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I. General

Commercial reagents were used without further purification. Dithiothreitol (DTT) was from Research Products International (Mt. Prospect, IL). Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) was from CHEM-IMPEX INT'L INC. (Wood Dale, IL). Pre-activated succinimidyl ester TentaGel resin with a particle size of 130 μm was from Rapp Polymere (Tübingen, Germany). N-Methyl-2-pyrrolidone (NMP), N,N-diisopropylethylamine (Hünig's base), cystamine dihydrochloride (**3**), selenocystamine dihydrochloride (**8**), racemic lipoic acid (**6**), 2-mercaptoethanol, 2,2′-dithiodiethanol (βME^{ox}, **4**), $trans$-4,5-dihydroxy-1,2-dithiane (DTT^{ox}, **2**), papain (lyophilized powder from papaya latex), N_α-benzoyl-L-arginine-4-nitroanilide hydrochloride, and S-methyl methanethiosulfonate were from Sigma Chemical (St. Louis, MO). DTBA, DTBA^{ox} (**1**), BMC, and BMC^{ox} (**5**) were synthesized as described previously.[1]

All glassware was oven or flame-dried, and reactions were performed under N_2(g) unless stated otherwise. Dichloromethane, diethyl ether, and tetrahydrofuran were dried over a column of alumina. Dimethylformamide and triethylamine were dried over a column of alumina and purified further by passage through an isocyanate scrubbing column. Flash chromatography was performed with columns of 40–63 Å silica, 230–400 mesh from Silicycle (Québec City, Canada). Thin-layer chromatography (TLC) was performed on plates of EMD 250-μm silica 60-F$_{254}$. The synthesis of immobilized DTBA was performed with a solid-phase peptide synthesis vessel from Chemglass (Vineland, NJ). Ellman's assay for sulfhydryl groups was performed with a Varian Cary 50 Bio UV-Vis spectrophotometer. Equilibrium and reduction potential studies were performed on an analytical HPLC (Waters system equipped with a Waters 996 photodiode array detector, Empower 2 software and a Varian C18 reverse phase column). Analytical samples of DTBA^{ox} (**1**), BMC^{ox} (**5**), selenoDTBA^{ox} (**7**), and selenoBMC^{ox} (**9**) were obtained by using a Shimadzu (Kyoto, Japan) preparative HPLC, equipped with a C18 reverse phase preparative column, a Prominence diode array detector, and fraction collector. Kinetic studies on proteins

were carried out using a Varian Cary 400 Bio UV-Vis spectrometer with a Cary temperature controller at the Biophysics Instrumentation Facility at Madison (BIF). All NMR spectra were acquired at ambient temperature with a Bruker Avance III 500ii with cryoprobe spectrometer at the National Magnetic Resonance Facility at Madison (NMRFAM), and were referenced to TMS or a residual protic solvent.
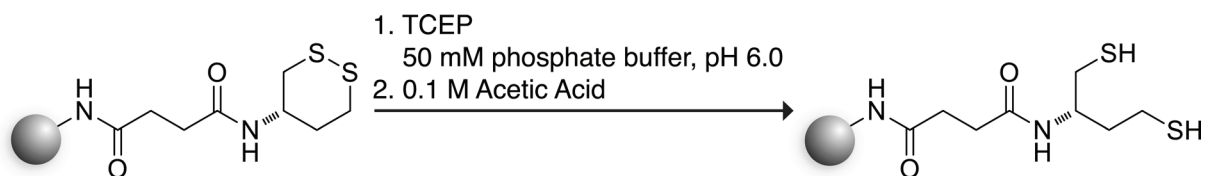
II. Synthesis

### Coupling of DTBA^{ox} to pre-activated TentaGel resin
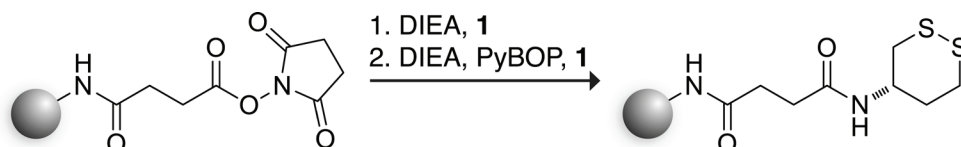
Preactivated succinimidyl ester TentaGel resin (1 g; ~0.21 mmol/g) with a particle size of 130 μm was placed in a solid-phase peptide synthesis vessel. As a pretreatment, the resin was allowed to swell in 5 mL of N-methyl-2-pyrrolidone (NMP) for 5 min while bubbling $N_2(g)$ through the solution. The NMP was then removed by vacuum filtration and the process was repeated two more times. To the resin was then added 10 mL of NMP, 0.32 mL (1.8 mmol) of N,N-diisopropylethylamine (DIEA), and 0.1224 g (0.7128 mmol) of DTBA^{ox} (1, prepared as described previously).[1b] The resulting mixture was allowed to react for 60 min while bubbling $N_2(g)$ through the solution. The solution was then removed by vacuum filtration, and the resin was washed with NMP (3 × 5 mL). In a second coupling step, 10 mL of NMP, 0.4701 g (0.9034 mmol) of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP), 0.32 mL (1.8 mmol) of DIEA, and 0.1224 mg (0.7128 mmol) of DTBA^{ox} were added to the resin and allowed to react for an additional hour while bubbling $N_2(g)$ through the solution. Then, the solution was removed by vacuum filtration. The coupling of DTBA to pre-activated TentaGel resin was performed multiple times with yields ranging from 75–93% as determined by Ellman's assay for sulfhydryl groups (see below).[2]

### Reduction/Regeneration of Immobolized DTBA^{ox}

To 1 g of immobilized DTBA^{ox}, in a solid-phase peptide synthesis vessel, was added 0.2603 g (0.9081 mmol) of tris(2-carboxyethyl)phosphine hydrochloride (TCEP–HCl) in 10 mL of 50 mM sodium phosphate, pH 6.0. The mixture was allowed to react for 60 min while bubbling $N_2(g)$ through the solution. Then, the solution was removed by vacuum filtration, and the resin was washed with 0.1 M acetic acid (5 × 5 mL) to ensure that the thiol groups were protonated completely. Finally, the resin was washed methanol (3 × 5 mL) and dried under vacuum overnight.
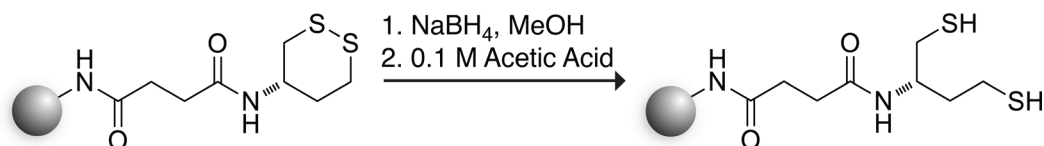
To 1 g of immobilized DTBA^{ox}, in a solid-phase peptide synthesis vessel, was added 79.41 mg (2.099 mmol) of NaBH$_4$ in 10 mL of methanol. The mixture was allowed to react for 60 min while bubbling N$_2$(g) through the solution. Then, the solvent was removed by vacuum filtration, and the resin was washed with 0.1 M acetic acid (5 × 5 mL) to ensure that the thiol groups were protonated completely. Finally, the resin was washed methanol (3 × 5 mL) and dried under vacuum overnight.
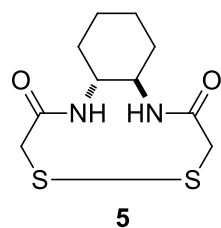
Synthesis of DTBA^{ox}
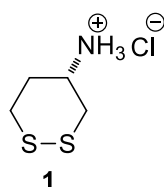
Compound **1** was synthesized from L-aspartic acid as described previously.[1b]

^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.29 (s, 3H), 3.43–3.37 (m, 1H), 3.15–3.08 (m, 2H), 3.02–2.96 (m, 1H), 2.88 (dd, J = 13.1, 10.6 Hz, 1H), 2.32–2.28 (m, 1H), 1.85–1.77 (m, 1H); **^{13}C NMR (125 Hz, DMSO-d_6)** δ = 48.8, 34.6, 32.9, 31.5; **HRMS** (ESI) calculated for [C$_4$H$_{10}$NS$_2$]$^+$ (M$^+$) requires m/z = 136.0250, found 136.0249.

Synthesis of BMC^{ox}

Compound **5** was synthesized from (±)-*trans*-1,2-diaminocyclohexane as reported previously.[1a]

^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.98 (d, J = 9.0 Hz, 1.63H), 7.76 (d, J = 8.8 Hz, 0.37H), 3.76−3.69 (m, 1.63H), 3.54–3.51 (m, 0.37H), 3.40 (d, J = 14.0 Hz, 1.63H), 3.32 (d, J = 11.4 Hz, 0.37H), 3.19 (d, J = 14.0 Hz, 1.63H), 3.08 (d, J = 11.4 Hz, 0.37H), 1.72–1.71 (m, 4H), 1.36–1.22 (m, 4H); **^{13}C NMR (125 Hz, DMSO-d_6)** δ = 169.9, 166.1, 56.6, 54.2, 44.6, 31.12, 31.05, 25.0, 24.9; **HRMS** (ESI) calculated for [C$_{10}$H$_{16}$N$_2$O$_2$S$_2$Na]$^+$ (M+Na$^+$) requires m/z = 283.0546, found 283.0532.

Synthesis of SelenoDTBA^ox
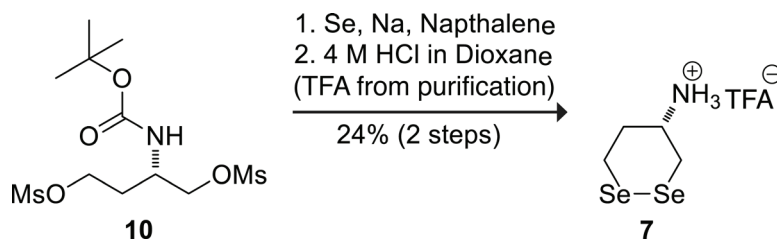
Sodium diselenide was generated *in situ* by closely following a previously described method.[3] 146.8 mg (1.859 mmol) of selenium powder and 238.3 mg (1.859 mmol) of naphthalene were placed in a flame-dried three-neck round-bottom flask. Anhydrous THF (5 mL) was added, and the resulting mixture was stirred at room temperature under $N_2(g)$. Freshly shaved sodium metal (42.74 mg, 1.859 mmol) was then added to the mixture under $N_2(g)$. The reaction mixture was allowed to stir for 2 h to enable consumption of all of the sodium metal. Compound **10** (331.0 mg, 0.9158 mmol), which was prepared as described previously,[1b] was dissolved in 10 mL of anhydrous DMF, and the resulting solution was added dropwise to the reaction mixture. After 24 h, the reaction mixture was filtered, concentrated under reduced pressure, and eluted through a silica plug with ethyl acetate (20% v/v) in hexanes. The resulting solution was then concentrated under reduced pressure, and the residue was dissolved in 4 M HCl in dioxane. After 2 h, TLC revealed the removal of the Boc group. Compound **7** was purified by reverse-phase HPLC using a preparatory C18 column and a linear gradient of 10–50% v/v acetonitrile (0.1% v/v TFA) in water (0.1% v/v TFA) over 55 min. Compound **7** eluted at 17 min and, after lyophilization, was isolated as a yellow powder (68.13 mg, 22% over two steps)

^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.02 (s, 3H), 3.43–3.36 (m, 2H), 3.25–3.21 (m, 1H), 3.14 (dd, J = 12.2 Hz, 1.9 Hz, 1H), 3.0 (dd, J = 10.9, 12.0, 1H), 2.38–2.35 (m, 1H), 1.87–1.80 (m, 1H); **^{13}C NMR (125 Hz, DMSO-d_6)** δ = 50.5, 33.6, 24.2, 23.9; **HRMS** (ESI) calculated for $[C_4H_{10}NSe_2]^+$ (M^+) requires m/z = 223.9192 or 227.9154, found 223.9196 or 227.9144.

Synthesis of SelenoBMC^ox
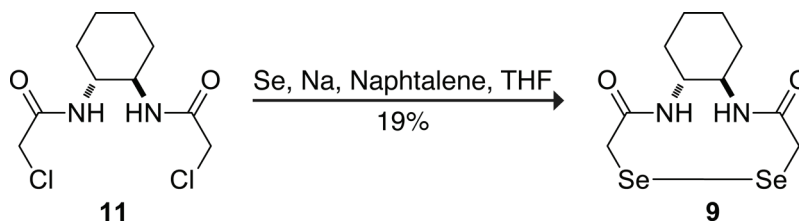
Sodium diselenide was generated *in situ* by following closely a method described previously.[3] Selenium powder (296.0 mg, 3.749 mmol) and naphthalene (480.5 mg, 3.749 mmol) placed in a flame-dried three-neck round-bottom flask. Anhydrous THF (10 mL) was added, and the resulting mixture was stirred under $N_2(g)$. Freshly shaved sodium metal (86.20 mg, 3.749 mmol) was then added to the reaction mixture under $N_2(g)$. The reaction mixture was allowed to stir for 2 h to enable consumption of all of the sodium metal. Compound **11** (497.6 mg, 1.863 mmol), which was prepared as described previously,[1a] was then dissolved in 20 mL of anhydrous THF, and the resulting solution was added dropwise to the reaction mixture. After 24 h, the reaction mixture was filtered, concentrated under reduced pressure, and purified by column

chromatography (20% v/v methanol in ethyl acetate) resulting in **9** as a red/orange solid (125.3 mg, 19%). An analytically pure sample of **9** was obtained by reverse-phase HPLC using a preparatory C18 column and a linear gradient of 10–80% v/v acetonitrile (0.1% v/v TFA) in water (0.1% v/v TFA) over 45 min. Compound **9** eluted at 23 min and, after lyophilization, was isolated as an off-white powder.

¹H NMR (500 MHz, DMSO-*d₆*) δ = 7.86−7.85 (m, 1.23H), 7.62−7.60 (m, 0.77H), 3.68−3.65 (m, 2.46H), 3.54 (d, *J* = 10.2 Hz, 0.77H), 3.50−3.45 (m, 0.77H), 3.41 (d, *J* = 12.5 Hz, 1.23H), 3.19 (d, *J* = 10.2 Hz, 0.77H), 1.73−1.68 (m, 4H), 1.35−1.18 (m, 4H); **¹³C NMR (125 MHz, DMSO-*d₆*)** δ = 170.7, 167.2, 55.8, 53.5, 33.7, 31.5, 31.4, 27.6, 24.9; **HRMS** (ESI) calculated for $[C_{10}H_{16}N_2O_2Se_2Na]^+$ (M+Na$^+$) requires *m/z* = 374.9474, found 374.9465.

III. Coupling yield determined by Ellman's assay for sulfhydryl groups

A reaction buffer (0.10 M sodium phosphate buffer, pH 8.0, containing 1 mM EDTA) was prepared by the Pierce protocol. A concentrated stock of Ellman's reagent (5,5′-dithio-*bis*-(2-nitrobenzoic acid) solution was prepared by adding Ellman's reagent (6 mg) to 2 mL of reaction buffer. 0.4 mL of Ellman's reagent solution was then added to two separate vials containing 10 mL of reaction buffer. One of these vials was used as a blank, and its absorbance was measured at 412 nm. Immobilized DTBA (1.3 mg) was then added to the other vial and allowed to react. After 30 min, its absorbance at 412 nm was recorded. Using Beer's law ($c = A/(\varepsilon\cdot l)$ with A = 0.690, l = 1.00 cm, and ε = 14,150 M^{-1}cm^{-1}) gave a thiol concentration of 4.88×10^{-5} M. Because DTBA contains two thiol groups, the assay mixture had an immoblized DTBA concentration of 2.44×10^{-5} M. Since the total volume of the reaction mixture was 10.4 mL, there were 2.54×10^{-7} moles of immobilized DTBA indicative of 0.195 mmol of immobilized DTBA per gram of resin and a ~93% coupling yield.

IV. Reduction potential of immobilized DTBA

The reduction potential ($E°'$) of immobilized DTBA was determined by using ¹H NMR spectroscopy to determine the equilibrium constant (K_{eq}) for its reaction with DTT^{ox}, and then inserting that value into a variation of the Nernst equation:

$$K_{eq} = \frac{[DTT][\text{oxidized immobilized DTBA}]}{[\text{immobilized DTBA}][\text{oxidized DTT}]} = \frac{[DTT]^2}{[\text{oxidized DTT}]^2} \tag{1}$$

$$E_{\text{immobilized DTBA}}°' = E_{DTT}°' - \frac{RT}{nF}\ln\frac{[DTT]^2}{[\text{oxidized DTT}]^2} \tag{2}$$

Data were obtained by a procedure similar to that described previously.[1b,4] Ellman's assay was performed on immobilized DTBA immediately prior to use. What was determined to be 1.56×10^{-3} mmol of immobilized DTBA was added to a 1.5-mL LoBind Eppendorf tube, and 0.9 mL of freshly degassed 50 mM sodium phosphate buffer, pH 7.0, was then added, followed by 156 µL (1.56×10^{-3} mmol) of a 10 mM stock solution of DTT^{ox} in the same buffer. The reaction mixture was agitated with a nutator for 24 h and then quenched by the addition of 3 N HCl (1:100 dilution). The resin was separated by centrifugation, and the solution was analyzed immediately by both ¹H NMR spectroscopy and analytical HPLC, as described previously.[1b] The equilibrium

concentrations of DTT and DTT^{ox} were obtained, and a value of K_{eq} = 0.421 ± 0.149 was determined. Assuming that DTT has $E°' = -0.327$ V, eq 2 was used to calculate $E°' = (-0.316 ±$ 0.002) V. This value is the mean ± SEM.
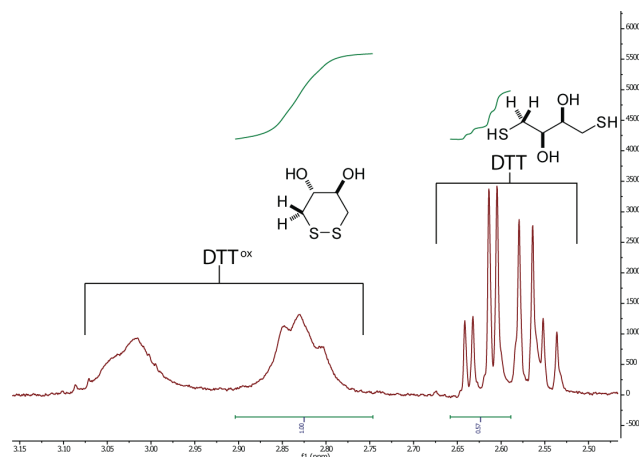

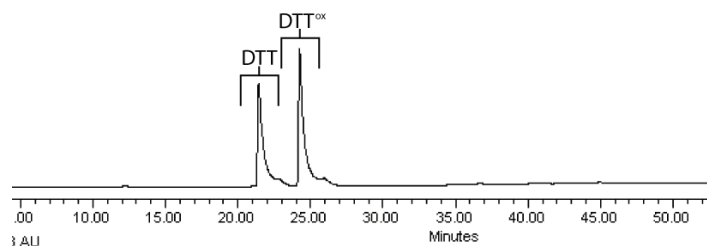**Figure S1.** Representative ^{1}H NMR spectrum of the redox equilibrium between immobilized DTBA and DTT.

Figure S2. Representative HPLC chromatogram of the redox equilibrium between DTBA and DTT^{ox}. Compounds were detected by their absorbance at 205 nm.

V. Reduction of small molecules with immobilized DTBA

A procedure similar to that described in Section IV was used to analyze the reduction of various small-molecule disulfides by a 10-fold molar excess of immobilized DTBA.

Reduction of cystamine

Ellman's assay was performed on immobilized DTBA immediately prior to its use. What was determined to be 13.4 × 10^{-3} mmol of immobilized DTBA was added to a 1.5-mL LoBind Eppendorf tube, and 0.6 mL of freshly degassed 50 mM sodium phosphate buffer, pH 7.0, was then added, followed by 134 µL (1.34 × 10^{-3} mmol) of a 10 mM stock solution of cystamine in the same buffer. The reaction mixture was agitated with a nutator for 24 h and then quenched by the addition of 3 N HCl (1:100 dilution). The resin was separated by centrifugation, and the solution was analyzed immediately ^{1}H NMR spectroscopy. The nearly complete reduction of cystamine was observed for this reaction.
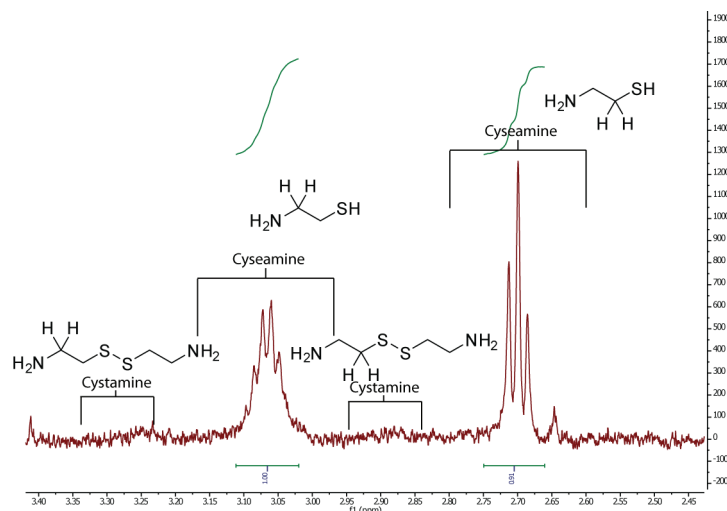
Figure S4. Representative ^{1}H NMR spectrum of the reaction between cystamine and a 10-fold excess of immobilized DTBA.

Reduction of βME^{ox}

Ellman's assay was performed on immobilized DTBA immediately prior to its use. What was determined to be 5.2×10^{-3} mmol of immobilized DTBA was added to a 1.5-mL LoBind Eppendorf tube, and 0.6 mL of freshly degassed 50 mM sodium phosphate buffer, pH 7.0, was then added, followed by 52 µL (5.20×10^{-4} mmol) of a 10 mM stock solution of βME^{ox} in the same buffer. The reaction mixture was agitated with a nutator for 24 h and then quenched by the addition of 3 N HCl (1:100 dilution). The resin was separated by centrifugation, and the solution was analyzed immediately by analytical HPLC using a Waters system equipped with a Waters 996 photodiode array detector, Empower 2 software, and a Varian C18 reverse-phase column. The column was eluted first at 1.0 mL/min with water (5.0 mL), followed by a linear gradient (0–40% v/v) of acetonitrile/water over 40 min. Compounds were detected by their absorbance at 205 nm. A single peak at 7 min was observed (Figure S8). Standards of βME and βME^{ox} showed that this peak corresponded to βME (βME^{ox} has a retention time of ~23 min), confirming that nearly complete reduction of βME^{ox} had taken place under the reaction conditions.
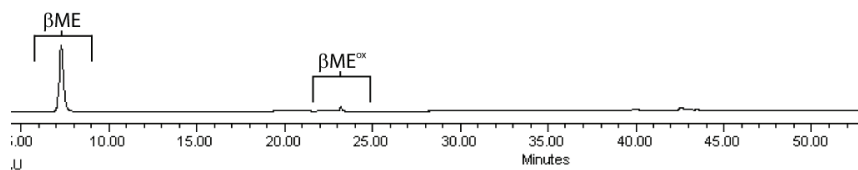

Figure S3. Representative HPLC chromatogram of the reaction between βME^{ox} and 10 fold excess of immobilized DTBA. Compounds were detected by their absorbance at 205 nm.

Reduction of DTBA^{ox}

Ellman's assay was performed on immobilized DTBA immediately prior to its use. What was determined to be 8.44×10^{-3} mmol of immobilized DTBA was added to a 1.5-mL LoBind Eppendorf tube, and 0.6 mL of freshly degassed 50 mM sodium phosphate buffer, pH 7.0, was

then added, followed by 84.4 µL (8.44×10^{-4} mmol) of a 10 mM stock solution of DTBA^{ox} in the same buffer. The reaction mixture was agitated with a nutator for 24 h and then quenched by the addition of 3 N HCl (1:100 dilution). The resin was separated by centrifugation, and the solution was analyzed immediately with ^{1}H NMR spectroscopy. Concentrations of DTBA and DTBA^{ox} were determined and revealed a 76% yield of DTBA.
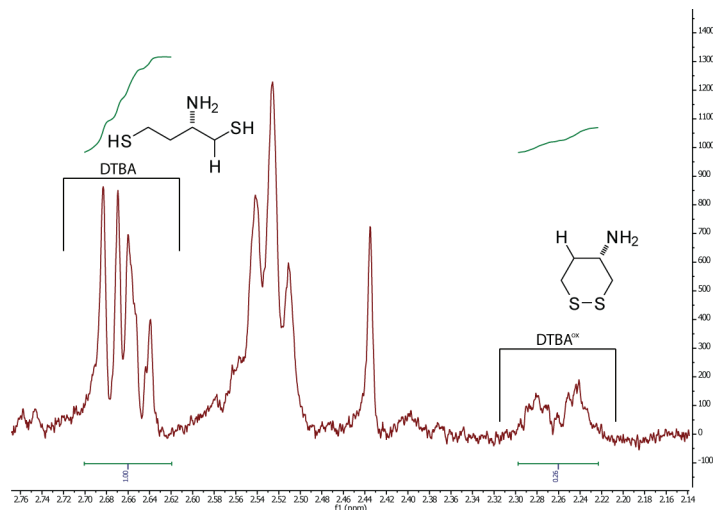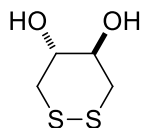

Figure S7. Representative ^{1}H NMR spectrum of the reaction between DTBA^{ox} and a 10-fold excess of immobilized DTBA.

Reduction of DTT^{ox}

Ellman's assay was performed on immobilized DTBA immediately prior to its use. What was determined to be 8.99×10^{-3} mmol of immobilized DTBA was added to a 1.5-mL LoBind Eppendorf tube, and 0.6 mL of freshly degassed 50 mM sodium phosphate buffer, pH 7.0, was then added, followed by 89.9 µL (8.99×10^{-4} mmol) of a 10 mM stock solution of DTT^{ox} in the same buffer. The reaction mixture was agitated with a nutator for 24 h and then quenched by the addition of 3 N HCl (1:100 dilution). The resin was separated by centrifugation, and the solution was analyzed immediately by both ^{1}H NMR spectroscopy and analytical HPLC, as described previously.[1b] Concentrations of DTT and DTT^{ox} were determined and revealed a 68% yield of DTT.
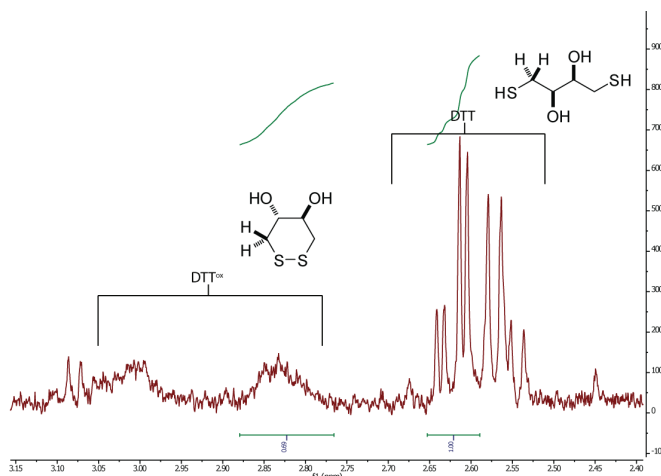
Figure S5. Representative ^{1}H NMR spectrum of the reaction between DTT^{ox} and a 10-fold excess of immobilized DTBA.
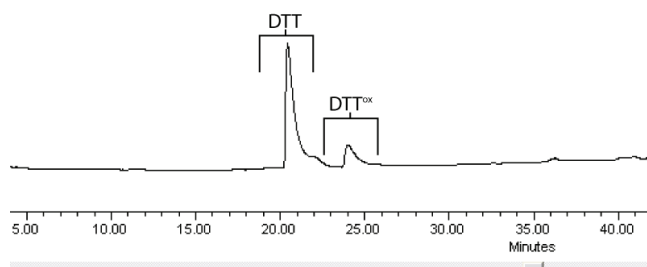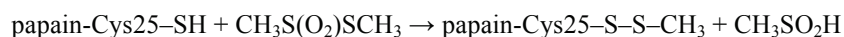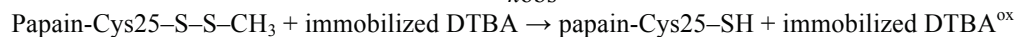

Figure S6. Representative HPLC chromatogram of the reaction between DTT^{ox} and a 10-fold excess of immobilized DTBA. Compounds were detected by their absorbance at 205 nm.
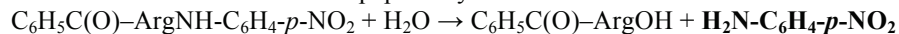
VI. Reactivation of papain with immobilized DTBA

$$papain\text{-}Cys25\text{–}SH + CH_3S(O_2)SCH_3 \rightarrow papain\text{-}Cys25\text{–}S\text{–}S\text{–}CH_3 + CH_3SO_2H$$

$$kobs$$
$$Papain\text{-}Cys25\text{–}S\text{–}S\text{–}CH_3 + immobilized\ DTBA \rightarrow papain\text{-}Cys25\text{–}SH + immobilized\ DTBA^{ox}$$

$$papain\text{-}Cys25\text{–}SH$$
$$C_6H_5C(O)\text{–}ArgNH\text{-}C_6H_4\text{-}p\text{-}NO_2 + H_2O \rightarrow C_6H_5C(O)\text{–}ArgOH + \textbf{H}_2\textbf{N-C}_6\textbf{H}_4\text{-}\textbf{\textit{p}}\textbf{-NO}_2$$

Ellman's assay was performed on immobilized DTBA immediately prior to setting up the assay. Papain was inactivated by oxidizing its active-site cysteine (Cys25) by treatment with *S*-methyl methanethiosulfonate following a procedure described previously.[1b] A 1.25-mL solution of papain-Cys25–S–S–CH$_3$ (1.25×10^{-5} M, 1.56×10^{-5} mmol) in degassed 0.10 M imidazole–HCl buffer, pH 7.0, containing EDTA (2 mM) was placed in a 1.5-mL LoBind Eppendorf tube. At time $t = 0$, a ~100-fold excess of immobilized DTBA (1.35×10^{-3} mmol) was added to the Eppendorf tube, which was then placed on a nutator. At various times, the resin was separated by centrifugation, and a 0.2-mL aliquot of the solution was removed and added to a cuvette containing 0.8 mL of 0.10 M imidazole–HCl buffer, pH 6.0, containing EDTA (2 mM) and *N*-

benzoyl-L-arginyl-*p*-nitroanilide (1.25 mM). The rate of change in absorbance at 410 nm was recorded at 25 °C. A unit of protein is defined as the amount of enzyme required to produce 4-nitroaniline at a rate of 1 μmol/min. The units of active papain at each time point was calculated by using an extinction coefficient for 4-nitroaniline of ε = 8,800 $M^{-1}cm^{-1}$ at 410 nm. To determine the possible number of units of active papain in the reaction mixture, enzymatic activity was assessed after the addition of a large excess of DTT (~10^3-fold) to an Eppendorf tube. As a control, the addition of DTT was shown to have no bearing on the assay data, other than in activating the enzyme. The enzymatic activity (%) at particular times was calculated by dividing the number of active units of enzyme by the possible number of units in the solution, and was plotted in Figure 1. The procedure described above was also performed using ~1,000 equiv of immobilized DTBA.

VII. Reactivation of papain using relay catalysts

The procedure described in Section VI was also performed in the presence of a relay catalyst. Stock solutions (1 mM) of **1–9** were prepared in degassed 0.10 M imidazole–HCl buffer, pH 7.0, containing EDTA (2 mM) or in DMSO, and 5 μL of this solution (5 × 10^{-6} mmol of catalyst, 30 mol%) of was added to the reaction mixture in the Eppendorf tube. The enzymatic activity (%) at particular times was calculated by dividing the number of active units of enzyme by the possible number of units in the solution, and was plotted in Figures 1–3. To determine the value of the second-order rate constant k_{obs}, the second-order rate equation (Eq 3) was transformed into Eq 4, which was fitted to the data with the program Prism 5.0 (GraphPad Software, La Jolla, CA). In Eq 3 and 4, A_o = [inactive protein]$_{t=0}$, A = [inactive protein]$_t$ = $A_o - A_o y$, B_o = [immobilized DTBA]$_{t=0}$, and B = [immobilized DTBA]$_t$ = $B_o - A_o y$.[1b] Values of k_{obs} were the mean ± SEM from at least 4 different experiments.

$$\frac{1}{B_o - A_o} \ln \frac{A_o B}{A B_o} = k_{obs} t \qquad (3)$$

$$y = \frac{B_o - B_o e^{k_{obs} t (A_o - B_o)}}{B_o - A_o e^{k_{obs} t (A_o - B_o)}} \qquad (4)$$

immobilized DTBA k_{obs} = (0.16 ± 0.01) $M^{-1}s^{-1}$
immobilized DTBA + DTBA^{ox} (**1**) k_{obs} = (0.25 ± 0.01) $M^{-1}s^{-1}$
immobilized DTBA + DTT^{ox} (**2**) k_{obs} = (0.17 ± 0.01) $M^{-1}s^{-1}$
immobilized DTBA + cystamine (**3**) k_{obs} = (0.30 ± 0.02) $M^{-1}s^{-1}$
immobilized DTBA + βME^{ox} (**4**) k_{obs} = (0.17 ± 0.01) $M^{-1}s^{-1}$
immobilized DTBA + BMC^{ox} (**5**) k_{obs} = (0.67 ± 0.01) $M^{-1}s^{-1}$
immobilized DTBA + lipoic acid (**6**) k_{obs} = (0.29 ± 0.04) $M^{-1}s^{-1}$
immobilized DTBA + selenoDTBA^{ox} (**7**) k_{obs} = (0.82 ± 0.03) $M^{-1}s^{-1}$
immobilized DTBA + selenocystamine (**8**, 1 mol%) k_{obs} = (0.40 ± 0.02) $M^{-1}s^{-1}$
immobilized DTBA + selenocystamine (**8**, 5 mol%) k_{obs} = (0.86 ± 0.05) $M^{-1}s^{-1}$
immobilized DTBA + selenocystamine (**8**, 30 mol%) k_{obs} = (4.7 ± 0.2) $M^{-1}s^{-1}$
immobilized DTBA + selenoBMC^{ox} (**9**) k_{obs} = (0.42 ± 0.02) $M^{-1}s^{-1}$

VIII. Reactivation of Papain with DTBA and BMC

A procedure similar to that described previously was used compare the rate at which DTBA and BMC reduce the active site cysteine of papain-Cys25–S–S–CH$_3$.[1b]
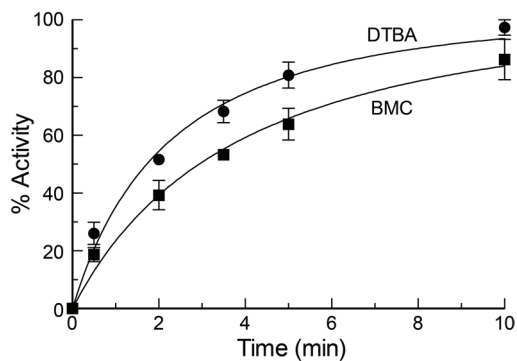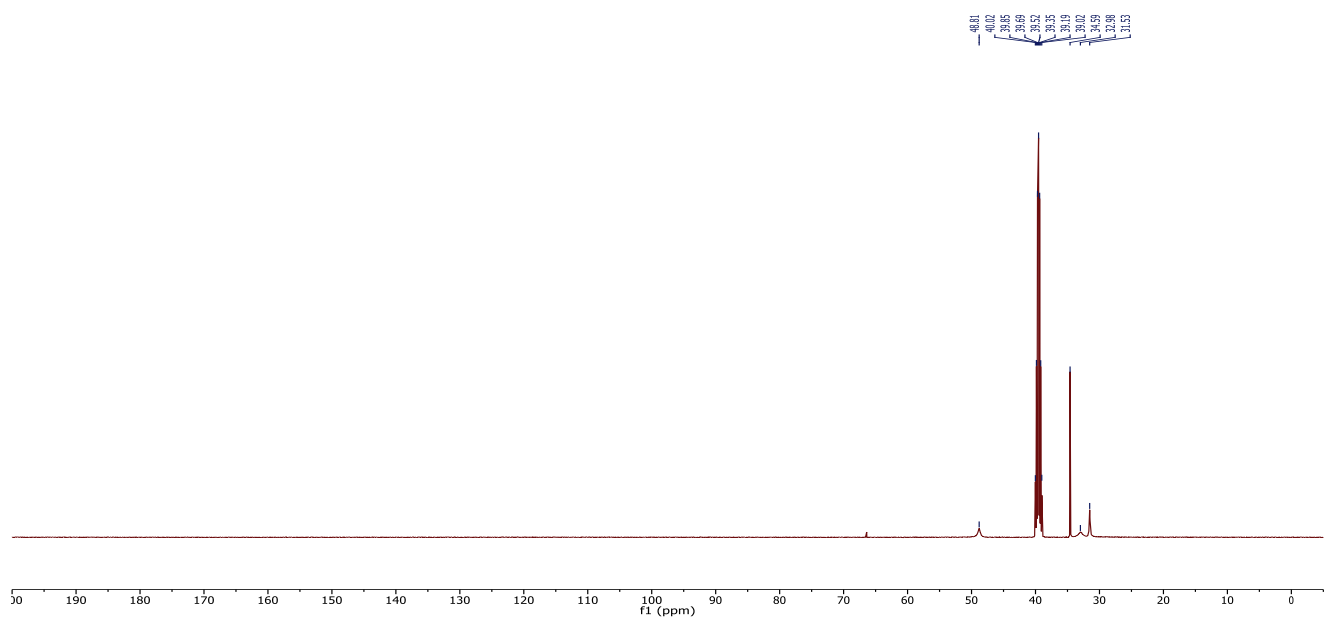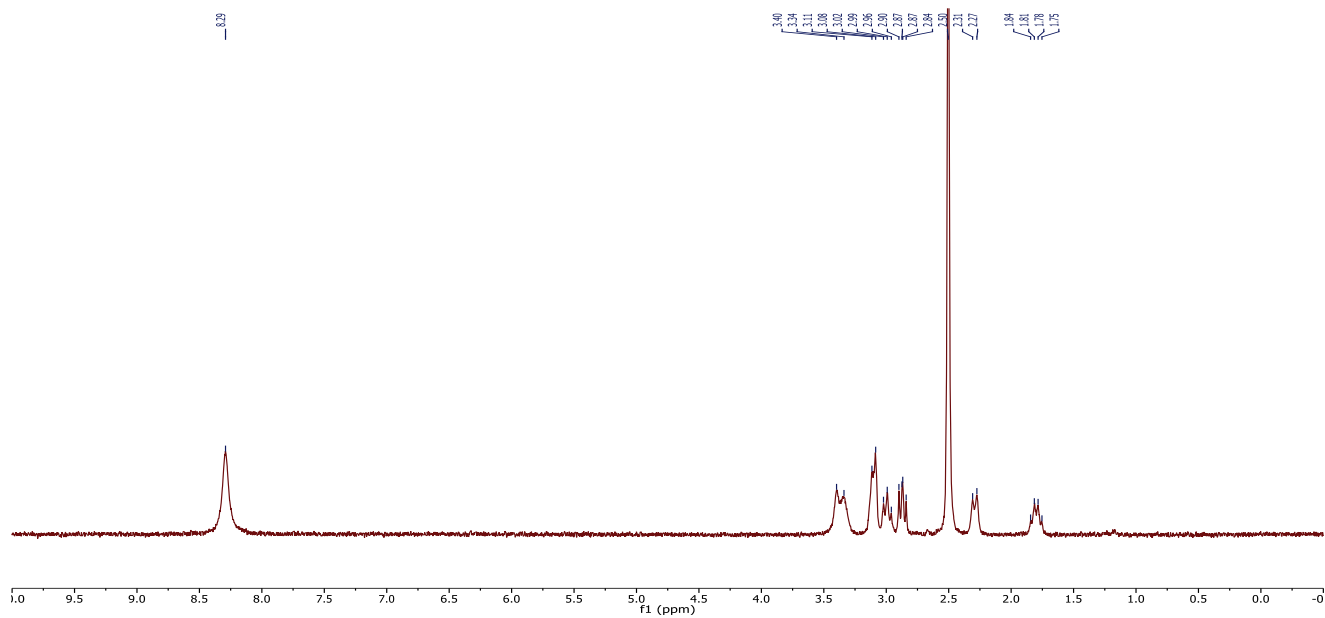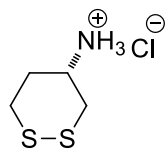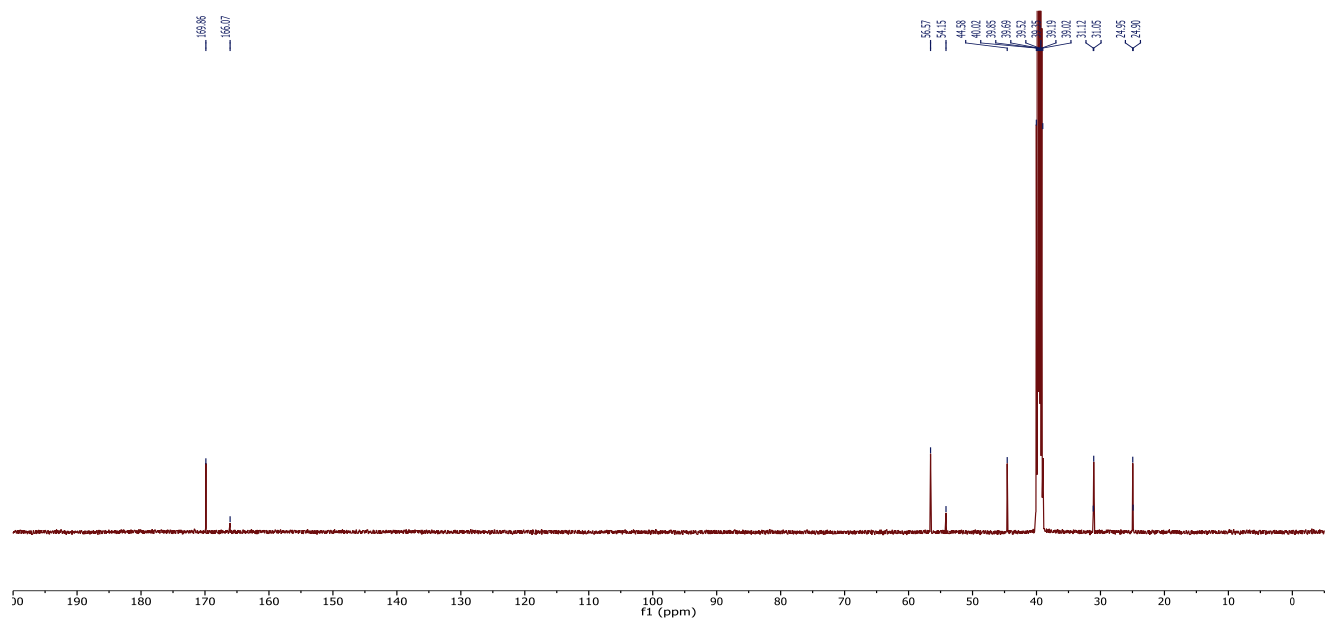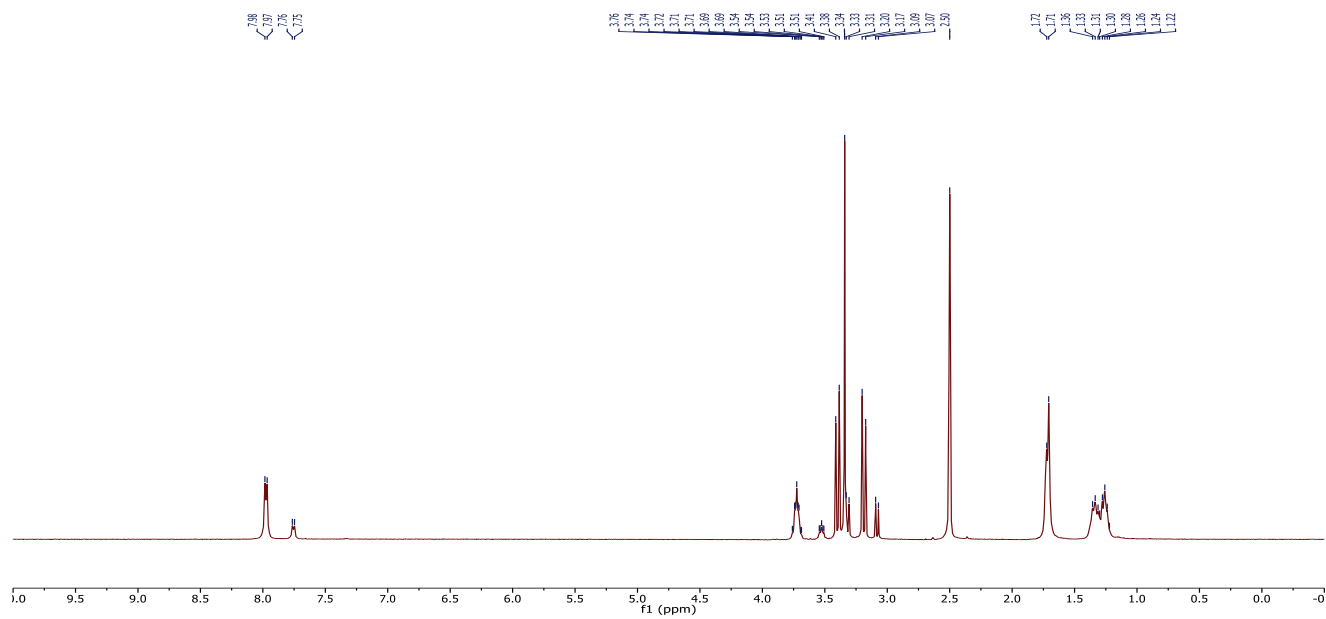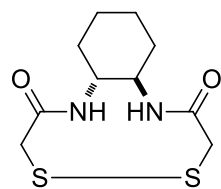

Figure S8. Time-course for the reactivation of papain-Cys25–S–S–CH$_3$ by dithiols (1.5 equiv) in 0.10 M imidazole–HCl buffer, pH 7.0, containing EDTA (2 mM). DTBA: k_{obs} = (1067 ± 68) M^{-1}s^{-1}; BMC: k_{obs} = (609 ± 42) M^{-1}s^{-1}.
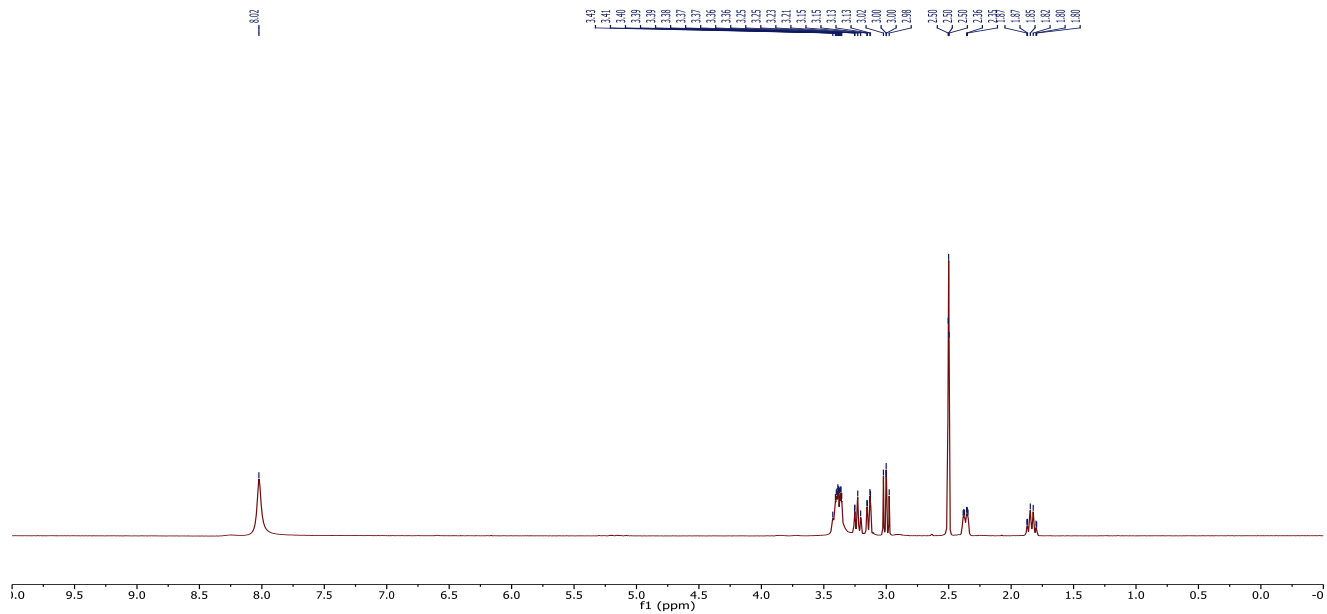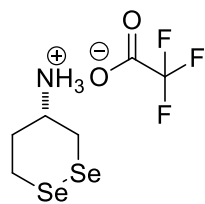
IX. References

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[2] G. L. Ellman, *Arch. Biochem. Biophys.* **1958**, *82*, 70–77.
[3] D. P. Thompson, P. Boudjouk, *J. Org. Chem.* **1988**, *53*, 2109–2112.
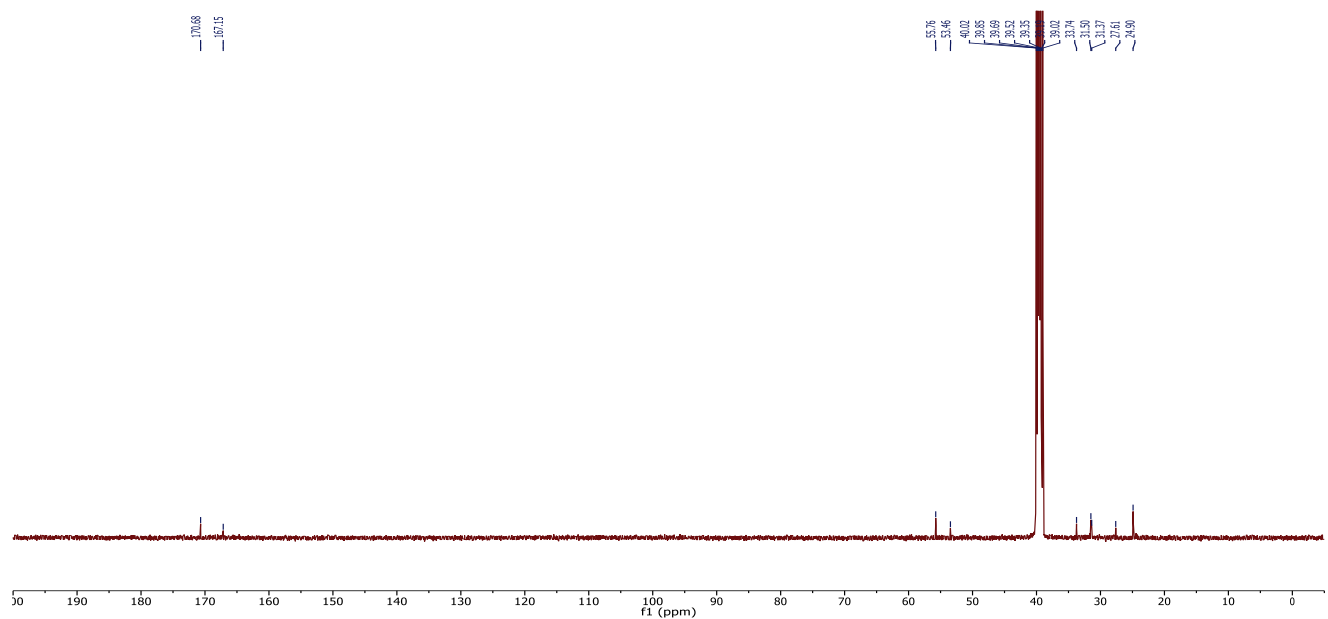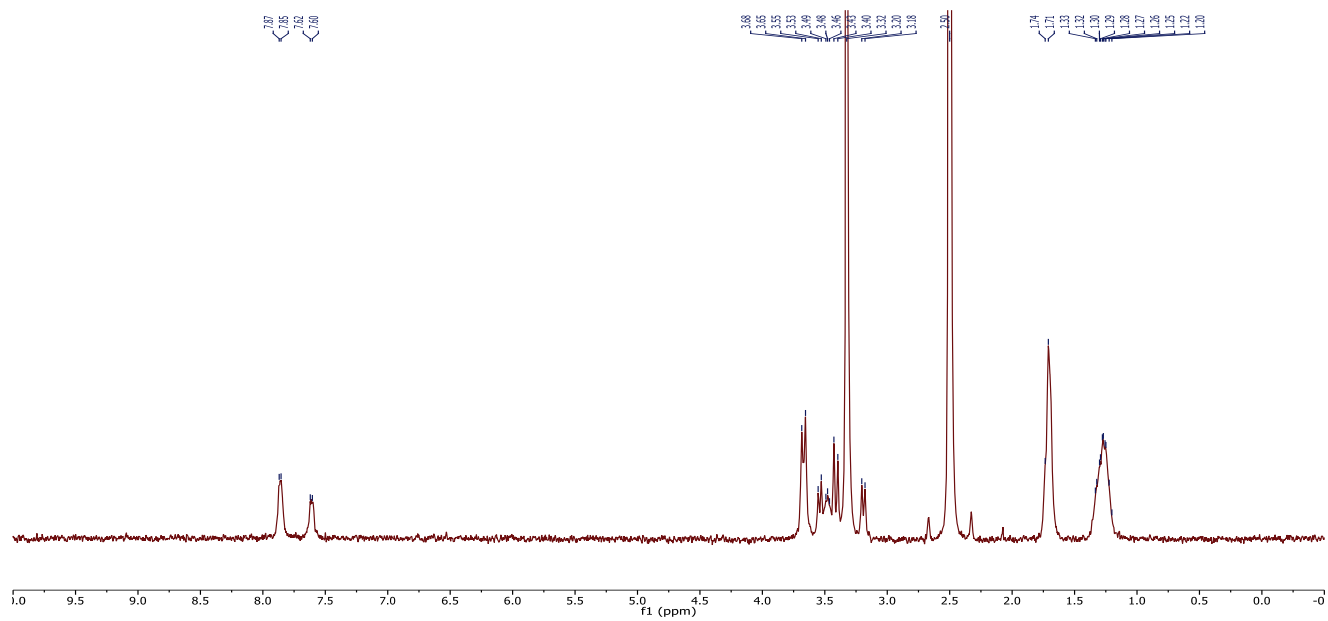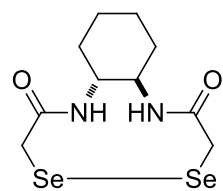[4] G. V. Lamoureux, G. M. Whitesides, *J. Org. Chem.* **1993**, *58*, 633–641.

X. NMR Spectra:

^{1}H NMR and ^{13}C NMR spectra (DMSO-d_6) of **1** with 1,4-dioxane

^{1}H NMR spectrum (50 mM sodium phosphate buffer, pH 7.0) of DTBA

^{1}H NMR spectrum (50 mM sodium phosphate buffer, pH 7.0) of **1**

^{1}H NMR and ^{13}C NMR spectra (DMSO-d_6) of **5**

¹H NMR and ¹³C NMR spectra (DMSO-d_6) of **7**
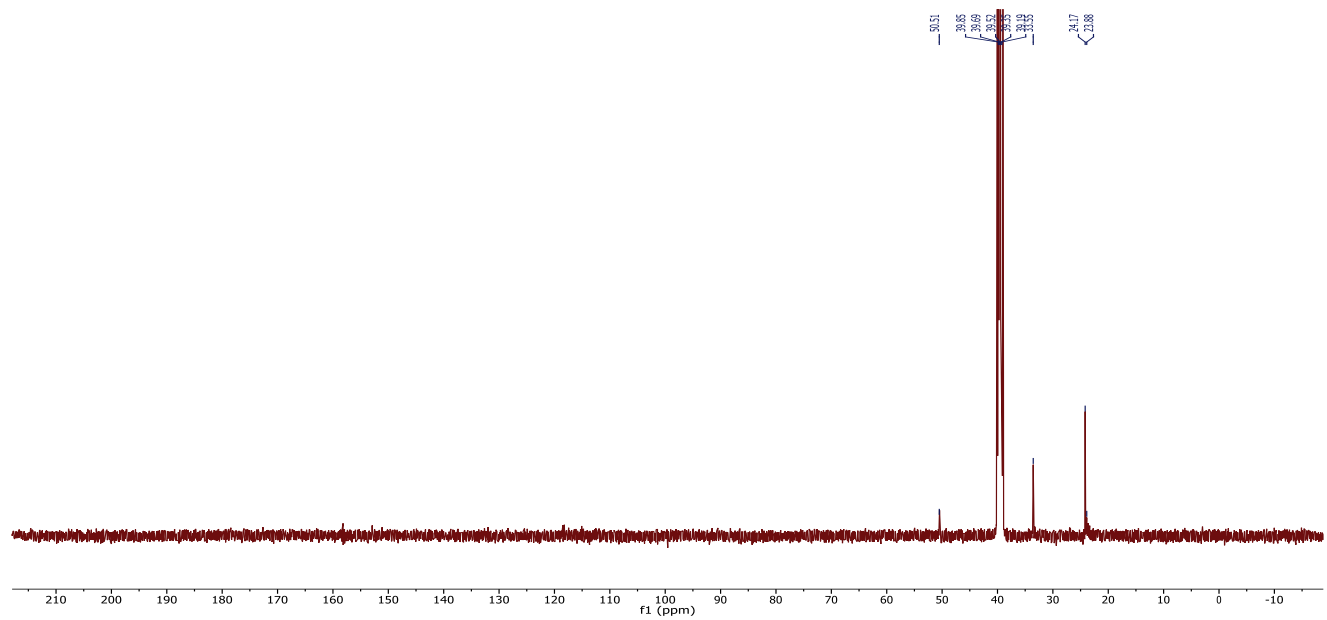
^{1}H NMR and ^{13}C NMR spectra (DMSO-d_6) of **9**

^{1}H NMR and ^{13}C NMR spectra (DMSO-d_6, 80 °C) spectra of **9**