Orthogonal Breaking and Forming of Dynamic Covalent Imine and Disulfide Bonds in Aqueous Solution

Supporting Information

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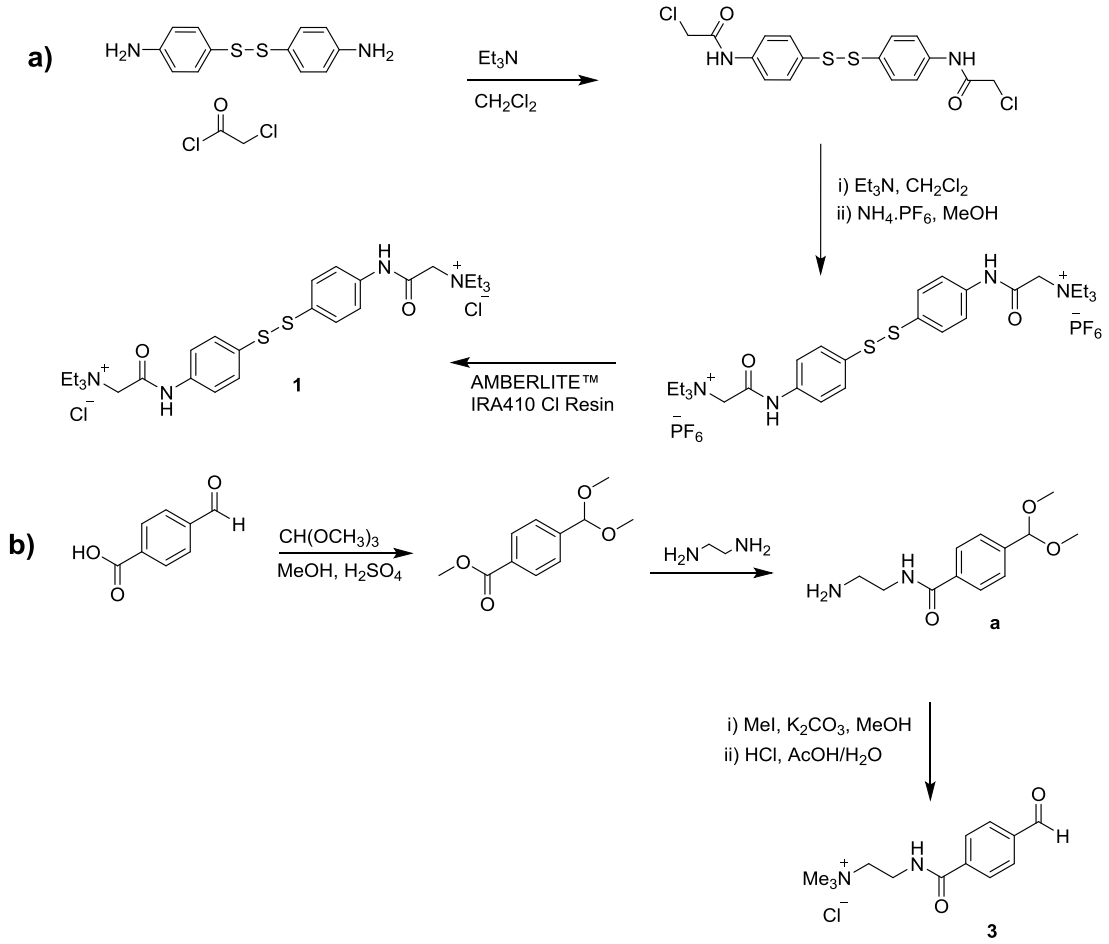
General Experimental: All chemicals were purchased from Sigma Aldrich or Alfa Aesar and were used as received without further purification. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 300 spectrometer. The NMR data are reported as follows: chemical shift in ppm, residual solvent signals were used as internal standards, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz) and integration. Aquired spectra were processed using MestReNova. Thin layer chromatography was used to monitor progress of all synthetic reactions using Merck 60 F254 silica gel plates on aluminum foil. Column chromatography was performed using Merck Geduran Si 60 40-63 µm mesh silica gel. FTIR spectroscopy was performed on a Varian 800 FTIR instrument (Varian Inc.). High resolution mass spectrometry was performed on a Waters Micromass LCT Premier with leucine enkephalin as a standard.

Synthesis of System Components 1 and 3



Scheme S1. **(a)** Synthesis of disulfide **1**, **(b)** synthesis of aldehyde **3**.

2,2'-((Disulfanediylbis(4,1-phenylene))bis(azanediyl))bis(N,N,N-triethyl-2-oxoethan-1-aminium) ***(1)****:* 4-Aminophenyl disulfide (500 mg, 2.01 mmol) was dissolved in CH_2Cl_2 (25 mL). Chloroacetyl chloride (450 mg, 3.62 mmol) was added dropwise over 30 min at 0 ^oC. The mixture was allowed to stir at 30 ^oC overnight, and the resulting white precipitate was filtered and the filtrate was evaporated to dryness. The crude solid obtained was dissolved in MeOH (25 mL) and Et_3N (784.9 mg, 10.5 mmol) was added followed by stirring at 60 ^oC for 72 h. The resulting dark brown precipitate was removed by filtration, then NH_4PF_6 was added to the filtrate in portions until the solution was saturated. This mixture was stirred at 60 ^oC for 16 h, concentrated, and then precipitated into cold water (250 mL). The precipitate was collected by filtration, washed with cold water and dissolved in MeOH. Amberlite® IRA-410 chloride form ion exchange resin beads (2 g) were added and the mixture was stirred at 40 ^oC for 18 h. The beads were then filtered and the filtrate evaporated to dryness. The resulting brown solid was dissolved in water (20 mL) and washed with CH_2Cl_2 (2 x 50 mL) to remove hydrophobic impurities. Removal of water under reduced pressure yielded disulfide **1** (385 mg, 0.73 mmol, 36 % yield). ^1H NMR (300 MHz, D_2O): δ = 1.22 (18H, t, J = 7.0 Hz), 3.47 (12H, q, J = 7.0 Hz), 4.01 (4H, s), 7.31 (4H, d, J = 8.5 Hz), 7.41 (4H, d, 8.5 Hz). ^{13}C NMR (75 MHz, D_2O): δ = 7.0, 54.5, 56.3, 121.7, 129.1, 133.0, 135.9, 162.3. FT-IR (wavenumber, cm^{-1}): 3226 (N–H), 3156 (C–H, aromatic), 2971 (C–H, alkyl), 1685 (C=O), 1490 (C=C, aromatic), 1406 (C=C, aromatic). $HRMS^+$ $C_{28}H_{44}N_4O_2S_2$: Theoretical: 266.1531. Actual: 266.1516.

N-(2-Aminoethyl)-4-(dimethoxymethyl)benzamide ***(a)****:* 4-Carboxybenzaldehyde (18.3 g, 0.121 mol) was dissolved in MeOH (120 mL) and acidified with concentrated H_2SO_4 (10 drops). Methyl orthoformate (37.7 mL, 0.344 mol) was added in one portion and the mixture was refluxed for 48 h with stirring. After this time, the mixture was transferred to a separating funnel with saturated $NaCHO_3$ (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 150 ml) and the organic extracts were combined and dried over $MgSO_4$, filtered and evaporated to dryness to afford a yellow oil (22.41 g, 0.11 mol, 88.1 %) which was used without further purification. ^1H NMR ($CDCl_3$, 400 MHz): δ 3.30 (s, 6H), 3.90 (s, 3H), 5.42 (s, 1H), 7.52 (d, 2H, J = 8 Hz), 8.03 (d, 2H, J = 8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 52.1, 52.6, 102.3, 126.8, 129.6, 130.2, 143.0, 166.4. Methyl-4-(dimethoxymethyl)-benzoate (2.5g, 12.0 mmol) was refluxed in ethylenediamine (35 mL) for 18h. Ethylenediamine was removed under pressure to afford a deep brown solid which was purified by column chromatography (SiO_2, CH_2Cl_2:EtOH:Et_3N, 80:15:5 v/v) to furnish intermediate ***a*** as a pale white solid (1.3 g, 5.5 mmol, 46 % yield). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.75 (s, 2H), 2.89 (t, 2H, J = 5.5 Hz), 3.25 (s, 6H), 3.45 (q, 2H, J = 5.5 Hz), 5.36 (s, 1H), 6.77 (br t, 1H), 7.45 (d, 2H, J = 8.0 Hz), 7.74 (d, 2H, J = 8.0 Hz). FT-IR (wavenumber, cm^{-1}): 3281 (N–H), 2947 (C–H, alkyl), 1634 (C=O), 1593(C=O), 1448 (C=C, aromatic), 1421 (C=C, aromatic).

2-(4-Formylbenzamido)-N,N,N-trimethylethan-1-aminium ***3****:* *N*-(2-Aminoethyl)-4-(dimethoxymethyl)benzamide ***(a)*** (370 mg, 1.6 mmol) was dissolved in MeOH (8 mL). K_2CO_3 (431 mg, 3.12 mmol) was added followed by addition of iodomethane (5.0 g, 35 mmol) in one portion, and this mixture was stirred at 8 h at 55 ^oC. Residual MeI was removed under reduced pressure and Et_2O (30 mL) was added and the suspension was agitated in a sonic bath for 30 min and filtered to afford a pale brown solid. This solid was dissolved in 1:1 acetone:water (20 mL) and acidified with 5 drops of concentrated HCl. After stirring at room temperature for 2 h the mixture was filtered and the filtrate evaporated to dryness. The resulting brown solid was purified by column chromatography (SiO_2, CH_3CN:H_2O, 8:2 v/v) to afford a pale brown solid (215 mg, 0.91 mmol, 69%)

^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.14 (s, 9H), 3.53 (t, 2H, J = 6.5 Hz), 3.82 (t, 2H, J = 6.5 Hz), 7.82 (d, 2H, J = 8.0 Hz), 7.95 (d, 2H, J = 8.5 Hz), 9.92 (s, 1H). ^{13}C NMR ($CDCl_3$, 75MHz) δ 34.3, 53.6, 64.1, 128.0, 130.3, 138.1, 138.4, 169.8, 195.7. FT-IR (wavenumber, cm^{-1}): 3391 (N–H), 3001 (C–H, alkyl), 2866 (C-H, CHO), 1659(C=O), 1593(C=O), 1490 (C=C, aromatic), 1455 (C=C, aromatic). HRMS^{+} $C_{13}H_{19}N_2O_2$: Theoretical: 235.1446. Actual: 235.1506.

Full ^{1}H NMR Spectra of Nodes A – D.

Complete spectra are presented below displaying the upfield regions of the spectra which are not shown in the main manuscript are displayed here.

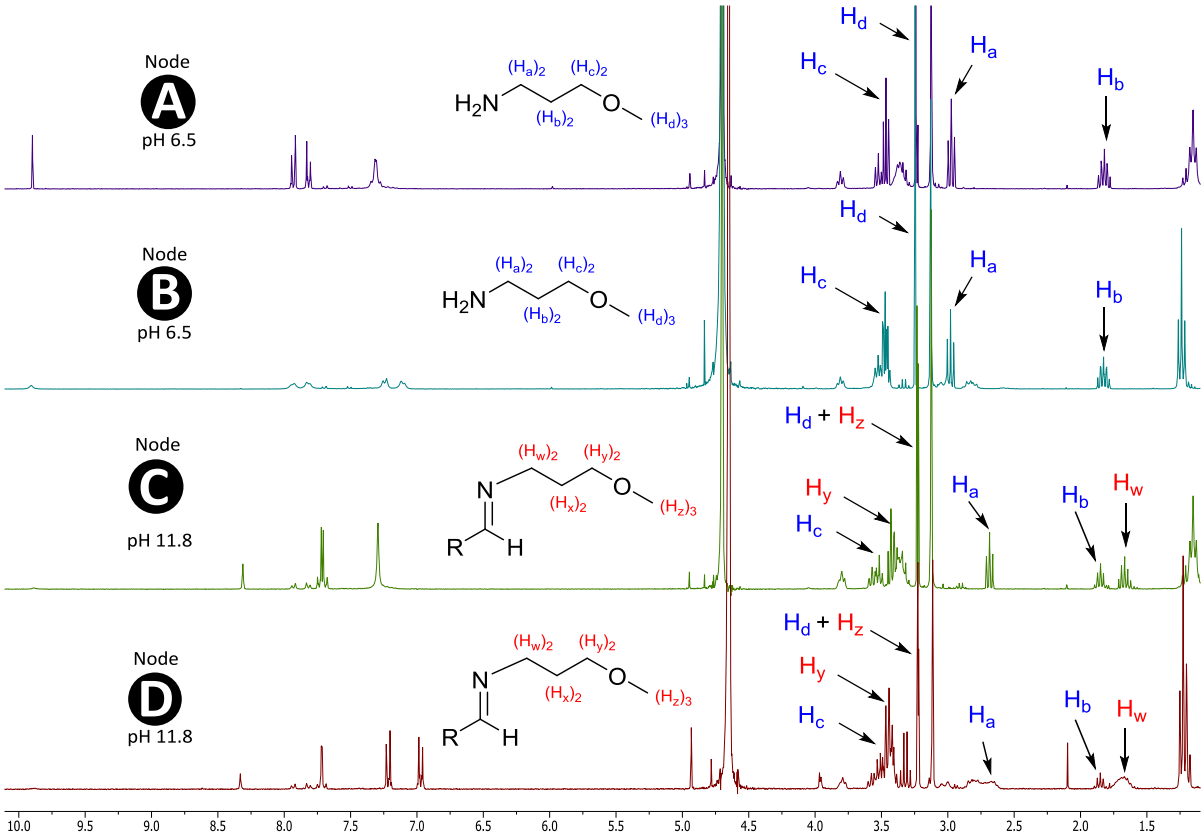


Figure S1. ^{1}H NMR spectra (300 MHz, D_2O) of network nodes **A** – **D** with focus on the upfield region (δ=0-5 ppm) not shown in the main manuscript. There is no change with respect to the signals corresponding to 3-methoxypropylamine **(2)** between node **A** and node **B** (S1 a, b), indicating no undesired side reactions involving **2** during this transition. In the transition of node **B** to node **D** (S1 d), new signals corresponding to imine **5** emerge. At pH 11.8 there is a small amount of amine **2** present and signals corresponding to amine **2** are still visible in the spectrum. Slight changes in chemical shift of signals corresponding to amine **2** between node **B** and node **D** is likely on account of a change in the state of hydrogen bonding with the methoxy oxygen on account of the system being at higher pH. The same changes in chemical shift are observed when comparing ^{1}H NMR spectra of amine **2** at pH 6.5 and pH 11.8 in the absence of the other system components. Identified signals for both imine **5** and amine **2** are present in both node **D** and node **C**, again suggesting no unwanted side reactions during this transition.

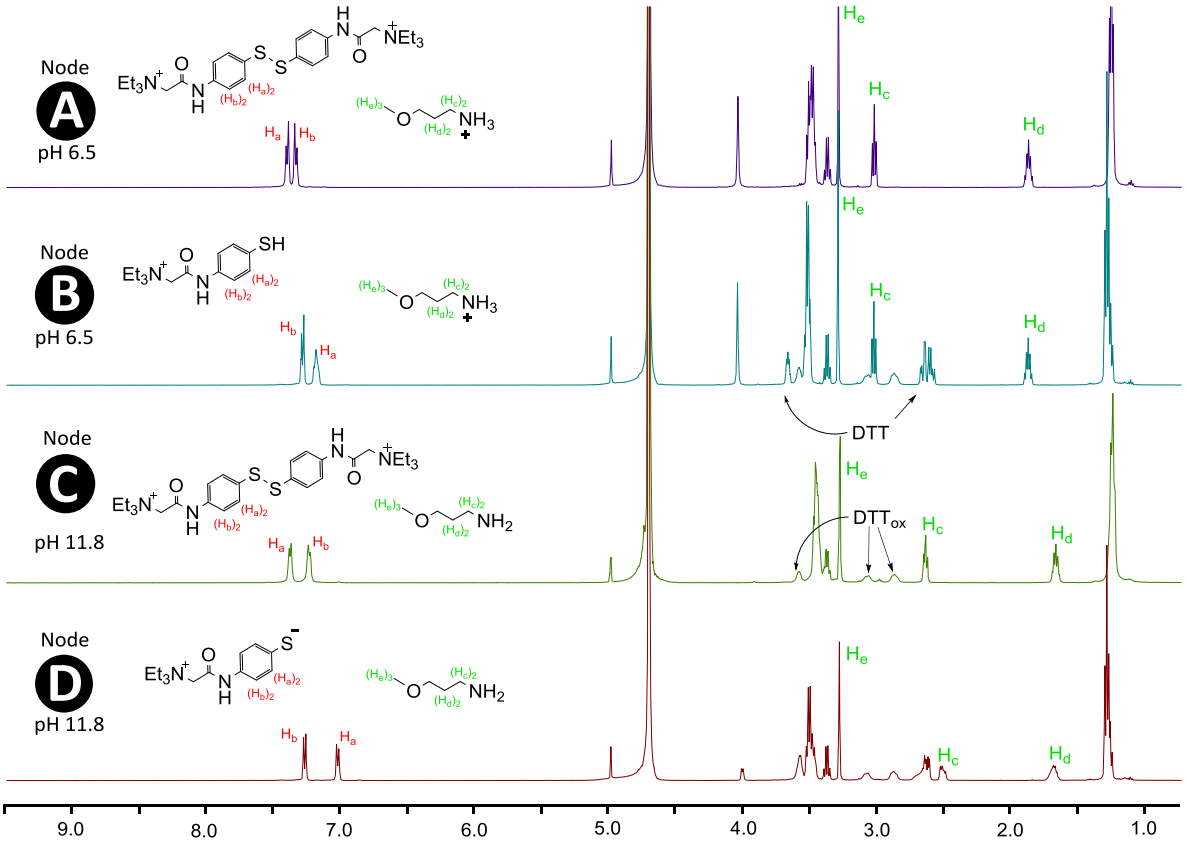


Figure S2a. ^{1}H NMR spectra (300 MHz, D_2O) of nodes **A** – **D** acquired in the absence of aldehyde component, **3**. The pair of doublets (**H_a** & **H_b**) in the aromatic region of the spectrum correspond to the thiol, thiolate or disulfide, **4**, **4'** or **1,** respectively. Signals corresponding to amine **2** remain near-identical at each node, differing only slightly in chemical shift on account of changes in pH between the nodes. In the transition between node **A** and node **B**, disulfide **1** is quantitatively reduced to form thiol **4** by an excess of DTT in presence of amine **2** at pH 6.5 and in the absence of unwanted competing processes. Between node **B** and node **D**, increasing the pH from 6.5 to 11.8 results in deprotonation of thiol **4** to form thiolate **4'** and amine **2** remains unprotonated. In the transition from node **D** to node **C** thiolate **4'** is oxidatively coupled with itself to form disulfide **1** in the presence of amine **2** whilst avoiding unwanted processes. These control experiments indicate that interconversion between disulfide **1** and thiol **4** or thiolate **4'**proceeds as expected in the absence of aldehyde **3** without competing side processes.

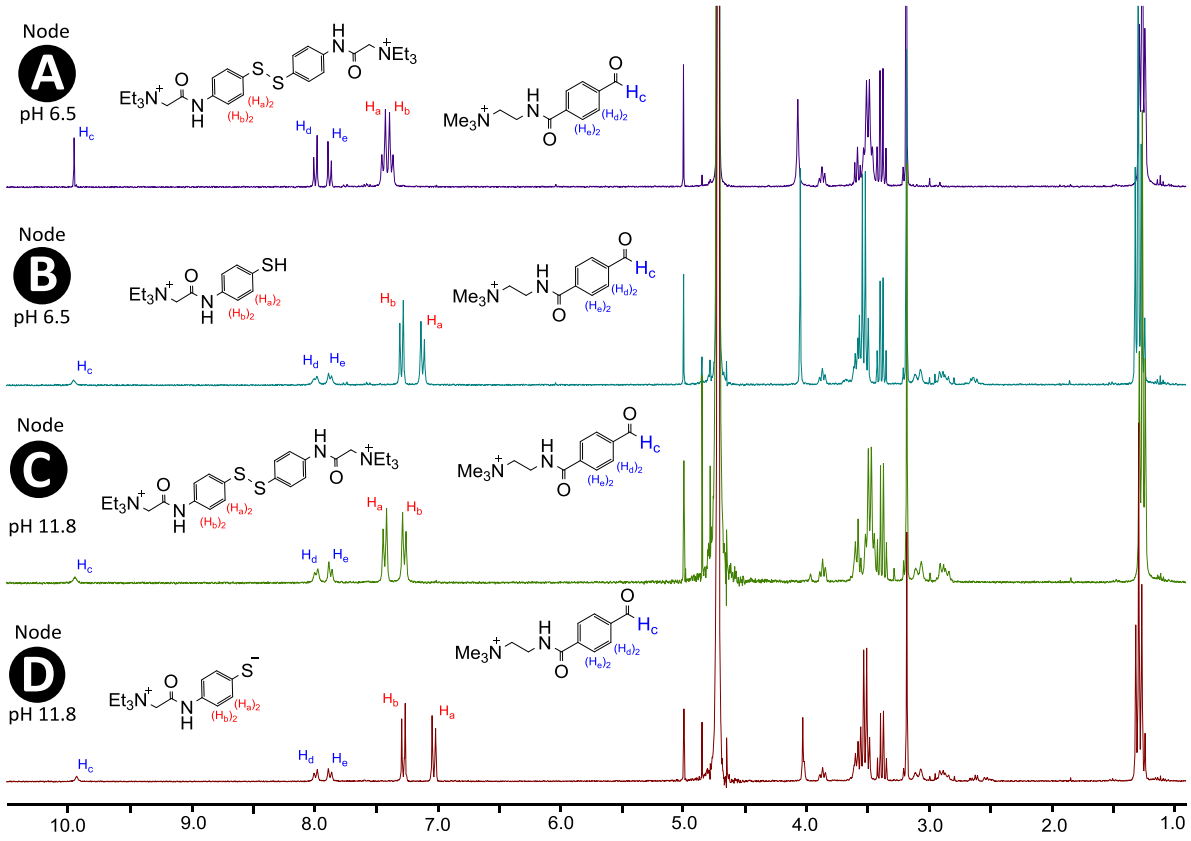


Figure S2b. ^{1}H NMR spectra (300 MHz, D_2O) of nodes **A** – **D** acquired in the absence of amine component, **2**. Signals $\mathbf{H_c}$ (δ = 9.0 ppm) and $\mathbf{H_d}$ & $\mathbf{H_e}$, which correspond to the C**H**O and the aromatic ring protons respectively in aldehyde **3**, remain unchanged throughout each node transition aside from line broadening whose origin is elaborated in *Figure S6*. Between nodes, the expected changes in signals corresponding to the thiol/disulfide component are observed. In the transition from node **A** to node **B** signals $\mathbf{H_c}$, $\mathbf{H_d}$ and $\mathbf{H_e}$ remain unchanged suggesting that the reduction of disulfide **1** by DTT to form thiol **4** proceeds in the absence of undesired side reactivity in the presence of aldehyde **3**. In the transition from node **B** to node **D**, increasing the pH from 6.5 to 11.8 results in deprotonation of thiol **4** forming thiolate **4'**, there is no change in signals $\mathbf{H_c}$, $\mathbf{H_d}$ and $\mathbf{H_e}$, suggesting there are no competing processes involving aldehyde **3**. During the transition from node **D** to node **C,** successful oxidative coupling of thiolate **4'** by H_2O_2 proceeds without a change to signals $\mathbf{H_c}$, $\mathbf{H_d}$ and $\mathbf{H_e}$. These control experiments indicate that interconversion between disulfide **1** and thiol **4** or thiolate **4'**proceeds as expected in the absence of amine **2** without undesired side reactivity.

ESI-Mass Spectra of Nodes A-D

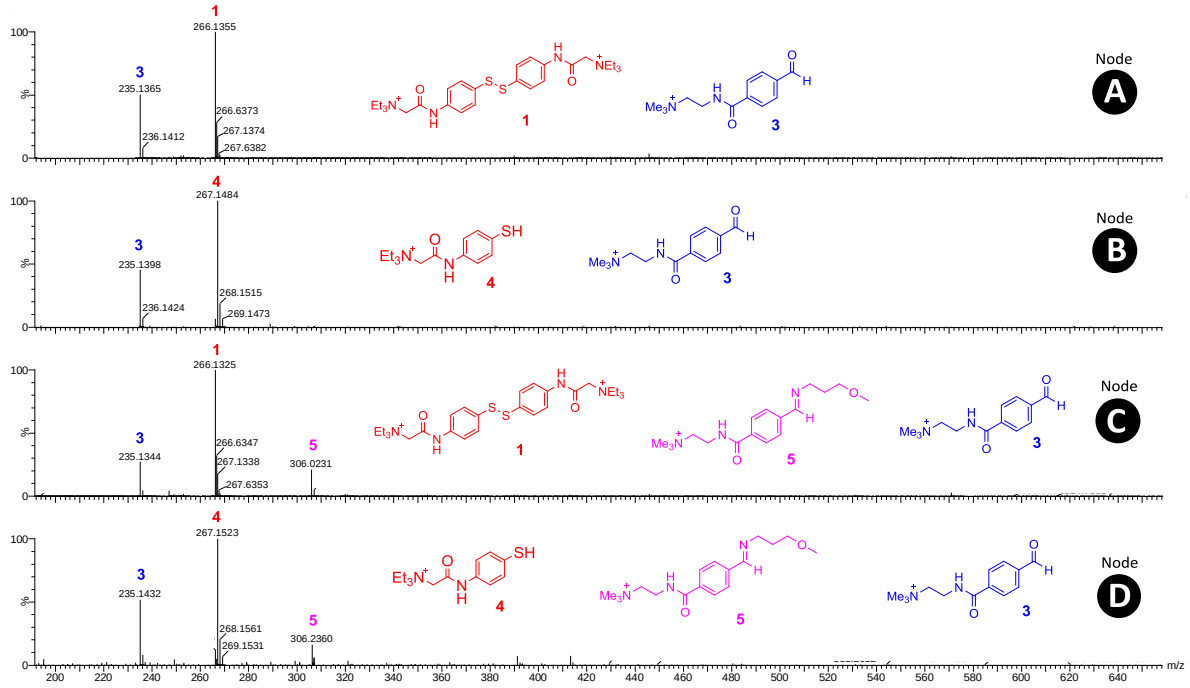


Figure S3. Expanded region of mass spectra (ESI-MS, positive ion mode) for nodes **A – D**. For each node (**A – D** as shown in Figure 2) 10µl was added to 10 ml H_2O and injected into mass spectrometer within 5 min. It is worth noting that amine **2** did not appear in the mass spectra on account of its molecular weight being lower than the cut-off threshold for the instrument. Imine **5**, disulfide **1**, thiol **4** and aldehyde **3** are all visible in the spectra. At node **A**, only aldehyde **3** and disulfide **1** appear in the spectra. As observed, spacing of ≈ 0.5 m/z between isotope peaks are characteristic of a doubly charged ion. In the node transition from node **A** to node **B** quantitative reduction to form thiol **4** from disulfide **1** by DTT is achieved in the presence of aldehyde **3** and amine **2.** Increasing the pH of the mixture from pH 6.5 to 11.8 drives the transition from node **B** to node **D**. A new peak corresponding to imine **5** is present at node **D**. This imine has hydrolysed to a significant extent on account of a reduction in pH after diluting the mixture for analysis. In *Figure 2,* we show by ^{1}H NMR spectroscopy that imine formation is near-quantitative at pH 11.8. The peak corresponding to thiol **4** remains unchanged. In the transition from node **D** to node **C** successful oxidation of thiol **4** to disulfide **1** is achieved. Peaks corresponding to aldehyde **3** and imine **5** are still present. These spectra indicate that conversion between disulfide **1** and thiol **4** is achieved in the presence of the other system components, there is evidence for imine **5** in nodes **C** and **D** as expected, however imine equilibrium is shifted on account of dilution for analysis by mass spectrometry therefore quantitative imine formation is not observed.

Procedures for Node Transitions (Clockwise Cycle):

***Node A to Node B**, reduction of disulfide* **1**: Aldehyde **3** (7.4 mg, 0.03 mmol), 3-methoxypropylamine (3.0 µL, 0.03 mmol) and disulfide **1** (15.6 mg, 0.03 mmol) were dissolved in D_2O (2 mL) at room temperature. The pH of the mixture was adjusted to pH 6.5 with aliquots of 1 M D_3PO_4 (5 µL, in D_2O). DL-dithiothreitol (DTT) (4.5 mg, 0.03 mmol) was added in one portion and stirred for 20 min. After this time, the pH of the mixture was re-adjusted to pH 6.5 and the mixture was analysed by ^{1}H NMR spectroscopy.

***Node B to Node D**, formation of imine* **5**: The mixture was raised from pH 6.5 to pH 11.8 with 5 µL aliquots of 1 M NaOH (in D_2O). The mixture was analysed by ^{1}H NMR spectroscopy.

***Node D to Node C**, oxidation of thiol* **4** *to disulfide* **1**: aliquots of H_2O_2 (2.5 µL, 0.25 M, in D_2O) were added 5 min intervals with stirring and the reaction was monitored by ^{1}H NMR spectroscopy until full oxidation had occurred. The pH was retained between pH 11.6 and pH 11.8 during the oxidation.

***Node C to Node A**, hydrolysis of imine* **4**: The pH of the mixture was decreased from pH 11.8 to pH 6.5 with aliquots of 1 M D_3PO_4 (5 µL, in D_2O). The mixture was analysed by ^{1}H NMR spectroscopy.

Procedures for Node Transitions (Anticlockwise Cycle):

Node A to Node C,** formation of imine* **5:* Aldehyde **3** (7.4 mg, 0.03 mmol), 3-methoxypropylamine (3.0 µL, 0.03 mmol) and disulfide **1** (15.6 mg, 0.03 mmol) were dissolved in D_2O (2 mL) at room temperature. The mixture was raised from pH 6.5 to pH 11.8 gradually with 5 µL aliquots of 1 M NaOH (in D_2O). The mixture was analysed by ^{1}H NMR spectroscopy.

***Node C to Node D**, reduction of disulfide* **1**: DL-dithiothreitol (DTT) (4.5 mg, 0.03 mmol) was added in one portion and stirred for 20 min. After this time, the pH of the mixture was re-adjusted to pH 6.5 with aliquots of 1 M D_3PO_4 (5 µL, in D_2O) and the mixture was analysed by ^{1}H NMR spectroscopy.

***Node D to Node B**, hydrolysis of imine* **4**: The pH of the mixture was decreased from pH 11.8 to pH 6.5 with aliquots of 1 M D_3PO_4 (5 µL, in D_2O). The mixture was analysed by ^{1}H NMR spectroscopy.

***Node B to Node A**, oxidation of thiol* **4** *to disulfide* **1**: aliquots of H_2O_2 (2.5 µL, 0.25 M, in D_2O) were added at 5 min intervals with stirring and the reaction was monitored by ^{1}H NMR spectroscopy until full oxidation had occurred. The pH was retained between pH 11.6 and pH 11.8 during the oxidation.

Expanded ^{1}H NMR Spectra of Nodes A – D (Anticlockwise cycle).

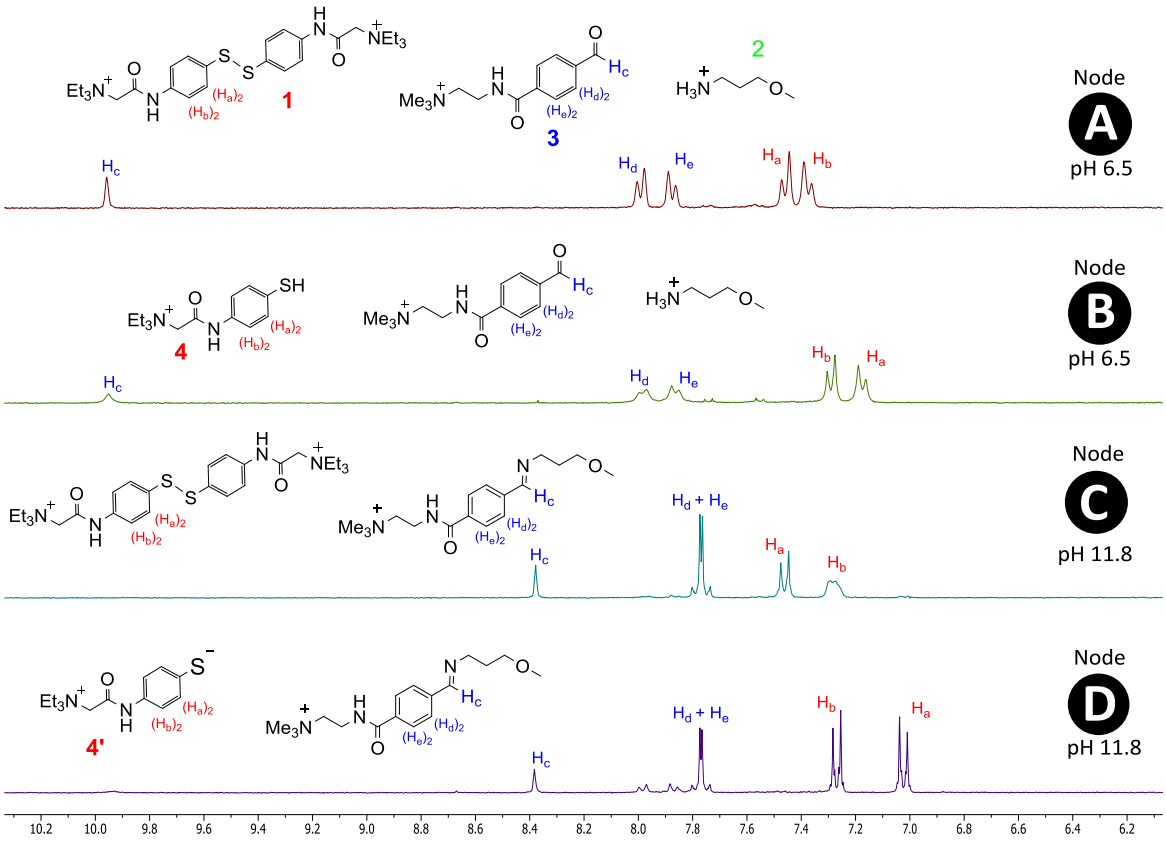


Figure S4. Expanded region of ^{1}H NMR spectra (300 MHz, D_2O) of nodes **A** – **D**. Node transitions were performed in the anticlockwise direction starting at node **A**. At node **A**, signals corresponding to disulfide **1** and aldehyde **3** are present. In the transition from node **A** to node **C** the condensation reaction of aldehyde **3** and amine **2** results in the appearance of signals corresponding to imine **5**. The chemical shift of signals corresponding to the aromatic protons H_a & H_b changes slightly on account of a change in hydrogen bonding caused by the significant change in pH. Imine **5** has been formed in the presence of aldehyde **3** and amine **2** in the absence of unwanted side reactivity. In the transition from node **C** to node **D**, quantitative reduction of disulfide **1** by DTT to form thiolate **4'** is reflected in a change in the chemical shift of the aromatic proton signals H_a & H_b. Signals corresponding to imine **5** remain unchanged suggesting that reduction of disulfide **1** by DTT at pH 11.8 is successfully achieved in the presence of imine **5** whilst avoiding unwanted side reactions. In the transition from node **D** to node **B** lowering the pH of the mixture from pH 11.8 to pH 6.5 results in hydrolysis of imine **5** to yield aldehyde **3** and amine **2**. Signals corresponding to protons in aldehyde **3** re-emerge and there are no signals corresponding to imine **5**. Protonation of thiolate **4'** to form the corresponding thiol **4** is evidenced by a change in chemical shift of signal H_a. In this transition imine **5** is hydrolysed while, other than protonation, thiol **4** remains chemically unchanged. In the transition from node **B** to node **A** oxidative coupling of thiol **4** to form disulfide **1** by H_2O_2 at pH 6.5 is indicated by a change in the chemical shift of the aromatic proton signals H_a & H_b. During this oxidative transformation, signals corresponding to protons in aldehyde **3** remain unchanged suggesting the absence of competing chemical processes. These spectra demonstrate orthogonal control over the breaking and forming of disulfide and imine dynamic covalent bonding motifs when traversing the '*4-node* network' in an anticlockwise fashion.

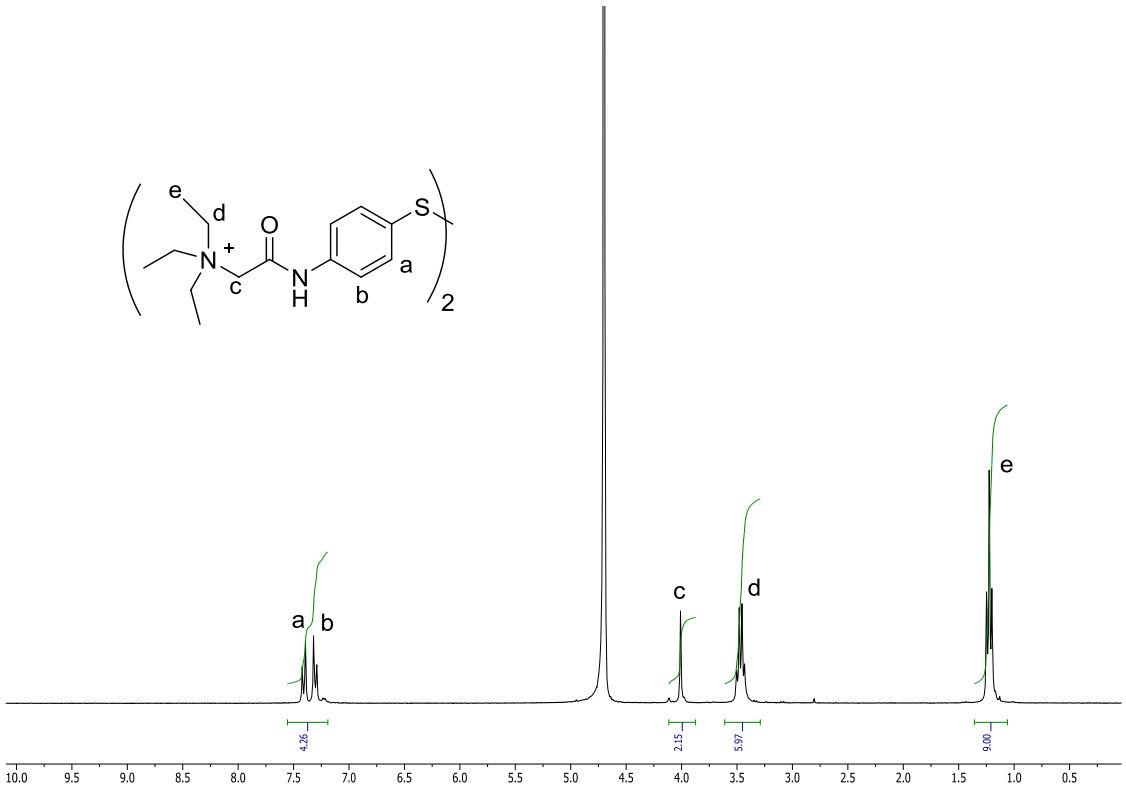


Figure S5a. ^{1}H NMR spectrum (300 MHz, D_2O) of disulfide.

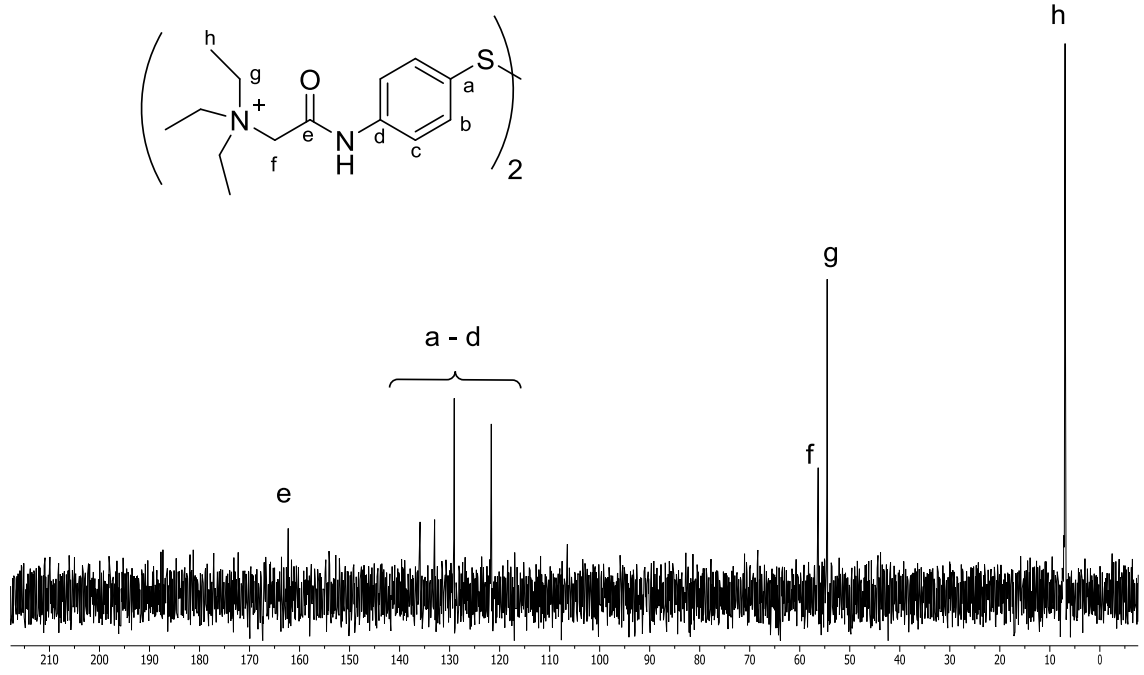


Figure S5b. ^{13}C NMR spectrum (75 MHz, D_2O) of disulfide **1.**

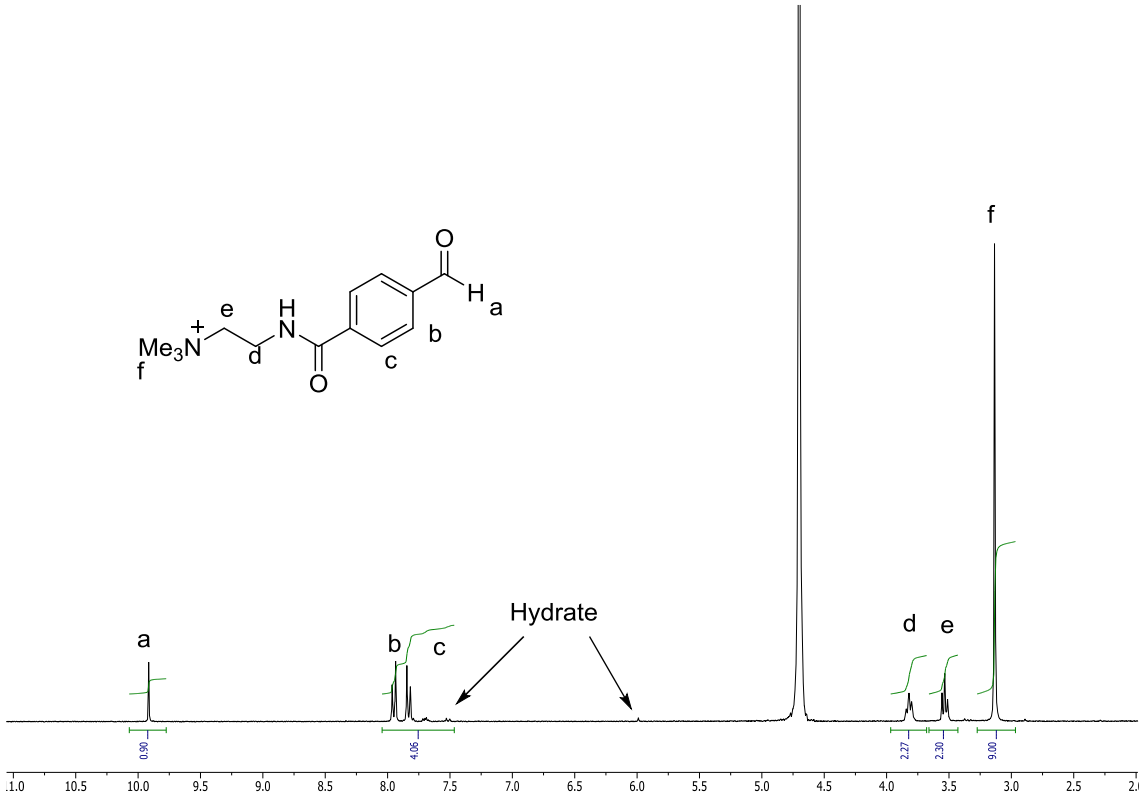


Figure S6a. ^{1}H NMR spectrum (300 MHz, D_2O) of aldehyde **3**.

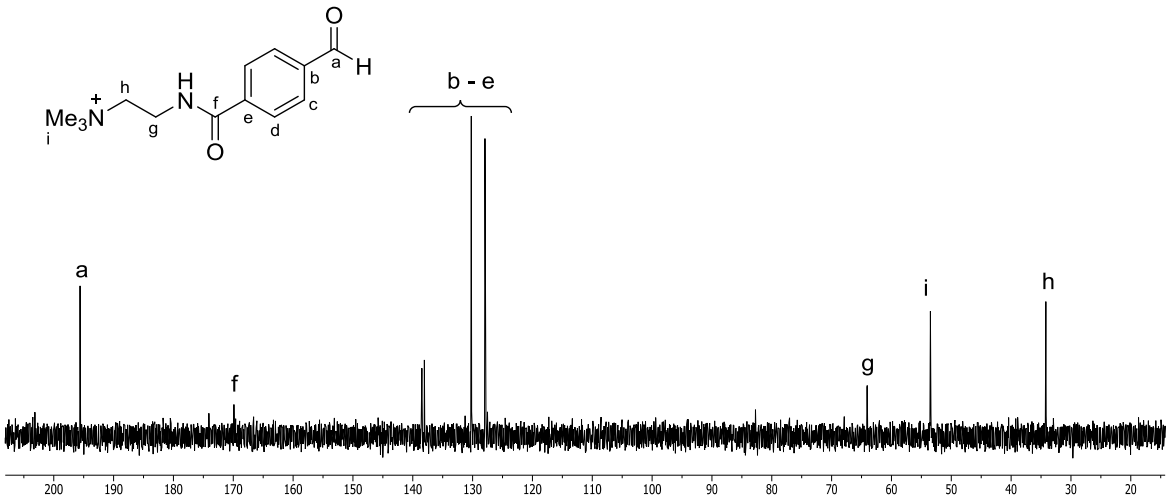


Figure S6b. ^{13}C NMR spectrum (75 MHz, D_2O) of aldehyde **3.**

Sources of Signal Broadening in ^{1}H NMR spectra

^{1}H 2D EXSY NMR spectroscopy was performed which suggests line broadening with respect to aromatic proton signals in disulfide **1** and thiol **4** is on account of thiol-disulfide interchange on the NMR timescale. The EXSY spectrum (Figure S4) displays two sets of cross peaks ***a*** and ***b*** indicating chemical exchange between aromatic protons H_a & H_c and H_b & H_d respectively. This observation suggests thiol-disulfide interchange between disulfide **1** and thiol **4** occurs on the NMR timescale in D_2O at pH 6.5. The line broadening present in node **A** and node **B** (Figure 3) with respect to disulfide/thiol aromatic signals is on account of a small amount of thiol-disulfide interchange. In the case of node **A** a small amount of thiol is present and in the case of node **B** a small amount of disulfide is present.

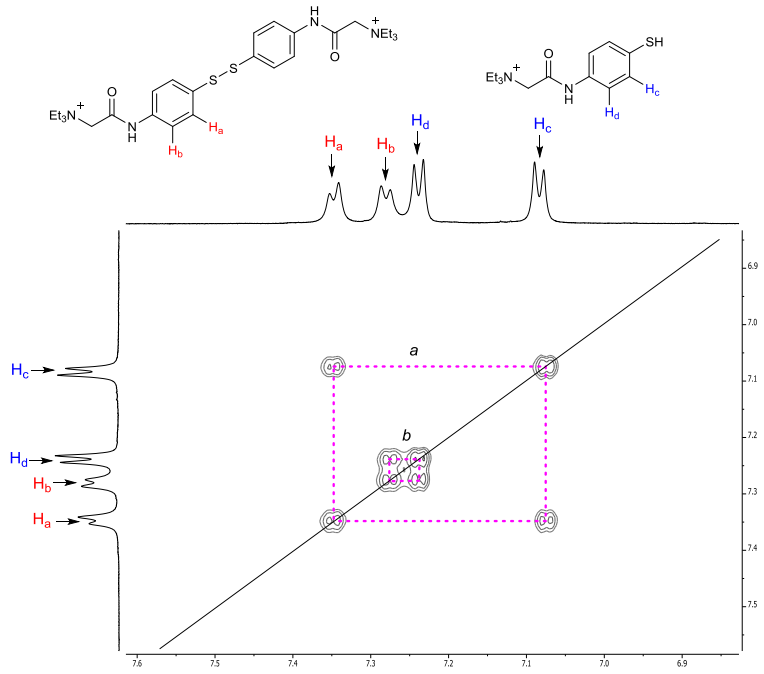


Figure S7. 2D Exsy ^{1}H NMR spectrum (700 MHz, D_2O, pH 6.5) at 100 ms mixing time of the expanded 'aromatic region' of a 1:1 mixture of disulfide **1** and thiol **4** (30 mM total concentration). A 1D spectrum of disulfide **1** and thiol **4** in D_2O (pH 6.5) is shown on the vertical and horizontal projections.

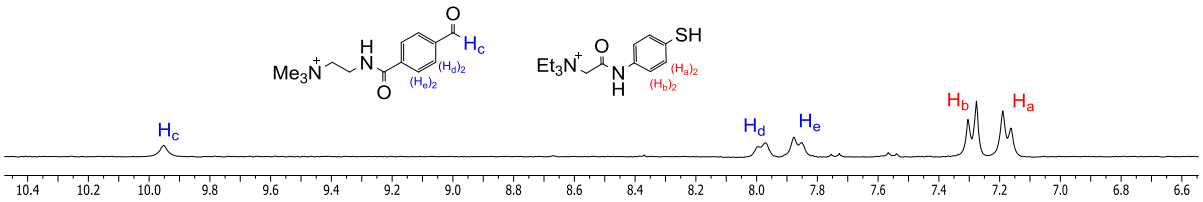


Figure S8. ^{1}H NMR (300 MHz, D_2O) spectrum of a 1:1 mixture of thiol **4** and aldehyde **3**. Thiol **4** was afforded by reduction of disulfide **1** with triphenylphosphine and purified by extraction. Signal broadening of signals corresponding to **3** suggest that there is chemical exchange involving reaction of aldehyde **3** and thiol **4** to form traces of hemithioacetal at pH 6.5 and this process is slow on the NMR timescale.