Supporting Information for:

Mass Spectrometry-Based Assay for the Rapid Detection of Thiol-Containing Natural Products

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Supporting Information Figure S13. TCEP resin was incubated with oxidized glutathione to yield reduced glutathione, which was then incubated with resin **2**.

General Procedures and Materials:

Unless otherwise noted, all chemicals were purchased from commercial sources and used as received without further purification. Water (dd-H_2O) used in all procedures was deionized using an EMD Millipore Milli-Q® purification system (Billerica, MA). Tentagel HL-NH_2 resin (110 μm, 0.4 mmol/g) was purchased from Chem Impex International, Inc. (Wood Dale, IL). The Fmoc-photolinker (4-(4-[1-(9-Fluoroenylmethyloxycarbonylamino)ethyl]-2-methoxy-5-nitrophenoxy)butanoic acid) was purchased from Advanced ChemTech (Louisville, KY). The 2-Fmoc-PEG_2-OH (8-(Fmoc-amino)-3,6-dioxaoctanoic acid) was purchased from Chem-Impex (Wood Dale, IL). The Fmoc-Ahx-OH (Fmoc-6-aminohexanoic acid) was purchased from Novabiochem. The Fmoc-*p*-chloro-Phe-OH (Fmoc-4-chloro-D-phenylalanine) was purchased from Bachem. Vasopressin (H-Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2 disulfide bridge: 1-6) was purchased from AnaSpec. Immobilized TCEP disulfide reducing gel was purchased from Pierce Thermo Fisher Scientific (Waltham, MA) and used according to the manufacturer's instructions.

Resin modification steps were performed in fritted disposable chromatography columns (Bio-Rad, Hercules, CA). During the reactions, the resin suspensions were rotated using a Labquake™ Shaker Rotisserie (Barnstead/Thermolyne, Dubuque, IA).

Mycobacterium smegmatis was purchased from ATCC (strain 19420). Middlebrook ADC growth supplement was purchased from Fluka. Middlebrook OADC growth supplement was purchased from Hinedia Laboratories.

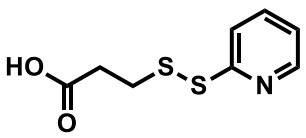
Instrumentation and Sample Analysis:

NMR. ^{1}H and ^{13}C spectra were measured with a Bruker Avance III HD NanoBay AX-400 (400 MHz) spectrometer.

Mass Spectrometry. Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on either an Applied Biosystems-SCIEX 4800 MALDI-TOF/TOF MS or an Applied Biosystems-SCIEX 5800 MALDI-TOF/TOF MS, and the data were analyzed using Data Explorer software. Samples were co-crystallized with one of two MALDI matrices. First, universal MALDI matrix (1:1 mixture of 2,5-dihydroxybenzoic acid:α-cyano-4-hydroxy-cinnamic acid) as a 10 mg/mL solution in 1:1 acetonitrile (MeCN) to H_2O with 2% formic acid. Second, 1:10 2-(4′-hydroxybenzenazo)benzoic acid(HABA):α-cyano-4-hydroxy-cinnamic acid (CHCA) as a 10 mg/mL solution in 7:3 MeCN to H_2O with 2% formic acid. In Figure 2c, resin **1** was co-crystallized with universal MALDI matrix and resin **2** was co-crystallized with 1:10 HABA:CHCA. In Figure 3b, samples were co-crystallized with 1:10 HABA:CHCA. In Figure 4b, samples were co-crystallized with universal MALDI matrix.

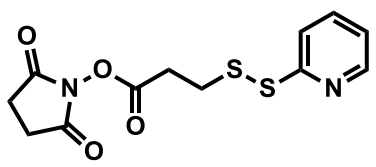
Accurate mass analysis for crude *M. smegmatis* cell lysis was performed on an Agilent 6450 LC-CID-MS-QTOF using a reverse phase column (ZORBAX Eclipse Plus C18, 1.8 μm, 2.1 x 50 mm) with electrospray ionization (positive ion mode). Reverse-phase UPLC of the cell lysate was accomplished using a H_2O:MeCN with 0.1% formic acid gradient mobile phase.

Experimental Procedures:



3-(Pyridyldithio)propionic acid. 3-(Pyridyldithio)propionic acid was synthesized following a previously reported procedure.[1] To a flask equipped with a stir bar was added 2,2′-dipyridyl disulfide

(2.08 g, 9.4 mmol, 2 equiv), ethanol (20 mL), and acetic acid (0.40 mL, 7.0 mmol, 1.3 equiv). After 20 min, the 2,2′-dipyridyl disulfide was fully dissolved and a solution of 3-mercaptopropionic acid (0.50 g, 4.7 mmol, 1 equiv) in ethanol (10 mL) was added dropwise over the course of 30 min, resulting in a clear, bright yellow solution that was stirred overnight at room temperature. After stirring overnight, the solvent was removed *in vacuo*. The resulting yellow oil was purified via silica gel chromatography. Impurities were eluted using 200:10:4 ethyl acetate:methanol:triethylamine and the desired product was eluted from the column using 200:20:1 ethyl acetate:methanol:acetic acid. To remove residual acetic acid, the light yellow oil was purified via silica gel chromatography with CH_2Cl_2:MeOH (50:1) as the eluent, yielding a light yellow solid (0.69 g, 68% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.63 (2H, t, J = 6.92 Hz), 3.01 (2H, t, J = 6.90 Hz), 7.21-7.29 (1H, m), 7.73-7.79 (1H, m), 7.79-7.87 (1H, m), 8.44-8.48 (1H, m), ^{13}C NMR (100 MHz, DMSO-d_6): δ = 33.4, 33.5, 119.3, 121.2, 137.8, 149.6, 159.0, 172.6. ESI-MS $[M + H]^+$ calculated for $C_8H_9NO_2S_2$ 216.0147, found $[M + H]^+$ 216.0147.



_N_-Succinimidyl-3-(2-pyridyldithio)-propionate (SPDP). SPDP was synthesized following previously reported procedures.[1-3] A flask charged with 3-(pyridyldithio)propionic acid (0.37 g, 1.7 mmol, 1.0 equiv) dissolved in anhydrous dichloromethane (20 mL) was combined with *N*-hydroxysuccinimide (0.22 g, 1.9 mmol, 1.1 equiv) and cooled to 0 ºC. To this was added a solution of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (0.36 g, 1.9 mmol, 1.1 equiv) in dichloromethane (15 mL) dropwise. The resulting solution was warmed to room temperature and stirred overnight. The solvent was then removed via rotary evaporation. The product was re-dissolved in ethyl acetate, washed with water, a 5% aqueous solution of

$NaHCO_3$, brine, and dried over $MgSO_4$. The solvent was then removed via rotary evaporation, and the product purified via silica gel chromatography with CH_2Cl_2:MeOH (50:1) as the eluent, yielding a light yellow solid (0.28 g, 53% yield). ^{1}H NMR (400 MHz, dichloromethane-d_2): δ = 2.78-2.83 (4H, s), 3.05-3.16 (4H, m), 7.09-7.16 (1H, m), 7.64-7.71 (2H, m), 8.42-8.51 (1H, m); ^{13}C NMR (100 MHz, dichloromethane-d_2): δ = 26.1, 31.5, 33.5, 120.2, 121.5, 137.7, 150.3, 159.7, 167.7, 170.0. ESI-MS $[M + H]^+$ calculated for $C_{12}H_{12}N_2O_4S_2$ 313.0311, found 313.0322.

General procedure for solid-phase synthesis of photocleavable resin linker (1). The photocleavable resin linker (1) was synthesized using standard Fmoc-based chemistry on Tentagel-NH_2 resin. In a typical synthesis, the resin (500 mg, 0.21 mmol, 1 equiv) was washed once with dimethylformamide (DMF) and then swollen in DMF for 30 min. The solvent was then drained and fresh DMF (5 mL) was added to the reaction vial. The first Fmoc-protected acid (Fmoc-photolinker, 320 mg, 0.62 mmol, 3 equiv) was dissolved in DMF (2 mL). To this was added a solution of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP, 309 mg, 0.59 mmol, 2.9 equiv) in DMF (2 mL) with *N,N*-diisopropylethylamine (DIPEA, 143 μL, 0.82 mmol, 4 equiv). This combined solution was then added to the resin and the coupling was allowed to proceed for 1 h. After 1 h, the resin was washed with DMF (5 x 10 mL). Any unreacted resin was then capped through incubation with a 1:1 CH_2Cl_2: acetic anhydride solution (1 x 10 mL for 3 min, 1 x 10 mL for 7 min). After capping, the resin was washed with DCM (5 x 10 mL) and DMF (5 x 10 mL). Deprotection of the Fmoc groups was performed with a 20 min incubation in a 20% v/v piperidine in DMF solution. Following deprotection, the resin was washed with DMF (5 x 10 mL). Subsequent coupling reactions were carried out for 30 min using 3 equivalents of Fmoc acid with 2.9 equivalents of PyBOP and 4

equivalents of DIPEA. Capping and deprotection steps were performed as outlined above. Throughout the synthesis, the reaction vial was protected from light to prevent premature cleavage of the linker from the solid phase. The linker (**1**) was kept on the resin and stored dry and protected from light at 4 ºC until use.

General procedure for extension of photocleavable resin linker, yielding protected thiol-terminated linker resin (2). In a typical synthesis, resin (**1**) was weighed (10 mg, 0.004 mmol, 1 equiv) into a 10 mL biospin column. The resin was rinsed with CH_2Cl_2 (3 x), swollen in CH_2Cl_2 (1 x 15 min), washed with CH_2Cl_2 (3 x), and DMF (5 x). The solvent was drained and a solution of SPDP (10 mg, 0.03 mmol, 8 equiv) in DMF (1 mL) was added. The solution was protected from light and agitated at room temperature overnight. Following overnight incubation, the solution was drained and the resin was washed with DMF (5 x) and CH_2Cl_2 (5 x). Modification was confirmed via MALDI-TOF MS.

General procedure for evaluation of solid phase reaction via MALDI-TOF MS. Resins (post-washing) were swollen (~1 mg) in 20 μL of 7:3 MeCN:H_2O for 10 min, protected from light. Resins were then spotted on a stainless steel MALDI plate and allowed to dry in air. The plate was then irradiated for 10 min with long wavelength UV light from a compact UV lamp (model UVGL-25, P/N 95-0021-12, 254/365 nm, 4-Watt, 115V-60 Hz, 0.16 Amps) from UVP, LLC (Upland, CA). The matrix (universal MALDI matrix or 1:10 HABA:CHCA as indicated) was then added to the plate and allowed to dry in air prior to MALDI-TOF MS analysis.

General procedure for thiol-containing compound capture with resin (2). A 1.7 mL Eppendorf tube was charged with 50 mM tris pH 7.5 containing 0.5 mM EDTA buffer (390 μL) and thiol-containing compound (1 μmol, 2.5 equiv, 10 μL of a 100 mM solution in H_2O or DMF). To this was added resin **2** (~1 mg, 0.4 μmol, 1 equiv). The mixture was agitated at room temperature protected from light for 3 h. The resulting resins were rinsed with CH_2Cl_2 (3 x), DMF (3 x), H_2O (3 x), DMF (1 x), and CH_2Cl_2 (3 x). Modification was evaluated via MALDI-TOF MS.

General procedure for disulfide bond reduction of 2 with TCEP. In a typical reaction, a 1.7 mL Eppendorf tube was charged with *tris*(2-carboxyethyl)phosphine (TCEP) in H_2O (1 mL of 10 mM pH adjusted to 7, 10 μmol, 2.5 equiv). To this was added resin *2* (10 mg, 4 μmol, 1 equiv). This mixture was agitated at room temperature protected from light for 30 min. The resin was rinsed in a biospin column with CH_2Cl_2 (3 x), DMF (3 x), H_2O (3 x), DMF (1 x), and CH_2Cl_2 (1 x). Modification was evaluated via MALDI-TOF MS.

General procedure for regeneration of pyridyl disulfide-terminated resin, 2, from sulfhydryl-terminated resin, 7. A 1.7 mL Eppendorf tube was charged with 2,2-dithiodipyridine (0.5 mL of 90 mM in DMF, 45 μmol, 11.3 equiv) and 100 mM tris pH 8.0 with 1 mM EDTA (0.5 mL). To this was added resin 2 (10 mg, 4 μmol, 1 equiv). This mixture was briefly vortexed and allowed to agitate at room temperature protected from light for 3 h. The resin was rinsed in a biospin column with CH_2Cl_2 (3 x), DMF (3 x), H_2O (3 x), DMF (1 x), and CH_2Cl_2. Modification was evaluated via MALDI-TOF MS.

General procedure for evaluating chemoselectivity of resin (2) using compounds (iv), (v), (vi), (viii), and (ix). A 1.7 mL Eppendorf tube was charged with compounds 1-5 (1 μmol, 2.5 equiv, 2 μL each of compounds (iv), (v), (viii), and (ix) as 500 mM solutions in DMF and 2 μL of compound (vi) as a 500 mM solution in H_2O), DMF (90 μL), and 100 mM tris pH 7.5 containing 1 mM EDTA buffer (100 μL). This mixture was briefly vortexed. Then, resin **2** (~1 mg, 0.4 μmol, 1 equiv) was added to the solution. This mixture was agitated at room temperature protected from light for 3 h. The resin was rinsed in a biospin column with CH_2Cl_2 (3 x), DMF (3 x), H_2O (3 x), DMF (1 x), and CH_2Cl_2. Modification was evaluated via MALDI-TOF MS.

General procedure for evaluating chemoselectivity of resin (2) using compound (vii). A 0.7 mL Eppendorf tube was charged with 50 mM tris pH 7.5 containing 0.5 mM EDTA buffer (90 μL) and L-serine (1 μmol, 2.5 equiv, 10 μL of a 100 mM solution in H_2O). To this was added resin **2** (~1 mg, 0.4 μmol, 1 equiv). The mixture was agitated at room temperature protected from light for 3 h. The resulting resin was rinsed with CH_2Cl_2 (3 x), DMF (3 x), H_2O (3 x), DMF (1 x), and CH_2Cl_2 (3 x). Modification was evaluated via MALDI-TOF MS.

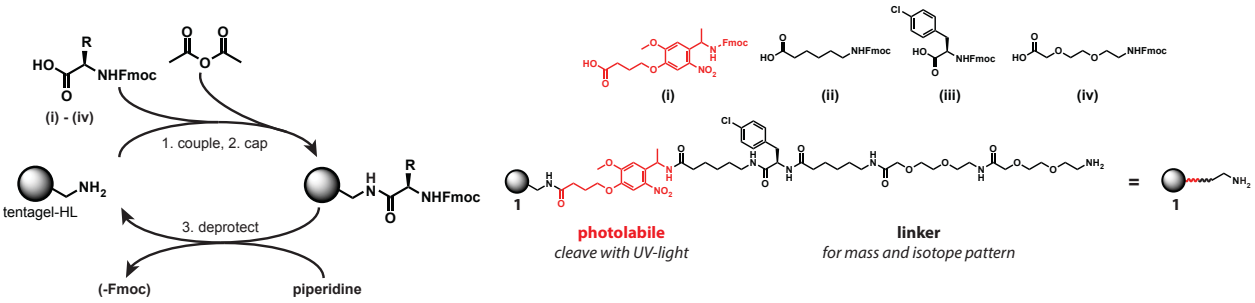
General procedure for evaluating chemoselectivity of resin (2) using oxidized glutathione (xi). A 0.7 mL Eppendorf tube was charged with 50 mM tris pH 7.5 containing 0.5 mM EDTA buffer (90 μL) and oxidized glutathione (1 μmol, 2.5 equiv, 10 μL of a 100 mM solution in H_2O). To this was added resin **2** (~1 mg, 0.4 μmol, 1 equiv). The mixture was agitated at room temperature protected from light for 3 h. The resulting resins were rinsed with CH_2Cl_2 (3 x), DMF (3 x), H_2O (3 x), DMF (1 x), and CH_2Cl_2 (3 x). Modification was evaluated via MALDI-TOF MS.

General procedure for evaluating chemoselectivity of resin (2) using maleimidobutyric acid (x). A 0.7 mL Eppendorf tube was charged with 50 mM tris pH 7.5 containing 0.5 mM EDTA buffer (90 μL) and maleimidobutyric acid (1 μmol, 2.5 equiv, 10 μL of a 100 mM solution in DMF). To this was added resin **2** (~1 mg, 0.4 μmol, 1 equiv). The mixture was agitated at room temperature protected from light for 3 h. The resulting resins were rinsed with CH_2Cl_2 (3 x), DMF (3 x), H_2O (3 x), DMF (1 x), and CH_2Cl_2 (3 x). Modification was evaluated via MALDI-TOF MS.

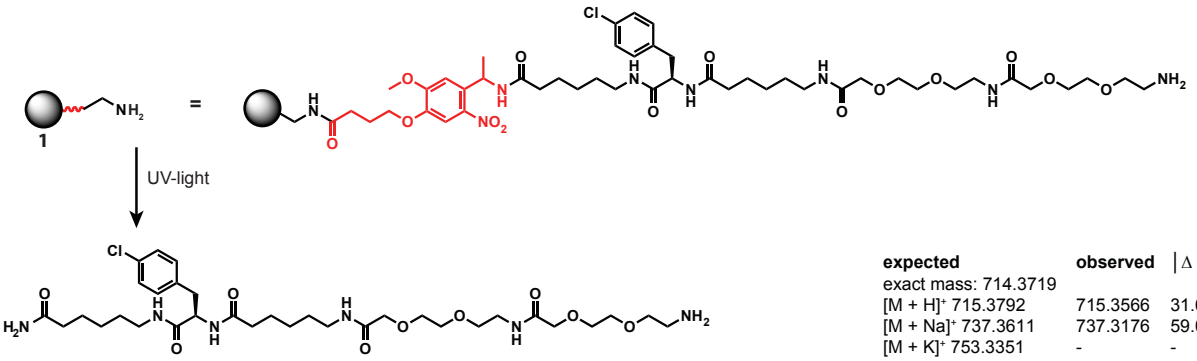
***Mycobacterium smegmatis* growth conditions.** *M. smegmatis* (ATCC strain 19420) was grown on either Middlebrook 7H10 agar with OADC enrichment or in Middlebrook 7H9 broth with ADC enrichment at 37 ºC and 150 rpm, according to the manufacturer's instructions.

Mycothiol production and isolation. *M. smegmatis* was grown in Middlebrook 7H9 broth with ADC enrichment at 37 ºC and 150 rpm for 2 days. The cultures were centrifuged (15,000 rpm for 10 min) to pellet the cells and the supernatant was discarded. The pelleted cells (~30 mg/1.5 mL Eppendorf tube) were re-suspended in 7:3 50 mM tris pH 7.5 with 0.5 mM EDTA:MeCN (150 μL) and then disrupted by sonication (Hielscher UP200st vial tweeter, set at 95% amplitude, interval of 60 s on/60 s off, 40 min total, on ice). Following cell lysis, the solution was again centrifuged (15,000 rpm for 10 min), and the supernatant was applied directly to the resin (**2**). The total thiol concentration in the cell lysate was quantified using Ellman's reagent.[4-6] For the capture of mycothiol shown in Figure 4b, the thiol concentration in the cell lysis was determined to be 0.26 μmol/g dry pellet weight. This corresponds to a resin capture concentration of 56 μM

(0.13 equiv relative to resin). The mixture was agitated at room temperature protected from light for 12 h. The resin was then washed with dd-H_2O (3 x), DMF (3 x), and DCM (3 x). Modification was confirmed via MALDI-TOF MS. The predicted isotope pattern shown in Figure 4 was created using ChemCalc (http://www.chemcalc.org/) with the FWHM set to 0.2.[7]

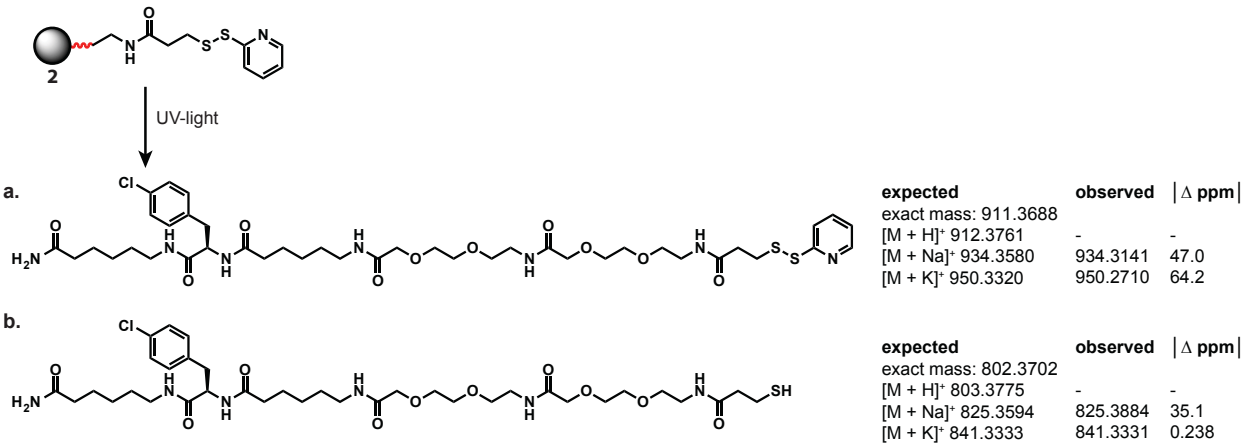


Supporting Information Figure S1. Synthesis of amine-terminated photocleavable linker. The amine-terminated photocleavable resin linker, **1**, was synthesized using standard Fmoc-based chemistry on Tentagel-NH_2 resin. Fmoc-protected acids were coupled in the following order: (i), (ii), (iii), (i), (ii), (iv), and (iv). Following each coupling, any remaining terminal amines were capped using acetic anhydride and the Fmoc protecting groups were removed using a solution of piperidine.



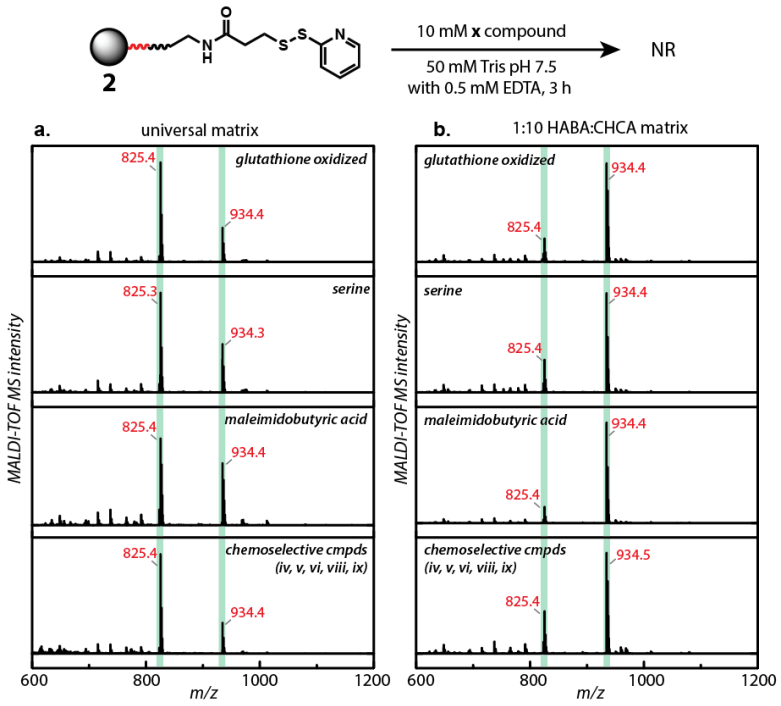
expected	observed	|Δ ppm|
exact mass: 714.3719		
$[M + H]^+$ 715.3792	715.3566	31.6
$[M + Na]^+$ 737.3611	737.3176	59.0
$[M + K]^+$ 753.3351	-	-

Supporting Information Figure S2. The expected *m/z* values for **1** cleaved from the solid support following irradiation with ultraviolet light (365 nm). The values given above correspond to the spectra shown in the main text.

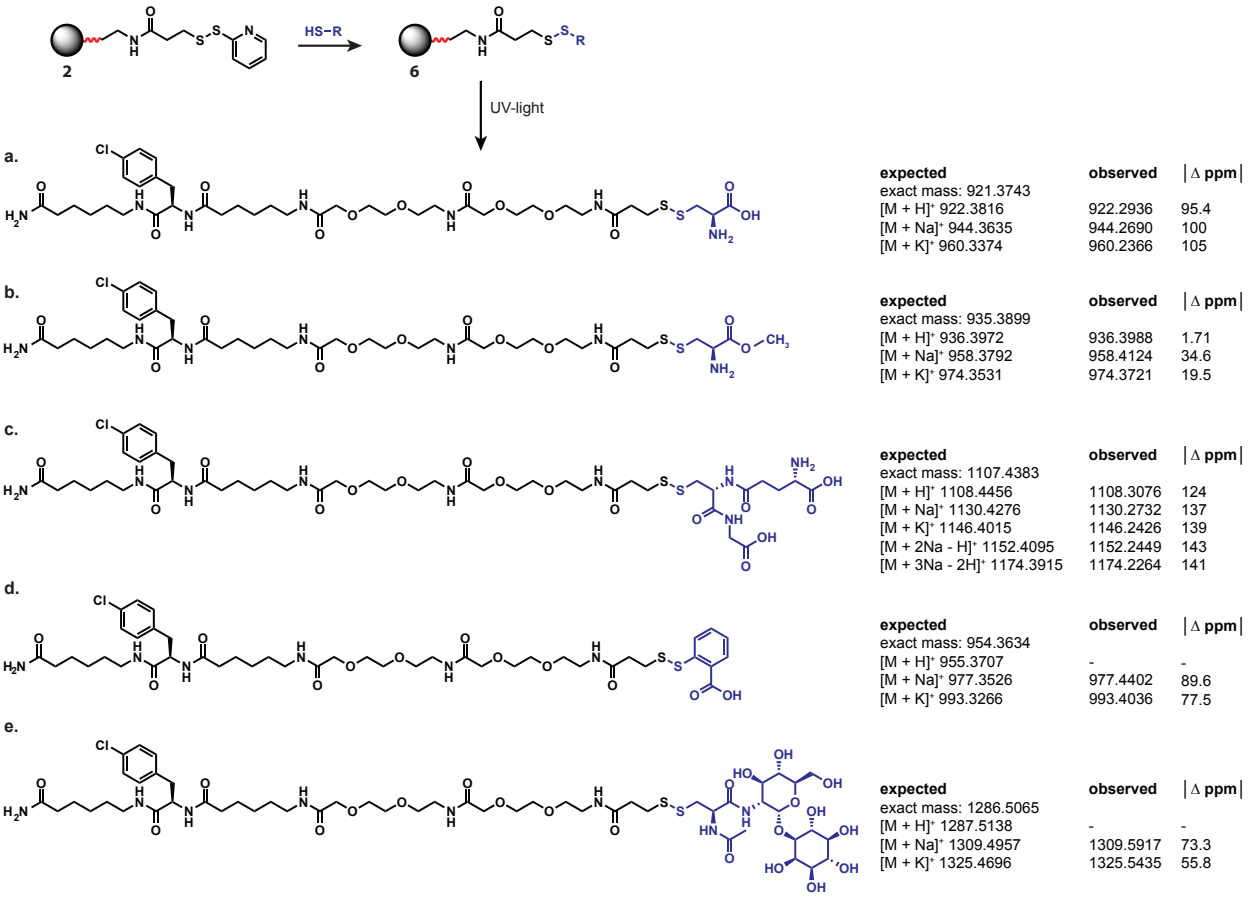


	expected	observed	|Δ ppm|
a.	exact mass: 911.3688		
	$[M + H]^+$ 912.3761	-	-
	$[M + Na]^+$ 934.3580	934.3141	47.0
	$[M + K]^+$ 950.3320	950.2710	64.2
b.	exact mass: 802.3702		
	$[M + H]^+$ 803.3775	-	-
	$[M + Na]^+$ 825.3594	825.3884	35.1
	$[M + K]^+$ 841.3333	841.3331	0.238

Supporting Information Figure S3. The expected *m/z* values for **2** cleaved from the solid support following irradiation with ultraviolet light (365 nm). Both the a) pyridyl disulfide- and b) sulfhydryl-terminated resin *m/z* values were observed. The values given above correspond to the spectra shown in the main text.

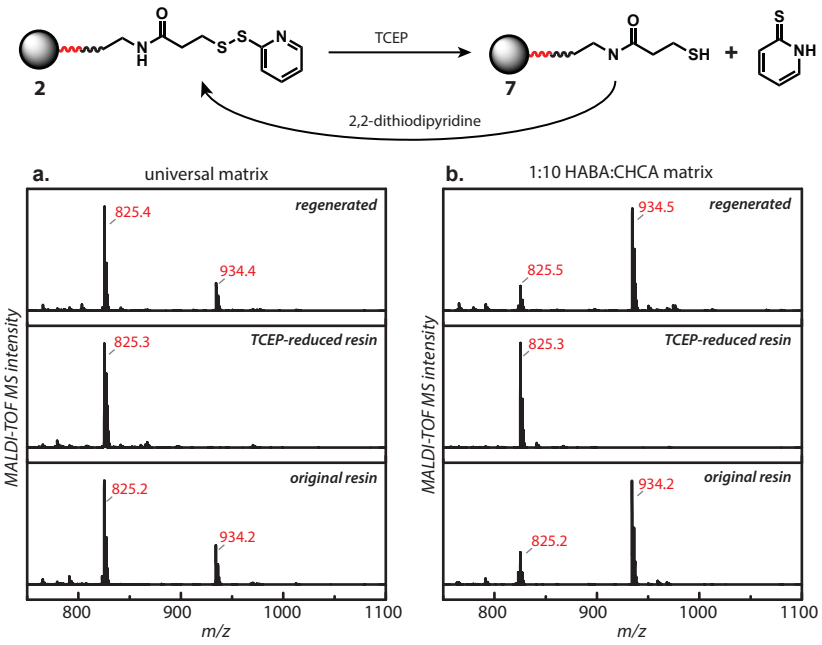


Supporting Information Figure S4. A variety of compounds (Figure 3 (iv)-(xi)) were incubated with resin **2** to determine the resin chemoselectivity. The extent of modification was evaluated following cleavage from the solid support by irradiation with ultraviolet light (365 nm). Samples were co-crystallized with either a) universal or b) 1:10 HABA:CHCA matrix and evaluated by MALDI-TOF MS. Compounds (iv)-(xi) were not immobilized on resin **2**, indicating the pyridyl disulfide-terminated resin, **2**, is chemoselective for sulfhydryl-containing compounds.

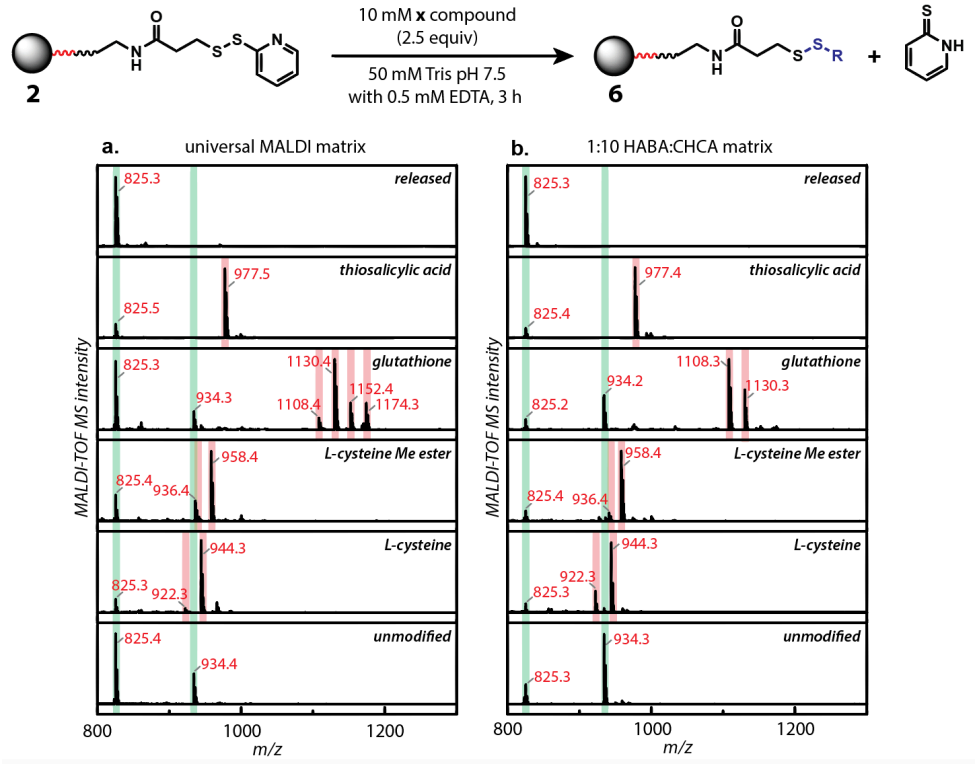


	expected	observed	|Δ ppm|
a.	exact mass: 921.3743		
	$[M + H]^+$ 922.3816	922.2936	95.4
	$[M + Na]^+$ 944.3635	944.2690	100
	$[M + K]^+$ 960.3374	960.2366	105
b.	exact mass: 935.3899		
	$[M + H]^+$ 936.3972	936.3988	1.71
	$[M + Na]^+$ 958.3792	958.4124	34.6
	$[M + K]^+$ 974.3531	974.3721	19.5
c.	exact mass: 1107.4383		
	$[M + H]^+$ 1108.4456	1108.3076	124
	$[M + Na]^+$ 1130.4276	1130.2732	137
	$[M + K]^+$ 1146.4015	1146.2426	139
	$[M + 2Na - H]^+$ 1152.4095	1152.2449	143
	$[M + 3Na - 2H]^+$ 1174.3915	1174.2264	141
d.	exact mass: 954.3634		
	$[M + H]^+$ 955.3707	-	-
	$[M + Na]^+$ 977.3526	977.4402	89.6
	$[M + K]^+$ 993.3266	993.4036	77.5
e.	exact mass: 1286.5065		
	$[M + H]^+$ 1287.5138	-	-
	$[M + Na]^+$ 1309.4957	1309.5917	73.3
	$[M + K]^+$ 1325.4696	1325.5435	55.8

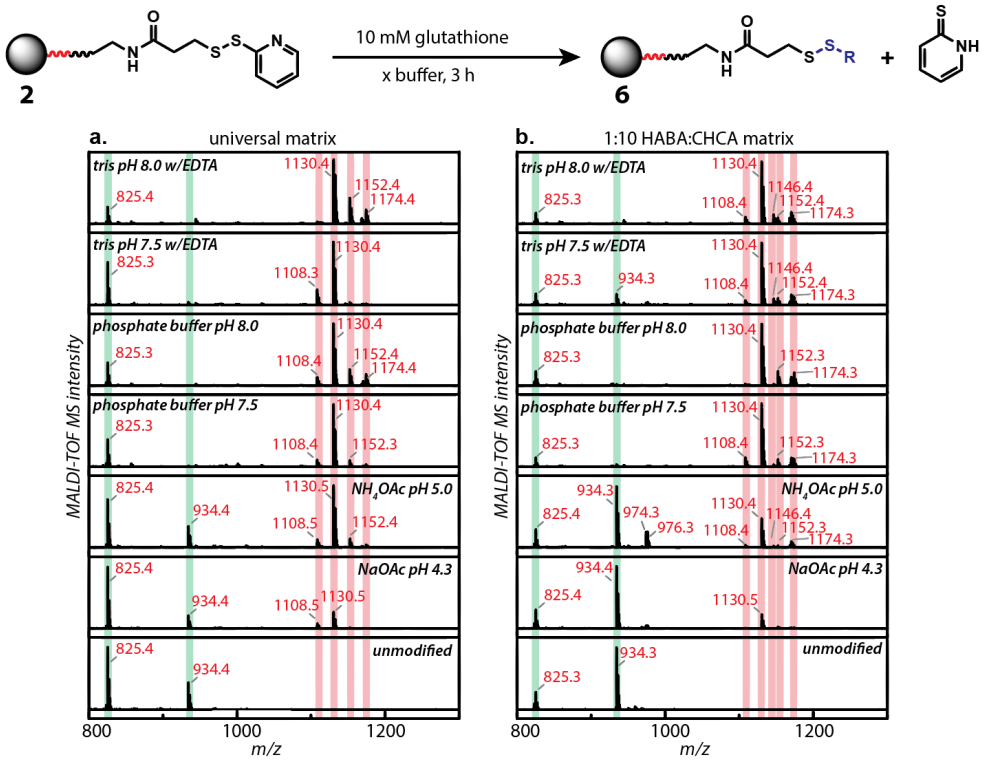
Supporting Information Figure S5. The expected *m/z* values for model sulfhydryl-containing compounds immobilized on **2** following cleavage from the solid support by irradiation with ultraviolet light. The model sulfhydryl-containing compounds immobilized on the solid support included: a) L-cysteine, b) L-cysteine methyl ester, c) glutathione, d) thiosalicylic acid, and e) mycothiol. The values given above correspond to the spectra shown in the main text.



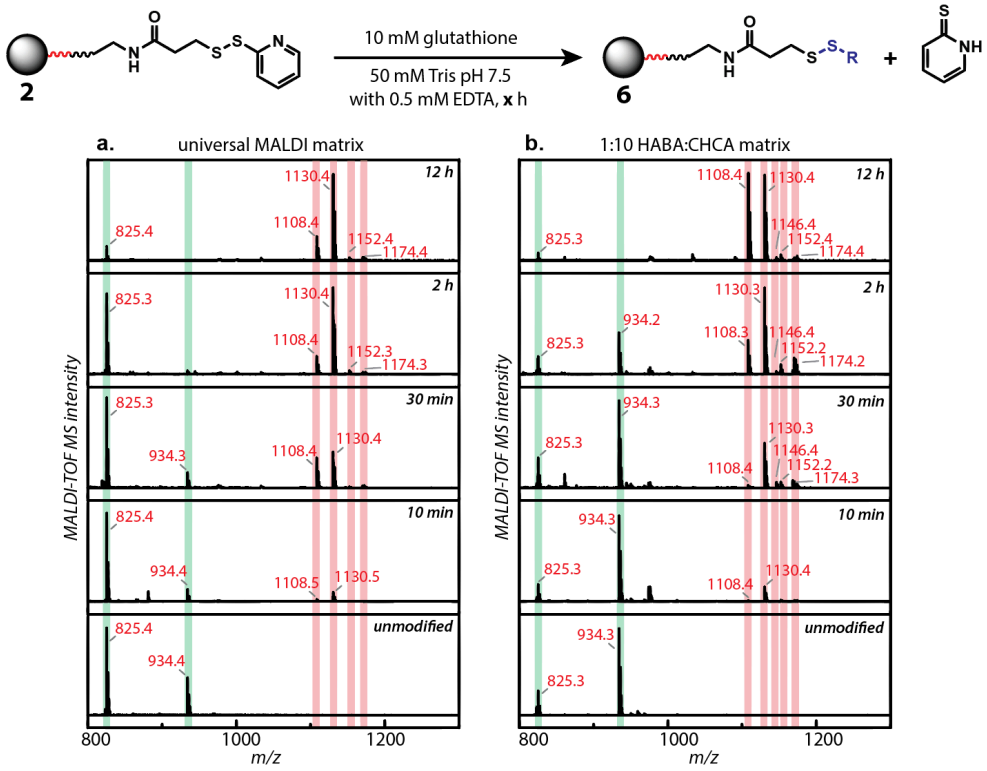
Supporting Information Figure S6. Pyridyl disulfide-terminated resin, **2**, can be reduced with TCEP to afford the sulfhydryl-terminated resin, **7**, and 2-thiopyridine. Incubation of resin **7** with 2,2-dithiodipyridine regenerates resin **2**. The original resin **2**, reduced resin **7**, and regenerated resin **2** were co-crystallized with either a) universal or b) 1:10 HABA:CHCA matrix and evaluated by MALDI-TOF MS.



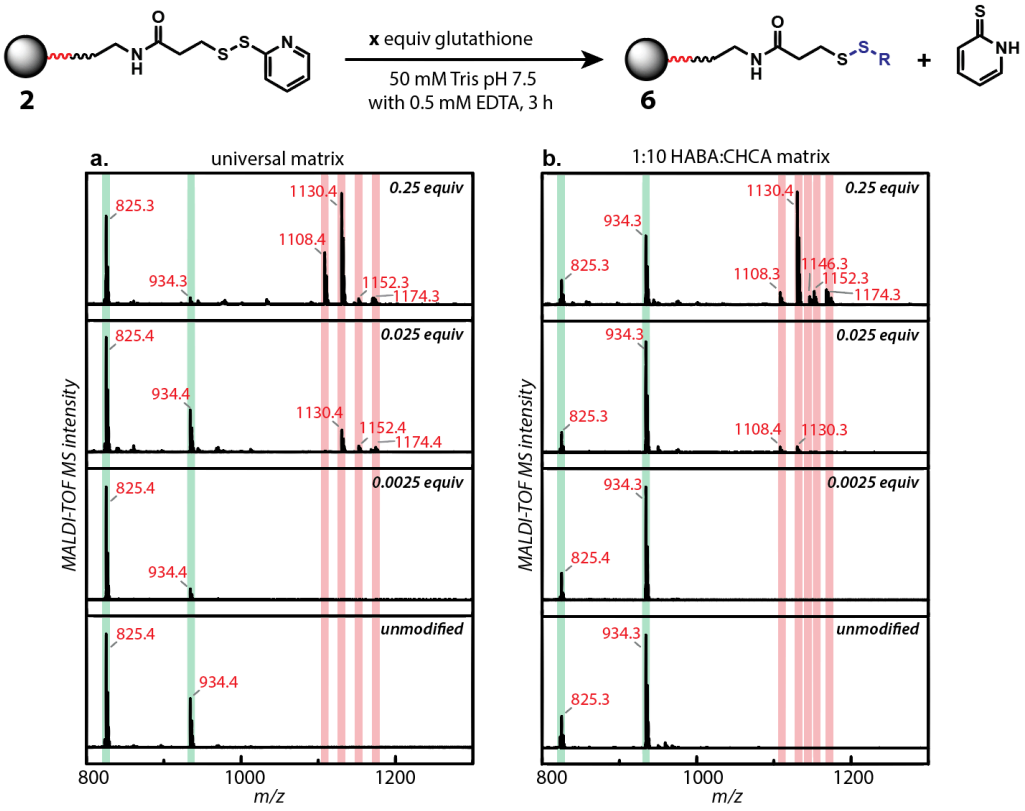
Supporting Information Figure S7. A variety of sulfhydryl-containing compounds were evaluated for their reactivity with resin **2** including L-cysteine, L-cysteine methyl ester, glutathione, and thiosalicylic acid. The extent of compound immobilization was evaluated following cleavage of **6** from the solid support by irradiation with ultraviolet light (365 nm). Samples were co-crystallized with either a) universal or b) 1:10 HABA:CHCA matrix and evaluated by MALDI-TOF MS. Each of the compounds resulted in the expected mass addition to the resin.



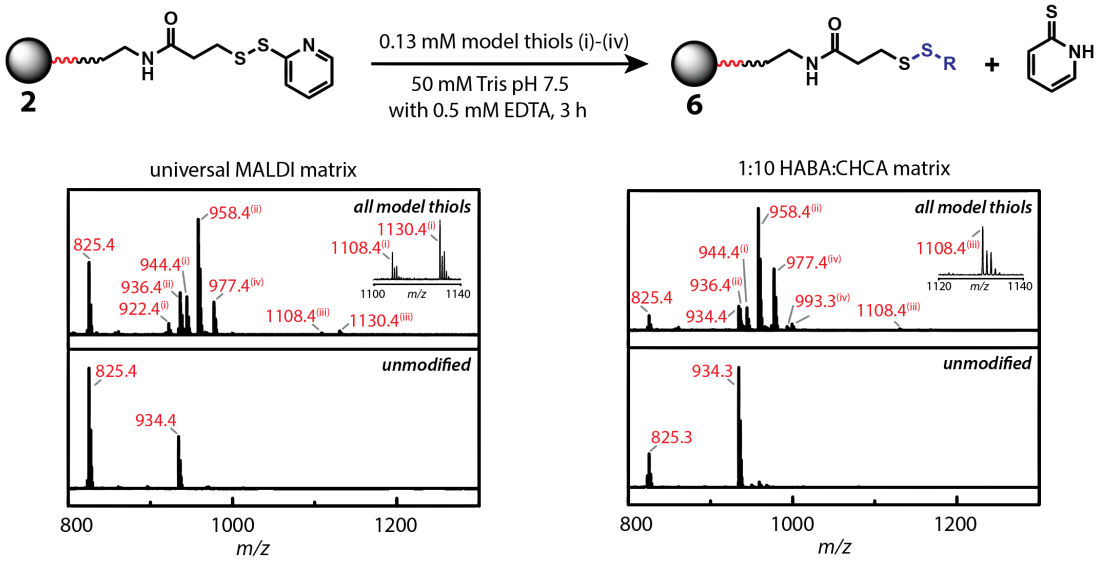
Supporting Information Figure S8. A variety of buffers were evaluated for their influence on the reactivity of glutathione with resin **2**, including 50 mM NaOAc pH 4.3, 50 mM NH4OAc pH 5.0, 50 mM sodium phosphate pH 7.5, 50 mM sodium phosphate pH 8.0, 50 mM tris pH 7.5 with 0.5 mM EDTA, and 50 mM tris pH 8.0 with 0.5 mM EDTA. The extent of glutathione immobilization was evaluated following cleavage of **6** from the solid support by irradiation with ultraviolet light (365 nm). Samples were co-crystallized with either a) universal or b) 1:10 HABA:CHCA matrix and evaluated by MALDI-TOF MS. From this screen, 50 mM tris pH 7.5 with 0.5 mM EDTA was selected as the capture buffer for subsequent reactions.



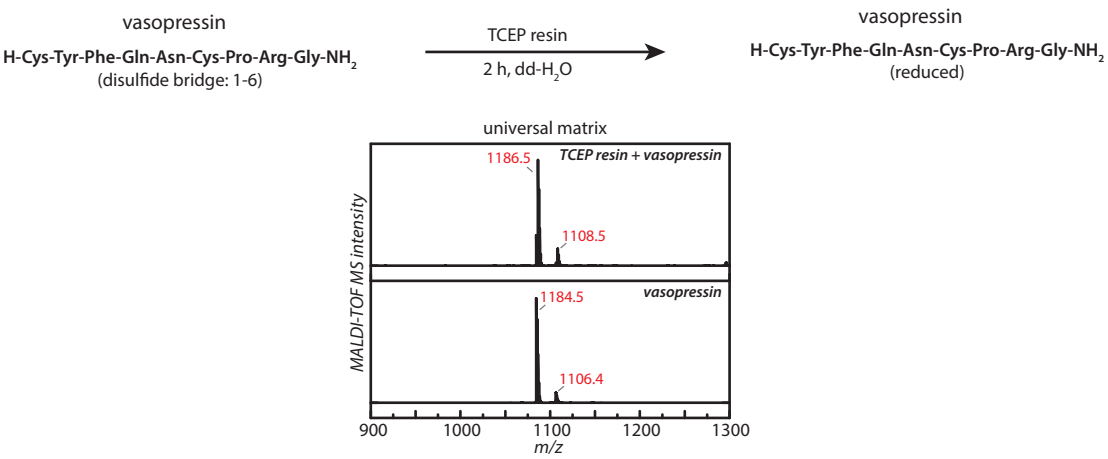
Supporting Information Figure S9. A variety of reaction times (10 min, 30 min, 2 h, and 12 h) were screened for the capture of glutathione with resin **2**. The extent of glutathione immobilization was evaluated following cleavage of **6** from the solid support by irradiation with ultraviolet light (365 nm). Samples were co-crystallized with either a) universal or b) 1:10 HABA:CHCA matrix and evaluated by MALDI-TOF MS. From this screen, 2 h was determined to be a sufficient time to observe the immobilization of glutathione at the indicated concentration.



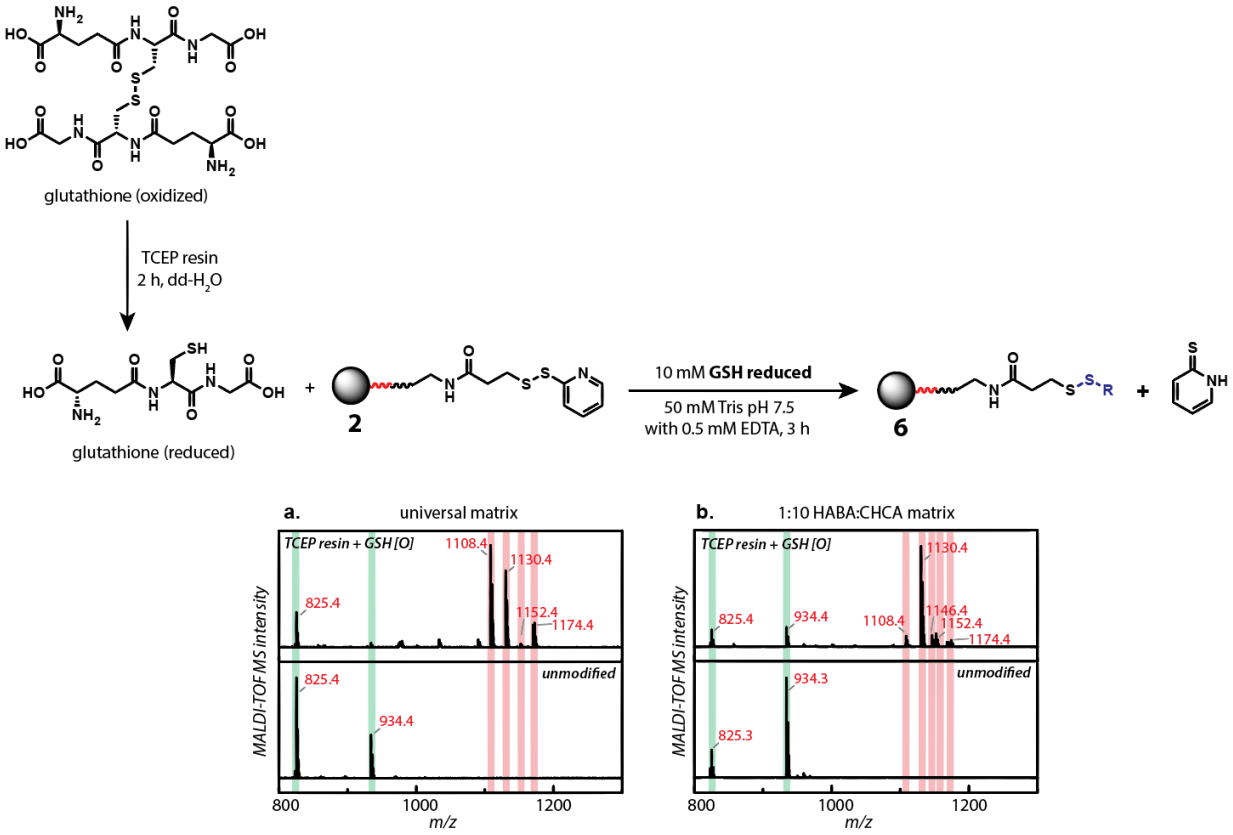
Supporting Information Figure S10. A variety of glutathione equivalents (0.0025 – 0.25) were evaluated for their influence on the reactivity of glutathione with resin **2**. The extent of glutathione immobilization was evaluated following cleavage of **6** from the solid support by irradiation with ultraviolet light (365 nm). Samples were co-crystallized with either a) universal or b) 1:10 HABA:CHCA matrix and evaluated by MALDI-TOF MS. From this screen, 0.025 equiv was determined to be a sufficient number of equivalents to observe the immobilization of glutathione.



Supporting Information Figure S11. Resin **2** with incubated with a mixture of the model sulfhydryl-containing compounds to afford resin **6** under capture conditions [10 mM model compound in 50 mM Tris pH 7.5 with 0.5 mM EDTA (3 h)]. MALDI-TOF MS evaluation of the captured thiols enable detection of all molecules, L-cysteine, L-cysteine methyl ester, glutathione, and thiosalicylic acid.



Supporting Information Figure S12. TCEP immobilized on a solid support was evaluated for its ability to reduce the disulfide bond in the peptide, vasopressin. Vasopressin (pre- and post-incubation with TCEP resin) was co-crystalized with universal matrix and evaluated by MALDI-TOF MS. Vasopressin incubated with TCEP resin resulted in the fully reduced dithiol version of vasopressin as revealed by MALDI-TOF MS.



Supporting Information Figure S13. TCEP resin was incubated with oxidized glutathione to yield reduced glutathione, which was then incubated with resin **2**. The extent of modification was evaluated following cleavage of **6** from the solid support by irradiation with ultraviolet light (365 nm). Samples were co-crystallized with either a) universal or b) 1:10 HABA:CHCA matrix and evaluated by MALDI-TOF MS. These data indicate that resin-bound TCEP can be used to reduce disulfide-containing compounds to allow for their immobilization on resin **2**.

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