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I. Supplemental Figures and Tables

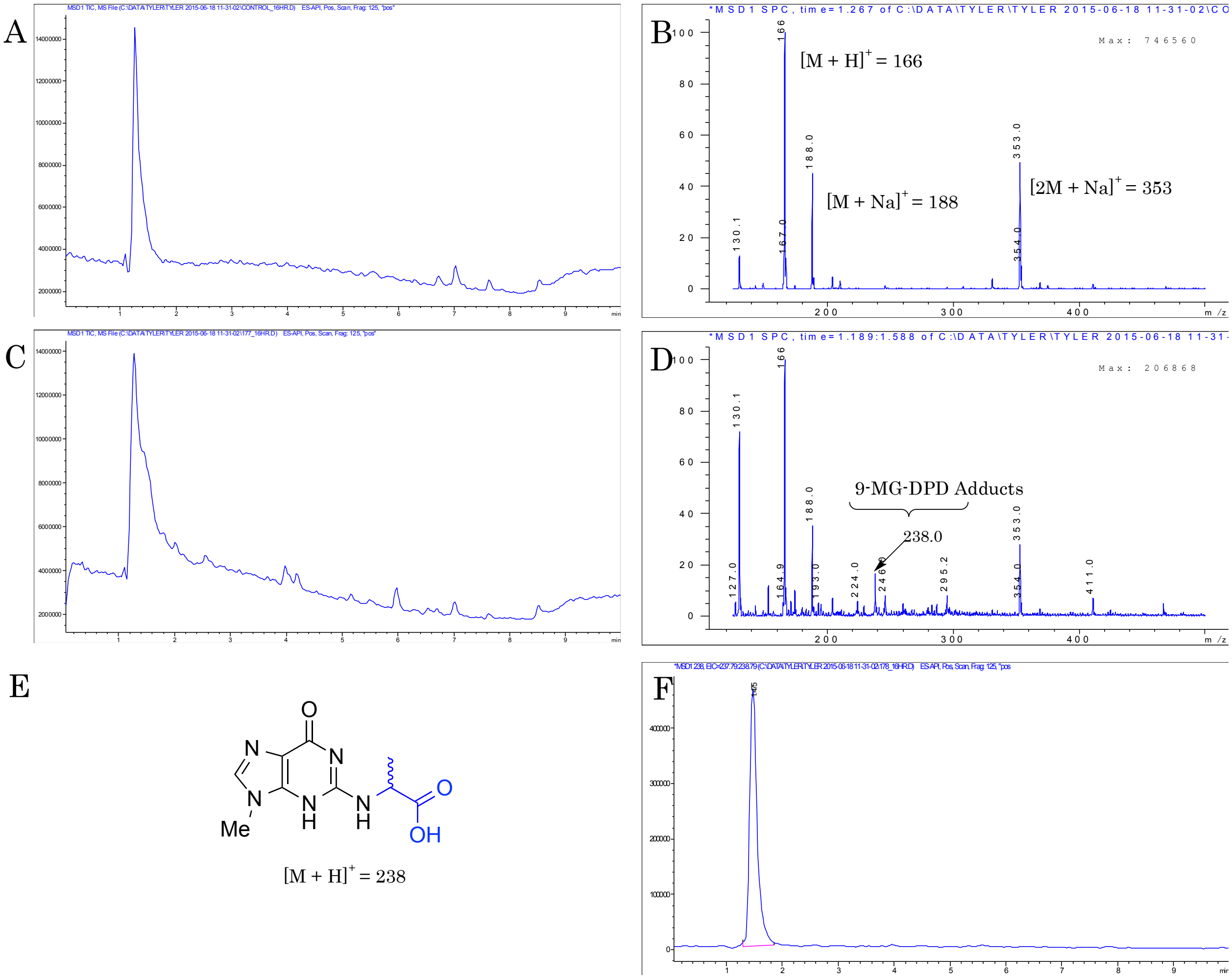


Figure S1. Reaction of 9-methylguanine (9-MG) with DPD monitored by LC/MS. A) Total ion chromatogram (TIC) and B) mass spectrum of control 9-MG. C) TIC and D) mass spectrum of 9-MG (1 mM) in PBS (pH 7.4) after refluxing for 16 h with DPD (10 mM). Panel D depicts a mixture of starting material (9-MG) and DPD adducts. No further attempt was made to isolate and characterize these adducts. E) *Proposed structure of major adduct, [M + H]$^+$ m/z = 238. F) Extracted ion chromatogram (EIC) at m/z = 238, ([M + H]$^+$).

* We speculate the peak at m/z = 238 to be the N^2-(1-carboxyethyl) adduct (Figure S1E), although the mechanism of its formation will still need to be elucidated. Incidentally, N^2-(1-carboxyethyl)-9-MG is the major product that is produced from 9-MG after treatment with glucose, G-6-P, G-6-P/lysine, the Schiff base 1-*n*-propylamino-*N*-D-glucoside, the Amadori product 1-*n*-propylamino-*N*-D-fructose, or methylglyoxal.[1]

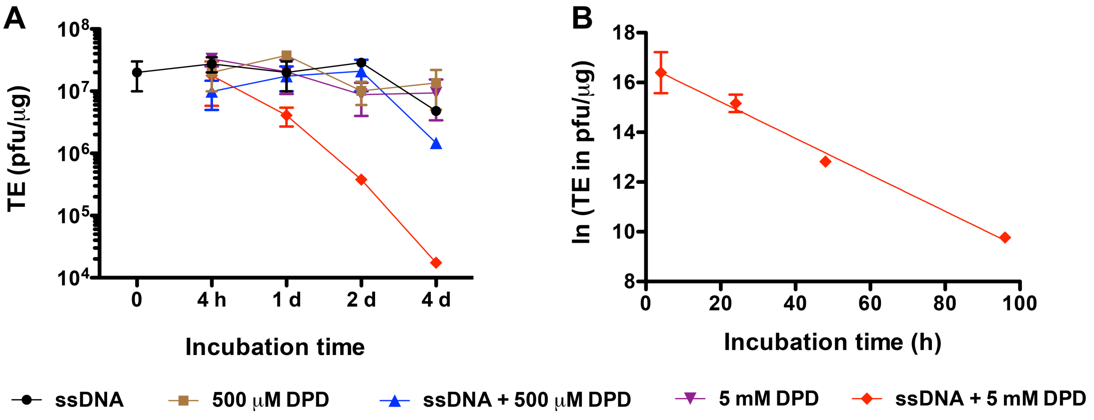


Figure S2. Transfection efficiency (TE) of VCSM13 ssDNA. A) VCSM13 ssDNA (100 ng/µL) was incubated with DPD at 37 °C, and subsequently transfected into TG1 electrocompetent cells. Control incubation mixtures contained ssDNA or DPD alone. Control incubated DPD was added to the transfection assay with unincubated ssDNA. Plaque-forming units (pfu) were quantitated after an overnight growth (n = 2). Loss of transfection efficiency was more pronounced after incubation with 5 mM DPD. B) First-order kinetics of the transfection efficiency of VCSM13 ssDNA incubated with 5 mM DPD. The rate constant was determined to be 0.073 ± 0.006 h^{-1} with an estimated half-life of 9.5 h.

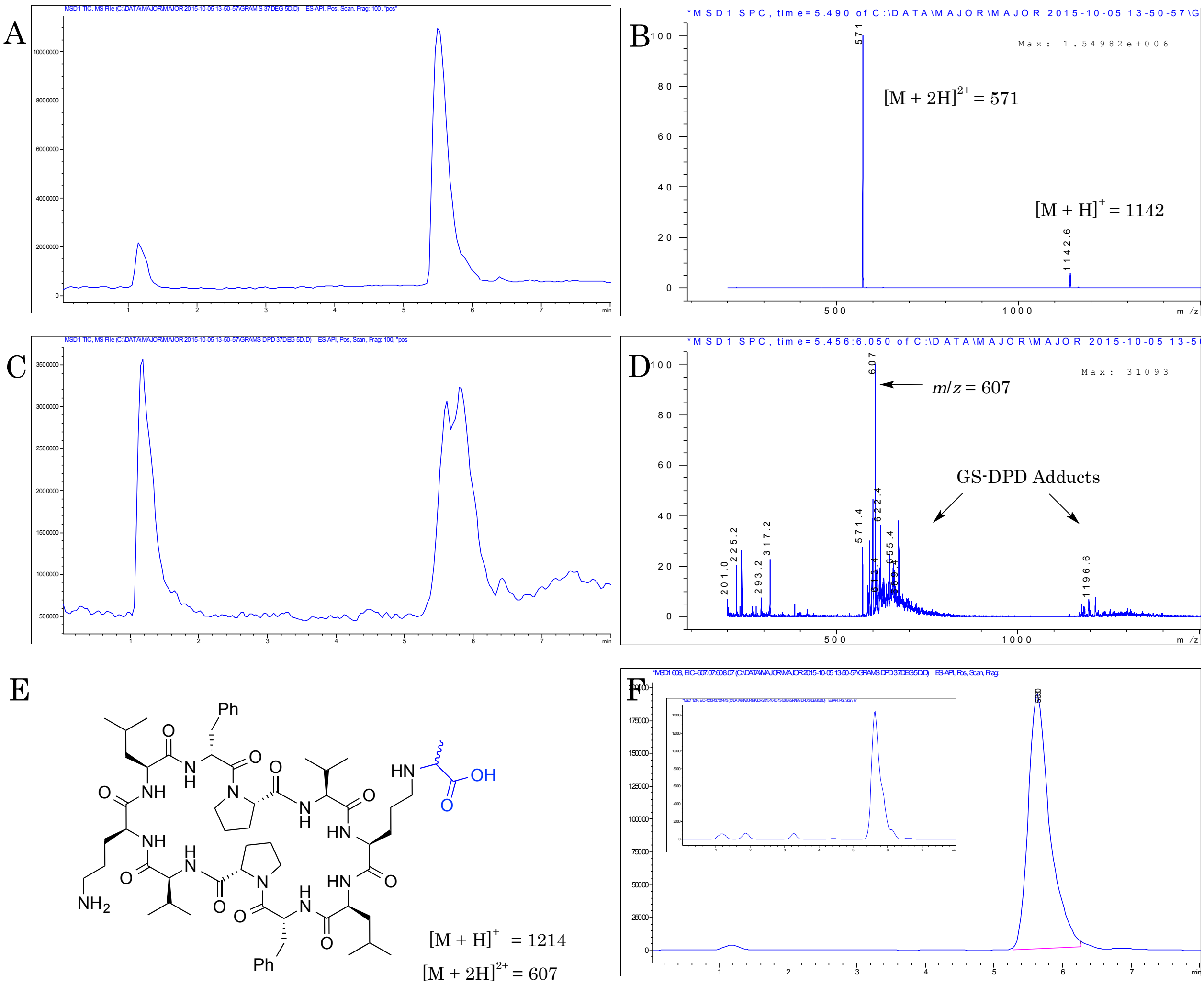


Figure S3. Reaction of Gramicidin S (GS) with DPD monitored by LC/MS. A) TIC and B) mass spectrum of control GS. C) TIC and D) mass spectrum of GS (50 μM) in bicarbonate buffer (~pH 8) after incubation with DPD (5 mM) at 37 °C for 5 d. Panel D depicts a mixture of starting material (GS) and DPD adducts. We note that no further attempt was made to isolate and characterize these adducts. E) Proposed structure of major adduct at *m*/*z* = 607, $[M + 2H]^{2+}$. The glycation reaction is assumed to occur at the ornithine residue(s). The major peak at *m*/*z* = 607 might be the N^2-(1-carboxyethyl) adduct, similar to what we propose for the reaction of DPD with 9-MG (see Figure S1E). F) EIC at *m*/*z* = 607 ($[M + 2H]^{2+}$) and *m*/*z* = 1214 ($[M + H]^{+}$, inset).

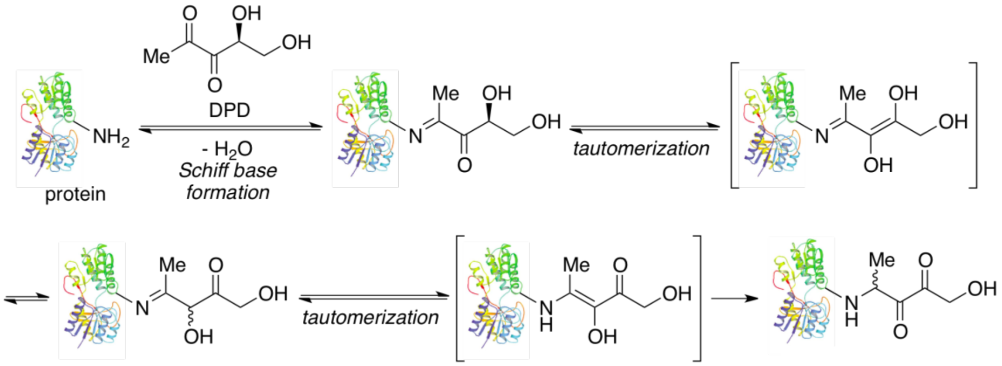


Figure S4. A proposed mechanism of Heyns rearrangement between protein and DPD. A nucleophilic primary amine of protein attacks the carbonyl group of DPD to form a Schiff base conjugate. The Schiff base subsequently undergoes sequential tautomerizations to yield the Heyns product with a 1,2-diketone moiety. The initial Schiff base formation may occur at the other carbonyl group, which affords other isoforms.

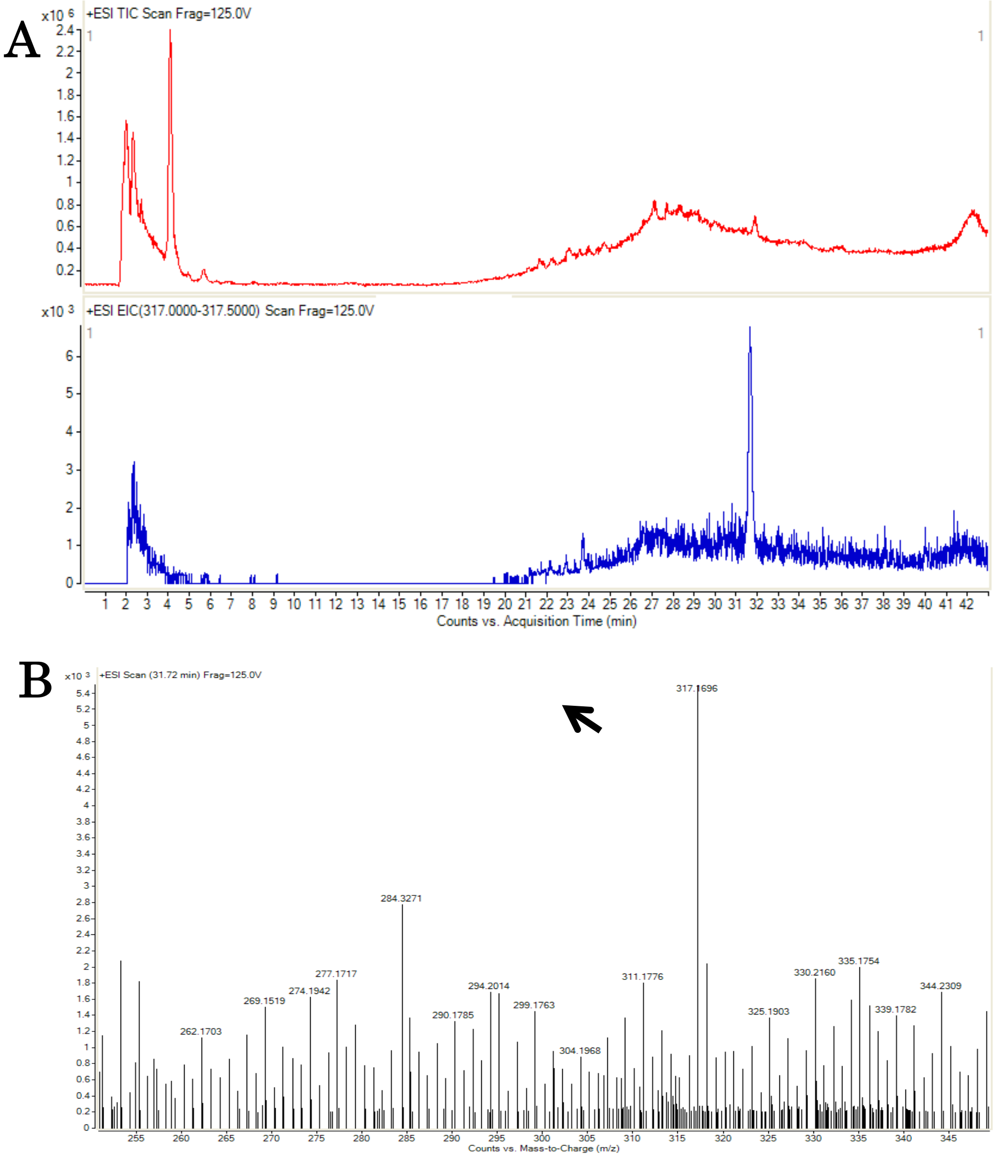


Figure S5. A) N_α-acetyl-L-lysine methyl ester (1 mM) with DPD (10 mM) at 70 °C in PBS (pH 8.0) for 48 h and monitored the reaction via LC/ESI-MS analysis. Experiment was analyzed on an Agilent MSD 1100 Series electrospray ionization mass spectrometer (Column: Agilent Zorbax SB-CN, 1 × 150 mm, 3.5 μm). Solvent system: water and acetonitrile supplemented with 0.1% formic acid (gradient elution with 1-99% acetonitrile). Extracted ion chromatogram indicates the presence of compound **2** (ESI, positive mode). B) Peak at 317.1696 *m/z* indicates Heyns product formation.

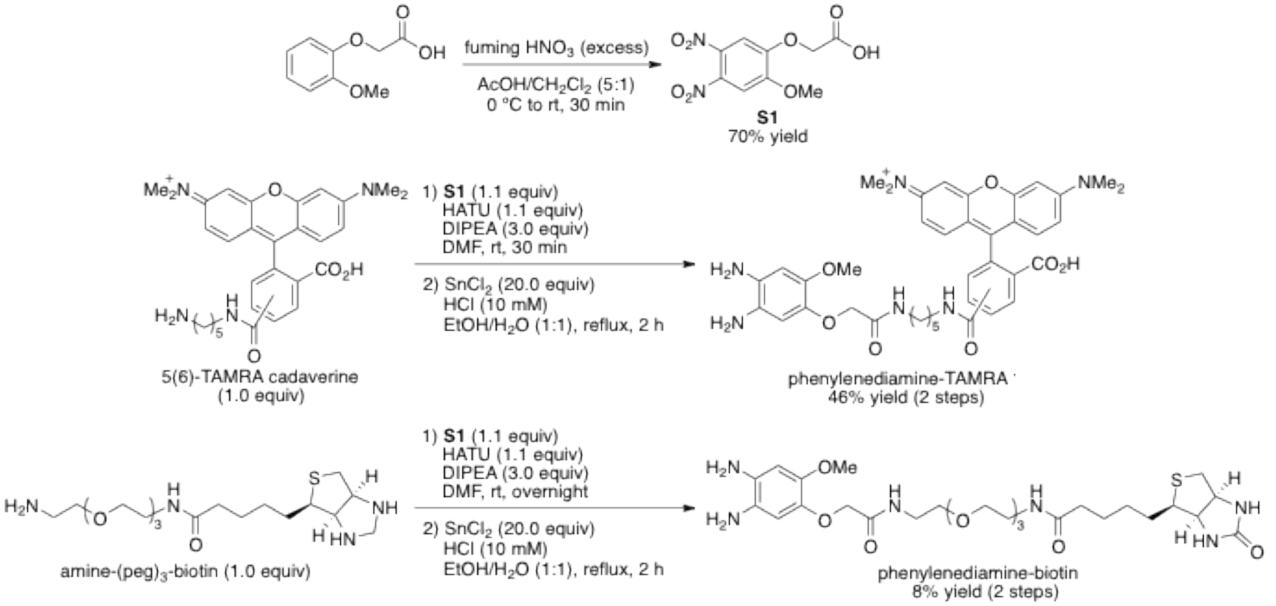


Figure S6. Synthesis of phenylenediamine-TAMRA **3** and -biotin **4** (See below for detailed experimental conditions).

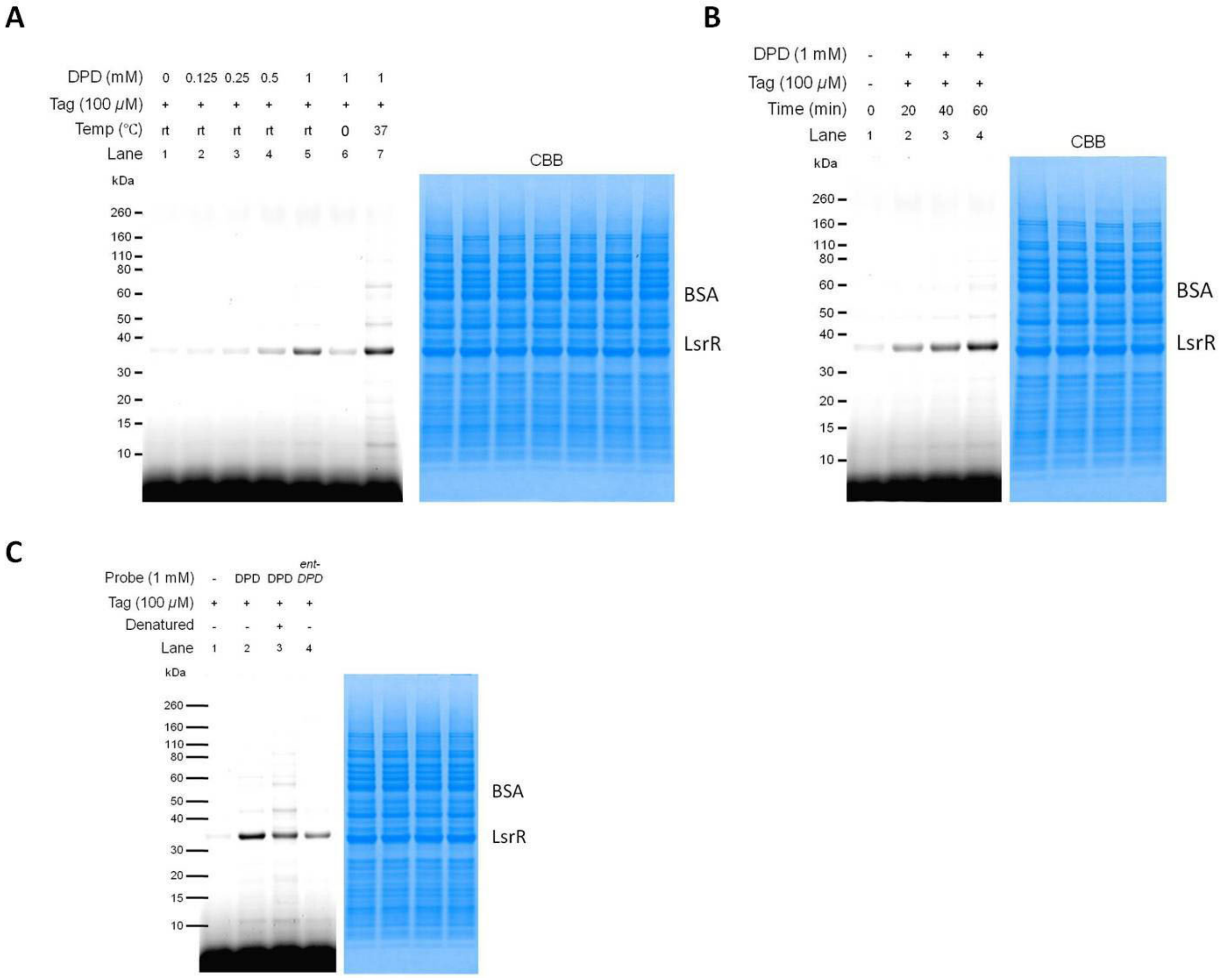


Figure S7. Full fluorescence images of the labeled whole cell lysate (1 mg/mL) including overexpressed LsrR and exogenous BSA (0.1 mg/mL). The lysate was incubated with DPD, precipitated, resolubilized in 1% SDS, and then incubated with phenylenediamine-TAMRA tag **3** (100 μM, rt, 1 h) under various conditions: (A) concentration of DPD and incubation temperature; (B) incubation time; (C) native and denatured LsrR, and enantiomer of DPD. The strong smear on the bottom of each fluorescence image is derived from unreacted tag **3**. Note that Figure 2 in the manuscript was generated from these full images.

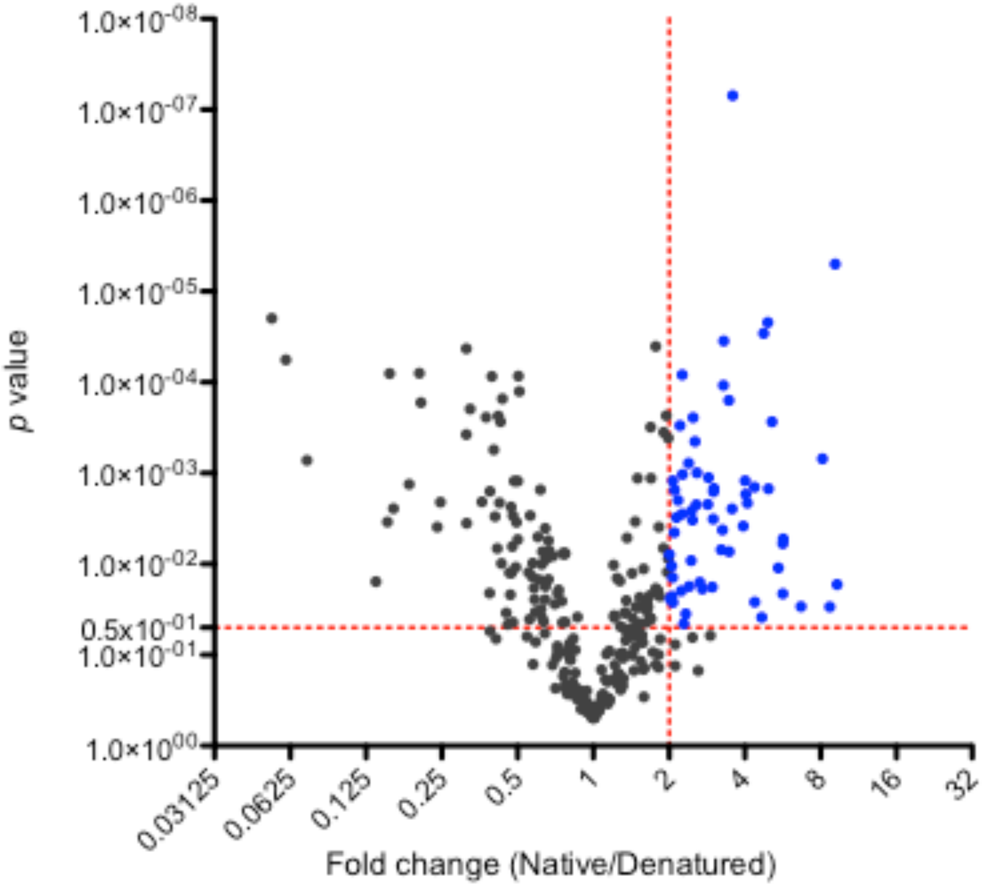


Figure S8. Volcano plot of proteins derived from *S. typhimurium* 14028 that were identified in native samples in proteomic experiments. Proteins identified with at least two unique peptides per replicate are plotted. Criteria for positive hits were defined as follows: (1) > 2-fold higher signal in native samples than in denatured samples and (2) *p* value < 0.05 (Student's *t*-test). To visualize the criteria for positive hits, horizontal and vertical red lines are drawn at a *p* value of 0.05 and at 2-fold change of signal, respectively. Of 296 data points, 63 proteins (blue dots) were determined to be potential target proteins of DPD.

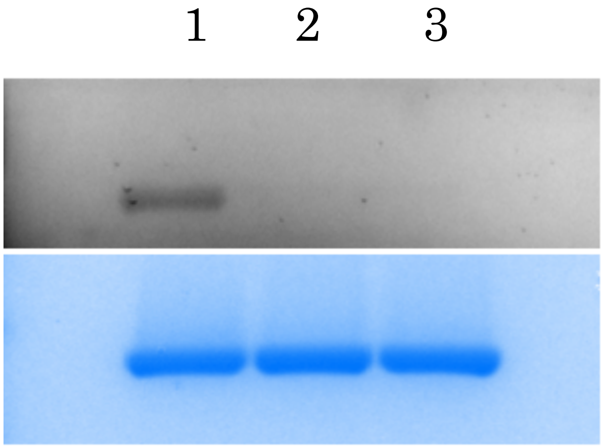


Figure S9. LsrR-His$_6$ (35375 Da) was overexpressed and purified as previously described.[2] Lane 1 - LsrR (450 μL, 1.1 mg/mL), neutralized DPD (50 μL, 40 mM, pH 7.0) and 2.5 mg CHAPS were incubated for 1 hr, protein was precipitated, resolubilized in 1% SDS and then incubated with compound **3** (100 μM, rt, 1 h). Sample preparation and gel conditions are described in Section II. Lane 2 - LsrR (450 μL, 1.1 mg/mL), glucose (50 μL, 40 mM, pH 7.0) and 2.5 mg CHAPS were incubated for 1 hr, protein was precipitated, resolubilized in 1% SDS and then incubated with compound **3** (100 μM, rt, 1 h). Sample preparation and gel conditions are described in Section II. Lane 3 - LsrR (450 μL, 1.1 mg/mL), fructose (50 μL, 40 mM, pH 7.0) and 2.5 mg CHAPS were incubated for 1 hr, protein was precipitated, resolubilized in 1% SDS and then incubated with compound **3** (100 μM, rt, 1 h). Sample preparation and gel conditions are described in Section II.

Table S1: Minimal Inhibitory Concentrations (MICs) of Gramicidin S

	MIC* (μg/mL)			
	Gramicidin S (GS) (unincubated)	GS (20 d at 37 °C)	GS + 500 μM DPD (20 d at 37 °C)	GS + 5 mM DPD (20 d at 37 °C)
A. baumannii M2	8	8	16	64
S. aureus RN6734	4	4	8	32
S. epidermidis 1457	4	4	8	32
P. aeruginosa PAO1	64	128	128	>128

* MIC determinations were performed in duplicate. Refer to Section II for experimental details.

Table S2. Hit proteins derived from *S. typhimurium* 14028 that could potentially recognize DPD. Proteins described in the manuscript are highlighted in yellow.

IDs	Protein Name	Symbol	Mol. Weight [kDa]	Fold Change Native/Denatured	*p* value (*t*-test)
P37426	Ribonucleoside-diphosphate reductase 1 subunit alpha	RIR1	85.7	2.3	0.000
P26973	DNA topoisomerase 4 subunit A	PARC	84.0	2.3	0.046
P0A1H3	Elongation factor G	EFG	77.6	3.3	0.000
Q8ZKX1	Glutamine--fructose-6-phosphate aminotransferase [isomerizing]	GLMS	66.9	2.5	0.000
Q8ZP20	Periplasmic trehalase	TREA	63.5	4.7	0.039
P0A249	Phosphoenolpyruvate-protein phosphotransferase	PT1	63.4	2.1	0.001
P66008	Soluble pyridine nucleotide transhydrogenase	STHA	51.6	2.3	0.003
Q8ZPC9	Gamma-aminobutyraldehyde dehydrogenase	ABDH	51.2	3.5	0.007
Q7CPR9	Bifunctional protein HldE	HLDE	51.1	2.5	0.000
Q7CPE2	ATP synthase subunit beta	ATPB	50.3	4.4	0.001
O30911	ATP-dependent protease ATPase subunit HslU	HSLU	49.7	2.1	0.026
Q8ZQM1	Ribosomal protein S12 methylthiotransferase RimO	RIMO	49.6	5.7	0.005
P19264	Ethanolamine ammonia-lyase heavy chain	EUTB	49.4	3.0	0.018
P26982	Periplasmic serine endoprotease DegP	DEGP	49.3	4.9	0.000
Q8ZP17	D-amino acid dehydrogenase small subunit	DADA	47.9	2.0	0.008
P24518	Trifunctional NAD biosynthesis/regulator protein NadR	NADR	47.1	4.1	0.002
P58525	N-methyl-L-tryptophan oxidase	MTOX	40.7	3.9	0.004
P77980	3-dehydroquinate synthase	AROB	38.7	3.5	0.000
P0A9X6	Rod shape-determining protein MreB	MREB	37.0	2.6	0.016
P26395	Protein RfbI	RFBI	36.6	2.1	0.014
Q8ZPV5	Selenide, water dikinase	SELD	36.4	2.4	0.001
P0A1P2	Glycerol-3-phosphate dehydrogenase [NAD(P)+]	GPDA (GPSA)	36.3	5.4	0.011
Q8ZM80	tRNA-modifying protein YgfZ	YGFZ	36.0	2.5	0.003
Q8ZL95	Glycine--tRNA ligase alpha subunit	SYGA	34.7	4.0	0.001
Q93IM5	Glucokinase	GLK	34.6	2.2	0.002
Q8ZKQ5	Transcriptional regulator LsrR	LSRR	34.4	8.7	0.030
Q8ZRT9	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase	LPXC	34.0	2.2	0.000
P60656	Agmatinase	SPEB	33.6	2.7	0.019
O85139	3-oxoacyl-[acyl-carrier-protein] synthase 3	FABH	33.5	3.3	0.000
Q8ZMD3	NADPH-dependent 7-cyano-7-deazaguanine reductase	QUEF	32.7	2.1	0.023
P67197	Phosphoenolpyruvate synthase regulatory protein	PSRP	31.1	3.0	0.002
Q8ZPM8	Pyridoxamine kinase	PDXY	31.1	3.6	0.002
P0A1F2	Diaminopimelate epimerase	DAPF	30.3	2.6	0.002
P60428	50S ribosomal protein L2	RL2	29.8	2.6	0.001

P29847	Serine acetyltransferase	CYSE	29.3	4.0	0.002
Q9L6M9	Putative carboxymethylenebutenolidase	DLHH	29.1	2.1	0.010
Q8ZQS2	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	GPMA	28.5	2.3	0.035
P26264	3(2),5-bisphosphate nucleotidase CysQ	CYSQ	27.5	4.4	0.026
P69936	NADP-dependent L-serine/L-allo-threonine dehydrogenase YdfG	YDFG	27.0	4.8	0.000
P63557	Acetylglutamate kinase	ARGB	27.0	6.7	0.029
P66541	30S ribosomal protein S2	RS2	26.8	9.1	0.000
P0A2C9	3-oxoacyl-[acyl-carrier-protein] reductase FabG	FABG	25.5	2.1	0.002
Q8ZQC4	Cytidylate kinase	KCY	24.8	2.1	0.004
P68738	Endonuclease V	NFI	24.7	5.7	0.021
P64068	Probable GTP-binding protein EngB	ENGB	23.6	8.2	0.001
O54297	30S ribosomal protein S4	RS4	23.5	3.6	0.000
Q8ZK71	Peptide methionine sulfoxide reductase MsrA	MSRA	23.4	3.0	0.003
P0A2H9	Thiol:disulfide interchange protein DsbA	DSBA	22.9	3.3	0.004
P67366	UPF0227 protein YcfP	YCFP	21.1	5.7	0.006
P65559	Uncharacterized Nudix hydrolase YfcD	YFCD	20.9	2.3	0.001
P33321	Translation initiation factor IF-3	IF3	20.6	2.9	0.002
P62405	50S ribosomal protein L5	RL5	20.3	3.0	0.001
Q7CQV9	DNA protection during starvation protein	DPS	18.7	2.1	0.003
Q9L9I6	Regulator of sigma D	RSD	18.7	9.3	0.017
P0A297	50S ribosomal protein L10	RL10	17.8	5.0	0.001
P0A7W4	30S ribosomal protein S5	RS5	17.6	2.9	0.001
P67091	Universal stress protein F	USPF	15.7	3.2	0.007
Q8ZKA3	HTH-type transcriptional repressor NsrR	NSRR	15.6	5.1	0.000
P0A1S2	DNA-binding protein H-NS	HNS	15.5	2.5	0.009
Q7CPL7	50S ribosomal protein L17	RL17	14.4	2.2	0.020
O54296	30S ribosomal protein S11	RS11	13.8	2.0	0.024
Q8ZLM1	30S ribosomal protein S13	RS13	13.2	2.4	0.018
Q7CR66	Iron-sulfur cluster insertion protein ErpA	ERPA	12.1	2.5	0.003

II. Biochemical Assays and Protein Labeling Protocols

DNA transfection

Infected VCSM13 cultures were grown according to Sambrook et al.[3] Single-stranded DNA was isolated using QIAprep Spin M13 Kit (Qiagen, Valencia, CA), following the manufacturer's procedure. VCSM13 ssDNA (100 ng/μL final concentration) was incubated with DPD (0.5 and 5 mM final concentration) in buffer (10 mM Tris, pH 7.5, 1 mM EDTA) at 37 ^{o}C, and at different time points, transfected into 40 μL of TG1 electrocompetent cells. Control incubation mixtures contained ssDNA or DPD alone. Control incubated DPD was added to the transfection assay with unincubated ssDNA. Plaque-forming units (pfu) were quantitated after an overnight growth. DNA transfection experiments were performed in duplicate.

Determination of Minimal-Inhibitory Concentration (MIC)

Gramicidin S (250 μM) was first reacted with DPD (0.5 mM and 5 mM final concentration) in PBS pH 7.4 at 37 ^{o}C. The mixtures were incubated for 20 d at the same temperature, and then assayed by LC/MS to determine the amount of unreacted GS. (For the reaction with 0.5 mM DPD, 90% unreacted GS remained in solution after 20 d, whereas only 30% of unreacted GS remained after reaction with 5 mM DPD). The incubation mixtures were then evaluated for antibiotic activity against *A. baumannii* M2, *S. aureus* RN6734, *S. epidermidis* 1457 and *P. aeruginosa* PAO1. MICs were determined by the microdilution method procedure in Mueller Hinton II Broth in accordance with recommendations of CLSI.[4] MIC determinations were performed in duplicate.

Preparation of the overexpression vector encoding the repressor LsrR (*S. typhimurium* LT2)

The repressor LsrR derived from *S. typhimurium* strain LT2 was overexpressed as a recombinant protein according to a known protocol with several modifications.[5] Whole genomic DNA of *S. typhimurium* strain LT2 was extracted using QIAamp DNA mini kit (Qiagen, Valencia, CA) and used as a template to amplify the *lsrR* gene. Primers designated as Nde I-LsrR-LT2-F (5'-GAT <u>CATATG</u> AGCGATAATACGTTGGTATCTG-3') and Xho I-LsrR-LT2-R (5'-CATG <u>CTCGAG</u> TTTTTCAATAATTTGAATTATTTTCCCTGCGG-3') were used to amplify the lsrR sequence from the genomic DNA by the polymerase chain reaction. The DNA fragments were then digested with restriction enzymes Nde I and Xho I (New England Biolabs, Beverly, MA) and cloned into the pET22b (+) expression vector (Novagen, La Jolla, CA) to generate the construct pET22-LsrR-LT2, which has a hexahistidine (His$_6$) tag at the C-terminus. The sequence of pET22-LsrR-LT2 was confirmed by standard sequencing analysis.

Cell culture: overexpression of the repressor LsrR (*S. typhimurium* LT2)

The pET22-LsrR-LT2 expression vector was transformed into *E. coli* strain BL21(DE3) (Invitrogen, NY). The cells were grown in LB medium (200 mL) supplemented with 100 μg/mL carbenicillin with shaking at 250 rpm at 37 °C until OD$_{600}$ reached 0.4. The cell culture was immediately cooled with an ice bath and then expression of LsrR protein was initiated by adding 0.1 mM isopropyl *β*-D-1-thiogalactopyranoside (IPTG) and culturing at 16 °C overnight. The cells were then centrifuged (4 °C, 4,000 x g, 20 min) and washed with cold PBS (2 x 25 mL). The cells were resuspended in 50 mM phosphate buffer (pH 7, 10 mL) containing 150 mM NaCl and the cOmplete protease inhibitor cocktail (Roche) and lysed with sonication under native conditions. Cell debris was removed from the obtained lysate by centrifugation (4 °C, 12,600 x g, 20 min) and total concentration of proteins in supernatant was determined by BCA assay (4.5 mg/mL). The supernatant was aliquoted and snap-frozen with liquid nitrogen and stored in a -80 °C freezer until protein labeling experiments.

Cell culture: preparation of the whole cell lysate from *S. typhimurium* strain 14028

The cells were grown in LB medium (5 mL) with shaking at 250 rpm at 37 °C overnight (OD_{600} = 4.1). The overnight culture was diluted 1:100 in LB medium (300 mL) and incubated at 37 °C until OD_{600} reached 3.6 (~ 8 h, stationary phase). The cells were then centrifuged (4 °C, 4,000 x g, 20 min) and washed with 2 x 25 mL cold PBS. The cells were resuspended in 50 mM phosphate buffer (pH 7, 10 mL) containing 150 mM NaCl and the cOmplete protease inhibitor cocktail (Roche) and lysed with sonication under native conditions. Cell debris was removed from the obtained lysate by centrifugation (4 °C, 12,600 x g, 20 min) and total concentration of proteins in supernatant was determined by BCA assay (5.0 mg/mL). The supernatant was aliquoted and snap-frozen with liquid nitrogen and stored in a -80 °C freezer until protein labeling experiments.

Preparation of stock solution of phenylenediamine-TAMRA 3 and -biotin 4

Phenylenediamine-TAMRA **3** and -biotin **4** are very air sensitive under neutral and basic conditions due to the electron-rich dialkoxyphenylenediamine moiety. To prevent undesired oxidative degradation, we prepared stock solutions of these compounds in 10% acetic acid containing five equivalent of tris(2-carboxyethyl)phosphine (TCEP) and stored in a -80 °C freezer until just before use. No significant degradation was observed after the stock solutions were stored for a month under these conditions.

A typical protocol of LsrR labeling using DPD and fluorescent imaging

Whole cell lysate containing overexpressed LsrR was adjusted to 1.11 mg/mL and supplemented with 0.11 mg/mL Bovine Serum Albumin (BSA, Sigma) and 0.55% CHAPS ("Native" sample) or 0.55% SDS ("Denatured" sample). For denatured sample, lysate was heated at 95 °C for 10 min, and cooled to rt. 25 mM DPD stock solution containing 0.1% H_2SO_4 (4 µL)[6] was neutralized with 1 M phosphate buffer (pH 7, 6 µL) and mixed with the Native/Denatured samples (90 µL). The resultant mixture (100 µL, 1.0 mg/mL proteins, final DPD concentration: 1 mM) was incubated at rt for 1 h. Methanol/chloroform (4:1, 500 µL) was added to the mixture, sample tubes were vigorously shaken for 10 sec, and DI water (300 µL) was added. After vigorously shaking for 10 sec, the sample tubes were centrifuged at 14,000 x g for 1 min at rt to yield a white protein solid between the aqueous and organic layers. The top layer was aspirated, and the protein solid was washed with methanol (400 µL). After discarding residual liquid, the protein pellet was dried in air for 5 min and resolubilized in 1% SDS (80 µL). The protein solution (20 µL) was acidified with 2% trifluoroacetic acid (1 µL) and 1 mM phenylenediamine-TAMRA **3** (2 µL) was then added to the solution. After incubating the mixture in the dark at rt for 1 h, samples were mixed with 500 mM TCEP (1.66 µL) and 4X LDS loading buffer (Life Technologies, 8.33 µL) without boiling and 15 µL sample separated using SDS-PAGE. Imaging was achieved using in-gel fluorescent detection on a ChemiDoc MP System (Bio-Rad) (excitation: 532 nm, emission filter: 605 ± 50 nm). The obtained fluorescence images were edited using Image Lab software (Bio-Rad).

Proteome labeling using DPD

Whole cell lysate from *S. typhimurium* strain 14028 was adjusted to 5 mg/mL and supplemented with 0.55% CHAPS ("Native" sample) or 0.55% SDS ("Denatured" sample). For denatured sample, lysate was heated at 95 °C for 10 min and cooled to rt. 100 mM DPD stock solution containing 0.1% H_2SO_4 (40 µL) was neutralized with 1 M phosphate buffer (pH 7, 60 µL) and mixed with the Native/Denatured samples (900 µL). The resultant mixture (1 mL, 1.0 mg/mL proteins, final DPD concentration: 4 mM) was incubated at rt for 2 h. Methanol/chloroform (4:1, 1.25 mL) was added to the mixture, and then sample tubes were vigorously shaken for 10 sec and centrifuged at 14,000 x g for 1 min at rt to yield a white protein solid between the aqueous and organic layers. The top layer was aspirated, and the protein solid was washed with methanol (1 mL). After discarding residual liquid, the protein pellet was dried in air for 5 min and resolubilized in 1% SDS (495 µL). This protein solution was acidified with 10% trifluoroacetic acid (5 µL) and 10 mM phenylenediamine-biotin **4** (5 µL) was then added to the solution. After incubating the mixture on a sample rotor at rt for 1.5 h, the proteins were precipitated using methanol/chloroform (4:1, 1 mL), 1 M Tris buffer (pH 7, 300 µL), and methanol (800 µL) as described above. The protein pellet was rinsed with 4:1 methanol/chloroform (1 mL) before resolubilized in PBS (2 mL) containing 0.25% SDS and 0.6 M urea. Streptavidin-agarose beads (Thermo Fisher Scientific, 50 µL bed volume) were added to the solution and incubated on a sample rotor at rt for 1 h. Beads were washed with PBS containing 0.5% SDS (3 x 1 mL) and PBS (2 x 1 mL), and resuspended in PBS containing 6 M urea (200 µL). The suspension was treated with 5 mM TCEP (neutralized to pH 7) at 37 °C for 1 h, and with 15 mM iodoacetamide in the dark at rt for 30 min. After discarding the solution, the beads were washed with 50 mM tetraethylammonium bicarbonate (TEAB) containing 1 M urea (pH 8.3, 1 mL), and transferred to clean Eppendorf tubes and resuspended in 50 mM TEAB containing 1 M urea (300 µL). The enriched proteins were submitted to on-bead digestion using mass spectrometry-grade trypsin/Lys-C mix (Promega, 0.2 µg) at 37 °C for 16-20 h. The solution containing digested peptides was desalted and concentrated to 50 µL using STAGETips[7] and acidified with 5% formic acid. The obtained peptide solution was stored at -20 °C until before mass spectrometry analysis.

Proteome profiling using mass spectrometry

Proteomic experiments were performed at the Sanford-Burnham proteomics facility (Sanford-Burnham Medical Research Institute). Prior to LC-MS/MS analysis, peptides were concentrated on a SpeedVac

(Thermo Fisher Scientific) and reconstituted with 2% acetonitrile containing 0.1% formic acid (90 µL). A total of 30 µL solution was then injected onto column for LC-MS/MS using an online Paradigm HPLC system (Michrom) coupled to an Orbitrap Velos Pro mass spectrometer (Thermo Fisher Scientific). Injected peptides were separated at a flow rate of 2000 nL/min with solvents A (0.1% formic acid in water) and B (100% acetonitrile) using three slopes of linear gradients (from 2% to 10% solvent B in 4 min, then to 30% solvent B in 80 min, and finally to 35% solvent B in 5 min). Peptide separation was followed by 20 minutes of column washing with 80% solvent B, in addition to two blank runs. Mass spectrometer was operated in positive data-dependent acquisition mode. MS1 Spectra were measured with a resolution of 60,000, an AGC target of 1.0×10^6 and a mass range from 350 to 1400 m/z. Up to five MS2 spectra per duty cycle were triggered, fragmented by collision-induced dissociation (CID), and acquired in the ion trap with an AGC target of 1.0×10^4, an isolation window of 2.0 *m*/*z* and a normalized collision energy of 35. Dynamic exclusion was enabled with duration of 20 sec.

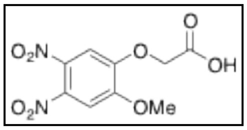
All mass spectra were analyzed with MaxQuant software (version 1.4.1.2).[8] MS/MS spectra were searched against *Salmonella typhimurium* in Uniprot-Swissprot database (version February/2014, contained 1,772 entries). Precursor mass tolerance was set to 20 ppm and 4.5 ppm for the first search where initial mass recalibration was completed and for the main search, respectively. Product ions were searched with a mass tolerance 0.5 Da. The maximum precursor ion charge state used for searching was 7. Carbamidomethylation of cysteines was searched as a fixed modification, while oxidation of methionines was searched as variable modification. Enzyme was set to trypsin in a semi-specific mode and a maximum of two missed cleavages was allowed for searching. The target-decoy-based false discovery rate (FDR) filter for spectrum and protein identification was set to 1%. One or more unique peptides were required for protein identification. Second peptide mode of MaxQuant software was also enabled. All six samples (three “Native” samples and three “Denatured” samples) were also analyzed together in label-free quantitative mode using MaxQuant LFQ algorithm. Features were matched between runs using a maximal 'match time window' of 1 min. See Figure S7 and Table S2 for a graphical summary of the obtained data and for a complete list of proteins identified in proteomic experiments, respectively. Potential targets of DPD were defined as proteins that meet criteria as follows: (1) >2-fold higher signal intensity (average of three replicates) in proteomic experiments with native samples than with denatured samples, (2) two or more unique peptides per replicate of experiments using native samples, and (3) *p* value (Student’s *t*-test) <0.05.

III. Synthetic Methods and Characterization of New Compounds

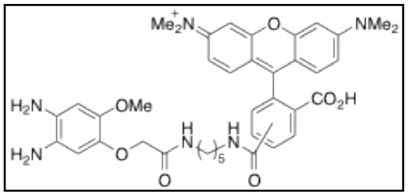
General Information

Unless otherwise noted, all materials were purchased from commercial suppliers and used as received. Anhydrous *N*,*N*-dimethylformamide (DMF) was purchased and stored over activated molecular sieves (4A) under argon atmosphere. Analytical thin-layer chromatography (TLC) was performed using silica gel plates (Merck Kieselgel 60 F_{254}, 0.25 mm for TLC), and spots were visualized with ultraviolet light (254 nm) or *p*-anisaldehyde stain.

Nuclear magnetic resonance (NMR) spectra were recorded on a DRX-600 equipped with a cryoprobe (Bruker, ^{1}H: 600 MHz, ^{13}C: 150 Hz) using dimethylsulfoxide-d_6 (DMSO-d_6) as a deuterated solvent. Chemical shifts (d) in ^{1}H NMR spectra are reported in parts per million (ppm) relative to DMSO-d_6 (δ = 2.50 ppm). Chemical shifts (d) in ^{13}C NMR spectra are reported in ppm relative to DMSO-d_6 (δ = 39.52 ppm). Coupling constants (*J*) in all NMR spectra are reported in Hz. Peaks in NMR spectra are assigned as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet, br = broad), coupling constant, and integration. LC-MS analysis was performed using a C8 column (4.6 x 50 mm) coupled with a HPLC system and 1100 MSD mass spectrometer (Agilent) in ESI positive mode. High-resolution mass spectra (HRMS) were measured on a VG ZAB-VSE or an ABI/SCIEX API-150 EX with electrospray ionization (ESI) method.

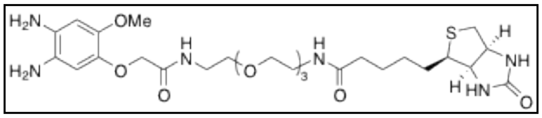


2-(2-Methoxy-4,5-dinitrophenoxy)acetic acid (S1). A solution of 2-(2-methoxyphenoxy)acetic acid[9] (800 mg, 4.39 mmol) in AcOH/CH_2Cl_2 (5:1, 6 mL) was added dropwise to fuming HNO_3 (15 mL) at 0 ºC. After being warmed up to rt and stirred for 30 min, the reaction mixture was poured into a flask filled with cold water (200 mL) and a light yellow solid precipitated. This solid was collected, washed with cold water and then cold 70% EtOH, and dried in vacuo to give analytically pure **S1** (832 mg, 70% yield). Light yellow powder. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.80 (s, 1H), 7.77 (s, 1H), 4.98 (s, 2H), 3.98 (s, 3H) ^{13}C NMR (125 MHz, DMSO-d_6): δ 169.0, 151.9, 150.1, 136.3, 135.5, 109.0, 108.3, 65.6, 57.2. HRMS (ESI+, *m*/*z*): calcd for $C_9H_7N_2O_8$ 271.0208, found 271.0209 (M - H).



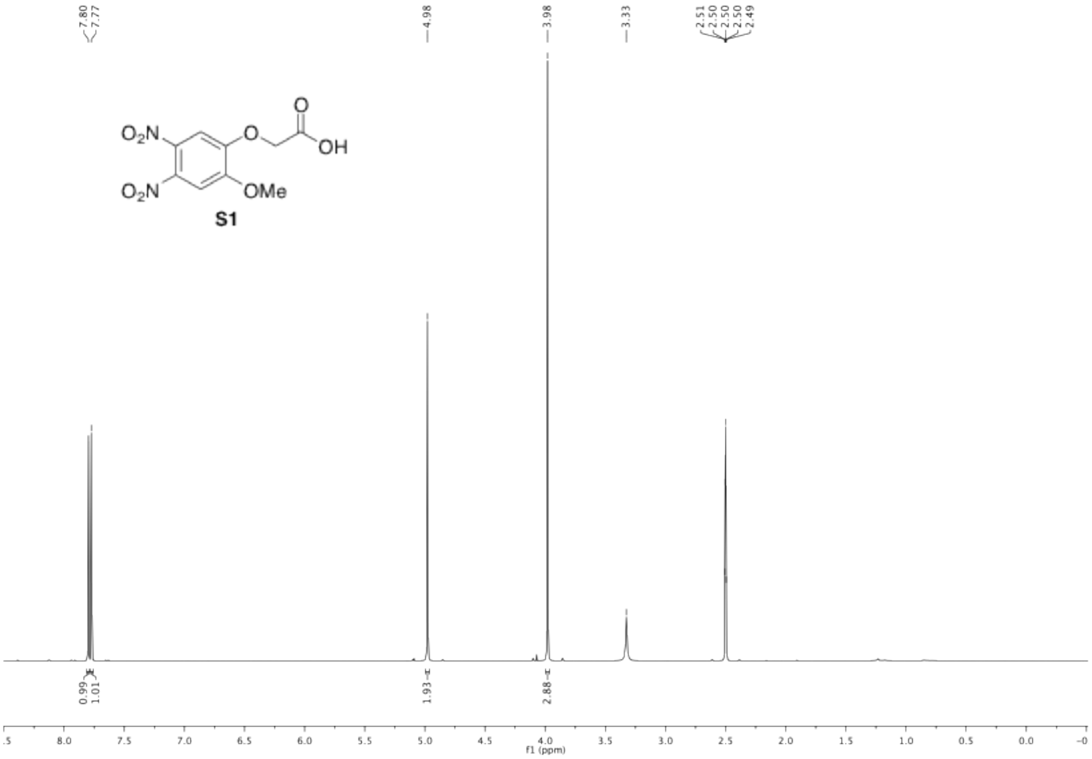
Phenylenediamine-TAMRA 3. *N*,*N*-diisopropylethylamine (17.2 μL, 0.099 mmol) was added to a solution of HATU (13.7 mg, 0.036 mmol), **S1** (9.8 mg, 0.036 mmol), and 5(6)-TAMRA cadaverine (Anaspec, 20 mg, 0.033 mmol) in DMF (2 mL) at rt. After being stirred 30 min, the reaction mixture was diluted with DI water (3 mL) and passed through a syringe filter. The solution was directly injected onto a preparative C18 column connected to a HPLC system (Agilent) and separated at a flow rate of 10 mL/min with solvents A (water + 0.1% trifluoroacetic acid) and B (acetonitrile + 0.1% trifluoroacetic acid) using a gradient program (solvent B: 1% for 7 min, from 1% to 80% in 43 min, from 80% to 99% in 5 min, and finally 99% for 10 min). Fractions were concentrated using a rotary evaporator to give dinitrophenyl-5(6)-TAMRA with inseparable impurities (26.5 mg, assumed as 0.033

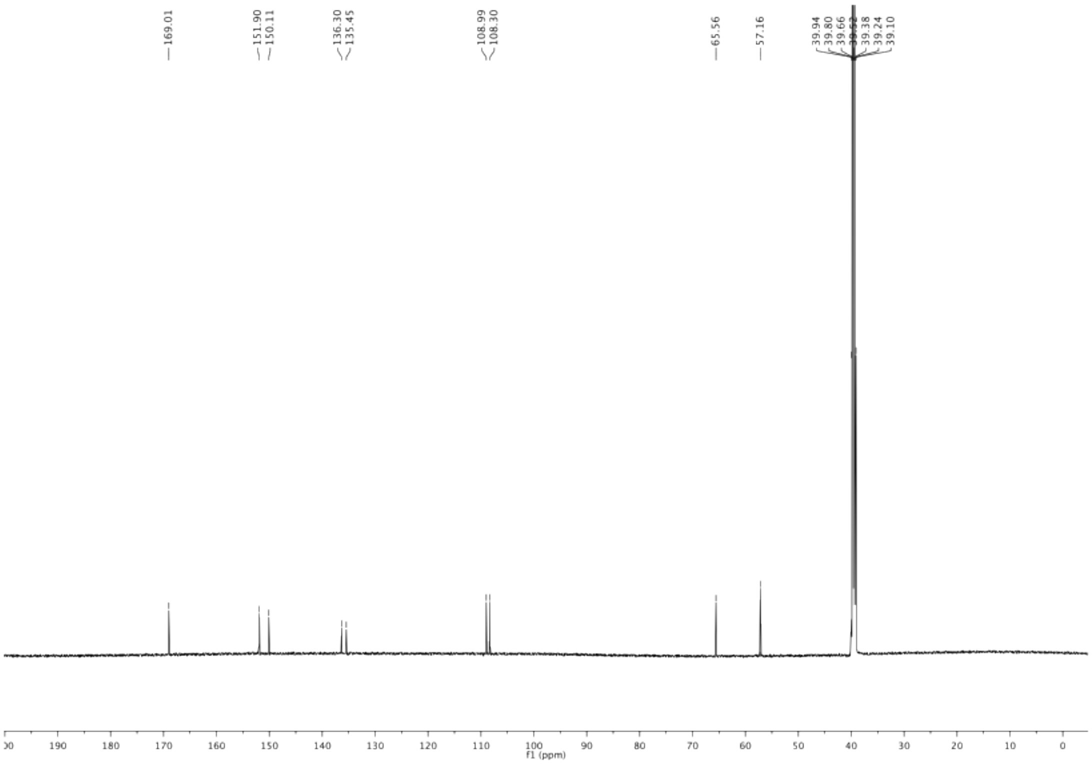
mmol). The obtained crude products were placed in a flask filled with argon and degassed EtOH/water (1:1, 10 mL) and 1 *N* HCl (132 µL) were added. Subsequently, $SnCl_2$ (dihydrate, 149.0 mg, 0.66 mmol) was added and the solution was refluxed for 2.5 h. After the reaction mixture containing a white solid was cooled to rt, the solution was transferred to Eppendorf tubes and centrifuged (14,000 x g, 30 min). Supernatant was collected and passed through a syringe filter. The solution was separated on a preparative HPLC system as described above (solvent B: 1% for 7 min, from 1% to 60% in 43 min, from 60% to 99% in 5 min, and finally 99% for 10 min). Fractions were concentrated on a Genevac EZ-2 (Genevac) and then freeze-dried on a FreeZone lyophilizer (Labconco) to give analytically pure phenylenediamine-5(6)-TAMRA **1** (2TFA salt, 13.6 mg, 46% yield). Dark red powder. ^{1}H and ^{13}C NMR spectra were complex as the product is a mixture of two isomers, and therefore purity was confirmed by LC-MS analysis. LC-MS: eluent: 1% Acetonitrile + 0.1% formic acid for 1 min, and from 1% to 90% Acetonitrile + 0.1% formic acid in 9 min, flow rate: 1 mL/min, retention time: 5.0 min and 5.2 min. HRMS (ESI+, *m*/*z*): calcd for $C_{39}H_{45}N_6O_7$ 709.3344, found 709.3342 (M + H).

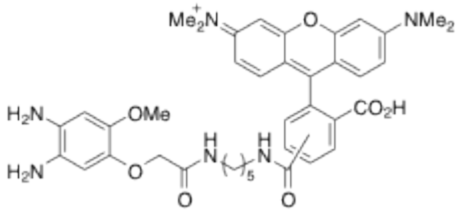


Phenylenediamine-biotin 4. This compound was prepared from **S1** (17.9 mg, 0.066 mmol) and amine-(peg)$_3$-biotin (Thermo Fisher Scientific, 25 mg, 0.060 mmol) by the same method as described above except reaction time (overnight) (2TFA salt, 4.0 mg, 8% yield). Pale yellow gummy solid. ^{1}H NMR (600 MHz, DMSO-*d*$_6$) *δ* 7.90 (t, *J* = 5.7 Hz, 1H), 7.83 (t, *J* = 5.7 Hz, 1H), 6.70 (s, 1H), 6.64 (s, 1H), 6.41 (s, 1H), 6.36 (br, 1H), 4.34 (s, 2H), 4.30 (dd, *J* = 7.8, 4.5 Hz, 2H), 4.12 (dd, *J* = 7.8, 4.5 Hz, 2H), 3.72 (s, 3H), 3.55-3.47 (m, 6H), 3.45 (t, *J* = 5.9 Hz, 2H), 3.38 (t, *J* = 6.0 Hz, 2H), 3.29 (dd, *J* = 12.8, 5.7 Hz, 2H), 3.17 (dd, *J* = 12.8, 5.7 Hz, 2H), 3.09 (m, 1H), 2.81 (dd, *J* = 12.5, 5.1 Hz, 1H), 2.57 (d, *J* = 12.4 Hz, 1H), 2.06 (dd, *J* = 7.4, 7.4 Hz, 2H), 1.66-1.55 (m, 1H), 1.55-1.38 (m, 3H), 1.35-1.21 (m, 2H). ^{13}C NMR (125 MHz, DMSO-*d*$_6$): *δ* 172.2, 167.8, 162.7, 158.3, 158.1, 142.9, 141.1, 117.1, 115.2, 69.7, 69.6, 69.2, 68.8, 61.0, 59.2, 55.9, 55.4, 38.4, 38.2, 35.1, 28.2, 28.0, 25.3. Peaks at 158.3, 158.1, 142.9, 141.1, 117.1, and 115.2 ppm were weak. LC-MS (ESI+): eluent: 1% Acetonitrile + 0.1% formic acid for 1 min, and from 1% to 90% acetonitrile + 0.1% formic acid in 9 min, flow rate: 1 mL/min, retention time: 4.1 min. HRMS (ESI+, *m*/*z*): calcd. for $C_{27}H_{44}N_6O_8S$ 613.3014, found 613.3010 (M + H).

^1H, ^{13}C NMR and/or LC-MS



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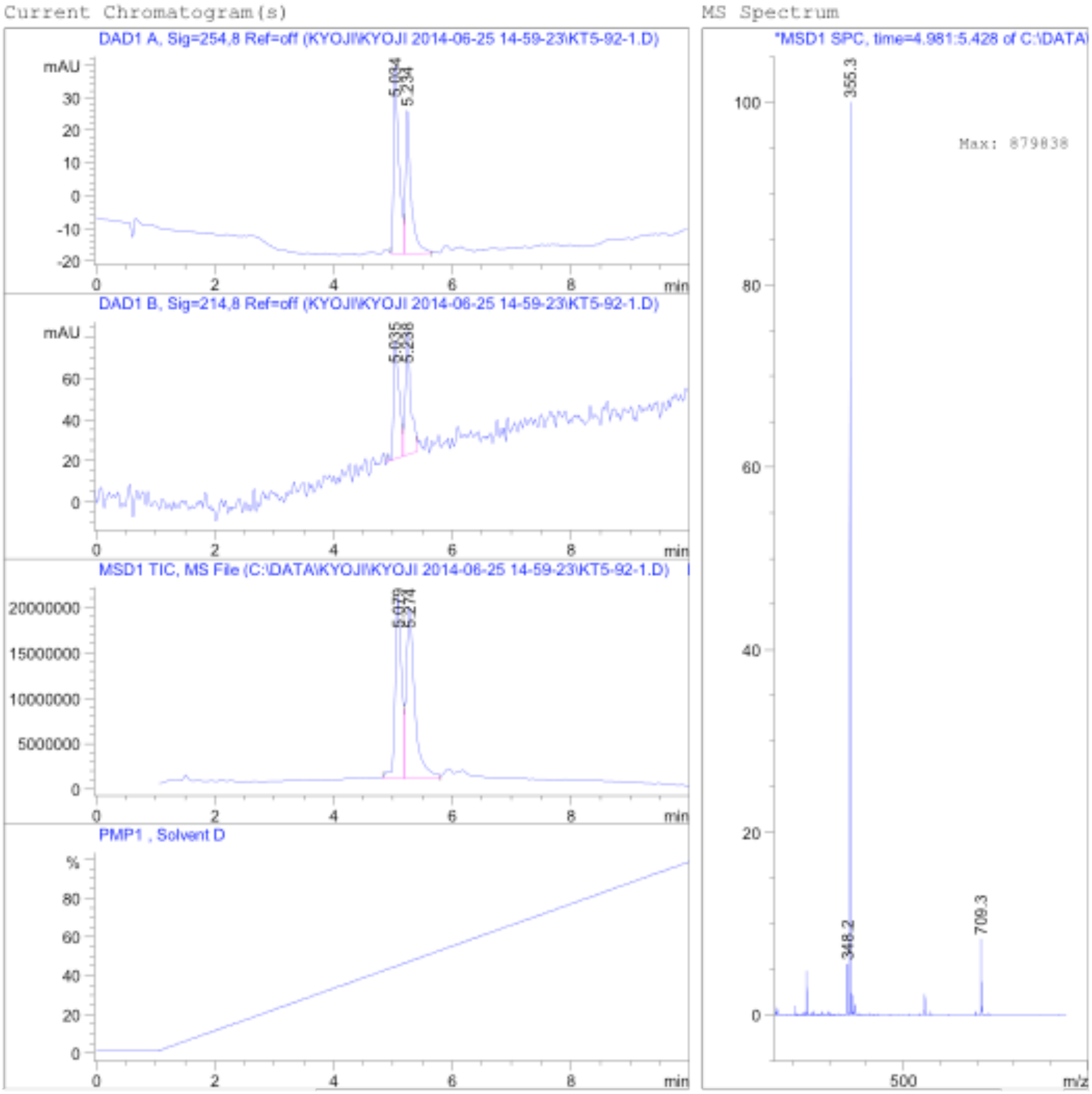
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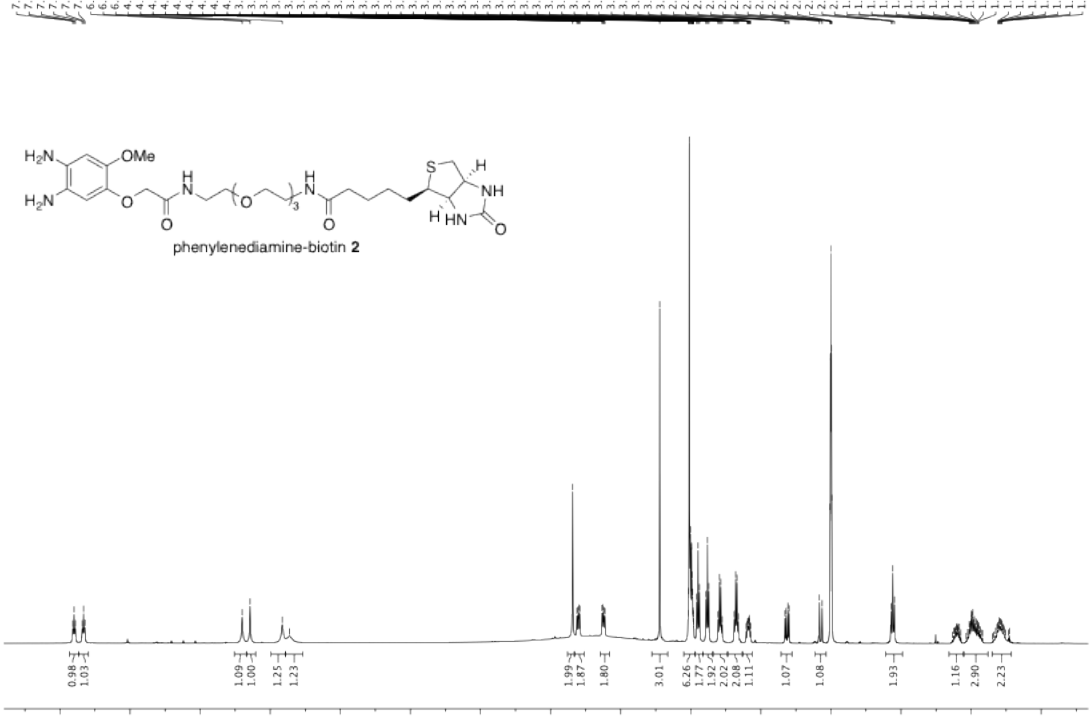
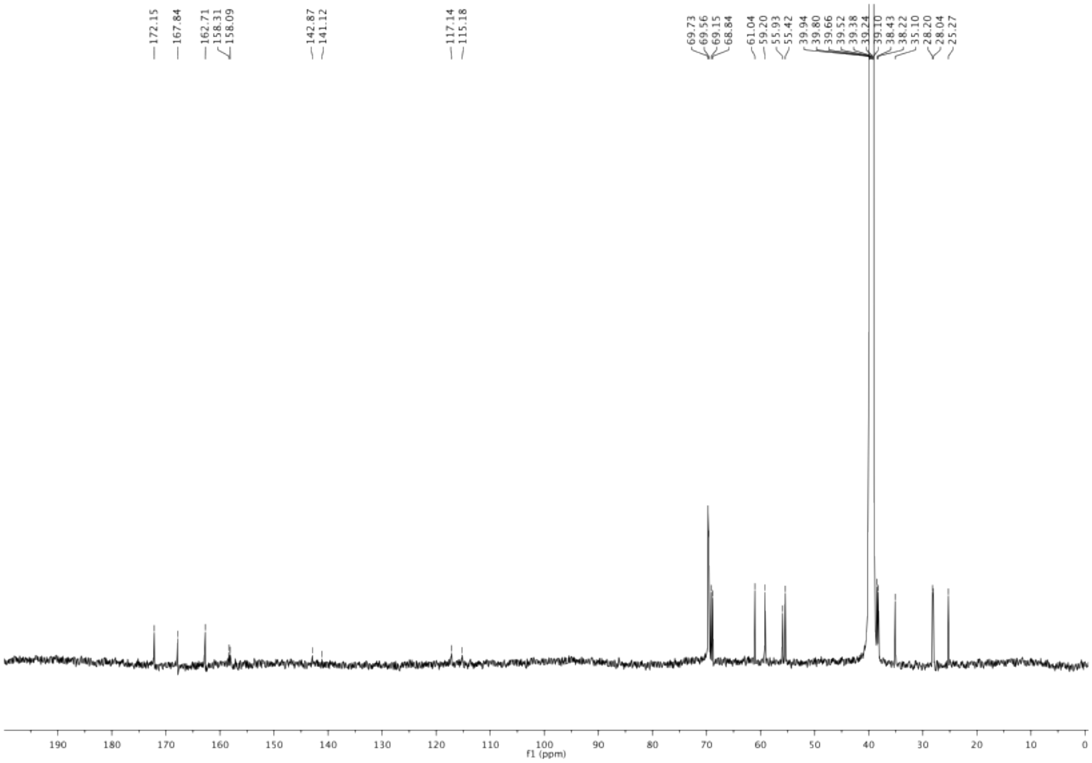
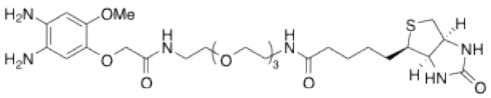


phenylenediamine-biotin **2**

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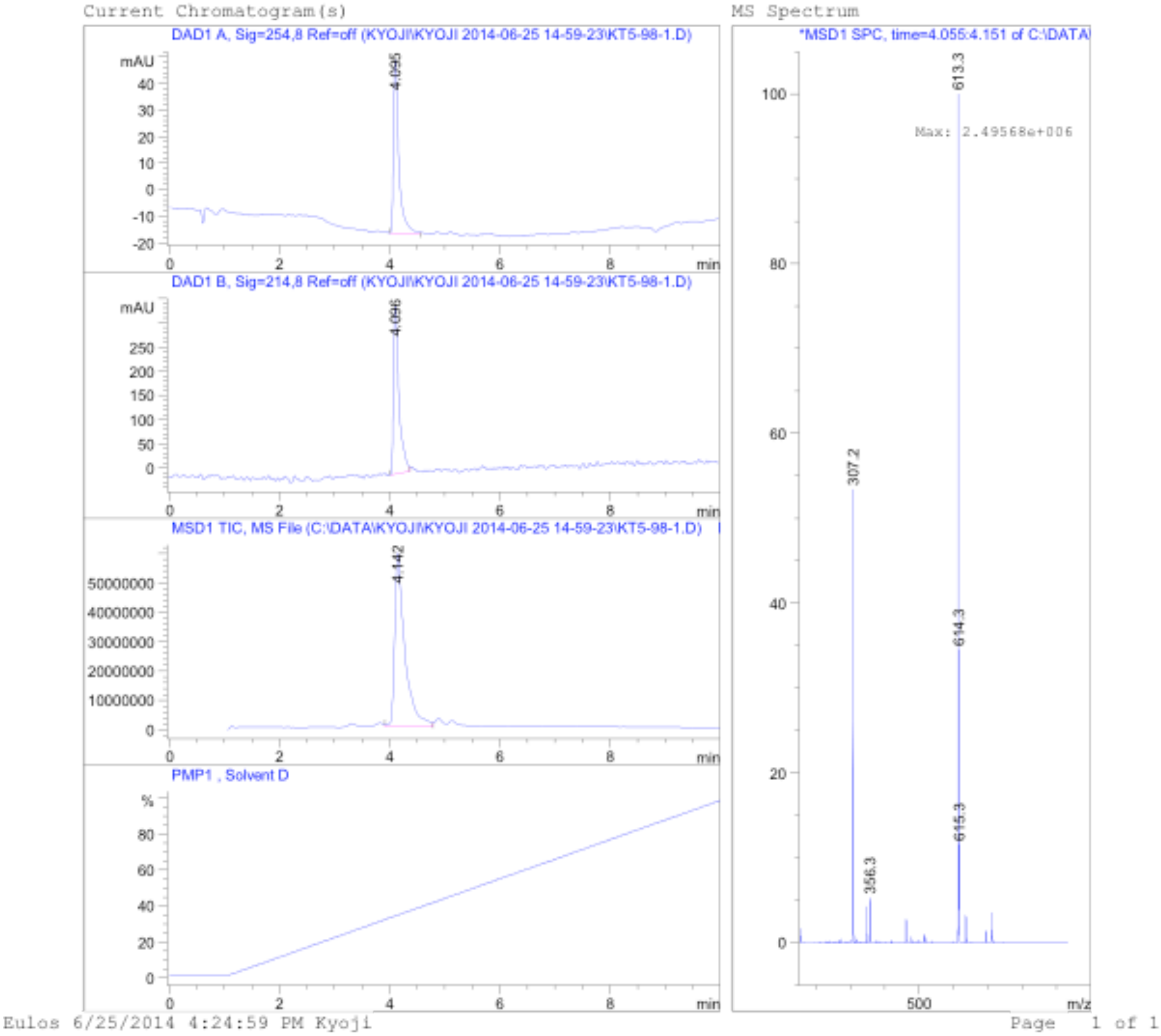
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IV. References

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