Covalent capture and electrochemical quantification of pathogenic *E. coli*

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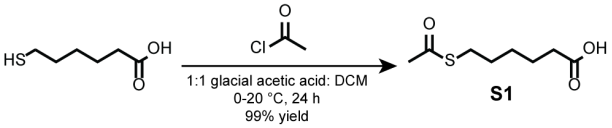
1 Small-molecule synthesis and characterization

1.1 Materials

Unless otherwise specified, reagents were used as received without further purification.

1.2 Small-molecule synthesis

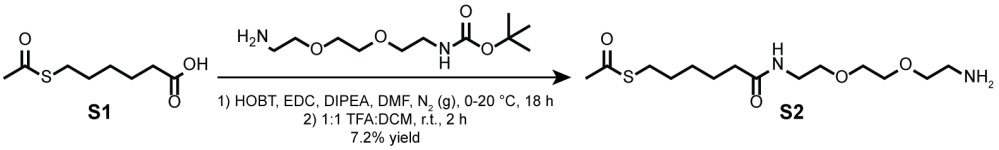
Synthesis of 6-(acetylthio)hexanoic acid (S1)



To a solution of 500 µL 6-mercaptohexanoic acid (3.12 mmol) in 1:1 glacial acetic acid: dichloromethane, 1.1 mL of acyl chloride was added dropwise as the reaction was stirred on ice. The solution was then warmed to room temperature and stirred for 24 h. The solvent was then removed by rotary evaporation, resulting in 592 mg (3.11 mmol) of a yellow oil (99% yield).

^{1}H NMR (300 MHz, Chloroform-d) δ = 11.51 (s, 1H), 2.86 (t, J = 7.1 Hz, 2H), 2.36 (m, 2H), 2.32 (s, 3H), 1.61 (m, 4 H), 1.42 (q, J = 7.2, 5.9 Hz, 2H).
LC-MS/MS calculated for $C_8H_{14}O_3S$ ([M+H]$^+$) 191.26 Da, found: 191.8 Da

Synthesis of *S*-(6-((2-(2-(2-aminoethoxy)ethoxy)ethyl)amino)-6-oxohexyl) ethanethioate (S2)



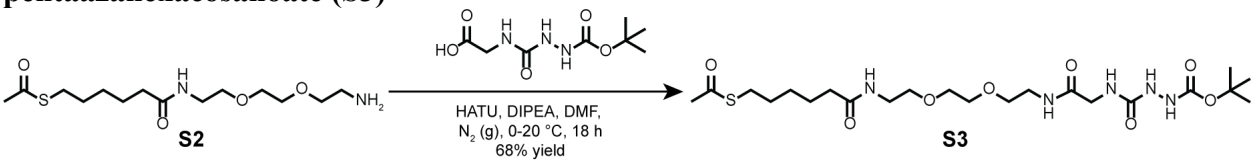
To a solution of 500 mg **S1** (2.6 mmol, 1 eq) in 20 mL of DMF at 0 °C under N_2 (g), 1.5 mL DIPEA (8.7 mmol, 3 eq), 718 mg *tert*-butyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (2.9 mmol, 1.1 eq), 603 mg EDC (3.2 mmol, 1.2 eq), and 402 mg HOBt (2.6 mmol, 1 eq) were added. The solution was warmed to room temperature and stirred for 18 h, followed by solvent removal by rotary evaporation. The crude material was purified by column chromatography using a gradient of 20 - 100% ethyl acetate in hexanes. The fractions containing the product were combined, and the solvent was removed by rotary evaporation, resulting in 80.6 mg of a clear oil (7.2% yield). Integration of the acyl-thiol peak (δ = 2.34) indicates that some deprotection of compound S2 has occurred.

^{1}H NMR (400 MHz, Chloroform-d) δ = 6.13 (s, 1H), 5.06 (s, 1H), 3.63 (s, 4H), 3.58 (m, 4H), 3.48 (m, 2H), 3.33 (m, 2H), 2.88 (m, 1H), 2.34 (s, 1.5H), 2.21 (t, 2H), 1.64 (m, 4H), 1.46 (m, 14H).

The full 80.6 mg (0.19 mmol) was then deprotected by resuspending the clear oil in a solution of 1:1 TFA:DCM and stirring under N_2 (g) for 2 h. The TFA and DCM were then removed by rotary evaporation.

LC-MS/MS calculated m/z for $C_{14}H_{28}N_2O_4S$ ([M+H]$^+$) 321.45 Da, found: 321.2 Da

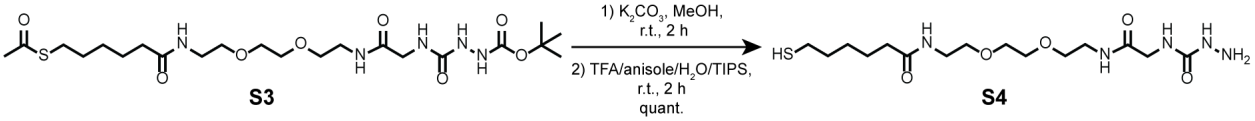
Synthesis of *tert*-butyl 4,7,18,25-tetraoxo-11,14-dioxa-24-thia-2,3,5,8,17-pentaazahexacosanoate (S3)



To a solution of 196 mg **S2** (0.6 mmol, 1 eq) in 10 mL dry DMF at 0 °C under N_2 (g), 0.32 mL DIPEA (1.8 mmol, 3 eq), 256 mg HATU (1.1 eq, 0.67 mmol), and 142.8 mg (0.6 mmol, 1 eq) of (2-(*tert*-butoxycarbonyl)hydrazine-1-carbonyl)glycine were added based on a previously-reported procedure.[3] The solution was stirred at room temperature for 18 h, followed by solvent removal with rotary evaporation. The crude material was extracted with EtOAc and washed with saturated NH_4Cl, H_2O, and brine, followed by drying over $MgSO_4$. The solvent was then removed by rotary evaporation, and the material was resuspended in a small amount of DCM and purified by column chromatography with a gradient of 0 - 20% MeOH in DCM. The fractions containing product were combined, and the solvent was removed by rotary evaporation, resulting in 221.1 mg (0.4 mmol) of a white foam (68 % yield).

^{1}H NMR (300 MHz, Chloroform-d) δ = 7.64 (s, 1H), 7.48 (s, 2H), 6.96 (s, 1H), 6.67 (s, 1H), 3.87 (s, 2H), 3.61 - 3.56 (m, 8H), 3.45– 3.32 (m, 6H), 2.33 (d, J = 1.6 Hz, 3H), 2.21 (t, J = 7.4 Hz, 2H), 1.69 – 1.50 (m, 4H), 1.46 (s, 9H), 1.44 – 1.30 (m, 2H).
LC-MS/MS calculated m/z for $C_{22}H_{41}N_5O_8S$ ([M+H]$^+$) 536.66 Da, found: 536.46. Da

Synthesis of *N*-(18-mercapto-2,13-dioxo-6,9-dioxa-3,12-diazaoctadecyl) hydrazinecarboxamide (S4)



To a solution of 206 mg **S3** (0.38 mmol, 1 eq) dissolved in 10 mL of MeOH, 106 mg (2 eq, 0.76 mmol) K_2CO_3 was added. The solution was stirred at room temperature for 2 h, followed by extraction into EtOAc and washing with sat. NH_4Cl, H_2O, and brine. The solvent was removed by rotary evaporation, and the resulting yellow oil was resuspended in a deprotection cocktail of TFA/anisole/H_2O/TIPS (80/8/2/10) and stirred at room temperature for 2 h under N_2 (g). The solvent was removed by rotary evaporation, resulting in a yellow oil in quantitative yield.
LC-MS/MS calculated *m/z* for $C_{15}H_{31}N_5O_5S$ ([M+H]$^+$): 394.50 Da, found: 394.70 Da

Synthesis of 2-acetylphenyl boronic acid (S5)-will change based on Chiral HPLC
The synthesis of 2-acetylphenylboronic acid was performed as described in Bandyopadhyay 2016,[1] starting with L-tyrosine. We observed racemization of our final compound **S5**, likely due to prolonged exposure to acidic conditions and high temperatures. As the D-enantiomer was found to incorporate into the peptidoglycan at higher levels than the L-enantiomer we thought it prudent

to determine the enantiomeric ratio of our final product. For reproducible results, we suggest that chiral HPLC be performed on the final product as well as a 2-acetylphenylboronic acid screen with *E. coli* and bacterium such as *S. aureus* to determine the concentration of 2-acetylphenylboronic acid that will result in low background labelling. This can be accomplished using the flow cytometry assay outlined in Section **3.4** and Figure **S7**. To confirm the enantiomer ratio, a derivatization method with H_2O_2 and subsequent analysis on a chiral HPLC column was performed.

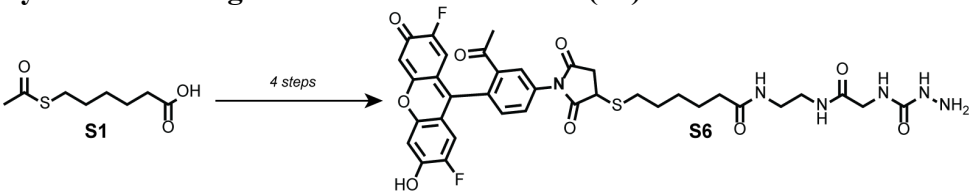
Evidence of enantiomeric impurity was first observed by splitting of the chiral alpha proton at δ = 4.04 and 4.25, which may be due to the ability of these compounds to participate in intermolecular interactions that would lead to the formation of diasteromeric complexes (Figure S4).

To determine if this was the case, chiral HPLC was performed on the purified 2-acetylphenylboronic acid. Multiple overlapping peaks were observed. The chiral column was then installed on an ESI-TOF-LC/MS and the mass of each peak was identified. The mass of all peaks corresponded to the boronic acid compound or its dimer product which is reversible. All peaks had the diagnostic boron isotope splitting pattern as well. However, this complicated our assignment of the enantiomeric ratio.

To determine if these multiple peaks were due to dimerization of the boronic acid head group, which was observed via NMR and ESI-TOF/LC-MS, we oxidized the boronic acid head group using hydrogen peroxide to generate a phenol, resulting in 2-acetyltyrosine (Figure S5a). 2-acetyltyrosine cannot form the dimerized products, and therefore separable peaks of the same mass should represent enantiomers. Chiral HPLC was performed on the oxidized product, and two distinct peaks were observed with a L:D ratio of 75: 25 (Figure S5b). The chiral column was installed on a ESI-TOF/LC-MS and the m/z and fragmentation patterns of the two peaks were examined. The oxidized compound again gave two distinct peaks with identical mass fragmentation patterns corresponding to 2-acetyltyrosine (Figure S5c). Based on chromatographic quantification, the percent of D-enantiomer in solution is approximately 25%. The enantiomers were not further purified, and stock solution of 3 mM S5 in Milli-Q H_2O was prepared and stored at -20 °C.

^{1}H NMR (300 MHz, D_2O) δ = 7.85 (t, 1H), 7.88 (t, 1H), 7.42 (t, 1H), 4.25 (t, 0.5H), 4.04 (t, 0.5), 3.27 (m, 2H), 2.61 (d, 3H).
LC-MS/MS calculated *m/z* for $C_{11}H_{14}BNO_5$ ($[M+Na]^+$): 274.04 Da, found : 274.32 Da

Synthesis of Oregon Green 488-Carbazide (S6)



To a solution of 357 mg **S1** (1.9 mmol, 1 eq) in dry THF at 0 °C stirring under N_2 (g), 992 µL DIPEA (5.7 mmol, 3 eq), 850 mg HATU (2.23 mmol, 1.2 eq), and 348 µL *tert*-butyl (2-aminoethyl)carbamate (2.2 mmol, 1.15 eq) were added. The reaction was warmed to room temperature and stirred overnight. The reaction was then extracted into EtOAc and washed with sat. NH_4Cl, H_2O, and brine, followed by drying over $MgSO_4$. The crude material was purified by

column chromatography using a gradient of 20 - 100% ethyl acetate in hexanes. The fractions containing product were combined, and the solvent was removed by rotary evaporation, resulting in a clear oil. The crude material was then dissolved in 1:1 TFA:DCM and stirred under N_2 (g) for 1 h. The solvent was removed by rotary evaporation, resulting in a clear oil **S6.1** (380 mg, 1.6 mmol, 87% yield).

^{1}H NMR (300 MHz, chloroform-d) δ = 6.72 (t, 1H), 5.37 (t, 1H), 3.17 (m, 4H), 2.74 (m, 2H), 2.22 (s, 3H), 2.08 (t, 2H), 1.5 (m, 4H), 1.32 (s, 12H).
LC-MS/MS calculated m/z for $C_{10}H_{20}N_2O_2S$ ($[M+H]^+$): 233.34 Da, found 233.2 Da

The intermediate **S6.1** (380 mg, 1.6 mmol, 1 eq) was dissolved in dry THF at 0 °C with stirring under N_2 (g). To this solution, 1106 µL DIPEA (6.3 mmol, 3.9 eq), 673 mg HCTU (1.6 mmol, 1 eq), and (2-(*tert*-butoxycarbonyl)hydrazine-1-carbonyl)glycine (380 mg, 1.6 mmol, 1 eq) were added. The reaction was allowed to warm to room temperature and stirred for 24 h. The crude material was then extracted with EtOAc and washed with sat. NH_4Cl, H_2O, and brine, along with drying over $MgSO_4$. The solvent was removed by rotary evaporation, and the material was resuspended in a small amount of DCM to be purified by column chromatography using a gradient of 0 - 15% MeOH in DCM. The fractions containing product were combined, and the solvent was removed by rotary evaporation, resulting in **S6.2** (300 mg, 0.7 mmol, 42% yield).

The intermediate **S6.2** (300 mg, 0.7 mmol, 1 eq) was dissolved in of 10 mL of MeOH, and 186 mg (1.4 mmol, 2 eq) K_2CO_3 was added. The solution was stirred at room temperature for 2 h. The solution was then extracted into EtOAc 3 times and washed with sat. NH_4Cl, H_2O, and brine, followed by drying over $MgSO_4$. The solvent was removed by rotary evaporation, and the resulting yellow oil was deprotected in TFA/anisole/H_2O/TIPS (80/8/2/10) with stirring at room temperature for 2 h under N_2 (g). The solvent was removed by rotary evaporation, followed by lyophilization to yield a white power **S6.3** (quantitative yield).

^{1}H NMR (300 MHz, D_2O) δ = 3.76 (s, 2H), 3.21 (s, 4H), 2.46 (dt, 2H), 2.12 (t, 2H), 1.57-1.14 (m, 9H).
LC-MS/MS calculated *m/z* for $C_{11}H_{23}N_5O_3S$ ($[M+H]^+$): 306.40 Da, found 306.8 Da

A 3 mg portion of **S6.3** was dissolved in 200 µL 1xPBS pH 7.4 with 100 µL TECP beads and incubated at room temperature with rotary shaking for 1 h. The solution was then filtered with a 0.22 µm cellulose spin column, and 10 µL of a 50 mM solution of Oregon Green maleimide in DMSO was added to the reduced thiol. The reaction proceeded for 3 h. The resulting solution was purified by HPLC using a gradient of 5-95% acetonitrile/H_2O over 30 min. The samples containing the appropriate product mass and fluorescence peak at 488 nm were combined and lyophilized.

LC-MS/MS calculated for ($[M+H]^+$): 769.75 Da, found: 769.02 Da

1.3 HPLC

HPLC was performed on an Agilent 1100 Series HPLC Systems (Agilent Technologies, USA). Sample analysis for all HPLC experiments was achieved with an in-line diode array detector (DAD) and in-line fluorescence detector (FLD). Analytical reverse-phase HPLC of small

molecules was accomplished using a C18 stationary phase and a mobile phase consisting of acetonitrile (Optima grade, 99.9%, Fisher, Waltham, MA) + 1% TFA and water purified to a resistivity of 18.2 MΩ·cm (at 25 ºC) using a Milli-Q Gradient ultrapure water purification system (Millipore, Billerica, MA) + 1% TFA. Following sample injection, a 5-95% elution gradient of acetonitrile + 1% TFA in water + 1% TFA was run over the course of 30 min.

1.4 Chiral HPLC

Chiral HPLC was performed on an Agilent 1100 Series HPLC Systems (Agilent Technologies, USA) with an in-line diode array detector (DAD) and in-line fluorescence detector (FLD). Separation of chiral compounds was achieved using a Chiradex (Agilent Technologies, USA) stationary phase chiral column (5µm, 100 A, 4.0 x 250 mm) and a mobile phase consisted of acetonitrile (Optima grade, 99.9%, Fisher, Waltham, MA) + 1% TFA and water purified to a resistivity of 18.2 MΩ·cm (at 25 ºC) using a Milli-Q Gradient ultrapure water purification system (Millipore, Billerica, MA) + 1% TFA. Following sample injection, a 0-1% elution gradient of acetonitrile + 1% TFA in water + 1% TFA was run over the course of 8 min followed by two washing gradients of 1-20% for 2 min and 20-95% for 2 min. Conversion of the boronic acid head group to the phenol was accomplished by treating a solution of 2 mM boronic acid in water with 20 mM of H_2O_2. The reaction was allowed to proceed for 1 h and then analysed by chiral HPLC and ESI-TOF-MS

1.5 Chiral HPLC-ESI-TOF-MS

Electrospray ionization mass spectrometry (ESI-MS) was performed using an Agilent 1260 series liquid chromatograph outfitted with an Agilent 6224 time-of-flight (TOF) LC-MS system (Santa Clara, CA). Separation of chiral compounds was achieved using a Chiradex (Agilent Technologies, USA) stationary phase chiral column (5µm, 100 A, 4.0 x 250 mm) and a mobile phase consisting of acetonitrile (Optima grade, 99.9%, Fisher, Waltham, MA), formic acid (1 mL ampules, 99+%, Pierce, Rockford, IL), and water purified to a resistivity of 18.2 MΩ·cm (at 25 ºC) using a Milli-Q Gradient ultrapure water purification system (Millipore, Billerica, MA). Solvent A was 99.9% water/0.1% formic acid and solvent B was 99.9% acetonitrile/0.1% formic acid (v/v). For each sample, 10 uL of a 2 mM analyte solution was injected onto the column. Following sample injection, a 0-1% elution gradient of acetonitrile + 1% TFA in water + 1% TFA was run over the course of 8 min followed by two washing gradients of 1-20% for 2 min and 20-95% for 2 min.

1.6 Liquid Chromatography Mass Spectrometry (LC-MS/MS) analysis

Acetonitrile (Optima grade, 99.9%, Fisher, Waltham, MA), formic acid, and water purified to a resistivity of 18.2 MΩ·cm (at 25 ºC) using a Milli-Q Gradient ultrapure water purification system (Millipore, Billerica, MA) were used to prepare mobile phase solvents for LC-MS/MS.

1.7 UV-Vis measurements

A NanoDrop 1000 (Thermo) was used to quantify the samples in this work based on absorbance values at 280 nm (or 488 nm for Oregon Green 488-containing samples).

1.8 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR ^{1}H spectra were measured with Bruker AV-300 (300 MHz, 75 MHz), AVB-400 (400 MHz, 100 MHz), or AV-600 (600 MHz, 151MHz) spectrometers. ^{1}H NMR chemical shifts are reported as δ in units of parts per million (ppm) relative to residual $CHCl_3$ (δ 7.26, singlet) or DMSO-d_6 (δ 2.50, pentet). Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multuplet or unresolved), or br (broad). Coupling constants are reported as a J_{value} in Hertz (Hz). The number of protons (n) for a given resonance is indicated as nH and is based on spectral integration values.

2 Electrochemical methods

2.1 Electrode Preparation

Disposable gold electrodes (1.6 mm diameter, Figure S6) on ceramic backing (C223BT, DropSens, Spain) were cycled in 0.5 M H_2SO_4 from 1.3 V to -0.2 V vs. the incorporated silver reference electrode for ten cycles until a consistent signal was observed. Electrodes were then washed with Milli-Q H_2O and dried under a stream of N_2 (g).

2.2 S4 (thiol-carbazide) monolayer formation on electrodes

S4 (thiol-carbazide)-functionalized electrodes were prepared by soaking the cleaned electrode in a 2 mM solution of **S4** in ethanol for 1-3 h at ambient temperature. Electrodes were then removed from solution and rinsed with ethanol and Milli-Q H_2O. The electrodes were air dried directly before use. For this study, self-assembled thiol-carbazide monolayers were always formed directly prior to use. All electrodes were subjected to a "zero scan" prior to the addition of analyte solutions.

2.3 Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy (EIS) was performed with a Gamry 600 Reference potentiostat (Gamry Instruments, Warminster, PA, USA). For EIS measurements, electrodes were placed in a buffer containing 4 mM each of $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ with 0.1 M KCl.[2] EIS spectra were acquired at the open circuit potential of the electrode, as measured for 60 s prior to EIS. EIS measurements were obtained at frequency ranges from 50,000 Hz to 0.2 Hz with 10 points per decade and a 10 mV potential range. Electrochemical data was analyzed using the Gamry Echem Analyst software. The charge transfer resistance (R_{CT}) was derived from a Warburg impedance model circuit fit. The data are reported as the normalized change in the charge transfer resistance ΔR_{CT} between the "zero scan" and the dilution series sample, as standardized to the initial R_{CT}.

3 Preparation of bacteria

3.1 Preparation of bacterial cultures for electrochemistry experiments

Escherichia coli (*E. coli*) O1:K1:H7 and *Staphylococcus aureus subsp. aureus* cultures were grown from -80 °C glycerol stocks incubated overnight with agitation at 37 °C in 10 mL culture tubes with sterilized Tryptic Soy or Nutrient Broth, respectively. A 10 µL aliquot was diluted into fresh broth, and cells were incubated for an additional 1-3 h until an OD_{600} of 0.1-0.3 was reached. This stock was diluted to generate 100 µL samples of a series of bacterial concentrations ranging from 10^7 cells/mL to 10^2 cells/mL. A control of 100 µL phosphate buffered saline (PBS) pH 7.4 was also included in the dilution series. To each dilution series, a 3 mM stock of 2-acetylphenylboronic acid was added to a final concentration of 50 µM.[3] The diluted cultures were incubated with agitation at 37 °C for 1 h in a benchtop incubator. The dilution series were centrifuged at 13,400 rpm and washed a total of three times with PBS. After the final wash, the cells were resuspended in 100 µL of PBS and heat-killed at 95 °C for 5 min on a heat block. A 10 µL portion of each culture was then added the thiol-carbazide-functionalized electrodes that had previously undergone a "zero scan" by directly pipetting the sample onto the working electrode. The type of electrode used is pictured in Figure S6. The samples containing the cells were allowed to incubate on electrodes for 30 min at ambient temperature. Electrodes were gently rinsed with PBS prior to EIS analysis. The limit of detection (LOD) was calculated based on three times the standard deviation of the blank. For detection in buffer, this represents a 49% increase in the R_{CT}.

For detection from the complex solutions of synthetic urine and milk, the sample was resuspended in the matrix of interest for both the 3-acetyl-4-borono-DL-phenylalanine incubation and washes, as well as for the final addition to the electrode surface. Milk samples were prepared from laboratory-grade dried milk powder (Sigma-Aldrich) and artificial urine containing phosphates and albumin (Innovating Science™).

3.2 Real-world sample preparations

Detection from real food and urine samples was accomplished through the isolation of solutions containing the microbes from those samples. To prepare chicken for detection, a chicken carcass obtained from the butcher counter at a local grocer was rinsed using protocols for chicken carcass rinsing based on those established by the USDA.[4] Briefly, the carcass was placed in a one-gallon sealable bag, and 30 mL of buffered peptone water (BPW) was added. The bag was sealed, and the contents shaken for one minute. The liquid was then recovered and used for detection. Eggs were obtained from a local farmer's market. Egg rinses were conducted similarly to chicken carcass rinses: six eggs were placed in a sealable bag, followed by 10 mL of BPW. The eggs were gently swirled in the sealed bag for one minute, followed by solution recovery. All eggs remained intact throughout the procedure. Spinach and romaine lettuce were also obtained from a local farmer's market. Rinsings were obtained similarly to the chicken and egg samples; 200 g of spinach or romaine lettuce was added to a sealable bag, along with 20 mL of phosphate-buffered

saline. The bag was agitated by hand for five minutes, followed by solution recovery. Feline urine was obtained following sample collection using hydrophobic cat litter and a specimen collection kit. 5 mL of urine were isolated for testing with this study, with a sample evaluated by a veterinarian as well. In all cases, 100 µL of sample was used for both synthetic amino acid treatment (with 10 µL of that used for electrochemical detection) and colony counting. One sample of each type was evaluated, with three individual aliquots of each rinsing treated independently with the synthetic amino acid, and three individual aliquots plated for CFU counting. Synthetic amino acid treatment and electrochemical detection were performed as previously described.

3.3 Colony counting on agar plates

To determine the colony-forming units (CFUs) present in each sample, 100 µL of sample was plated in triplicate on agar plates containing no antibiotics. The plates were allowed to grow for 36 h at 37 °C, followed by imaging of the plates using an iPhone 7 camera and manual counting of the colonies formed.

3.4 Flow cytometry

Bacterial samples for flow cytometry were grown as previously described for electrochemical experiments.[3] Cells were grown overnight then diluted to OD_{600} ~0.3. The 2-acetylphenylboronic acid was then added to 100 µL aliquots of cells to final concentrations of 0-300 µM. The cultures were incubated with agitation at 37 °C for 1 hour then centrifuged at 13,400 rpm and washed a total of three times with PBS. After the final wash, the cells were resuspended in 100 µL of PBS, and Cy5-modified carbazide was added to a final concentration of 50 µM. Cells were incubated at ambient temperature for 30 min, followed by centrifugation at 13,400 rpm and washing a total of three times with PBS. Samples were analyzed on a BD LSRFortessa X-20 (BD Biosciences). CytoBank was used to perform data analysis to extract the mean fluorescent intensities of each sample of 10,000 analyzed cells.

3.5 16S Sequencing

Two representative colonies from each agar plate were grown overnight in LB broth, and genomic DNA was extracted using phenol-$CHCl_3$ as previously described.[5] The V4 hypervariable region of the 16S rRNA gene was amplified using primers that contained Gibson overhangs to clone into the pET24a vector at the NdeI/XhoI cut sites using Gibson assembly.[6] Resultant colonies were picked and the insert region sequenced to determine the V4 region sequence of isolates, and all sequences were found to be *Escherichia* from BLAST similarity search.

4. Supplemental Figures

Figure S1. 6-(acetylthio)hexanoic acid (S1)
^{1}H NMR (300 MHz, Chloroform-d) δ = 11.51 (s, 1H), 2.86 (t, J = 7.1 Hz, 2H), 2.36 (m, 2H), 2.32 (s, 3H), 1.61 (m, 4 H), 1.42 (q, J = 7.2, 5.9 Hz, 2H).

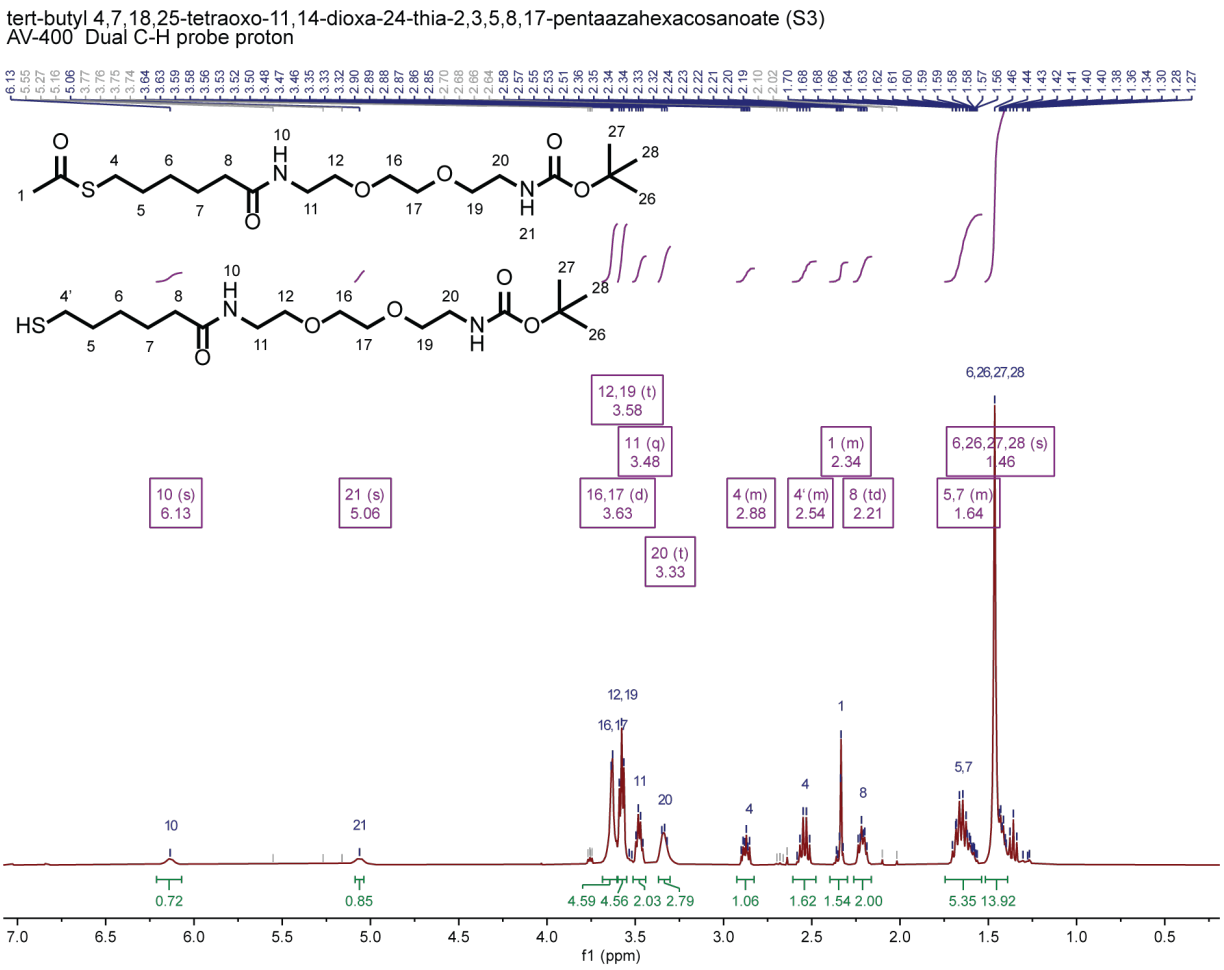


Figure S2. 6-(acetylthio)hexanoic acid (S2)
^{1}H NMR (400 MHz, Chloroform-d) δ = 6.13 (s, 1H), 5.06 (s, 1H), 3.63 (s, 4H), 3.58 (m, 4H), 3.48 (m, 2H), 3.33 (m, 2H), 2.88 (m, 1H), 2.34 (s, 1.5H), 2.21 (t, 2H), 1.64 (m, 4H), 1.46 (m, 14H).

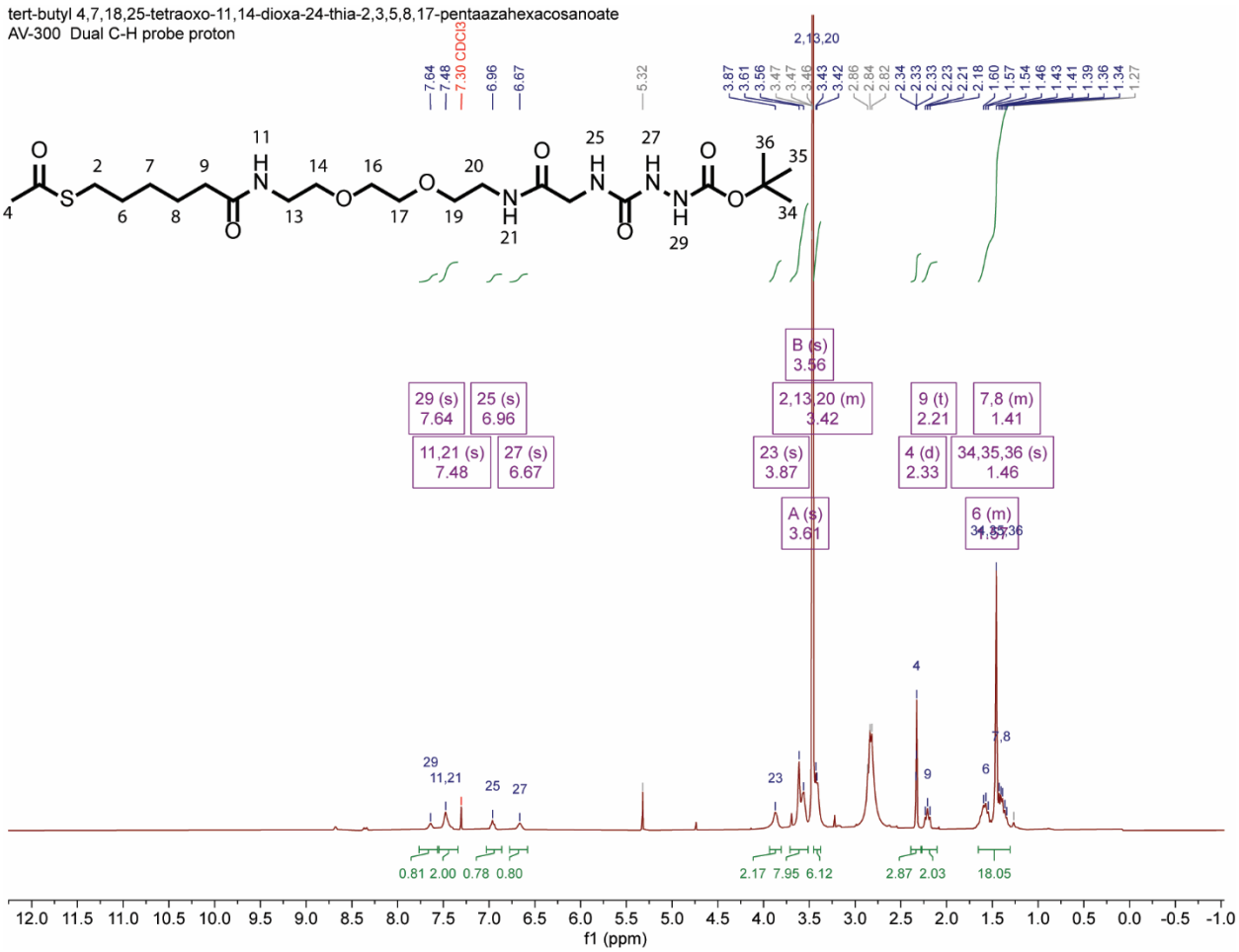


Figure S3. tert-butyl 4,7,18,25-tetraoxo-11,14-dioxa-24-thia-2,3,5,8,17-pentaazahexacosanoate (S3)

^{1}H NMR (300 MHz, Chloroform-d) δ = 7.64 (s, 1H), 7.48 (s, 2H), 6.96 (s, 1H), 6.67 (s, 1H), 3.87 (s, 2H), 3.61 - 3.56 (m, 8H), 3.45– 3.32 (m, 6H), 2.33 (d, J = 1.6 Hz, 3H), 2.21 (t, J = 7.4 Hz, 2H), 1.69 – 1.50 (m, 4H), 1.46 (s, 9H), 1.44 – 1.30 (m, 2H).

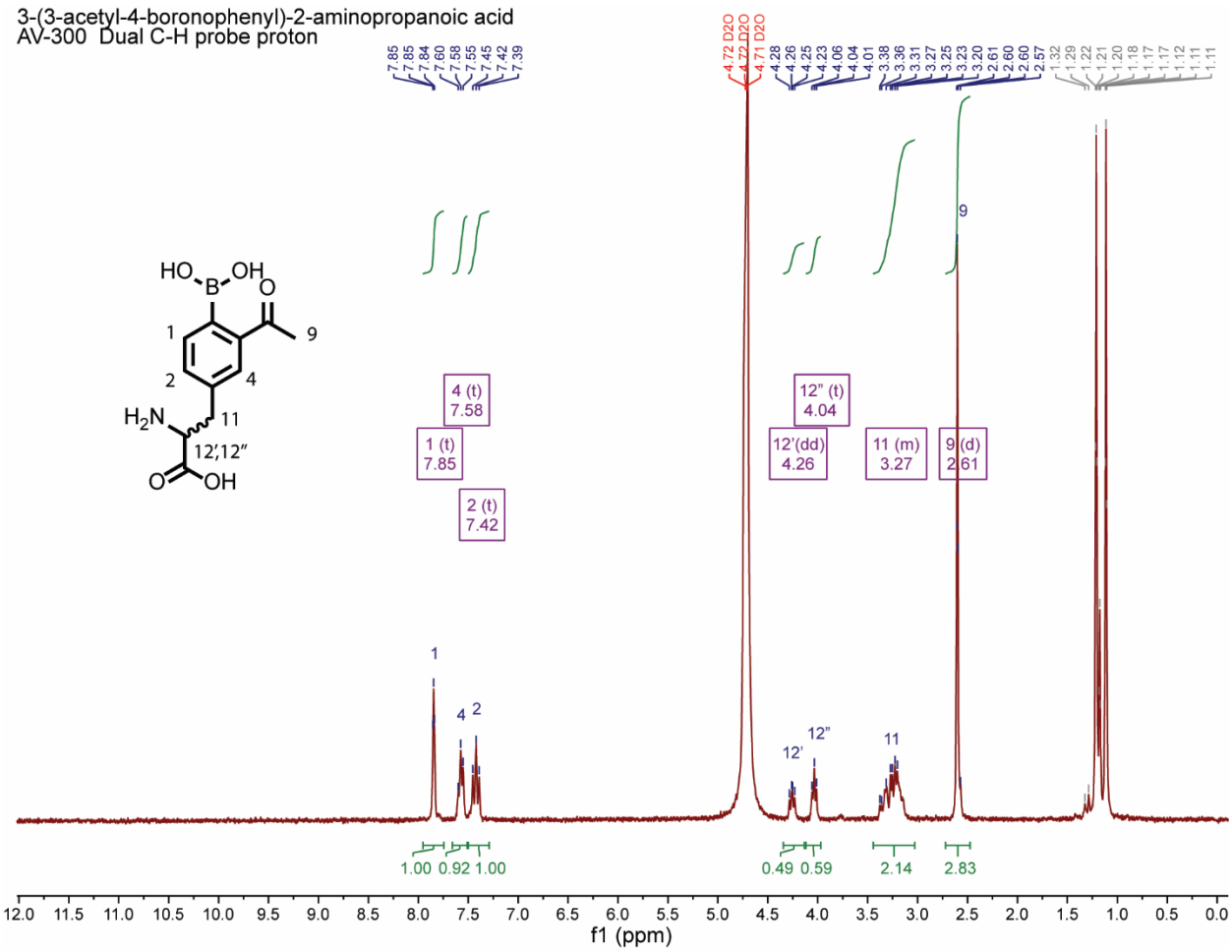


Figure S4. 2-acetylphenylboronic acid (S5)
^{1}H NMR (300 MHz, D_2O) δ = 7.85 (t, 1H), 7.88 (t, 1H), 7.42 (t, 1H), 4.25 (t, 0.5H), 4.04 (t, 0.5), 3.27 (m, 2H), 2.61 (d, 3H)

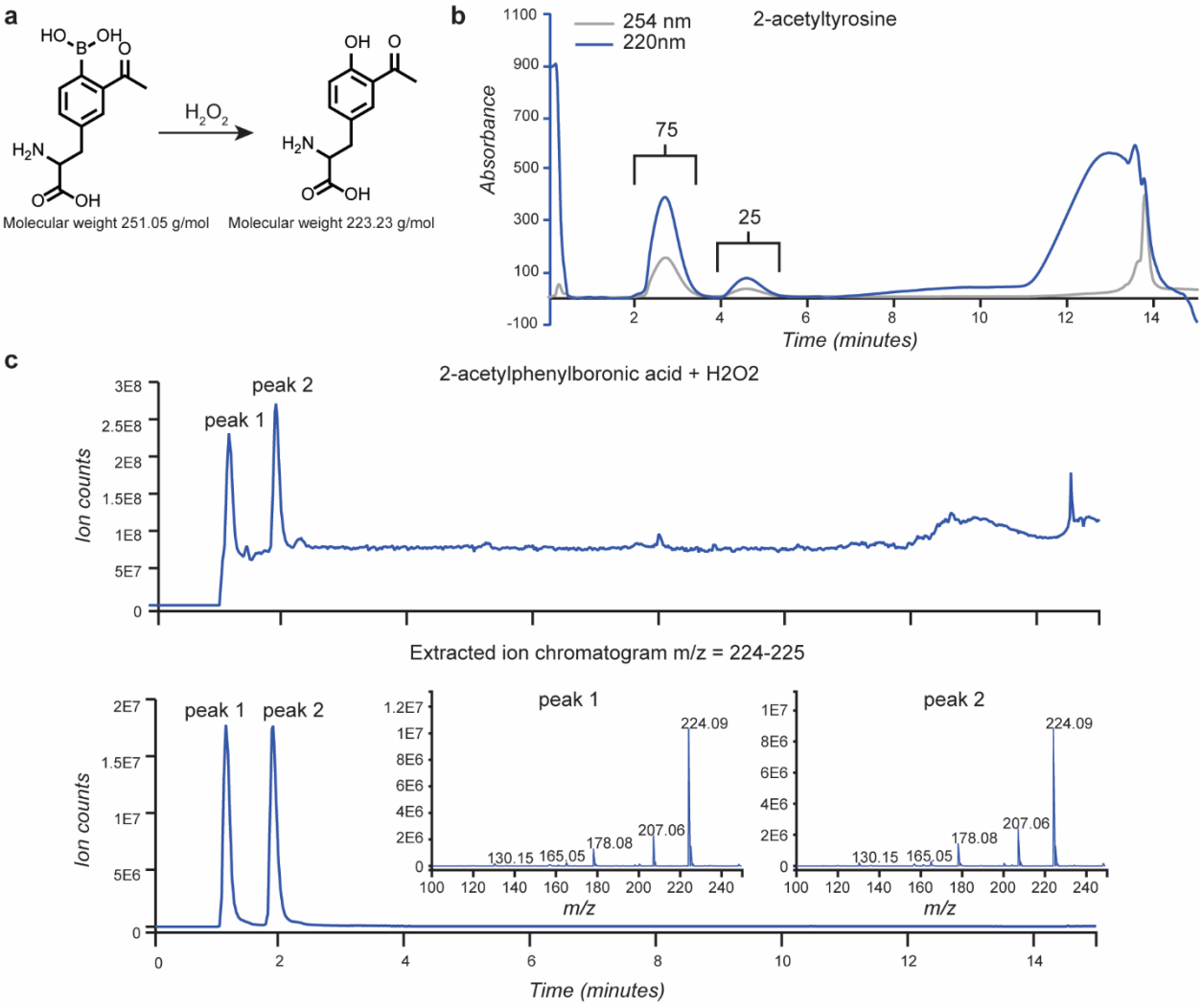


Figure S5. (a) oxidation of 2-acetylphenylboronic acid with 10 equivalents of H_2O_2 resulting in the conversion to 2-acetyltyrosine. (b) Chiral HPLC trace of 2-acetyltyrosine results in the separation of two peaks that both elute a 1% acetonitrile in water + 0.1% TFA. Integration of the peaks indicates a 75:25 ratio of L:D enantiomers. (c) ESI-TOF\Chiral HPLC-MS of 2-acetyltyrosine also results in the separation of two peaks with identical masses and fragmentation patterns. Expected m/z: 224.23 Da, observed m/z: 224.09 Da.

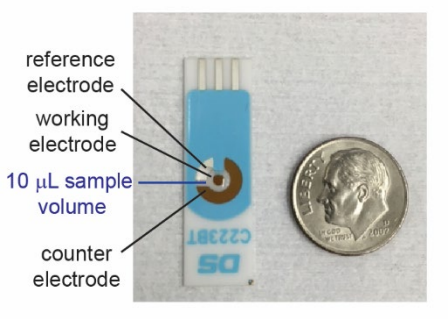


Figure S6. Image of disposable electrode used for studies. The working electrode (center) requires only 10 μL of sample volume.

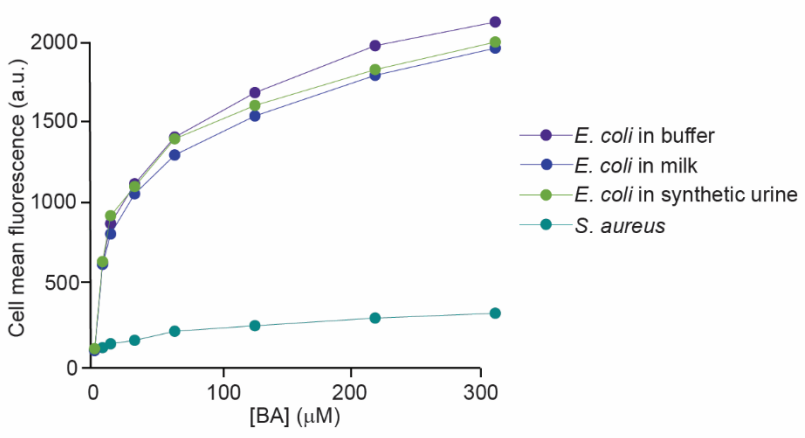


Figure S7. Flow cytometry data from labelling experiment. The concentration of 2-acetylphenylboronic acid was varied for incubation with either *E. coli* in buffer, milk, or synthetic urine, or with *S. aureus* in buffer. Oregon green-modified carbazide was used to report on the degree of labelling. For each measurement, the mean fluorescence intensity is reported for 10,000 cells.

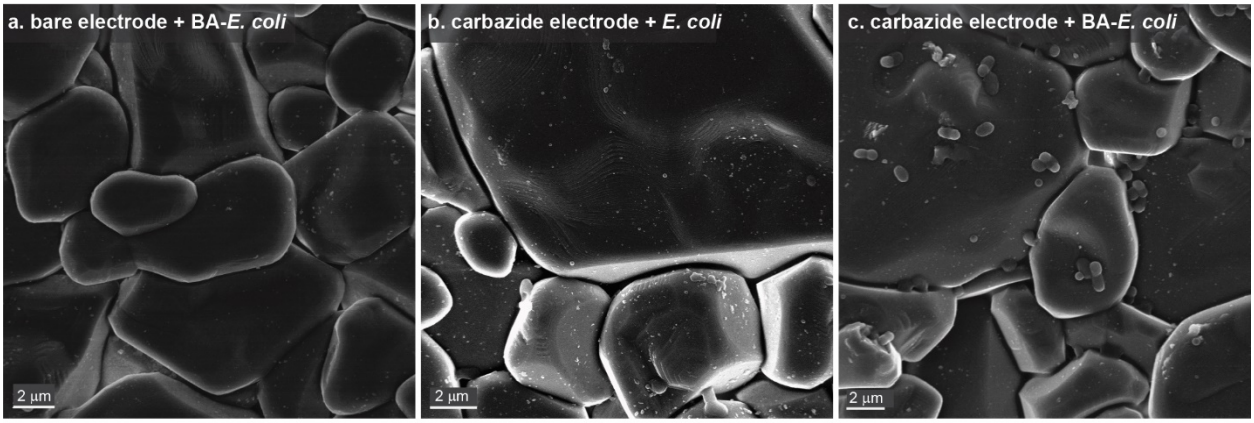


Figure S8. Scanning electron microscopy images of electrodes. (a) Bare gold electrodes without a self-assembled thiol-carbazide monolayer treated with boronic acid-modified *E. coli* (BA-*E. coli*). (b) Electrode modified with a thiol-carbazide self-assembled monolayer treated with unmodified *E. coli*. (c) BA-*E. coli* can be seen on the electrode surface of an electrode modified with a thiol-carbazide self-assembled monolayer.

5. SI References

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