


Figure S59. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 80 °C. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

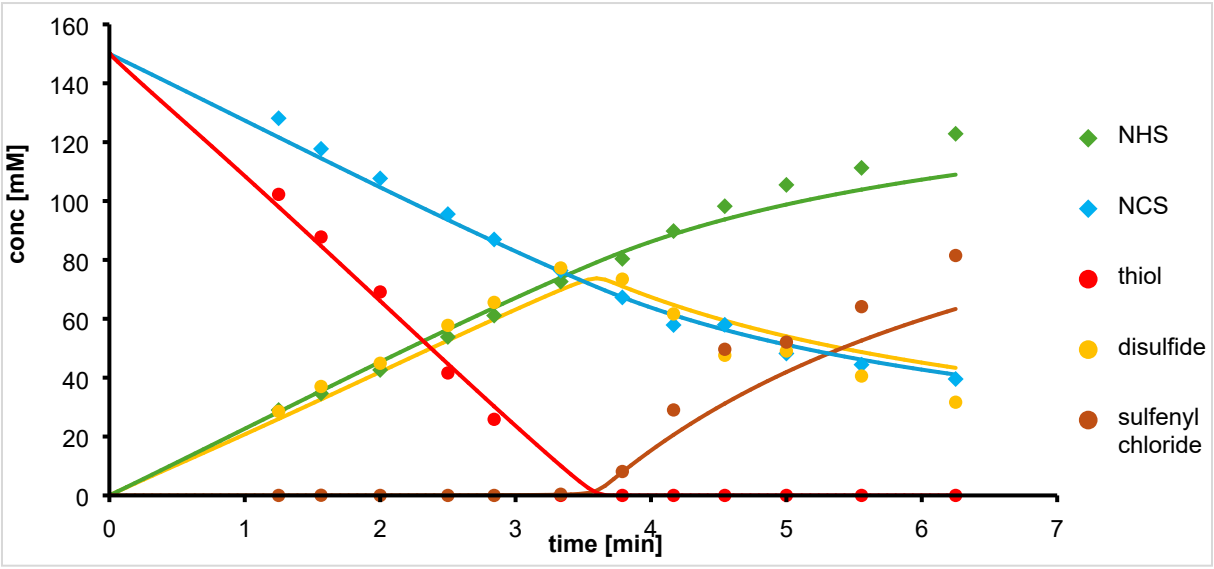


Figure S60. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 70 °C. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

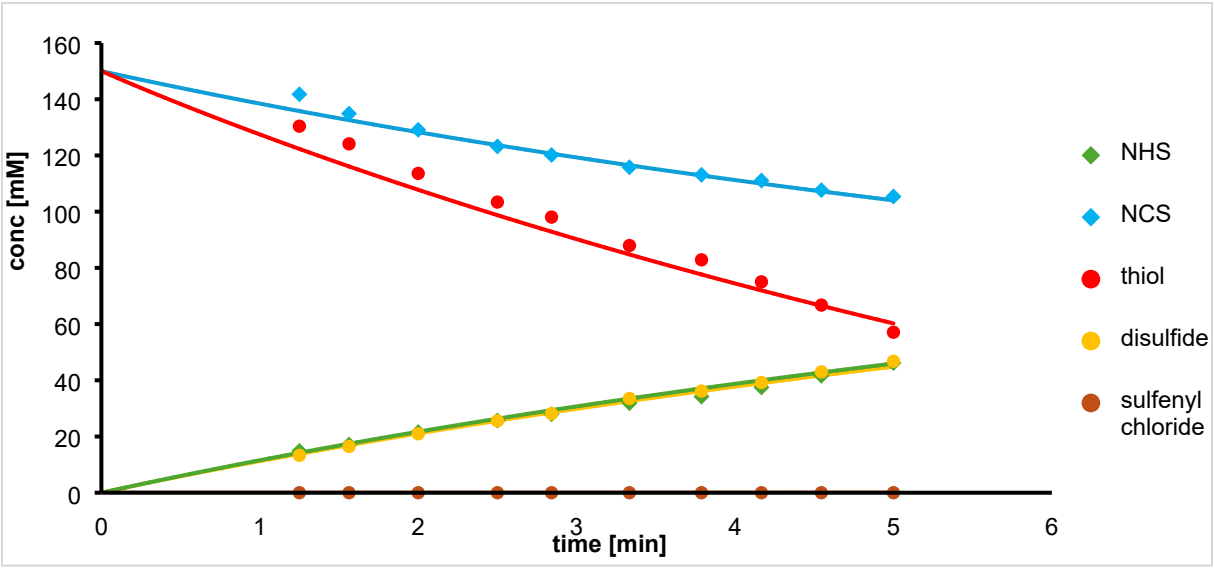


Figure S61. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 150 mM NCS (**2**), 150 mM thiol **1**, 0 mM H_2O, temperature = 50 °C. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

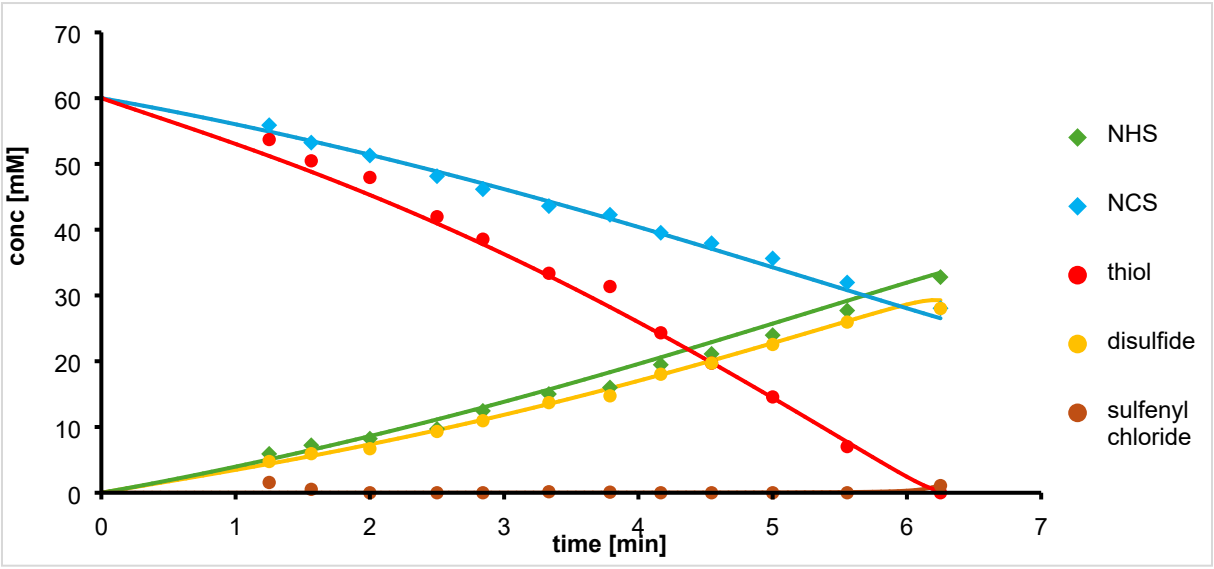


Figure S62. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 6 mM H_2O, temperature = 70 °C. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

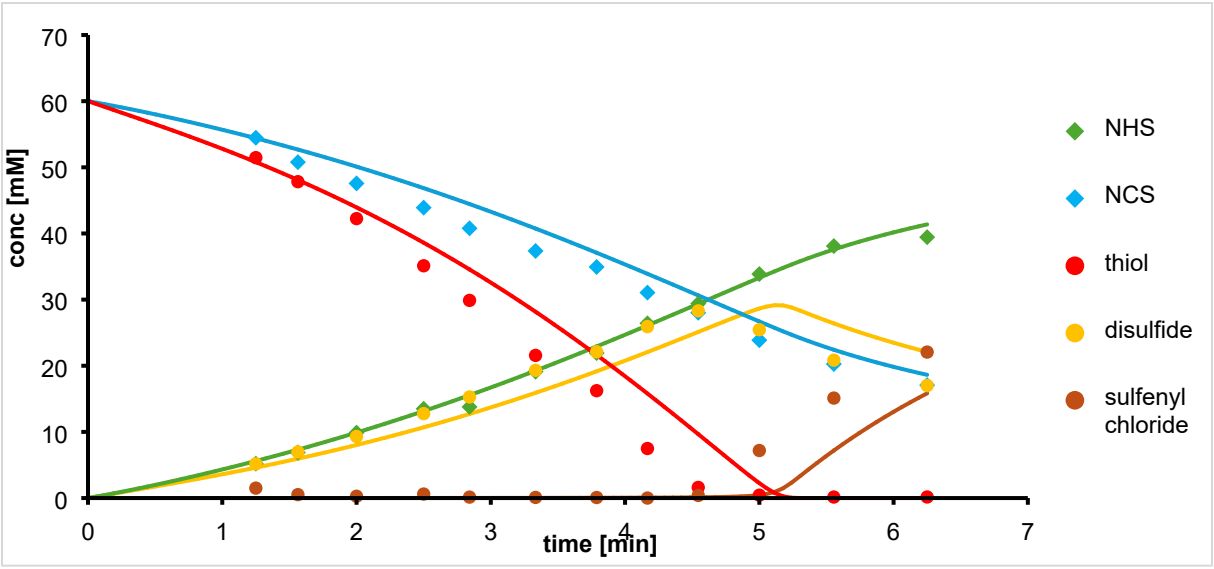


Figure S63. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 12 mM H_2O, temperature = 70 °C. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

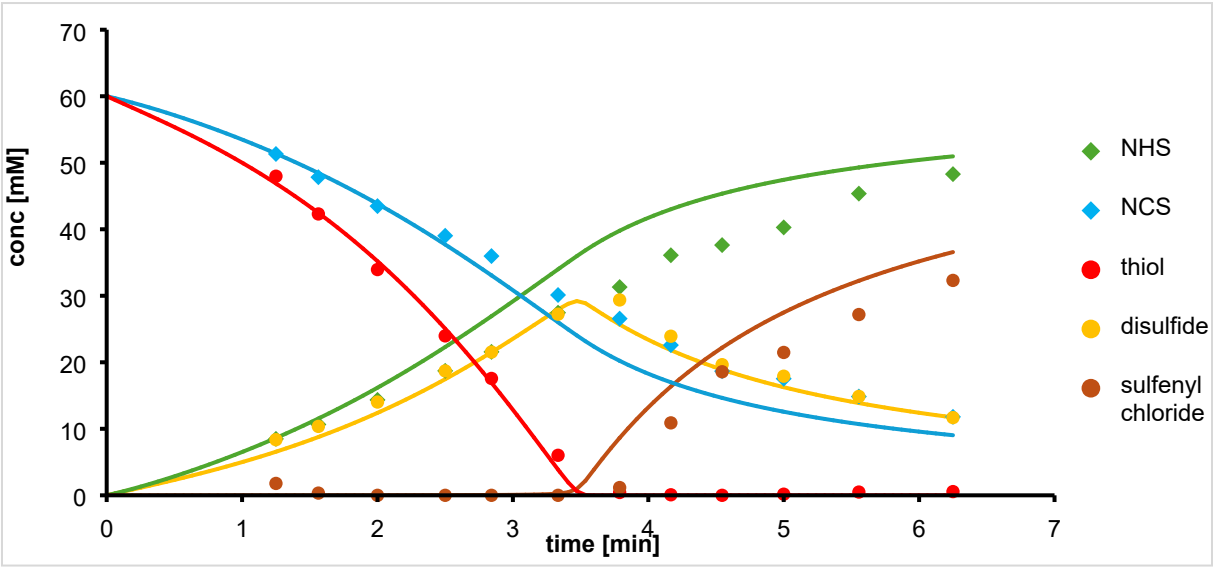


Figure S64. Reaction profile of the reaction of **1** and **2** performed in single pass flow. Initial concentrations: 60 mM NCS (**2**), 60 mM thiol **1**, 12 mM H_2O, temperature = 80 °C. Experimental data depicted as dots and diamonds, model predicted fit depicted as lines of the same color.

8 Comparison Data from ^{1}H and ^{19}F data

As mentioned in the manuscript, accurate quantification of compounds **1**, **3** and **4** was very hard to achieve due to the noisy baseline of the ^{19}F spectra. This is a known limitation of the used benchtop NMR device, that has since been solved by the manufacturer in newer instruments. To compare the obtainable reaction profiles, the chlorination of **1** with **2** was carried out in an NMR tube at initial concentrations of 120 mM and 150 mM respectively. The reaction was monitored with an alternating ^{1}H (4 scans, 10 s delay) and ^{19}F protocol (4 scans, 15 s delay) with two seconds delay in between measurements, resulting in one ^{1}H and ^{19}F spectrum every 104 seconds. A moving average of three was applied for smoothing. The reaction profile obtained from indirect hard modeling of the ^{1}H spectra (Fig. S65) and integration of the ^{19}F spectra (Fig. S66) are depicted below.

Both profiles show a similar trend, the decrease of thiol **1** and increase of sulfenyl chloride **3,** over the course of the reaction. However, the reaction profile obtained from integration of the ^{19}F spectra is less stable, due to the noisy baseline, a problem that we were also unable to solve through IHM. Consequently, we continued to quantify all species through the ^{1}H spectrum, which had the additional benefit of a 2.5 times increase in measurement points, due to a shorter NMR protocol.

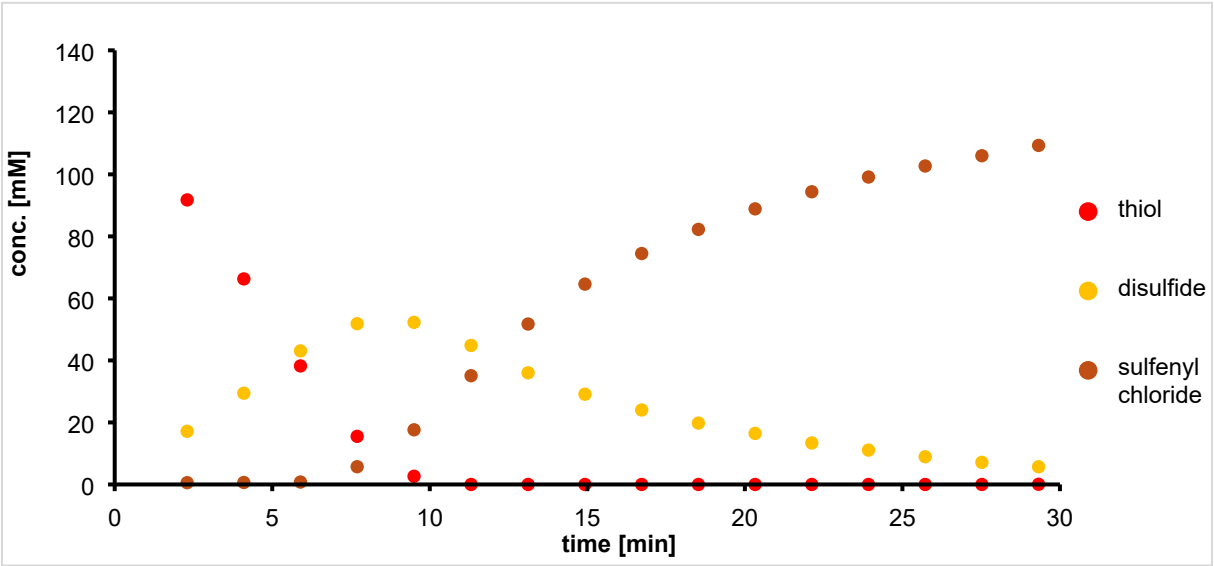


Figure S65. Reaction profile of the reaction of **1** and **2** in an NMR tube at ambient conditions, depicting **1**, **3** and **4**. Initial concentrations: 150 mM NCS (**2**), 120 mM thiol **1**. Concentration values derived from IHM of ^{1}H spectra.

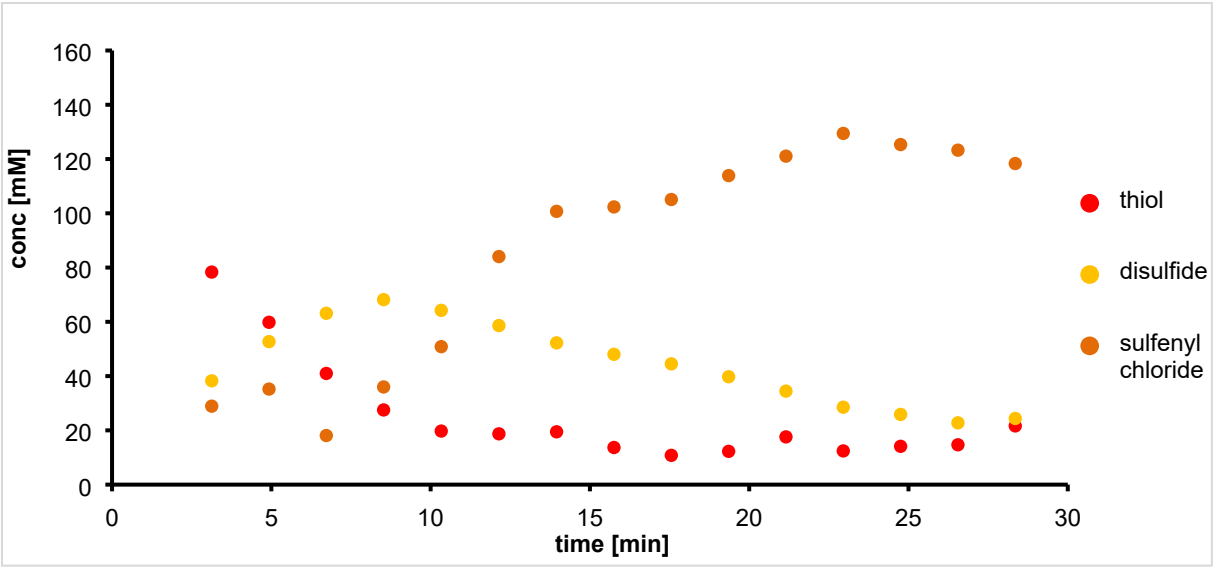


Figure S66. Reaction profile of the reaction of **1** and **2** in an NMR tube at ambient conditions, depicting **1**, **3** and **4**. Initial concentrations: 150 mM NCS (**2**), 120 mM thiol **1**. Concentration values derived from integration of ^{19}F spectra.

9 References

[1] M. S. Abaee, M. M. Mojtahedi and S. Navidipoor, *Synth. Commun*., 2010, **41**, 170-176.

[2] M. C. Baluja, R. Bravo, M. Pintos, M. I. Paz Andrade, G. Roux-Desgranges and J.-P. E.Grolier, *Calorim. Anal. Therm*., 1985, **16**, 138-144.