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Supporting Information

Profiling Protein S-Sulfination with Maleimide-Linked Probes

Yu-Hsuan Kuo, Aaron M. Konopko, Nicholas B. Borotto, Jaimeen D. Majmudar, Sarah E. Haynes, and Brent R. Martin*[a]

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Supporting Information

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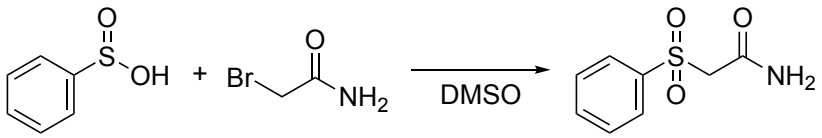
I. Experimental Procedures

General Procedures

NMR analysis was performed using a Varian 400 MHz NMR instrument. NMR integrations and coupling constants were computed using MestreNova 10.02_15465. Small molecule high-resolution mass spectrometry was performed using an electrospray Agilent Q-TOF mass spectrometer (accuracy 1-5 ppm) and analyzed using the Agilent MassHunter software suite version B.06.00. Compounds and reaction mixtures were injected in 5 µL volumes on an Agilent Zorbax Eclipse plus C18 rapid resolution column (2.1 x 50 mm, 1.8 µ), and separated using the 10-minute gradient starting at 5% and increasing to 100% acetonitrile in 8 minutes at room temperature. All the MS data was collected in ESI positive mode. Low-resolution mass spectrometry was performed using an electrospray Micromass LCT time- of-flight mass spectrometer coupled to a HPLC pump with a rheodyne loop injector. HPLC purifications were performed using a Waters semi-preparative 1525 binary pump system coupled to a photodiode array detector, an autosampler, and an automatic fraction collector. Separations were carried out on using the Waters Atlantis prep T3 C18 column (10 x 250 mm), in 95/5 water/acetonitrile 0.1% formic acid for 2 minutes, followed by a 40 min gradient increasing the mobile phase to 5/95 water/acetonitrile with 0.1% formic acid. HPLC assays were performed (5% - 95% ACN over 18 min) and analyzed using a photodiode array detector. Data were analyzed using the Waters Empower software. Resulting HPLC fractions were lyophilized using a Labconco FreeZone2.5-Plus freeze-drying system.

Scheme 1. Synthesis of 2-(phenylsulfonyl)acetamide (3)

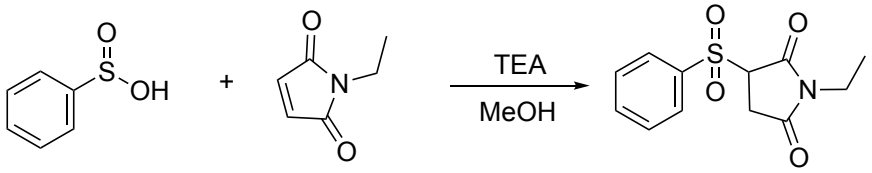


1 **14** **3**

Sodium phenylsulfinic acid (**1**, 1.2 equiv, 100 mg, 0.7 mmol) and 2-bromoacetamide (**14**, 1 equiv, 80.6 mg, 0.58 mmol) were mixed in DMSO (5 mL) and stirred overnight. The product was purified by semi-preparative HPLC, and fractions were analyzed by high-resolution mass spectrometry. The product fractions were lyophilized to afford compound **3**. ^{1}H NMR (401 MHz, DMSO-d_6): δ 7.86 (dd, J = 7.5, 1.7 Hz, 2H), 7.75 – 7.68 (m, 1H), 7.62 (t, J = 7.8 Hz, 2H), 7.55 (s, 1H), 7.31 (s, 1H), 4.21 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.10, 139.92, 134.24, 129.54,

128.38, 61.28. HRMS (ESI positive), $[M+Na]^+$ Calculated *m/z*: 222.0195, Observed *m/z* = 222.0199.

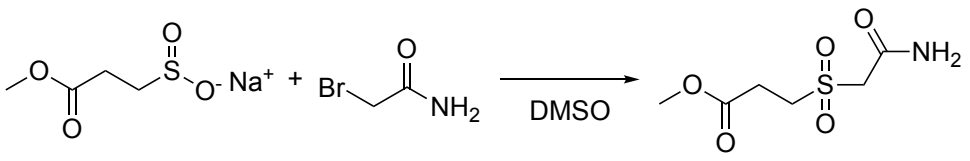
Scheme 2. Synthesis of 1-ethyl-3-(phenylsulfonyl)succinimide (5)



1 **2** **5**

Benzene sulfinic acid (**1**, 1 equiv, 100 mg, 0.7 mmol) and *N*-ethylmaleimide (**4**, 1 equiv, 130 mg, 0.7 mmol) were mixed in methanol (12 mL). TEA (2 equiv, 150 µL) was then added and the reaction was left at ambient temperature for 1 hr. The product was purified by semi-prep HPLC and fractions were analyzed by low-resolution mass spectrometry. The product fractions were lyophilized to afford compound **5**. ^{1}H NMR (401 MHz, Chloroform-*d*) δ 7.94 – 7.86 (m, 2H), 7.71 (ddt, *J* = 8.0, 6.9, 1.3 Hz, 1H), 7.64 – 7.54 (m, 2H), 4.31 (dd, *J* = 9.6, 3.7 Hz, 1H), 3.54 – 3.40 (m, 2H), 3.28 (dd, *J* = 19.1, 3.7 Hz, 1H), 3.03 (dd, *J* = 19.0, 9.6 Hz, 1H), 1.03 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.63 , 168.27 , 136.45 , 134.92 , 129.38 , 129.36 , 63.32 , 34.51 , 29.86 , 12.56. HRMS (ESI positive), $[M+Na]^+$ Calculated *m/z*: 290.0457, Observed *m/z* = 290.0500.

Scheme 3. Synthesis of methyl 3-((2-amino-2-oxoethyl)sulfonyl)propanoate (7)

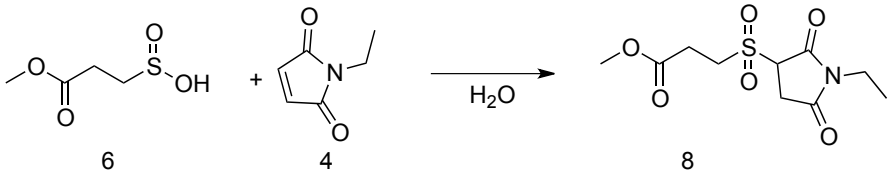


6 **14** **7**

Sodium 1-methyl 3-sulfinopropanoate (**6**, 1.2 equiv, 100 mg, 0.57 mmol) and 2-bromoacetamide (**14**, 1 equiv, 65.4 mg, 0.475 mmol) were mixed in degassed DMSO (5 mL), allowing the reaction to proceed at ambient temperature under nitrogen atmosphere overnight. The product was purified by semi-preparative HPLC, and fractions were analyzed by high-resolution mass spectrometry. The product fractions were lyophilized to afford compound **7**. ^{1}H NMR (401 MHz, Methanol-d_4): δ 4.08 (s, 2H), 3.72 (s, 3H), 3.64 (t, *J*=7.4 Hz, 2H) 2.88 (t, *J*=7.4 Hz, 2H) ^{13}C NMR (101 MHz, Methanol-d_4) δ 171.08, 164.97, 57.75, 48.67, 48.20, 47.99, 47.78, 47.57, 47.35,

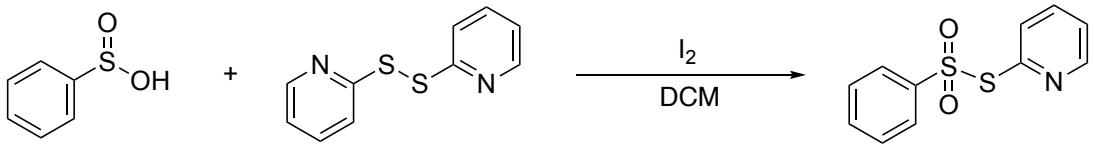
47.14, 46. 93, 26.35. HRMS (ESI positive), $[M+Na]^+$ Calculated *m/z*: 232.0250, Observed *m/z* = 232.0251.

Scheme 4. Synthesis of methyl 3-((*N*-ethyl-succinimid-3-yl)sulfonyl)propanoate (8)



Sodium 1-methyl 3-sulfinopropanoate (**6**, 1.2 equiv, 100 mg, 0.57 mmol) and *N*-ethylmaleimide (**4**, 1 equiv, 59.4 mg, 0.475 mmol) were mixed in degassed water with 0.1% formic acid (5 mL), allowing the reaction to proceed at rt for overnight under inert atmosphere. The product was purified by semi-preparative HPLC, and fractions were analyzed by high-resolution mass spectrometry. The product fractions were lyophilized to afford compound **8**. ^{1}H NMR (401 MHz, Methanol-d_4); δ 4.76 (dd, *J*=7.1, 6.4 Hz, 1H), 3.87 (ddd, *J*= 14.7, 7.8, 7.0 Hz, 1H), 3.78 (dd, *J* = 7.8, 6.8 Hz, 1H), 3.73 (s, 3H), 3.56 (q, *J*=7.2 Hz, 2H), 3.11-3.06 (m, 2H), 3.01-2.87 (m, 2H), 1.14 (t, *J*=7.2 Hz, 3H). ^{13}C NMR (101 MHz, Methanol-d_4); δ 173.82, 170.96, 169.81, 60.32, 48.21, 48.00, 47.78, 47.57, 47.36, 47.14, 46.93, 33.86, 27.49, 26.06, 11.38. HRMS (ESI positive), $[M+Na]^+$ Calculated *m/z*: 300.0512, Observed *m/z* = 300.0513.

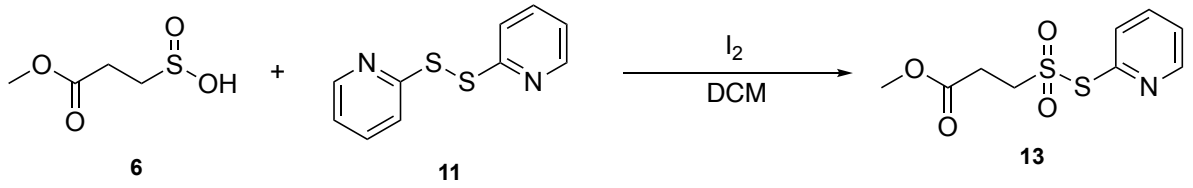
Scheme 5. Synthesis of *S*-pyridin-2-yl benzenesulfonothioate (12)



Benzene sulfinic acid (**1**, 3.2 equiv, 114 mg, 0.8 mmol) and 2, 2'-Dipyridyldisulfide (**11**, 1 equiv, 55.2 mg, 0.25 mmol) were dissolved in dichloromethane (2 mL). Iodine (2 equiv, 127.3 mg, 0.5 mmol) was then added, allowing the reaction to proceed for 1 hr. The reaction solution was diluted with dichloromethane (2 mL), and a 1 M aqueous sodium thiosulfate solution was added drop by drop until the iodine color disappeared. The organic layer was washed with water for twice and dried over anhydrous magnesium sulfate, and filtered, and the solvent was evaporated. The resulting residue was purified by silica gel column chromatography

(hexane:ethyl acetate=10:1 and then 4:1), collecting fractions for high-resolution mass spectrometry confirmation. Fractions containing the product were lyophilized to afford compound **12**. ^{1}H NMR (401 MHz, Chloroform-*d*); δ 8.51 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.79-7.73 (m, 2H), 7.69 (dd, *J*= 8.2, 1.5 Hz, 2H), 7.61-7.56 (m, 1H), 7.45 (t, *J*= 7.7 Hz, 2H), 7.35 (ddd, *J* = 7.0, 4.9, 2.6 Hz, 1H). ^{13}C NMR (101 MHz, Chloroform-*d*); δ 150.64, 137.82, 133.83, 131.93, 129.00, 127.37, 124.90. HRMS (ESI positive), $[M+H]^+$ Calculated *m/z*: 252.0147, Observed *m/z* = 252.0165.

Scheme 6. Synthesis of methyl 3-((pyridin-2-ylthio)sulfonyl)propanoate (13)



6 **11** **13**

Sodium 1-methyl 3-sulfinopropanoate (**6**, 3.2 equiv, 140 mg, 0.8 mmol) and 2, 2'-dipyridyldisulfide (**11**, 1 equiv, 55 mg, 0.25 mmol) were dissolved in dichloromethane (2 mL). Iodine (2 equiv, 126.7 mg, 0.5 mmol) was then added, allowing the reaction to proceed for 1 hr. The solvent was then evaporated. The resulting residue was purified by silica gel column chromatography (hexane:ethyl acetate=10:1 and then 3:1), collecting fractions for high-resolution mass spectrometry. Fractions containing the product were lyophilized to afford compound **13**. ^{1}H NMR (401 MHz, Chloroform-*d*); δ 8.68-8.58 (m, 1H), 7.79 (tt, *J*=7.7, 1.4 Hz, 1H), 7.73-7.64 (m, 1H), 7.39 (ddt, *J* = 7.3, 4.9, 1.1 Hz, 1H), 3.94-3.87 (m, 2H), 3.73 (s, 3H), 3.01 (t, *J* = 7.7, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*); δ 170.22, 150.88, 150.84, 138.21, 131.25, 124.96, 56.75, 52.36, 28.48. HRMS (ESI positive), $[M+H]^+$ Calculated *m/z*: 262.0202, Observed *m/z* = 262.0220.

Biochemical analysis of DJ-1 oxidation

Recombinant human DJ-1 was prepared as previously described[5], and diluted to 0.02 mg / mL in PBS buffer. Next, protein samples were incubated with either vehicle, 5 equivalents, or 100 equivalents of hydrogen peroxide at room temperature. After 30 min, excess reagents were removed by passing through one Vivaspin 500 PES 5k MWCO column (Sartorius) equilibrated with 6 M urea / PBS at pH 7.4. Spins were repeated to achieve complete buffer exchange. Recombinant human DJ-1 was then treated with 2.5 mM DTT for 30 min, followed by alkylation

with 50 mM iodoacetamide / 10 mM EDTA in the dark for 1 hour at room temperature. Excess reagents were removed using a Vivaspin 500 PES 5k MWCO column (Sartorius) equilibrated with 6 M urea / citrate-phosphate buffer at pH 4.5. For gel-based analysis, DyLight 800 Maleimide (Thermo Scientific) was added for 1 hour. Protein samples were resolved by SDS-PAGE (12% gel) and images using an Azure c600 imager. For proteomic analysis, 5 mM N-ethylmaleimide was added for 1 hour, quenched by passing through one Vivaspin 500 PES 5k MWCO column (Sartorius) equilibrated with potassium chloride-hydrochloric acid buffer at pH 1.5. Spins were repeated to achieve complete removal and buffer exchange. The samples were incubated with mass spectrometry grade pepsin (Promega) with enzyme:protein ratios of 1:50 (w:w) for 2 hrs at 37 °C with agitation. After digestion, additional salts were removed using a Waters Oasis HLB µElution plates (30 µm) following the manufacturer's protocol. The eluted peptide samples were dried using a Savant SPD1010 concentrator (Thermo) and reconstituted in 1-D LC-MS 0.1% formic acid in 3% acetonitrile/water.

Mass spectrometry analysis of DJ-1 oxidation

DJ-1 digests were separated using a Thermo Scientific Acclaim PepMap RSLC C18 column (75 µm x 15 cm, 2 µm, 100 Å) on a Thermo Scientific Dionex Ultimate 3000 RSLCnano system configured for nano flow. The peptides were eluted using a 30 min linear gradient ranging from 5 to 45% acetonitrile acidified with 0.1 % formic acid. The flow rate was set to 0.3 µl/min and the column was heated to 35 °C for the duration of the gradient. The eluant was directly introduced into the mass spectrometer. MS analyses were completed using a Thermo Scientific Orbitrap Fusion Lumos Tribrid Mass Spectrometer mass spectrometer. The nESI source was operated at a needle voltage of 1600 V, and the ion transfer tube temp was set to 275 °C. Tandem mass spectra were collected in the ion trap using CID for doubly charged peaks or ETD for all higher charger states. For CID experiments the collision energy was set to 30% and for ETD the calibrated charge-dependent ETD feature was utilized. For both fragmentation techniques three microscans were collected and an isolation window of 2 Daltons was used. An exclusion limit of 60 s was activated after one spectrum was collected for any given peak. The resolution of the Orbitrap was set to 120000 and the ion trap was run in enhanced resolution mode. Raw mass spectral files were analyzed using Sequest HT from within Proteome Discoverer 2.2.0.386. The spectra were searched against the cRAP database (http://www.thegpm.org/crap/) with DJ-1 added. The tolerance was set to 10 ppm and 0.3 Da for ions measured in the orbitrap and ion trap, respectively. Variable modification by NEM on cysteine residues was added as a user

modification (157.038 Da). Additionally, variable modifications for methionine oxidation, cysteine trioxidiation, and cysteine carbamidomethylation were also searched. The protein was set to pepsin (semi-specific cleavage at F, L, Q, W, or Y residues), three missed cleavages were allowed, and all PSMs were filtered to a FDR of 0.01% using Percolator. Quantification is based off of precursor ion abundance and was calculated using Proteome Discoverer.

II. Supporting Figures

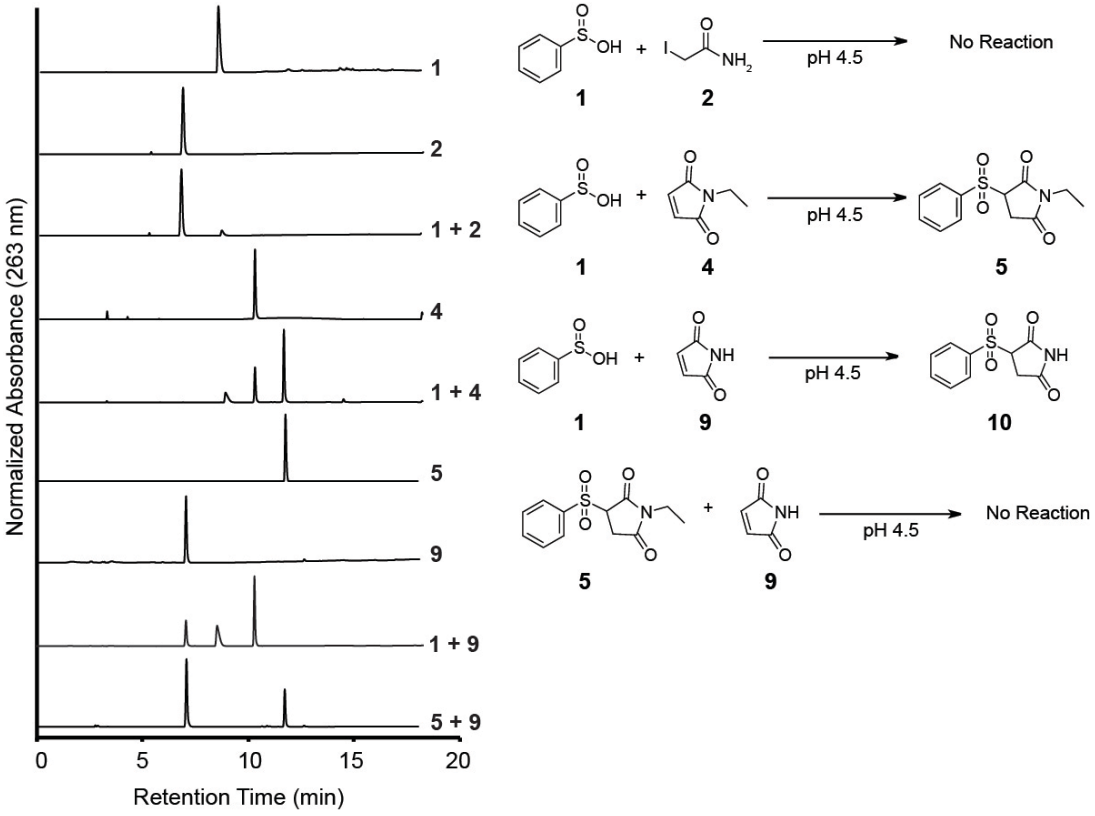


Figure S1. HPLC analysis of sulfinic acid reactivity and reversibility.

A 10-fold excess of electrophile (**2**, **4**, or **9**) was added to phenylsulfinate (**1**) for 60 minutes at room temperature in citrate-phosphate buffer supplemented with 6 M urea (pH 4.5). Samples were analyzed by HPLC using a photodiode array detector, extracting data elution spectra at 263 nm. The maximal absorbance value for each trace is shown normalized to 1.

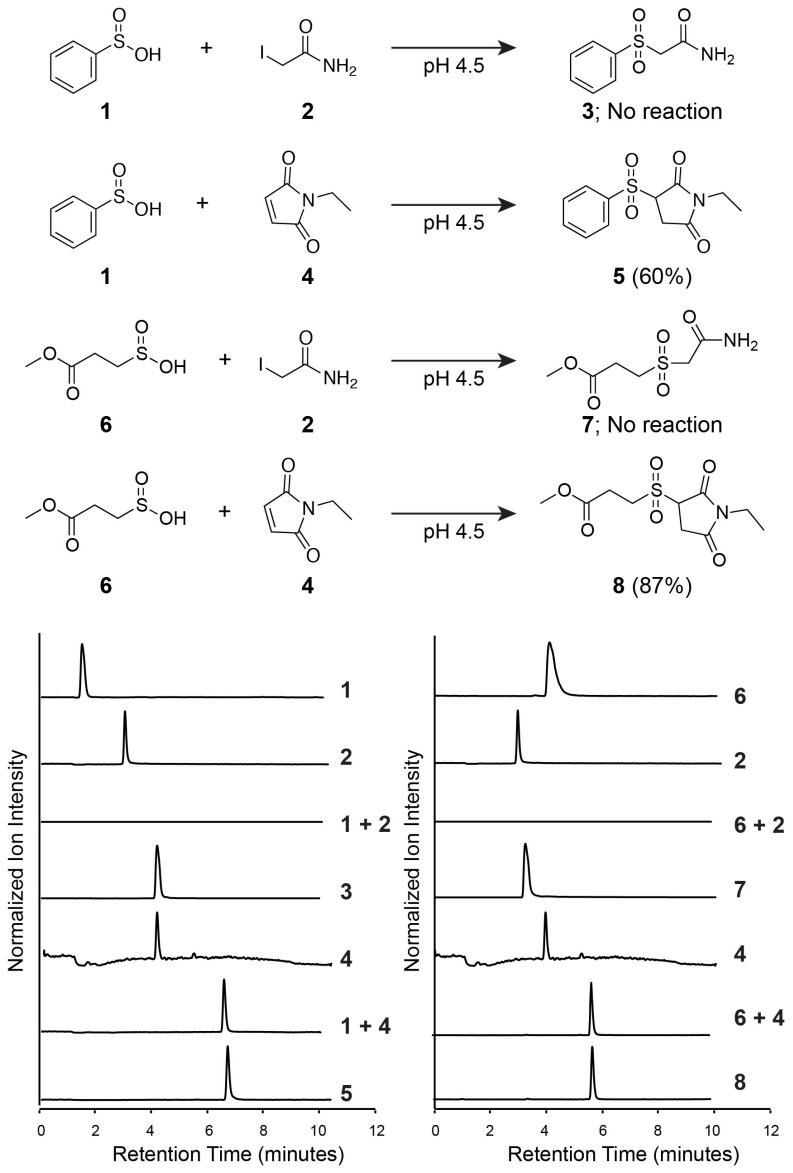


Figure S2. LC-MS analysis of sulfinic acid reactivity.

Reactions were carried out in 10:1 (electrophile : nucleophile) in 6 M urea / PBS (pH 7.4) for 2 hours and assayed by high resolution LC-MS. **1**: $[M+H]^+$; calculated *m/z* = 143.0161, observed *m/z* = 143.0149, **2**: $[M+H]^+$; calculated *m/z* = 185.941, observed m/z = 185.9406, **3**: [M+H]; calculated *m/z* = 200.0376, observed *m/z* = 200.0429, **4**: $[M+H]^+$; calculated *m/z* = 126.055, observed *m/z* = 126.0546, **5:** $[M+H]^+$; calculated *m/z* = 290.0457, observed *m/z* = 290.0499, **6**: $[M+Na]^+$; calculated *m/z* = 175.0036, observed *m/z* = 175.0024, **7**: $[M+H]^+$; calculated *m/z* = 210.0431, observed *m/z* = 210.0451, and **8**: $[M+Na]^+$; calculated *m/z* = 300.0512, observed *m/z* = 300.0503. Standard curves were generated with **5** and **8** to calculate the relative reaction efficiencies.

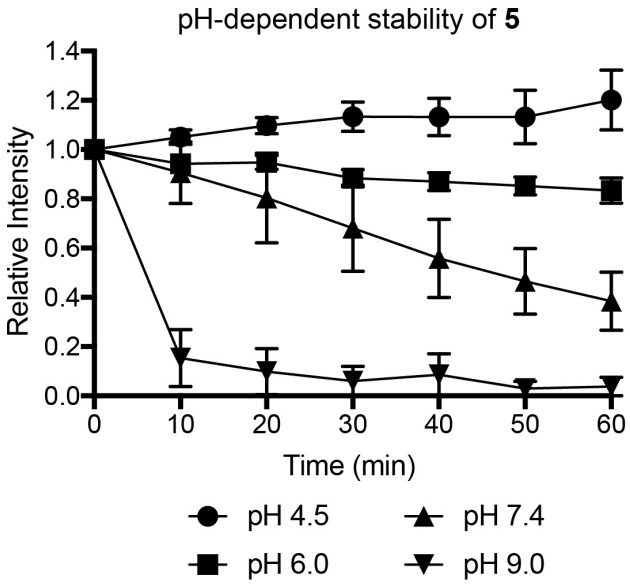


Figure S3. The sulfonyl succinimide product (5) is stable in acidic buffers.

5 was added in 6 M urea citrate/phosphate buffer at pH 4.5, 6, 7.4, and 9 and analyzed by high resolution LC-MS every 10 min for 1 hr. **5**: $[M+H]^+$; calculated *m/z* = 268.0638, observed *m/z* = 268.0629. Ion counts were normalized to the initial value (t = 0 min). Error bars represent standard error from three replicates.

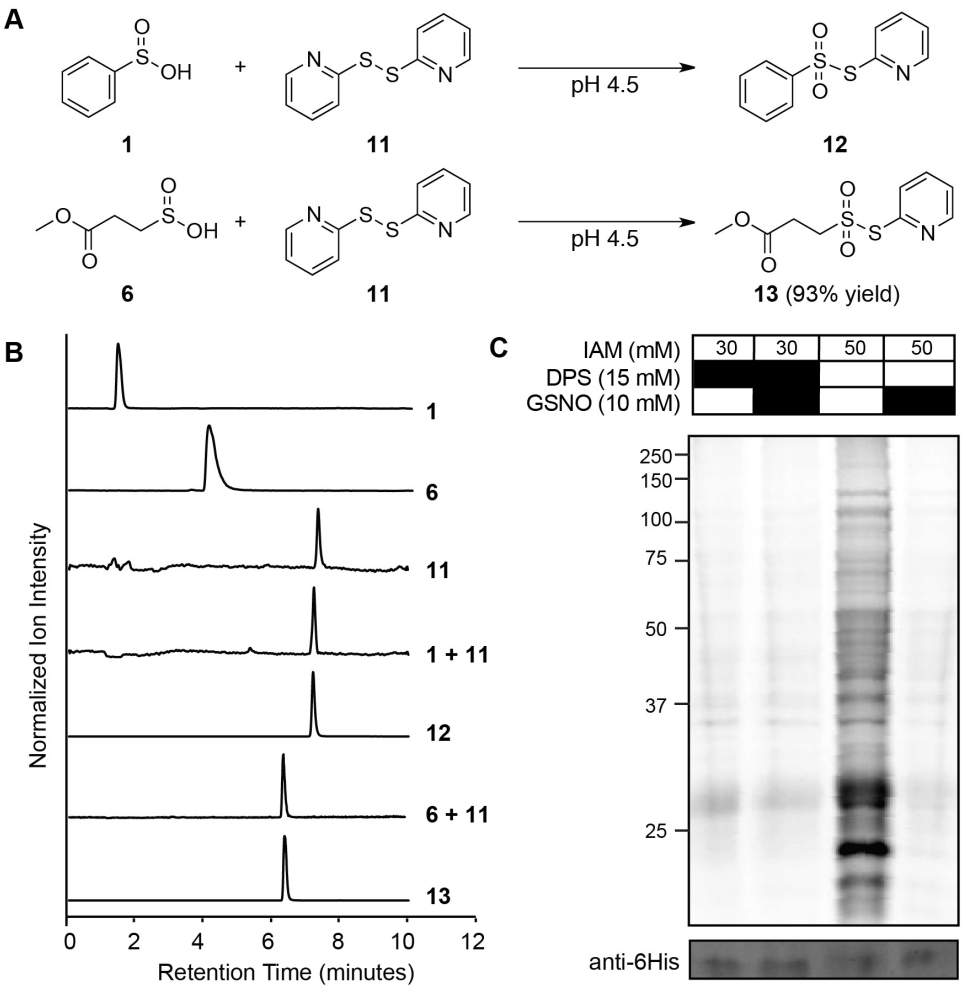


Figure S4. 2, 2'-Dipyridyldisulfide (DPS) reacts with sulfinic acids.

(A) 10 mM sulfinic acid (**1** or **6**) was mixed with 100 mM solution of DPS (**15**) in degassed 6 M urea / citrate-phosphate buffer (pH 4.5) for 2 hr, and assayed by high resolution LC-MS. (B) Normalized extracted ion chromatographs of each species are shown. **1**: $[M+H]^+$; calculated *m/z* = 143.0161, observed *m/z* = 143.0149, **6**: $[M+Na]^+$; calculated *m/z* = 175.0036, observed *m/z* = 175.0023, **11**: $[M+H]^+$; calculated *m/z* = 221.0202, observed *m/z* = 221.0279, **12**: $[M+H]^+$; calculated *m/z* = 252.0147, observed *m/z* = 252.0141, and **13**: $[M+H]^+$, calculated *m/z* = 262.0202, observed *m/z* = 262.0205. A standard curve was created with **13** to quantify the reaction efficiency. (C) DPS blocks iodoacetamide-insensitive maleimide labeling in E.coli lysates expressing human His_6-DJ-1. Lysates were labeled with 50 μM maleimde-TAMRA for in-gel fluorescence analysis.