Direct Quantitation of the Quorum Sensing Signal, Autoinducer-2, in Clinically Relevant Samples by Liquid Chromatography – Tandem Mass Spectrometry

Supporting Information

Shawn R. Campagna*, Jessica R. Gooding, and Amanda L. May

Department of Chemistry
The University of Tennessee, Knoxville, Tennessee 37996

Contents

Supporting Information Discussion:

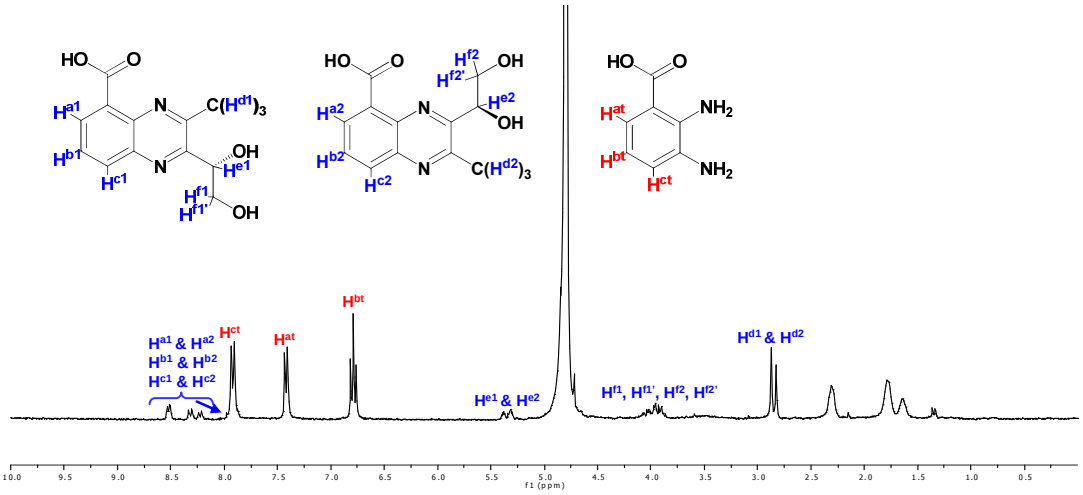
Selection of appropriate SRMs and error correction for internal standard calibration. Selection of the appropriate SRMs for the detection of the **(^{13}C)DPD-M1CQ** is critical for proper quantitation. The fragmentation of the molecule is not significantly affected by the inclusion of ^{13}C isotopes, but care must be taken to insure that the proper reactions are monitored for the internal standard as each reaction has differing efficiencies that could lead to quantitative errors if non-analogous reactions are compared. For **DPD-M1CQ**, the SRMs 381-201, 381-202, 381-231, and 381-363 were the most sensitive. This leads to 7 SRM options for the detection of **(^{13}C)DPD-M1CQ**. If the ^{13}C-methyl group remains in the fragment, the appropriate SRMs are 382-202, 382-203, 382-232, and 382-364. However, if the fragment loses the ^{13}C-methyl, the corresponding SRMs will be 382-201, 382-202*, and 382-231. The 382-364 SRM will remain unchanged as it corresponds to loss of water and cannot lose the ^{13}C -label. Each of the four SRMs for **DPD-M1CQ** and all seven possible SRMs for **(^{13}C)DPD-M1CQ** were monitored in cell-free supernatants to which **(^{13}C)DPD** and tag **7** had been added, and the ratio of intensities for sets of **DPD-M1CQ** and **(^{13}C)DPD-M1CQ** SRMs were then determined. For **DPD-M1CQ** measured from cultures of WT *E. coli*, the ratio of intensities was consistently 22:43:47:100 for SRMs 381-201:381-202:381-231:381-363, respectively (Supporting Information Figure S7, panel A). If the ^{13}C -label is retained in all fragmentation events for **(^{13}C)DPD-M1CQ**, then the ratio of the SRM intensities for 382-202:382-203:382-232:382-364 should be the same as those measured for the corresponding SRMs of **DPD-M1CQ** (Supporting Information Figure S7, panel B). However, if one or more of the fragments lose the ^{13}C -methyl group, then an altered ratio of SRM intensities would be observed (Supporting Information Figure S7, panels D-F). Confirmation of the presence of the ^{13}C-label in the fragments was most critical for SRMs 382-203 and 382-202, which retain the ^{13}C -methyl, and 382-202* and 382-201, which lose the ^{13}C-methyl, as the observation of signal for event 382-202 could be attributed to either of two fragmentation reactions. The measurement of the SRM intensity ratios for **(^{13}C)DPD-M1CQ** from *luxS*$^{-}$ *E. coli* supernatants was 22:43:41:100 for SRMs 382-202:382-203:382-232:382-364, respectively (Supporting Information Figure S7, panel C). This is nearly identical to the ratio measured for analogous SRMs of **DPD-M1CQ**. These data indicate that the methyl group is retained during the monitored fragmentation events.

Of the two widely utilized chemical syntheses of **DPD,**[1, 2] only one allows for the efficient incorporation of an isotopic label into the molecule. This route relies on the methylation of a terminal alkyne with $^{13}CH_3I$ to incorporate the labeled-C1 methyl of **DPD** (Supporting Information Scheme S1).[1] Unfortunately, this synthesis does not facilitate the incorporation of further ^{13}C or D at non-exchangeable positions. The inclusion of only one isotopic label in the internal standard introduces undesirable, although easily managed, complications during analysis. Specifically, two quantitative artifacts are noted, the first resulting from overlap of the M+1 isotope peak of **DPD-M1CQ** with the molecular ion peak of **(^{13}C)DPD-M1CQ** and the second from unavoidable contamination of **(^{13}C)DPD** with ~1% unlabeled material resulting from ^{12}C isotopic impurities in

the $^{13}CH_3I$. The use of SRM techniques diminishes the observed signal from naturally occurring isotopes of **DPD-M1CQ** as the heavy atom must be contained in both the parent and the product ion for signal to be observed. By analyzing samples of **DPD-M1CQ** in water, the intensity of the $[M+1+H]^+$ peak for this molecule was measured to be 14.4% that of the $[M+H]^+$ peak in SRM 381-202 (Supporting Information Table S3, panel A). Each of these artifacts becomes more or less significant at differing ratios of **(^{13}C)DPD-M1CQ** to **DPD-M1CQ** (see Supporting Information Table S4 for further details). The error introduced into the measurement from naturally occurring isotopes of **DPD-M1CQ** is most significant when endogenous [**DPD**] is a 30% or higher that of the **(^{13}C)DPD** internal standard. For measurements in which this was the case, 14.4% of the signal seen for **DPD-M1CQ** was subtracted from the observed signal for **(^{13}C)DPD-M1CQ**. The error from ^{12}C impurity in the **(^{13}C)DPD** is most significant when the concentration of the internal standard is greater than or equal to ten times that of the endogenous **DPD**. For analyses in which this occurred, 0.8% of the signal observed for **(^{13}C)DPD-M1CQ** was subtracted from that measured for **DPD-M1CQ**, as this was the average percentage of unlabeled contaminate measured in the internal standard used for these experiments (Supporting Information Table S3, panel B). For analyses containing observed peak ratios for **DPD-M1CQ** and **(^{13}C)DPD-M1CQ** between 20:100 and 30:100, corrections were not needed as the two sources of error nearly cancel in these cases.

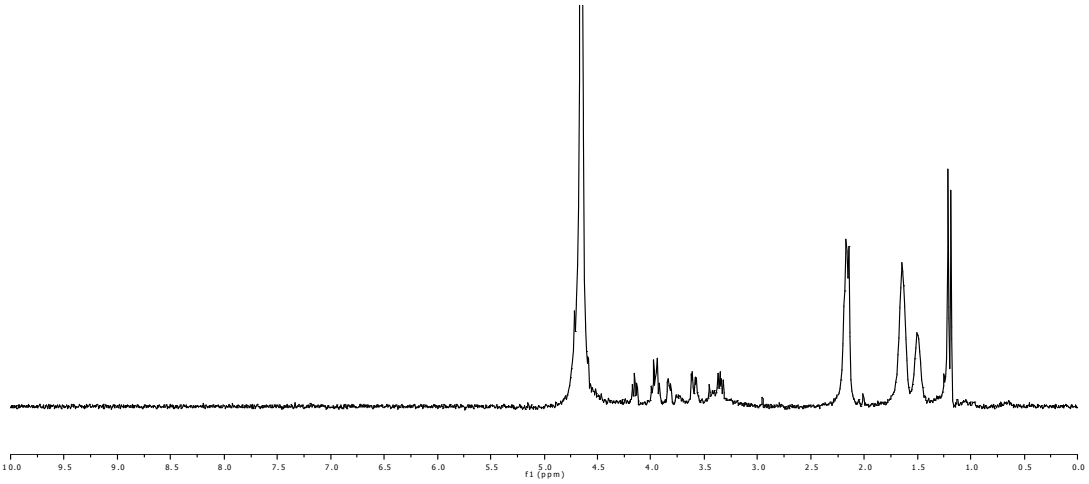
(A)

^{1}H NMR spectrum of **DPD-BAQ** in D_2O (pD = 1.8)



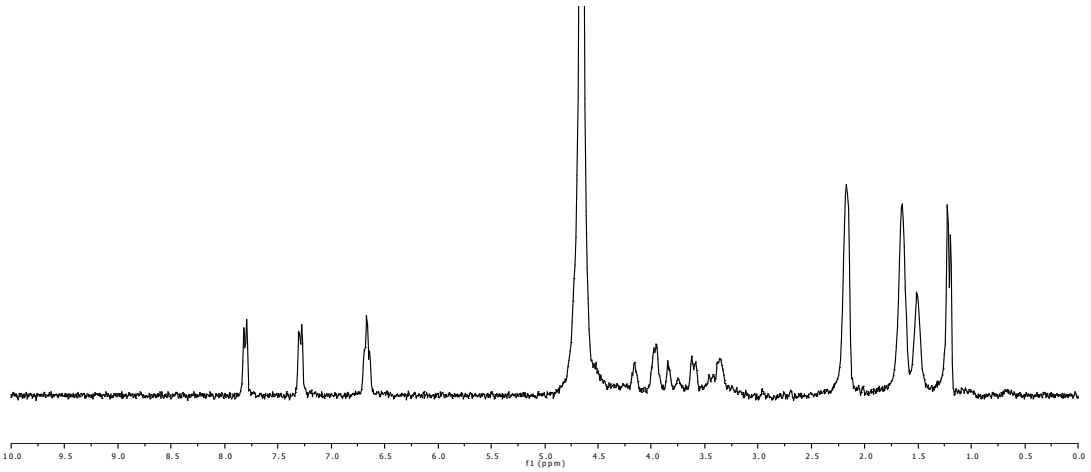
(B)

^{1}H NMR spectrum of **DPD** in D_2O (pD = 1.8)



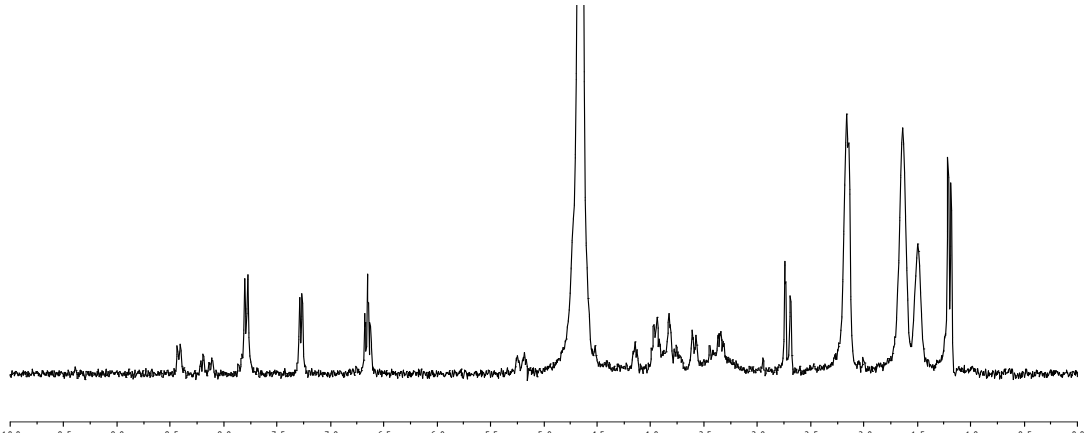
(C)

^{1}H NMR spectrum of **DPD** + 1 eq. **2** in D_2O (pD = 1.8, total t = 0 h)



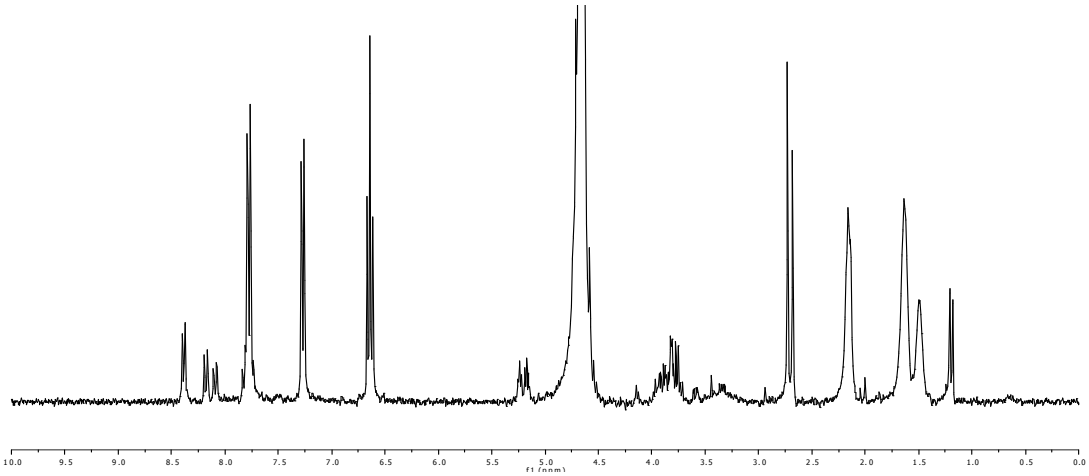
(D)

^{1}H NMR spectrum of **DPD** + 1 eq. **2** added at t = 0 h in D_2O (pD = 1.8, total t = 1 h)



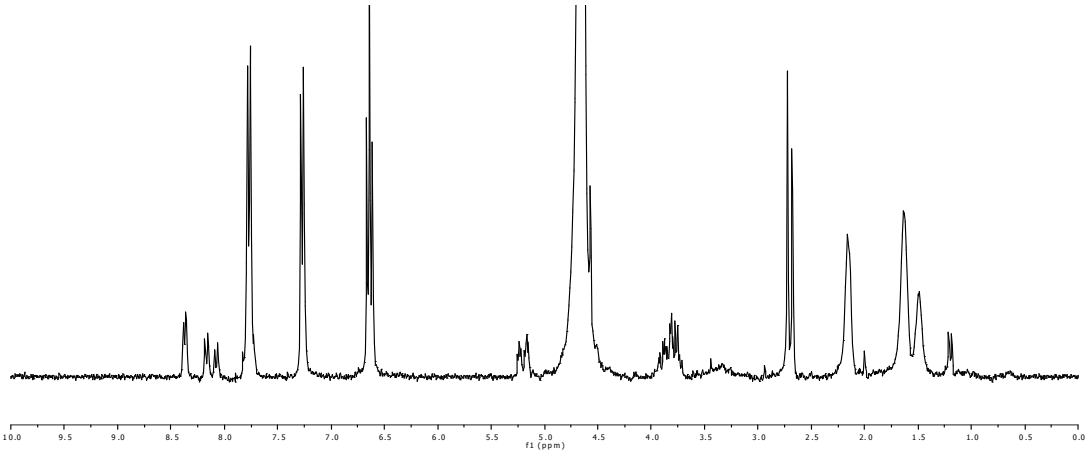
(E)

^{1}H NMR spectrum of **DPD** + 1 eq. **2** added at t = 0 h and 1 eq. **2** added at t = 1 h in D_2O (pD = 1.8, total t = 2 h)



(F)

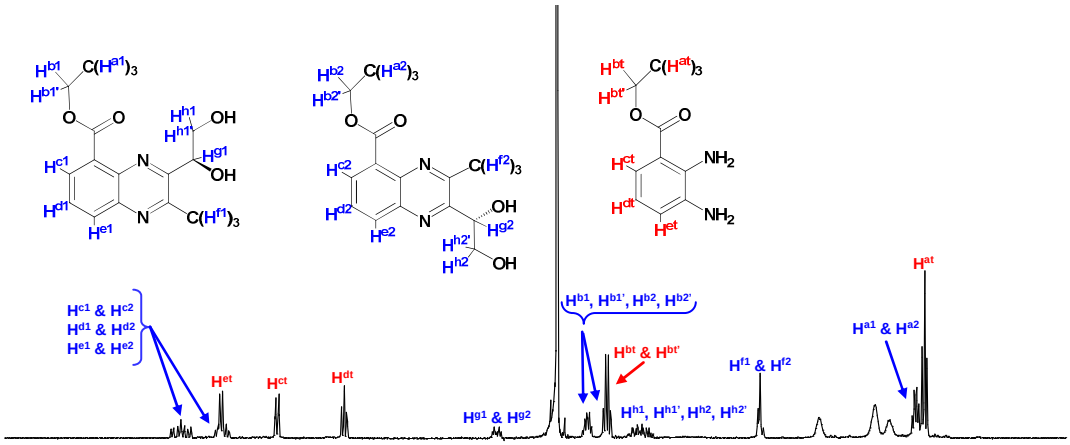
^{1}H NMR spectrum of **DPD** + 1 eq. **2** added at t = 0 h, 1 eq. **2** added at t = 1 h and 1 eq. **2** added at t = 2 h in D_2O (pD = 1.8, total t = 2.5 h)



Supporting Information Figure S1. Monitoring the conversion of **DPD** and **2** to **DPD-BAQ** in D_2O by ^1H NMR. Extraneous peaks in these spectra arise from cyclohexanone present as a byproduct of **DPD** deprotection. (**A**) The ^1H NMR spectrum of 4.7 mM **DPD-BAQ** in D_2O (pD = 1.8); (**B**) The ^1H NMR spectrum of 4.7 mM **DPD** in D_2O (pD = 1.8); (**C**) The ^1H NMR spectrum of 4.7 mM **DPD** and 1 mol eq. **2** in D_2O (pD = 1.8, total t = 0 h); (**D**) The ^1H NMR spectrum of 4.7 mM **DPD** + 1 mol eq. **2** in D_2O (pD = 1.8, total t = 1 h); (**E**) The ^1H NMR spectrum of 4.7 mM **DPD** + 1 mol eq. **2** added at t = 0 h and 1 mol eq. **2** added at t = 1 h in D_2O (pD = 1.8, total t = 2 h); (**F**) The ^1H NMR spectrum of 4.7 mM **DPD** + 1 mol eq. **2** at t = 0 h, 1 mol eq. **2** at t = 1 h and 1 mol eq. **2** at t = 2 h in D_2O (pD = 1.8, total t = 2.5 h).

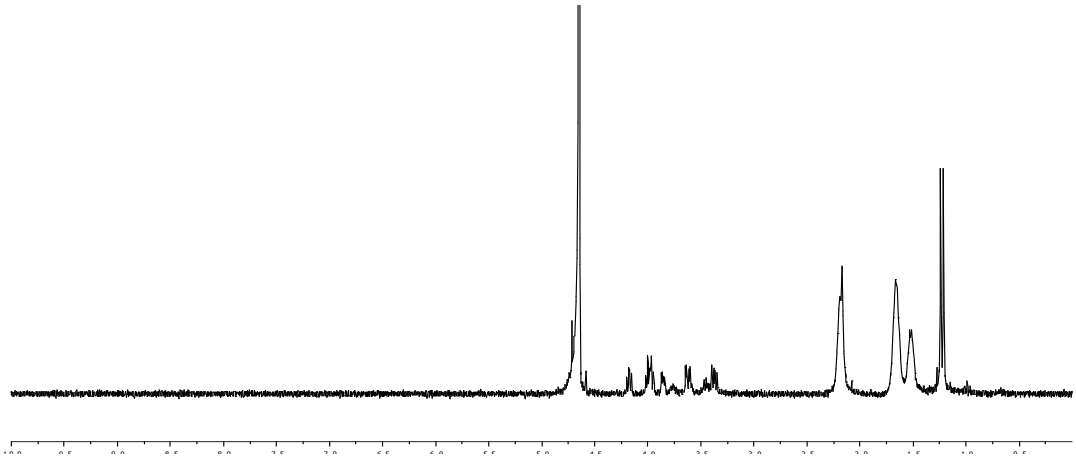
(A)

^{1}H NMR spectrum of **DPD-EBAQ** in D_2O (pD = 1.8)



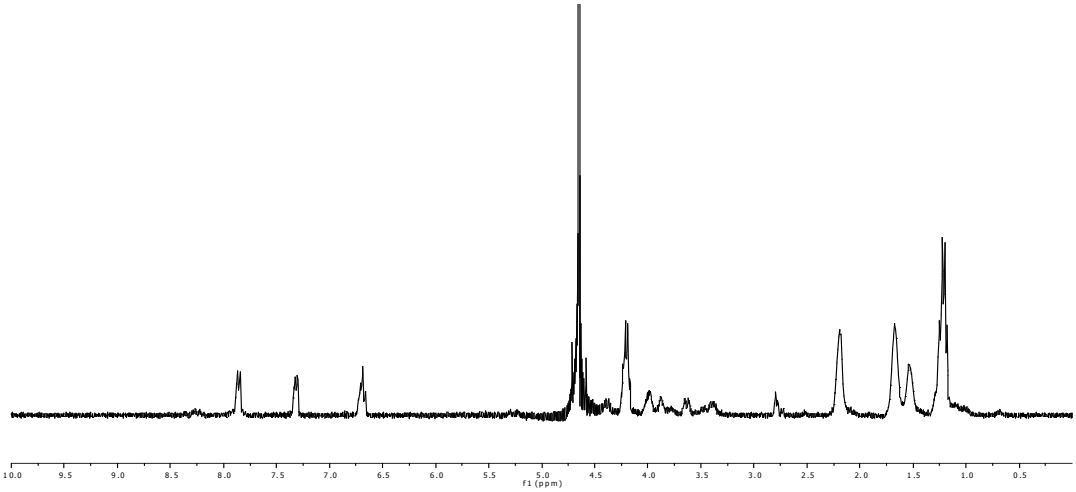
(B)

^{1}H NMR spectrum of **DPD** in D_2O (pD = 1.8)



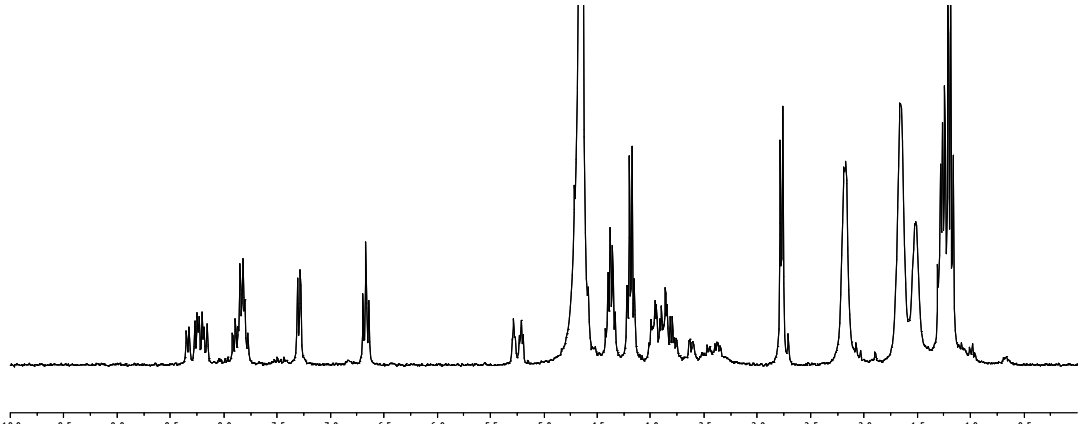
(C)

^{1}H NMR spectrum of **DPD** + 1 eq. **3** in D_2O (pD = 1.8, total t = 0 h)



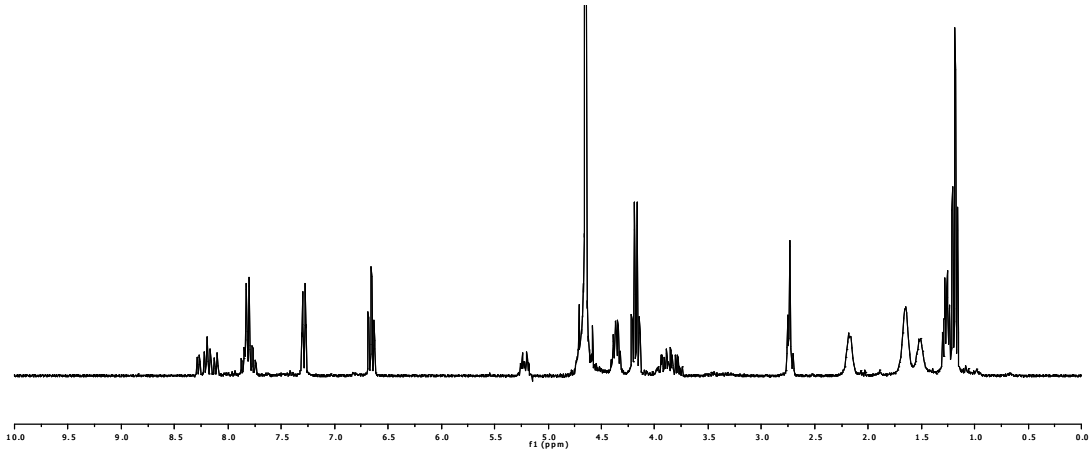
(D)

^{1}H NMR spectrum of **DPD** + 1 eq. **3** added at t = 0 h in D_2O (pD = 1.8, total t = 1 h)



(E)

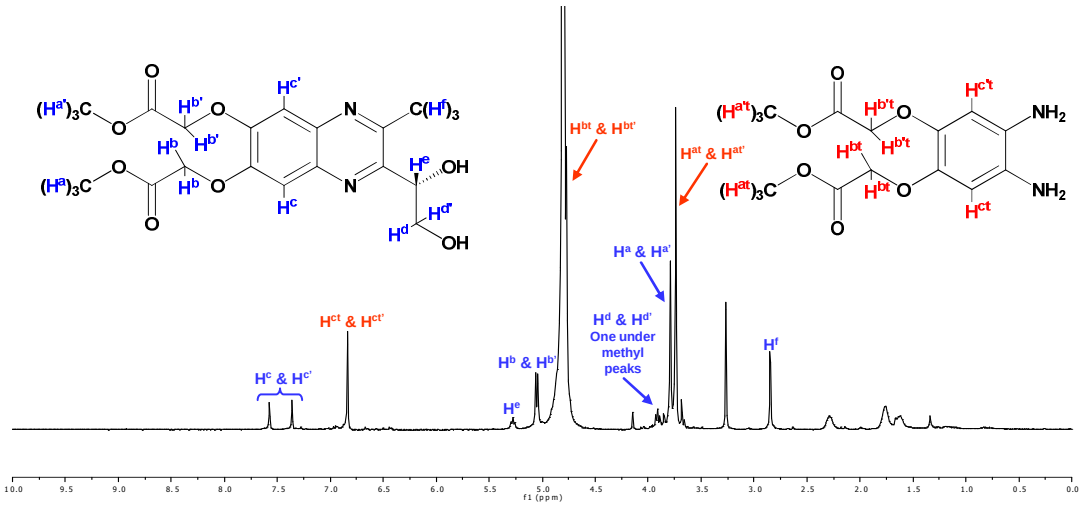
^{1}H NMR spectrum of **DPD** + 1 eq. **3** added at t = 0 h and 1 eq. **3** added at t = 1 h in D_2O (pD = 1.8, total t = 2 h)



Supporting Information Figure S2. Monitoring the conversion of **DPD** and **3** to **DPD-EBAQ** in D_2O by ^{1}H NMR. Extraneous peaks in these specta arise from cyclohexanone present as a byproduct of **DPD** deprotection. (**A**) The ^{1}H NMR spectrum of 4.7 mM **DPD-EBAQ** in D_2O (pD = 1.8); (**B**) The ^{1}H NMR spectrum of 4.7 mM **DPD** in D_2O (pD = 1.8); (**C**) The ^{1}H NMR spectrum of 4.7 mM **DPD** + 1 mol eq. **3** in D_2O (pD = 1.8, total t = 0 h); (**D**) The ^{1}H NMR spectrum of 4.7 mM **DPD** + 1 mol eq. **3** in D_2O (pD = 1.8, total t = 1 h); (**E**) The ^{1}H NMR spectrum of 4.7 mM DPD + 1 mol eq. **3** added at t = 0 h and 1 mol eq. **2** added at t = 1 h in D_2O (pD = 1.8, total t = 2 h).

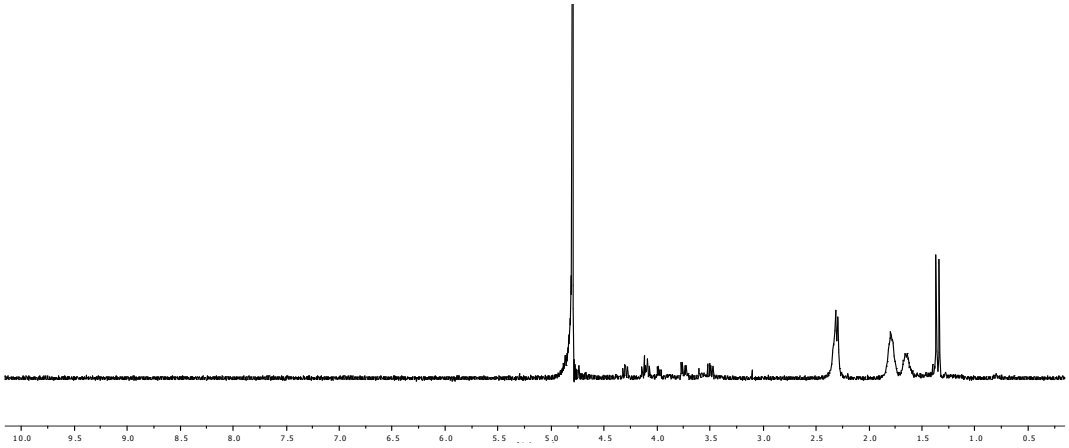
(A)

^{1}H NMR spectrum of **DPD-M1CQ** in D_2O (pD = 1.8)



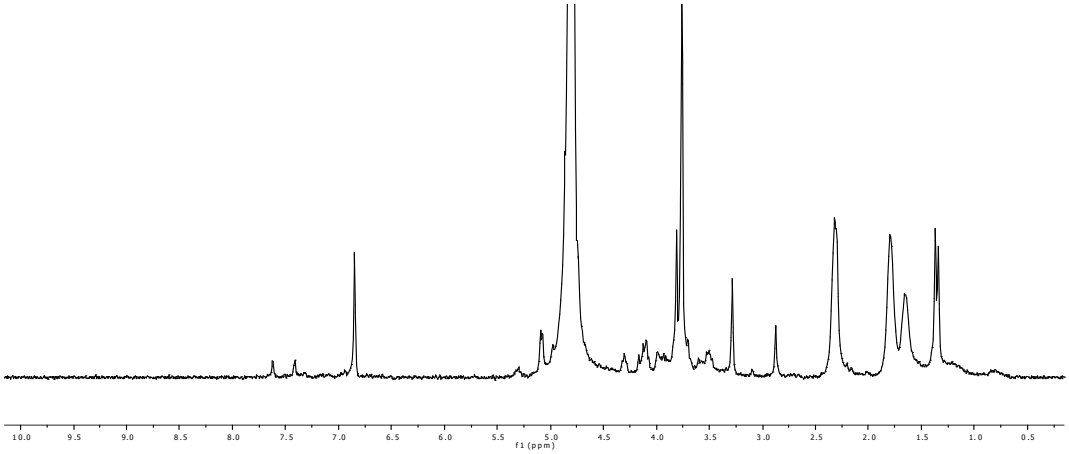
(B)

^{1}H NMR spectrum of **DPD** in D_2O (pD = 1.8)



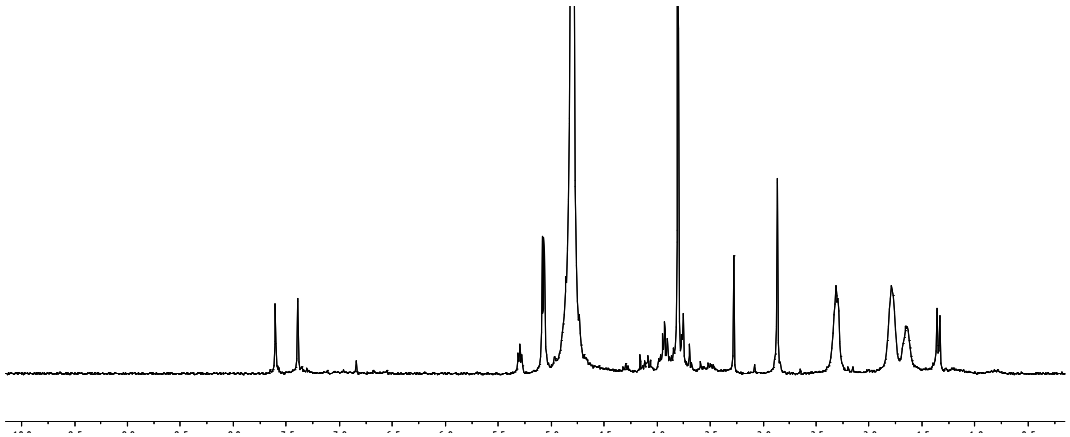
(C)

^{1}H NMR spectrum of **DPD** + 1 eq. **7** in D_2O (pD = 1.8, total t = 0 h)



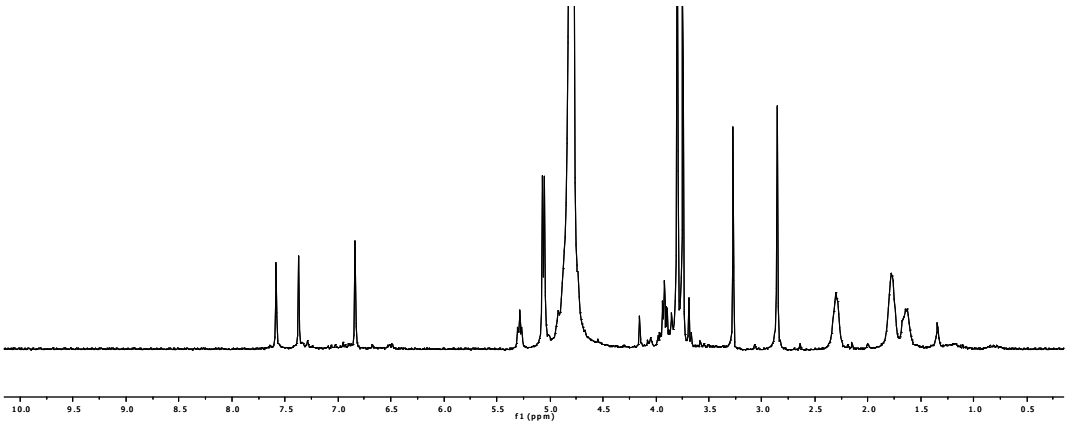
(D)

^{1}H NMR spectrum of **DPD** + 1 eq. **7** added at t = 0 h in D_2O (pD = 1.8, total t = 0.5 h)



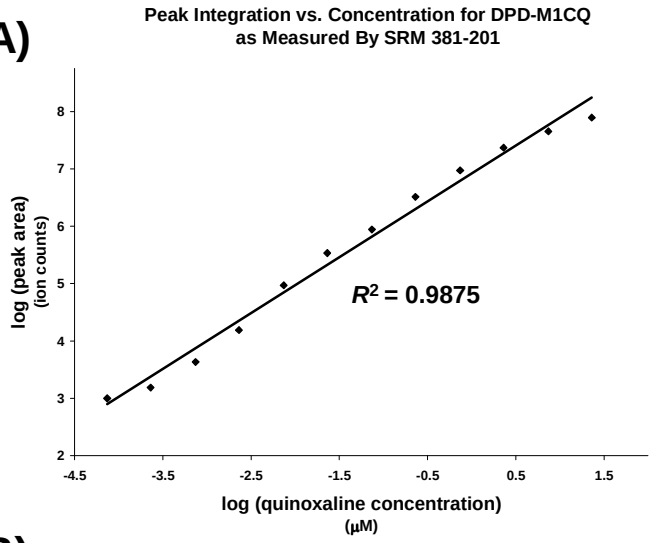
(E)

^{1}H NMR spectrum of **DPD** + 1 eq. **7** added at t = 0 h and 1 eq. **7** added at t = 0.5 h in D_2O (pD = 1.8, total t = 1 h)



Supporting Information Figure S3. Monitoring the conversion of **DPD** and **7** to **DPD-M1CQ** in D_2O by ^{1}H NMR. Extraneous peaks in these spectra arise from cyclohexanone present as a byproduct of **DPD** deprotection. (**A**) The ^{1}H NMR spectrum of 4.7 mM **DPD-M1CQ** in D_2O (pD = 1.8); (**B**) The ^{1}H NMR spectrum of 4.7 mM **DPD** in D_2O (pD = 1.8); (**C**) The ^{1}H NMR spectrum of 4.7 mM **DPD** + 1 mol eq. **7** in D_2O (pD = 1.8, total t = 0 h); (**D**) The ^{1}H NMR spectrum of 4.7 mM **DPD** + 1 mol eq. **7** in D_2O (pD = 1.8, total t = 0.5 h); (**E**) The ^{1}H NMR spectrum of 4.7 mM **DPD** + 1 mol eq. **7** added at t = 0 h and 1 mol eq. **7** added at t = 0.5 h in D_2O (pD = 1.8, total t = 1 h).

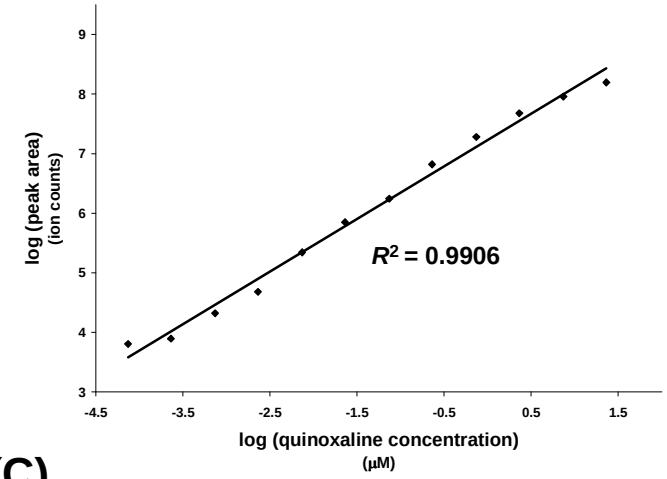
(A)



Data Used to Construct Calibration Curve for SRM 381-201

[DPD-M1CQ] (μM)	Intensity (Ion Counts)	Log of [DPD-M1CQ]	Log of Intensity
7.43×10^{-5}	1.00×10^{3}	-4.13	3.00
2.35×10^{-4}	1.54×10^{3}	-3.64	3.19
7.43×10^{-4}	4.31×10^{3}	-3.13	3.63
2.35×10^{-3}	1.55×10^{4}	-2.64	4.19
7.43×10^{-3}	9.36×10^{4}	-2.13	4.97
2.35×10^{-2}	3.42×10^{5}	-1.64	5.53
7.43×10^{-2}	8.73×10^{5}	-1.13	5.94
2.35×10^{-1}	3.27×10^{6}	-0.64	6.51
7.43×10^{-1}	9.43×10^{6}	-0.13	6.97
2.35	2.34×10^{7}	0.36	7.37
7.43	4.50×10^{7}	0.87	7.65
2.35×10^{1}	7.82×10^{7}	1.36	7.89

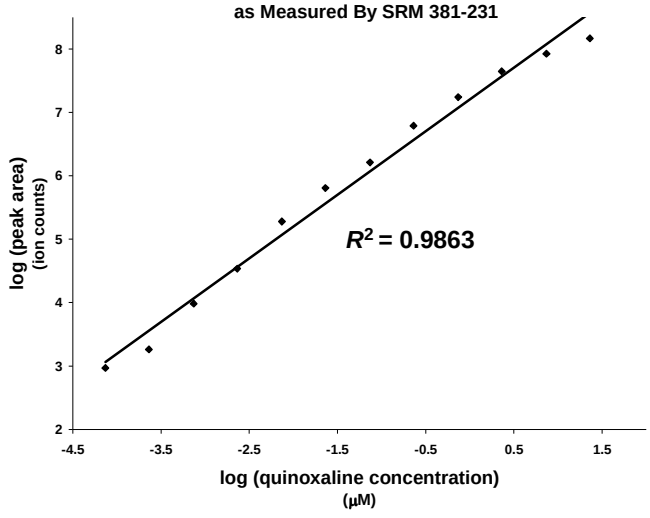
(B)



Data Used to Construct Calibration Curve for SRM 381-202

[DPD-M1CQ] (μM)	Intensity (Ion Counts)	Log of [DPD-M1CQ]	Log of Intensity
7.43×10^{-5}	6.41×10^{3}	-4.13	3.81
2.35×10^{-4}	7.84×10^{3}	-3.64	3.89
7.43×10^{-4}	2.09×10^{4}	-3.13	4.32
2.35×10^{-3}	4.78×10^{4}	-2.64	4.68
7.43×10^{-3}	2.20×10^{5}	-2.13	5.34
2.35×10^{-2}	7.07×10^{5}	-1.64	5.85
7.43×10^{-2}	1.76×10^{6}	-1.13	6.25
2.35×10^{-1}	6.60×10^{6}	-0.64	6.82
7.43×10^{-1}	1.91×10^{7}	-0.13	7.28
2.35	4.77×10^{7}	0.36	7.68
7.43	9.07×10^{7}	0.87	7.96
2.35×10^{1}	1.57×10^{8}	1.36	8.20

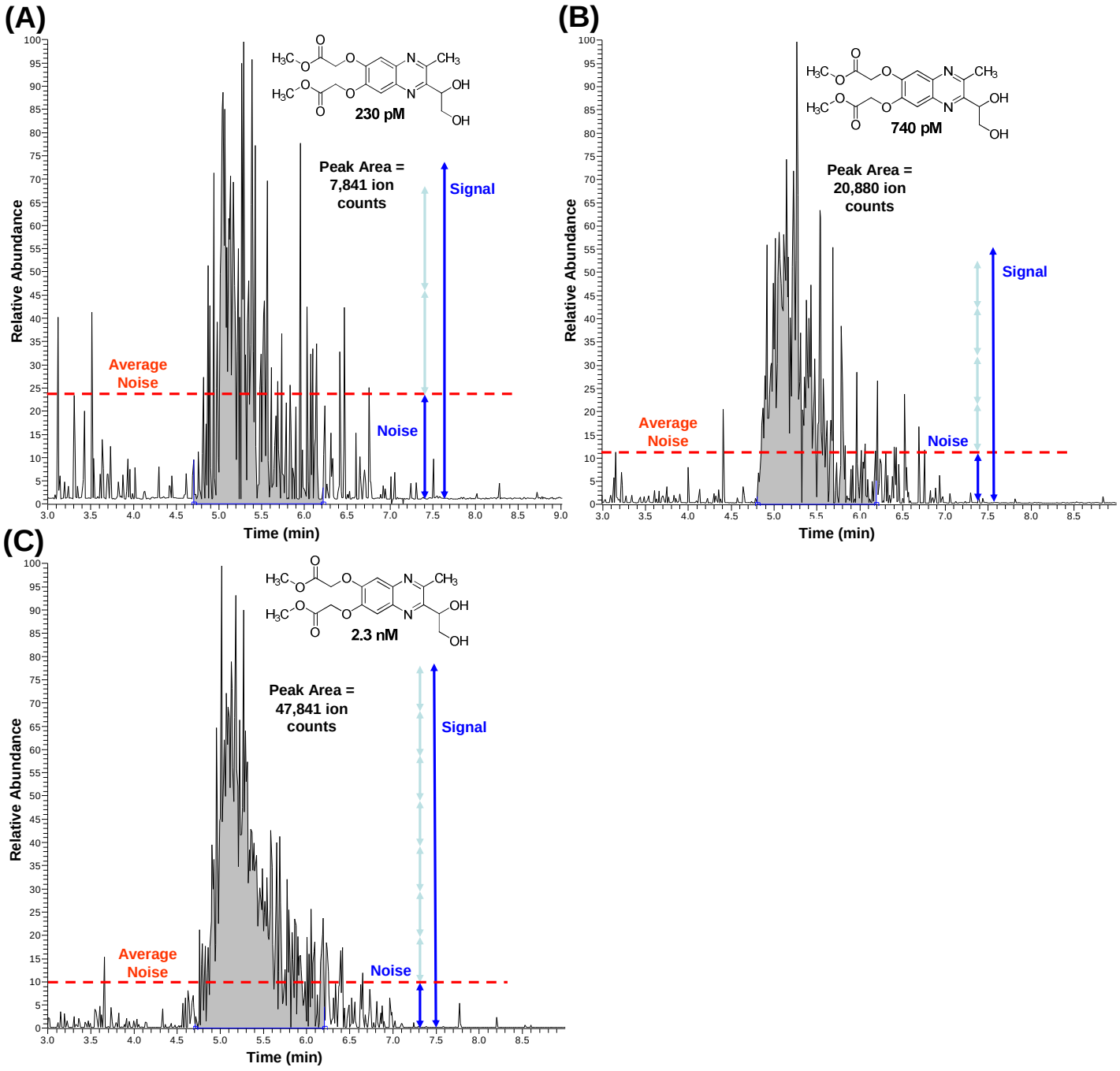
(C)



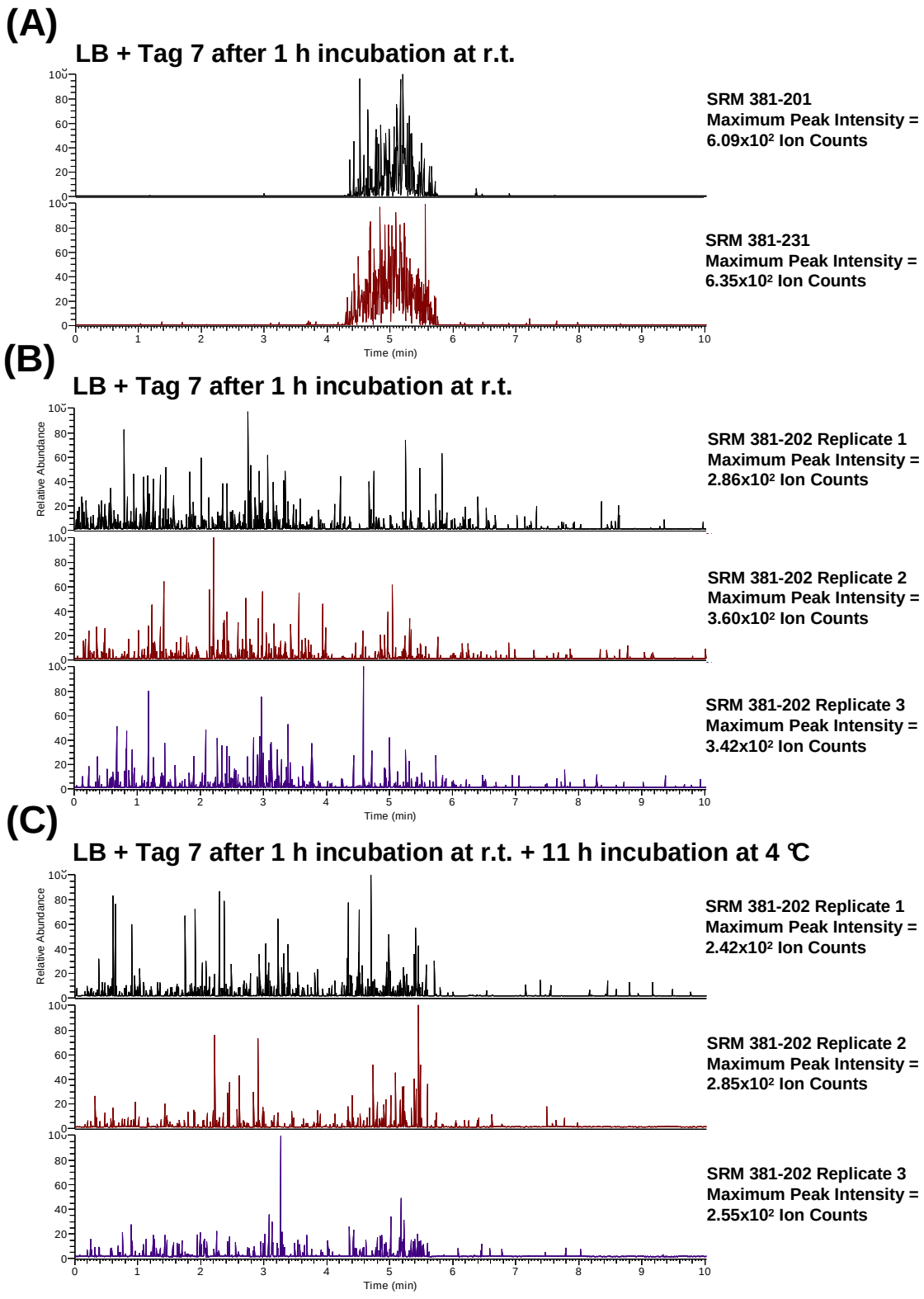
Data Used to Construct Calibration Curve for SRM 381-231

[DPD-M1CQ] (μM)	Intensity (Ion Counts)	Log of [DPD-M1CQ]	Log of Intensity
7.43×10^{-5}	9.29×10^{2}	-4.13	2.97
2.35×10^{-4}	1.82×10^{3}	-3.64	3.26
7.43×10^{-4}	9.62×10^{3}	-3.13	3.98
2.35×10^{-3}	3.41×10^{4}	-2.64	4.53
7.43×10^{-3}	1.90×10^{5}	-2.13	5.28
2.35×10^{-2}	6.41×10^{5}	-1.64	5.81
7.43×10^{-2}	1.63×10^{6}	-1.13	6.21
2.35×10^{-1}	6.14×10^{6}	-0.64	6.79
7.43×10^{-1}	1.75×10^{7}	-0.13	7.24
2.35	4.44×10^{7}	0.36	7.65
7.43	8.43×10^{7}	0.87	7.93
2.35×10^{1}	1.47×10^{8}	1.36	8.17

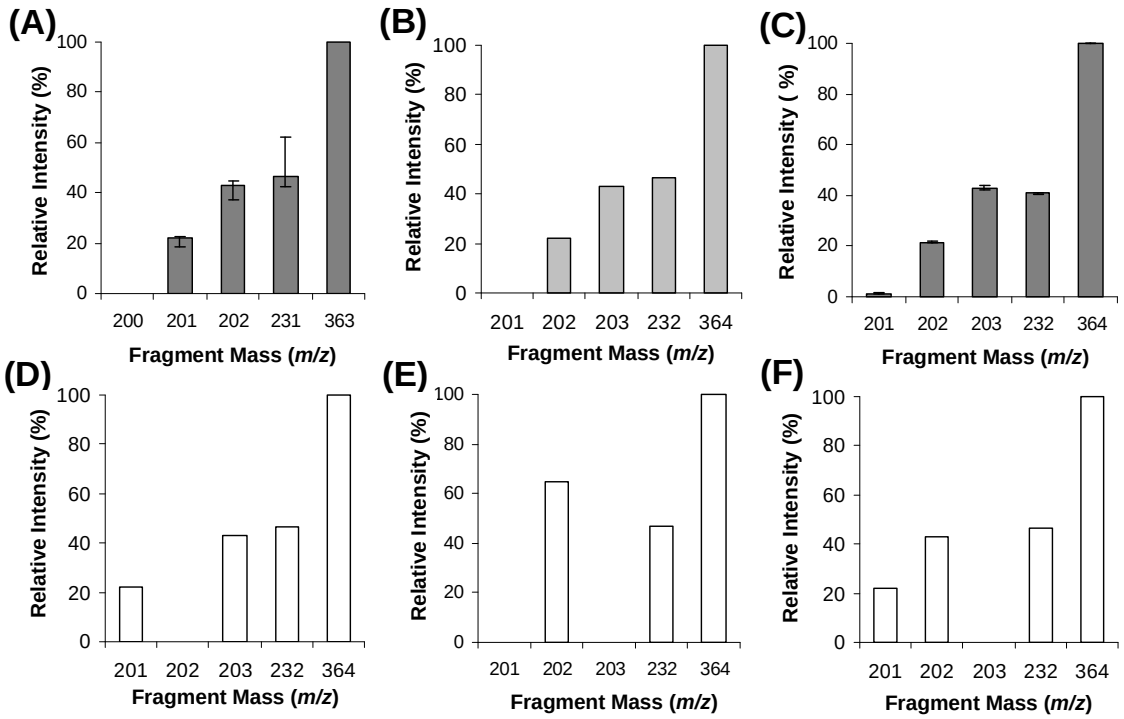
Supporting Information Figure S4. Calibration curves to determine linearity of **DPD-M1CQ** signal in water. Each plot is linear across the range of measured [**DPD-M1CQ**], with the possible exception of the point corresponding to the measurement at the lowest concentration. (**A**) Plot constructed from signal monitored by SRM 381-201. (**B**) Plot constructed from signal monitored by SRM 381-202. (**C**) Plot constructed from signal monitored by SRM 381-231. R^2 values were calculated using the data analysis tool pack in Excel and include all data displayed on each graph.



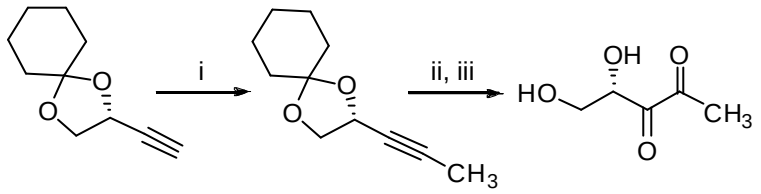
Supporting Information Figure S5. Chromatograms for **DPD-M1CQ** in water used to determine LOD. The approximate S/N for each is graphically displayed by stacking of double headed bars equal in height to the average noise next to a double-headed bar representing the maximum average signal. (**A**) Peak observed by monitoring SRM 381-202 with **[DPD-M1CQ**] = 230 pM. The approximate S/N for this plot is 3:1. (**B**) Peak observed by monitoring SRM 381-202 with **[DPD-M1CQ**] = 740 pM. The approximate S/N for this plot is 5:1. (**C**) Peak observed by monitoring SRM 381-202 with **[DPD-M1CQ**] = 2.3 nM. The approximate S/N for this plot is 8:1. Note, the data used to generate these mass chromatograms were also used to determine the linear range as outlined in Supporting Information Figure S4B. The peak areas were determined by manually identifying the beginning of each peak and then integrating over 1.5 min.



Supporting Information Figure S6. Test for unwanted reactivity of tag 7 with media. Chromatograms of LB to which 1.3 mM tag **7** had been added and allowed to react. (**A**) Signal of SRM 381-201 and 381-231 from unwanted reactivity of tag **7** with carbohydrates present in LB after 1 h incubation at room temperature. (**B**) Three replicate samples showing that SRM 381-202 does not have signal attributable to unwanted reactivity with LB matrix components after 1 h incubation at room temperature. (**C**) Further incubation for 11 h at 4 ℃ also does not lead to unwanted reactivity.



Supporting Information Figure S7. Ratios of SRM intensities for **DPD-M1CQ** and **(^{13}C)DPD-M1CQ**. The intensity of either SRM 381-363 or 382-364 was normalized to 100% for each sample, and the signal for the other SRMs are reported as a percent of the relative intensity of that peak. (**A**) Measured ratio of the intensities for the most sensitive SRMs used for **DPD-M1CQ** concentration determination. (**B**) Expected ratio of intensities for the SRMs needed to detect **(^{13}C)DPD-M1CQ** if the ^{13}C-methyl remains in the fragment. (**C**) Measured ratio of the SRM intensities found during **(^{13}C)DPD-M1CQ** concentration determination. (**D**) Expected ratio of intensities for **(^{13}C)DPD-M1CQ** SRMs if fragment 202 has lost the ^{13}C-label. (**E**) Expected ratio of intensities for **(^{13}C)DPD-M1CQ** SRMs if fragment 203 has lost the ^{13}C-label. (**F**) Expected ratio of intensities for **(^{13}C)DPD-M1CQ** SRMs if both fragment 202 and 203 have lost the ^{13}C-label.



Supporting Information Scheme S1. Chemical synthesis of **DPD**. Reagents and conditions (i) nBuLi, THF, -78 ℃, 30 min, then CH_3I, -78 ℃ to r.t., 4 h; (ii) $NaIO_4$, RuO_2, CCl_4, MeCN, H_2O, 15 min; (iii) H_2SO_4, H_2O, 3 h.

Most Sensitive SRMs for Each Quinoxaline

Quinoxaline	Parent Ion (*m/z*)	Product Ion (*m/z*)	Collision Energy (V)
DPD-BAQ	249	144	35
		173	27
		201	24
		231	17
DPD-EBAQ	277	144	36
		173	29
		201	25
		231	17
DPD-M1CQ	381	201	36
		202	43
		231	29
		363	17
DPD-1CQ	353	175	31
		202	42
		231	30
		335	18
DPD-2CQ	381	272	23
		291	31
		291	24
		363	14
DPD-3CQ	409	190	49
		219	28
		237	25
		305	20

Supporting Information Table S1. Fragment masses and collision energies for the SRMs of each quinoxaline were automatically determined on a Thermo Discovery Max triple quadrupole MS using the automated compound detection and SRM determination parameters of the Thermo Quantum Tune software. Each compound was introduced into the MS at concentrations ranging from 23 μM - 2.3 mM using direct infusion via a syringe pump at 20 μl/min. The selectivity of the technique relies on the proper choice of parent mass ─ fragment mass pairs, and the frequency of reactions leading to these transitions are maximized by proper choice of collision energy.

(A)

Measurement of [DPD] using 10 μM (^{13}C)DPD-M1CQ as Internal Standard

	Intensity for DPD-M1CQ (ion counts)	Intensity for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)
Replicate 1	5.08 x10^5	1.00 x10^6	5.48
Replicate 2	2.97 x10^5	5.71 x10^5	5.63

(B)

Measurement of [DPD] using 10 μM (^{13}C)DPD as Internal Standard

	Intensity for DPD-M1CQ (ion counts)	Intensity for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)
Replicate 1	1.30 x10^6	1.22 x10^6	12.46
Replicate 2	9.04 x10^5	8.74 x10^5	12.12

Supporting Information Table S2. Assessment of the utility of **(^{13}C)DPD-M1CQ** and **(^{13}C)DPD** as internal standards for the quantitation of DPD concentrations in cell-free supernatants. A culture of *E. coli* was grown for 9h in LB, and aliquots (300 μL) were then collected and mixed with either **(^{13}C)DPD-M1CQ** or **(^{13}C)DPD** (341 μM in 10 μL H_2O). After centrifugation to remove cells and other particulates, a portion of the supernatants (200 μL) were mixed with a solution of tag **7** (14 μM in 20 μL H_2O) and incubated 1 h at ambient temperature. (**A**) Data collected for the measurement of [**DPD**] utilizing pre-formed **(^{13}C)DPD-M1CQ** as the internal calibrant. The average measured [**DPD**] from these experiments was 5.56 ±0.08 μM. (**B**) Data collected for the measurement of [**DPD**] utilizing pre-formed **(^{13}C)DPD** as the internal calibrant. The average [**DPD**] measured during these experiments was 12.29 ±0.17 μM. The observation that the [**DPD**] concentration is lower when calculated via comparison with **(^{13}C)DPD-M1CQ** via comparison with **(^{13}C)DPD** indicates that either the molecule of interest and the internal standard partition into the pelleted biomass at different efficiencies or that the reaction to form **DPD-M1CQ** fails to reach completion in media.

(A)

Measurement of the percentage of naturally occurring isotopes for DPD-M1CQ

Concentration DPD-M1CQ (μM)	Signal for $[M+H]^+$ SRM 381-201 (ion counts)	Signal for $[M+1+H]^+$ via SRM 382-202 (ion counts)	Percent naturally occurring M+1 isotopes of DPD-M1CQ
0.074	1.78×10^6	2.59×10^5	14.57
0.235	6.64×10^6	9.62×10^5	14.48
0.740	1.93×10^7	2.75×10^6	14.24
2.35	4.79×10^7	6.76×10^6	14.10
7.40	9.23×10^7	1.32×10^7	14.35
23.5	1.61×10^8	2.33×10^7	14.49
		Average	14.37

(B)

Measurement of the percentage of ^{12}C contaminate in [^{13}C]DPD-M1CQ

Data Collected from	Signal from DPD-M1CQ SRM 381-201 (ion counts)	Signal for [^{13}C]DPD-M1CQ SRM 382-202 (ion counts)	% isotopic error
luxS⁻ E. coli	9.64×10^3	1.16×10^6	0.83
luxS⁻ E. coli	7.27×10^3	1.02×10^6	0.72
luxS⁻ E. coli	7.12×10^3	1.02×10^6	0.70
luxS⁻ E. coli	6.26×10^3	1.02×10^6	0.61
luxS⁻ E. coli	3.61×10^3	4.70×10^5	0.77
luxS⁻ E. coli	1.48×10^4	1.58×10^6	0.94
		Average	0.76

Supporting Information Table S3. Measurement of isotopic contaminants. (**A**) During detection of **DPD-M1CQ**, some signal is seen at the same parent and fragment *m/z* expected for **(^{13}C)DPD-M1CQ**. This signal is mainly derived from the unavoidable presence of **(^{15}N)DPD-M1CQ** and **(^{13}C)DPD-M1CQ** resulting from naturally occurring isotopes in both tag **7** and **DPD**. The intensity for the peak observed by SRM 382-203 is routinely measured to be 14.4% that of the intensity seen for the molecular ion of **DPD-M1CQ** as monitored via SRM 381-202. The data used in this table were collected from the samples of **DPD-M1CQ** in water used to determine the LOD for this molecule. (**B**) When **(^{13}C)DPD** is used as the internal standard, a small [**DPD**] is introduced into the sample. This is unavoidable since the $^{13}CH_3I$ used to synthesize the isotope labeled internal standard contains ~1% ^{12}C contaminant. As the percent contaminant is different for each batch of $^{13}CH_3I$, measurement of the percent unlabeled **DPD** is measured frequently. Usually, this occurs by careful examination of SRM 381-202 and 382-203 during analysis of bacterial strains that do not produce **DPD** as these data are routinely collected. For experiments where this is not possible, such as for the measurement of the [**DPD**] in saliva, care is taken to insure the use of a batch of **(^{13}C)DPD** for which the percent ^{12}C contaminant has been measured.

(A)

Prediction of Error in Measuring (^{13}C)DPD-M1CQ Arising from Naturally Occurring Isotopes of DPD-M1CQ

Ratio of Observed SRM 381-202 Intensity	to	SRM 382-203 Intensity	Actual SRM 382-203 Intensity	% Over Estimation SRM 382-203 Intensity
0.9	:	100	99.87	0.13
1	:	100	99.86	0.14
2	:	100	99.71	0.29
3	:	100	99.57	0.43
4	:	100	99.42	0.58
5	:	100	99.28	0.73
6	:	100	99.14	0.87
7	:	100	98.99	1.02
8	:	100	98.85	1.17
9	:	100	98.70	1.31
10	:	100	98.56	1.46
20	:	100	97.12	2.97
30	:	100	95.68	4.52
40	**:**	**100**	**94.24**	**6.11**
50	**:**	**100**	**92.80**	**7.76**
60	**:**	**100**	**91.36**	**9.46**
70	**:**	**100**	**89.92**	**11.21**
80	**:**	**100**	**88.48**	**13.02**
90	**:**	**100**	**87.04**	**14.89**
100	**:**	**100**	**85.60**	**16.82**
110	**:**	**100**	**84.16**	**18.82**
120	**:**	**100**	**82.72**	**20.89**
130	**:**	**100**	**81.28**	**23.03**
140	**:**	**100**	**79.84**	**25.25**
150	**:**	**100**	**78.40**	**27.55**
160	**:**	**100**	**76.96**	**29.94**
170	**:**	**100**	**75.52**	**32.42**
180	**:**	**100**	**74.08**	**34.99**
190	**:**	**100**	**72.64**	**37.67**
200	**:**	**100**	**71.20**	**40.45**
210	**:**	**100**	**69.76**	**43.35**
220	**:**	**100**	**68.32**	**46.37**
230	**:**	**100**	**66.88**	**49.52**
240	**:**	**100**	**65.44**	**52.81**
250	**:**	**100**	**64.00**	**56.25**
260	**:**	**100**	**62.56**	**59.85**
270	**:**	**100**	**61.12**	**63.61**
280	**:**	**100**	**59.68**	**67.56**
290	**:**	**100**	**58.24**	**71.70**
300	**:**	**100**	**56.80**	**76.06**

(B)

Prediction of Error in Measuring DPD-M1CQ Arising from ^{12}C contaminants in (^{13}C)DPD-M1CQ

Ratio of Observed SRM 381-202 Intensity	to	SRM 382-203 Intensity	Actual SRM 381-202 Intensity	% Over Estimation SRM 381-202 Intensity
0.9	**:**	**100**	**0.10**	**800.00**
1	**:**	**100**	**0.20**	**400.00**
2	**:**	**100**	**1.20**	**66.67**
3	**:**	**100**	**2.20**	**36.36**
4	**:**	**100**	**3.20**	**25.00**
5	**:**	**100**	**4.20**	**19.05**
6	**:**	**100**	**5.20**	**15.38**
7	**:**	**100**	**6.20**	**12.90**
8	**:**	**100**	**7.20**	**11.11**
9	**:**	**100**	**8.20**	**9.76**
10	**:**	**100**	**9.20**	**8.70**
20	:	100	19.20	4.17
30	:	100	29.20	2.74
40	:	100	39.20	2.04
50	:	100	49.20	1.63
60	:	100	59.20	1.35
70	:	100	69.20	1.16
80	:	100	79.20	1.01
90	:	100	89.20	0.90
100	:	100	99.20	0.81
110	:	100	109.20	0.73
120	:	100	119.20	0.67
130	:	100	129.20	0.62
140	:	100	139.20	0.57
150	:	100	149.20	0.54
160	:	100	159.20	0.50
170	:	100	169.20	0.47
180	:	100	179.20	0.45
190	:	100	189.20	0.42
200	:	100	199.20	0.40
210	:	100	209.20	0.38
220	:	100	219.20	0.36
230	:	100	229.20	0.35
240	:	100	239.20	0.33
250	:	100	249.20	0.32
260	:	100	259.20	0.31
270	:	100	269.20	0.30
280	:	100	279.20	0.29
290	:	100	289.20	0.28
300	:	100	299.20	0.27

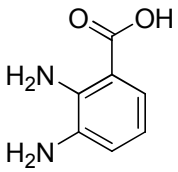
Supporting Information Table S4. Isotopes of **DPD-M1CQ** and **(^{13}C)DPD-M1CQ** are detected as signal in the SRMs used for detection of the other molecule. (**A**) Naturally occurring isotopes of **DPD-M1CQ** lead to the observation of extra signal in SRM 382-203 used to detect **(^{13}C)DPD-M1CQ**. The error from this is most significant when the [**DPD**] concentration is 20% or higher than that of the internal standard. The percent by which this overestimates the signal arising from **(^{13}C)DPD** is shown in the table. The bold text indicates relative concentrations of **DPD** to **(^{13}C)DPD** for which the data were corrected by subtraction of 14.4% of the signal of SRM 381-202 from SRM 382-203. (**B**) The unavoidable presence of ~1% ^{12}C isotope in the **(^{13}C)DPD** internal standards leads to extra intensity in SRM 381-202 used to detect **DPD-M1CQ**. The error from this is most significant when the [**DPD**] is lower than that of the internal standard. The percent by which this over estimates the actual [**DPD**] is shown in the above table, and the bold text indicates relative concentrations relative concentrations of **DPD** to **(^{13}C)DPD** for which the data were corrected by subtraction of 0.8% of the signal of SRM 382-203 from SRM 381-202.

Determination of Measurement Variability via Triplicate Injections of Two Samples

Sample Source: WT *V. harveyi* grown for 8 h in LM	DPD-M1CQ Intensity, SRM 381-202 (ion counts)	(^{13}C)DPD-M1CQ Intensity, SRM 382-203 (ion counts)	[DPD] (μM)
Replicate 1	1.89×10^6	1.28×10^6	18.71
Replicate 2	1.06×10^6	7.08×10^5	19.19
Replicate 3	5.31×10^5	3.65×10^5	18.41
		Average = 18.77 μM	**Range = 0.78 μM**

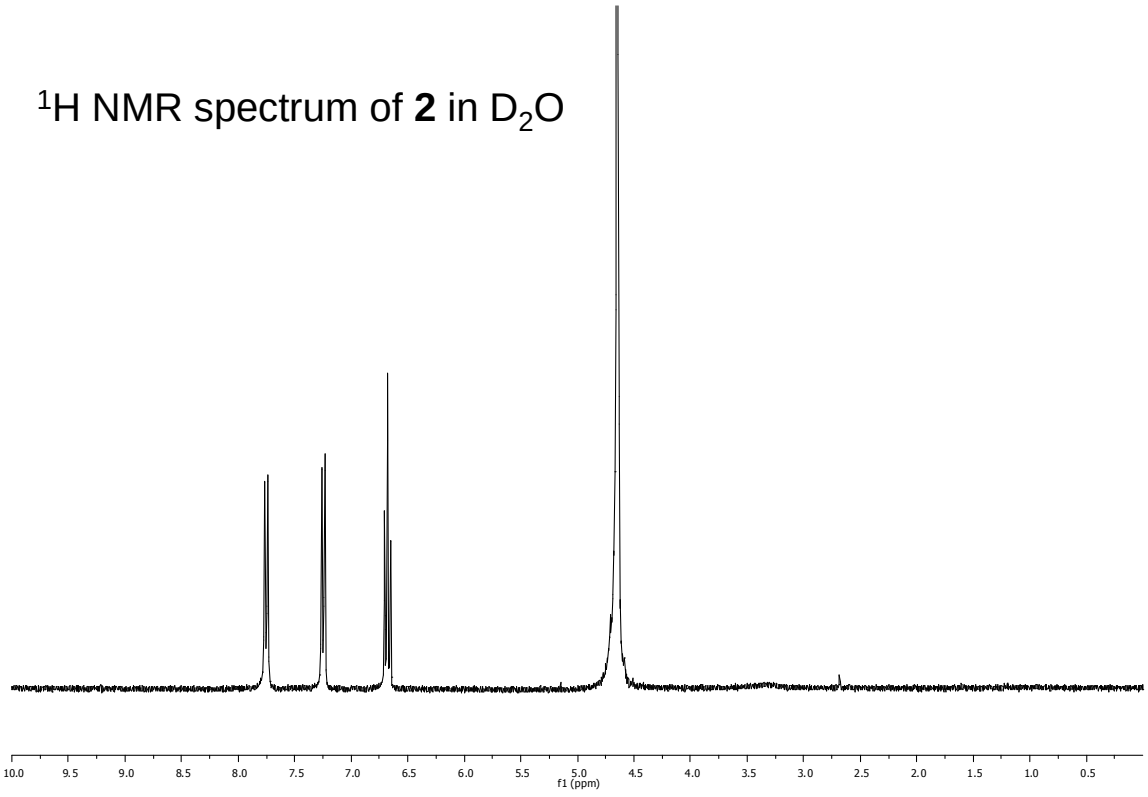
Sample Source: WT *V. harveyi* grown for 12 h in LM	DPD-M1CQ Intensity, SRM 381-202 (ion counts)	(^{13}C)DPD-M1CQ Intensity, SRM 382-203 (ion counts)	[DPD] (μM)
Replicate 1	5.83×10^5	8.91×10^5	7.23
Replicate 2	1.91×10^5	2.87×10^5	7.38
Replicate 3	1.67×10^5	2.59×10^5	7.12
		Average = 18.77 μM	**Range = 0.78 μM**

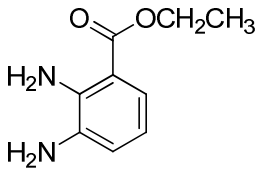
Supporting Information Table S5. Measurement of the error for multiple LC-MS/MS analyses of a single sample. The [**DPD**] from two of the time points of an experiment utilizing *V. harveyi* BB120 was determined via three separate injections of the same sample into the LC-MS. The average [**DPD**] for the three injections as well as the range is shown above for both samples. From these data, it was determined that the measurement variability in the LC-MS/MS analysis was ~4% for measurements in the μM range.



2

2,3-Diaminobenzoic acid, 2. The known compound **2** was synthesized following a previous procedure[3] with only a slight modification to the solvent used. A solution of 2-amino-3-nitro-benzoic acid (0.9609 g, 5.28 mmol) in ethanol (56 mL) was hydrogenated in a PARR apparatus using 5% Pd/C (0.7011 g, 6.59 mmol) catalyst at 35 bar H_2 for 1.5 h. The reaction was then filtered and concentrated *in vacuo* to give **2** as an impure solid which was then redissolved in water (30 mL) and acidified to pH 2 using concentrated HCl. The resulting mixture was filtered through celite, and the filtrate was concentrated *in vacuo* to give pure **2** as a red solid. ^{1}H NMR (300 MHz, D_2O) δ 7.75 (d, J = 8.1 Hz, 1H), 7.25 (d, J = 7.8 Hz, 1H), 6.68 (t, J = 7.9 Hz, 1H).

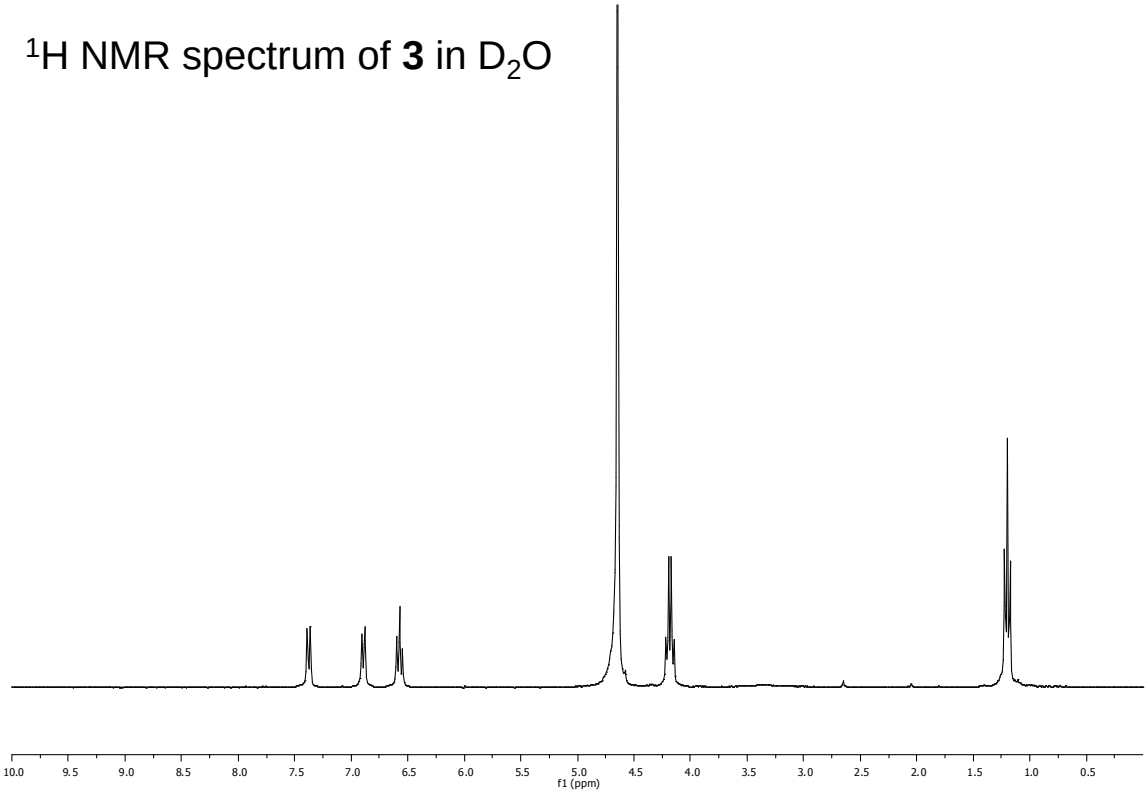


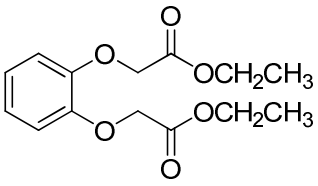


3

Ethyl 2,3-diaminobenzoate, 3. Synthesis of known compound **3** [4, 5] was performed by the modified procedure below. A solution of **2** (0.482 g, 3.17 mmol) and 95% ethanol (40 mL) in a pressure vessel was cooled to 0 °C. Thionyl chloride (2.4 mL, 32.9 mmol) was then added dropwise. The reaction vessel was then sealed, and the reaction was heated with stirring to 100 °C for 24 h. At this time, the reaction was cooled to room temperature and extracted with dichloromethane (3 x 20 mL). The resulting organic layers were combined and dried with $MgSO_4$. Following the removal of the $MgSO_4$, the dried organic layer was concentrated *in vacuo* to give a dark brown/black oil. This oil was then redissolved in dichloromethane (10 mL) and added to a pad of silica on a filtration apparatus. After elution of impurities with a 7:3 v/v hexanes:ethyl acetate (50 mL), the product is recovered via elution with 100% ethyl acetate. A yellow band of product is noted during this process. Concentration *in vacuo* yields clean **3** as a yellowish oil. (0.249 g, 1.382 mmol, 43.6%). ^{1}H NMR (300 MHz, D_2O) δ 7.37 (d, J = 8.1 Hz, 1H), 6.89 (d, J = 7.5 Hz, 1H), 6.57 (t, J = 7.9 Hz, 1H), 4.18 (q, J = 7.1 Hz, 2H), 1.20 (t, J = 7.1 Hz, 3H).

^{1}H NMR spectrum of **3** in D_2O

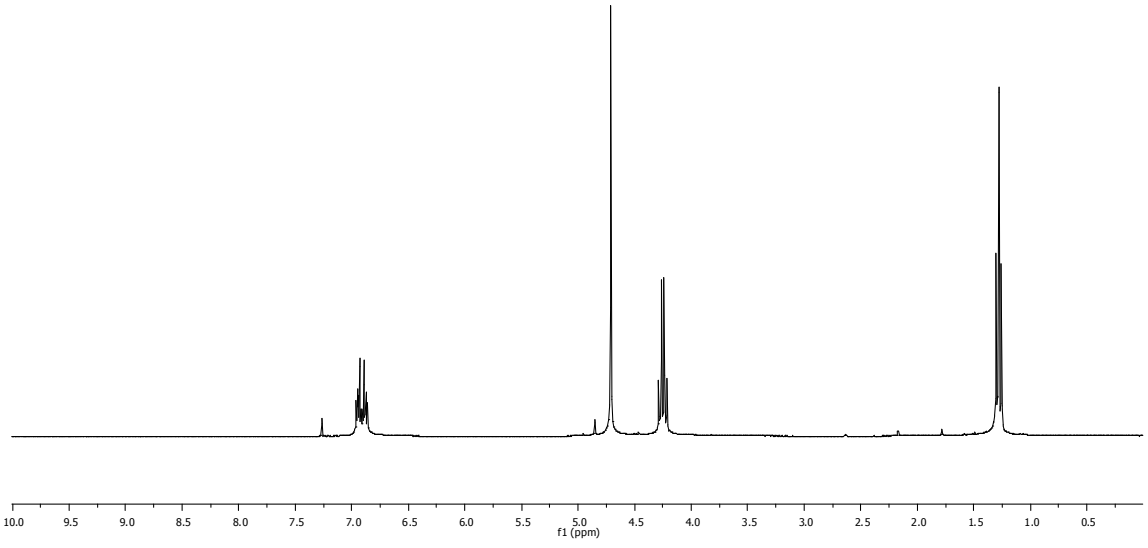


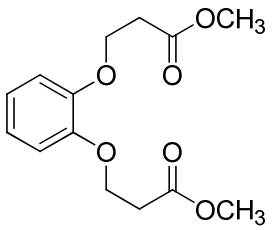


8a

Diethyl 2,2'-(1,2-phenylenebis(oxy))diacetate, 8a. Known compound **8a** was synthesized via slight modification of a previously reported procedure.[6] Under inert atmosphere, potassium carbonate (25.048 g, 182 mmol) was added to a solution of catechol (5.030 g, 45.4 mmol) in dry acetone (100 mL). Ethyl bromoacetate (9.97 mL, 90 mmol) was then added to the suspension, and the mixture was refluxed for 48 h. The excess carbonate was removed by filtration, and the filtrate was concentrated *in vacuo* to give a brown oil. This oil was then dissolved in diethyl ether (40 mL). The resulting solution was then washed with 5% sodium hydroxide in water (80 mL) and brine (2 x 80 mL), and the organic layer was dried with $MgSO_4$ and concentrated *in vacuo* to give 6.222 g (48.5%) crude **8a** as a light brown oil which was used in subsequent reactions without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91 (m, 4H), 4.25 (q, J = 7.1 Hz, 4H), 1.28 (t, J = 7.1 Hz, 6H).

^{1}H NMR spectrum of **8a** in $CDCl_3$

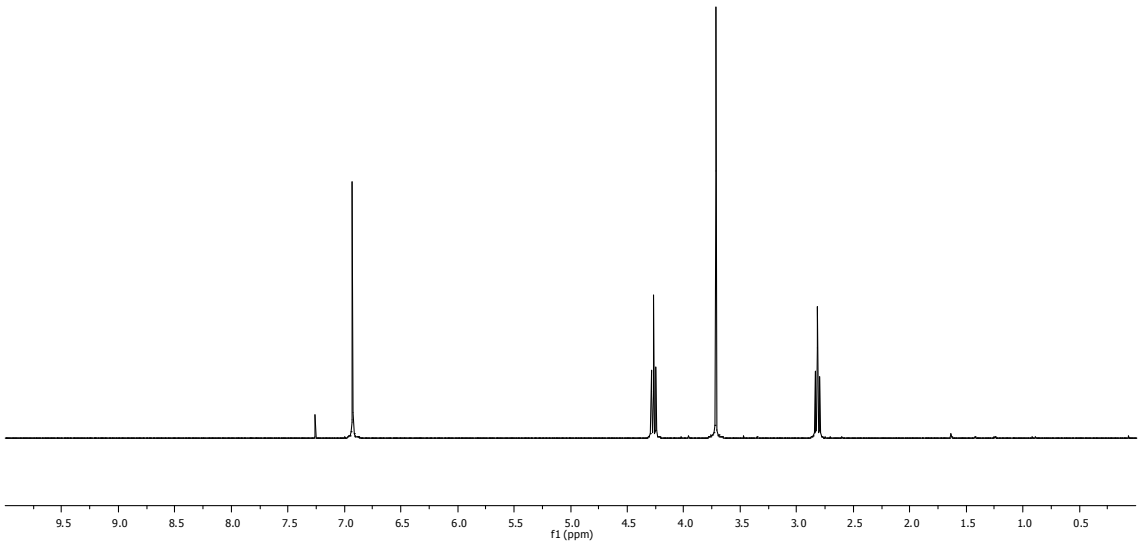


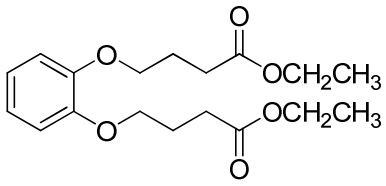


8b

Dimethyl 3,3'-(1,2-phenylenebis(oxy))dipropanoate, 8b. Catechol (10.00 g, 91.00 mmol) was placed in a pressure vessel and completely dissolved in methyl acrylate (32.70 mL, 363.00 mmol). Sodium methoxide (0.49 g, 9.08 mmol) was then added to the solution, and shaking was continued until all solid was dissolved. The reaction was then sealed, stirred, and heated to 100° C for 24 h. Methyl acrylate was removed via distillation *in vacuo* yielding a brown residue which was then dissolved in diethyl ether (40 mL). The resulting solution was washed with 5% sodium hydroxide in water (40 mL) and brine (2 x 40 mL). The organic layer was then dried with $MgSO_4$ and concentrated *in vacuo* to give **8b** as and pale white, oily solid (1.21 g, 4.29 mmol, 4.72%). mp: 43 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.93 (s, 4H), 4.27 (t, J = 6.6 Hz, 4H), 3.72 (s, 6H), 2.82 (t, J = 6.6 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.76, 148.96, 122.27, 115.63, 65.29, 52.06, 34.73; HRMS-DART (*m/z*): $[M+H]^+$ calculated for $C_{14}H_{19}O_6$ 283.11816; found, 283.11879.

^{1}H NMR spectrum of **8b** in $CDCl_3$

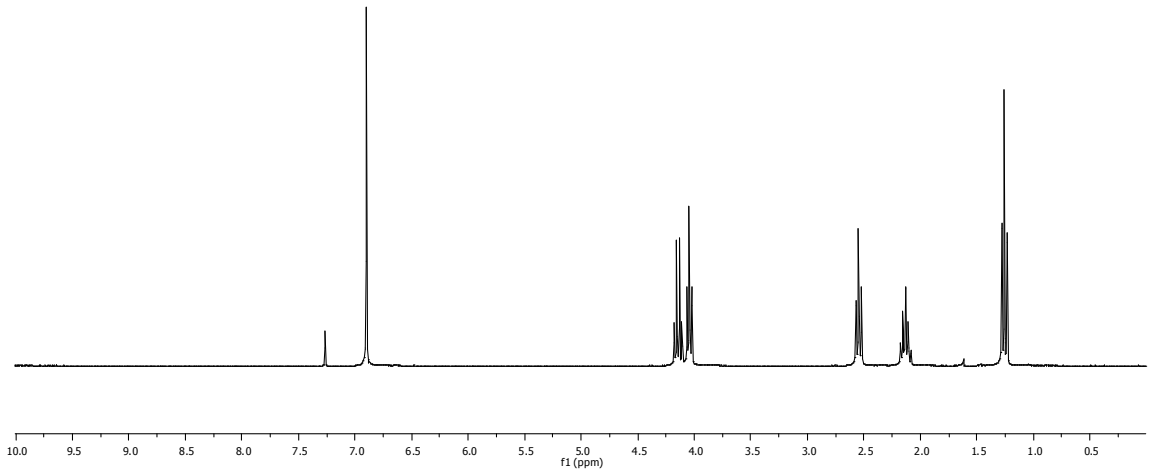


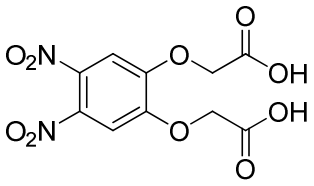


8c

Diethyl 4,4'-(1,2-phenylenebis(oxy))dibutanoate, 8c. Known compound **8c** was synthesized according to a previously reported literature procedure.[7] Briefly, potassium carbonate (25.224 g, 182 mmol) was added to a solution of catechol (5.056 g, 45.4 mmol) in dry acetone (100 mL) under inert atmosphere. Ethyl 4-bromobutyrate (12.87 mL, 90 mmol) was then added to the suspension and the reaction was refluxed for 48 h. The excess carbonate was removed by filtration, and the filtrate was concentrated *in vacuo* to give a brown oil. This oil was then dissolved in diethyl ether (40 mL). After washing with 5% sodium hydroxide in water (80 mL) and brine (2 x 80 mL), the organic layer was dried with $MgSO_4$ and concentrated *in vacuo* to give 12.169 g (79%) crude **8c** as a light brown oil which was used in subsequent reactions without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (s, 4H), 4.14 (q, J = 7.1 Hz, 4H), 4.04 (t, J = 6.2 Hz, 4H), 2.54 (t, J = 7.3 Hz, 4H), 2.13 (m, 4H), 1.26 (dd, J = 9.3 Hz, 4.9 Hz, 6H).

^{1}H NMR spectrum of **8c** in $CDCl_3$

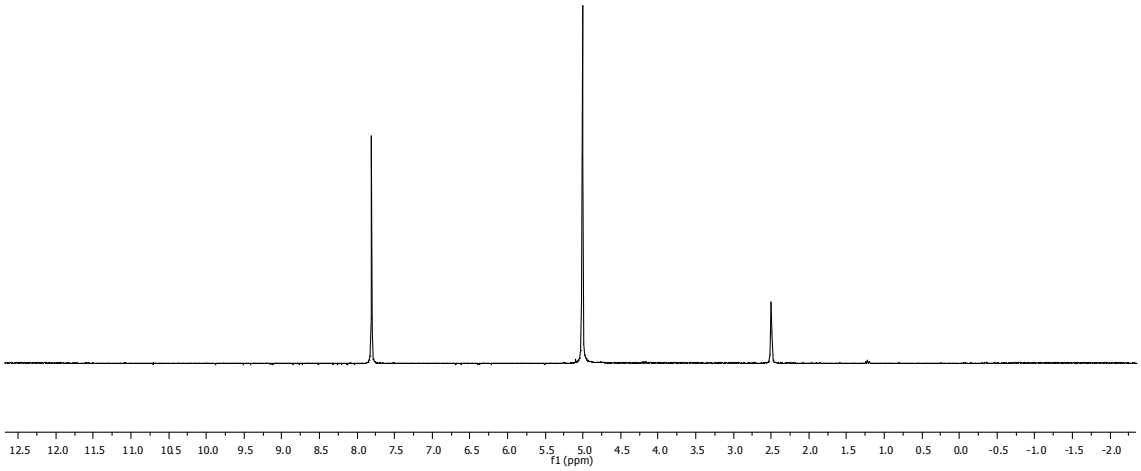


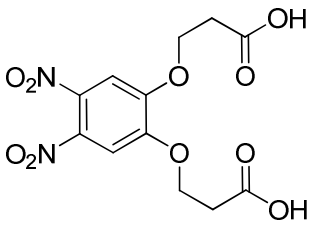


9a

2,2'-(4,5-dinitro-1,2-phenylene)bis(oxy)diacetic acid, 9a. Concentrated HNO_3 (4 mL) was added dropwise to a solution of **8a** (0.685 g, 2.427 mmol) in sulfuric acid (4 mL) at 0 °C. The reaction flask was then sealed, and the reaction was stirred at room temperature. After 24 h a precipitate had formed, and water (4 mL) was added dropwise to the reaction. The reaction was frozen and thawed to facilitate precipitation of a white solid which was then collected via filtration and washed with cold water (3 x 25 mL) to yield **9a** as a white solid (0.488 g, 1.543 mmol, 63.6%). mp: 182 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (s, 2H), 4.99 (s, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.93, 150.52, 136.39, 110.19, 66.29; HRMS-DART (*m/z*): $[M-H]^-$ calculated for $C_{10}H_7N_2O_{10}$ 315.01007; found, 315.01348.

^{1}H NMR spectrum of **9a** in DMSO-d_6

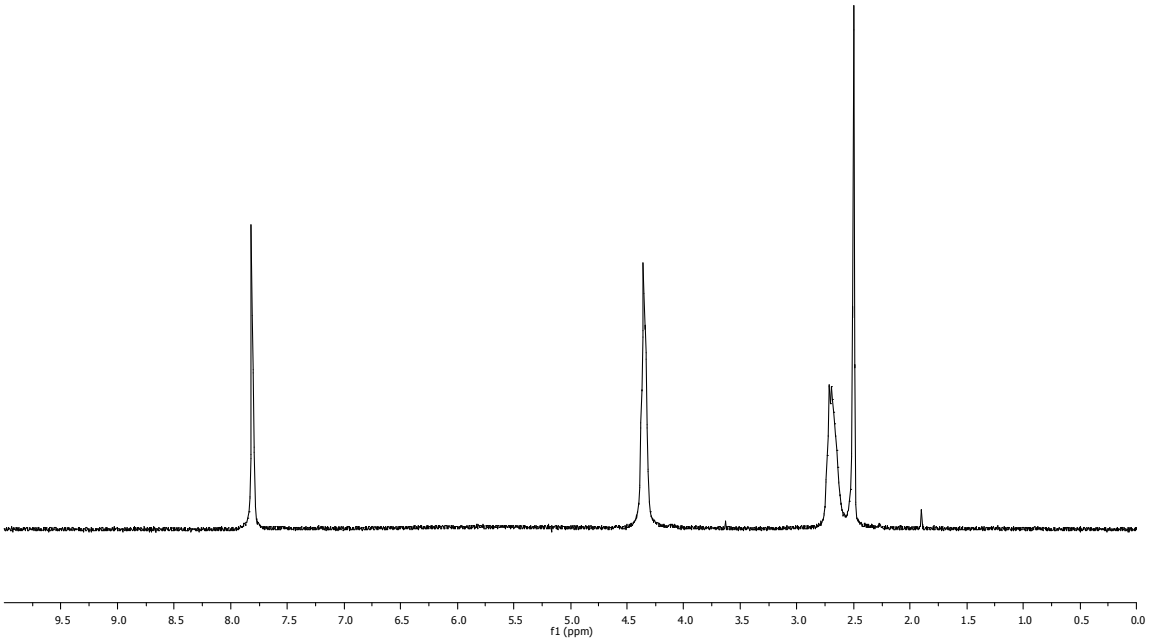


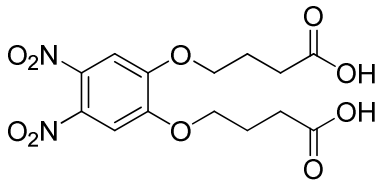


9b

3,3'-(4,5-dinitro-1,2-phenylene)bis(oxy)dipropanoic acid, 9b. Water (198 µL) was added to concentrated HNO_3 (6.5 mL) and the resulting solution was added dropwise to a solution of **8b** (0.517 g, 1.831 mmol) in acetic acid (6.5 mL) at 0 °C. The reaction flask was then sealed and stirred at 50° C for 11 h. At this time, water (6.7 mL) was added, and the reaction was allowed to stir for 1.5 h. The reaction was then frozen and thawed to facilitate precipitation of a yellow solid. This precipitate was collected via filtration and washed with cold water (3 x 30 mL) to yield **9b** as a yellow solid (0.375 g, 1.089 mmol, 59.5%). mp: 155 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.82 (s, 2H), 4.37 (t, J = 5.9 Hz, 4H), 2.75 (t, J = 5.9 Hz, 4H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.24, 151.12, 136.64, 109.86, 66.61, 34.39; HRMS-DART (*m/z*): $[M-H]^-$ calculated for $C_{12}H_{11}N_2O_{10}$ 343.04137; found, 343.03795.

^{1}H NMR spectrum of **9b** in DMSO-d_6

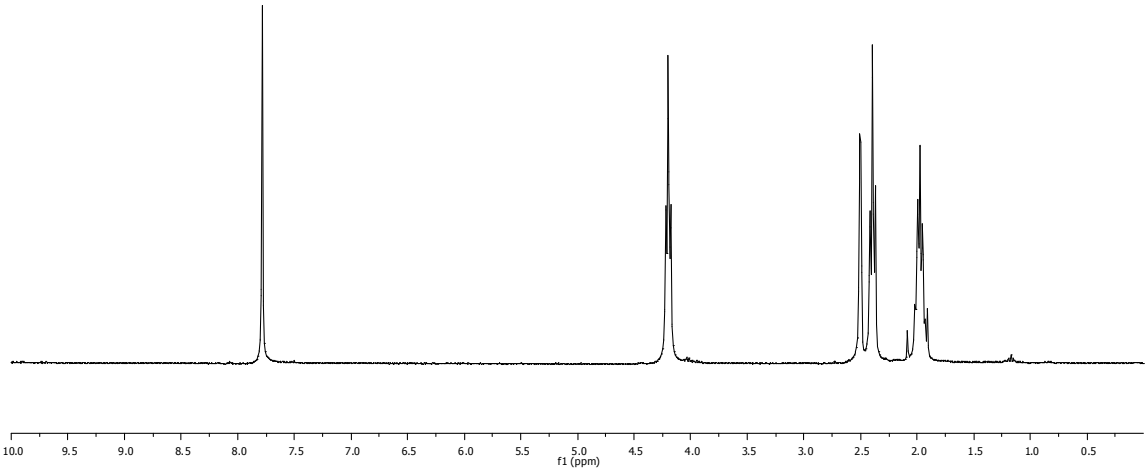


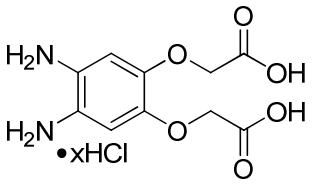


9c

4,4'-(4,5-dinitro-1,2-phenylene)bis(oxy)dibutanoic acid, 9c. Water (121 µL) was added to concentrated HNO_3 (4.0 mL) and the resulting solution was added dropwise to a solution of **8c** (0.318 g, 0.940 mmol) in acetic acid (4.0 mL) at 0 °C. The reaction flask was then sealed and stirred at 50° C for 11 h. Water (4.0 mL) was then added, and the reaction was stirred for 1.5 h. At this time, the reaction was frozen and thawed to facilitate precipitation of a yellow solid. This precipitate was collected via filtration and was washed with cold water (3 x 25 mL) to yield **9c** as a yellow solid (0.335 g, 0.900 mmol, 96.0%). mp: 162.3 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 2H), 7.83 (s, 2H), 4.36 (t, J = 6.1 Hz, 4H), 2.75 (t, J = 6.1 Hz, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 174.37, 151.65, 136.37, 109.30, 69.41, 30.19, 24.25; HRMS-DART (*m/z*): $[M-H]^-$ calculated for $C_{14}H_{15}N_2O_{10}$ 371.07267; found, 371.07083.

^{1}H NMR spectrum of **9c** in DMSO-d_6

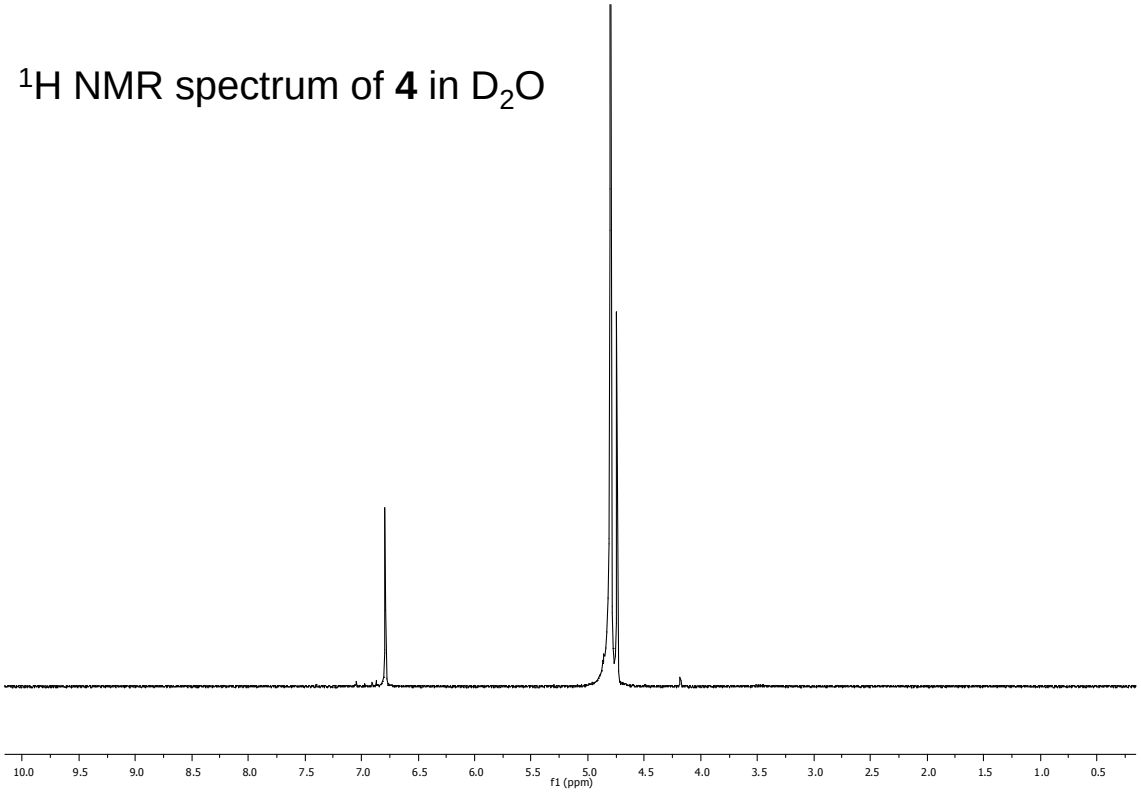


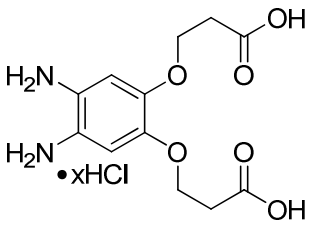


4

2,2'-(4,5-diamino-1,2-phenylene)bis(oxy)diacetic acid ● xHCl, 4. To a solution of tin (II) chloride (1.905 g, 8.44 mmol) in concentrated HCl (20 mL) was added **9a** (0.402 g, 1.271 mmol) in one portion. The reaction was then heated to 50 °C and stirred in air for 2.5 h. Concentration of the reaction *in vacuo* gave a brown oil that was then coevaporated with methanol (3 x 30 mL) to give a brown solid. The resulting solid was dissolved in water (50 mL), and hydrogen sulfide was bubbled through the solution to precipitate the tin as an insoluble brown sulfide. The mixture was then filtered through celite with water (2 x 15 mL), and the filtrate was collected. Further treatment of the filtrate with hydrogen sulfide followed by another filtration through celite was performed to insure complete removal of tin from the product. Concentration of the solution *in vacuo* yielded the hydrochloride salt, **4,** as a purple solid (0.421 g, 1.280 mmol, quantitative yield). mp: 195 °C dec; ^{1}H NMR (300 MHz, D_2O) δ 6.64 (s, 2H), 4.58 (s, 4H); ^{13}C NMR (101 MHz, D_2O) δ 173.03, 144.56, 122.61, 108.75, 66.43; HRMS-DART (*m/z*): $[M+H]^+$ calculated for $C_{10}H_{13}N_2O_6$ 257.07736; found, 257.07842.

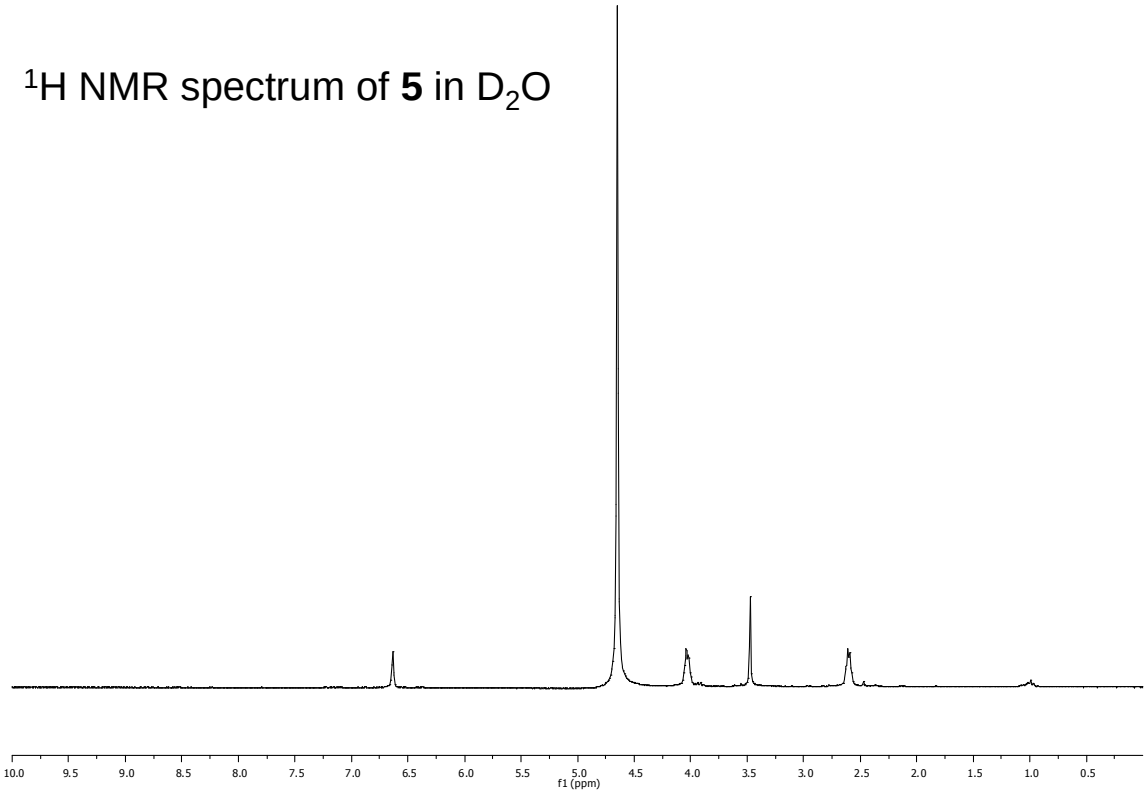
^{1}H NMR spectrum of **4** in D_2O

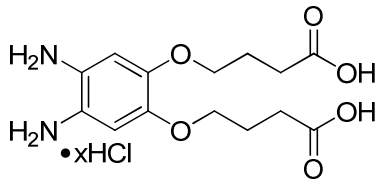




5

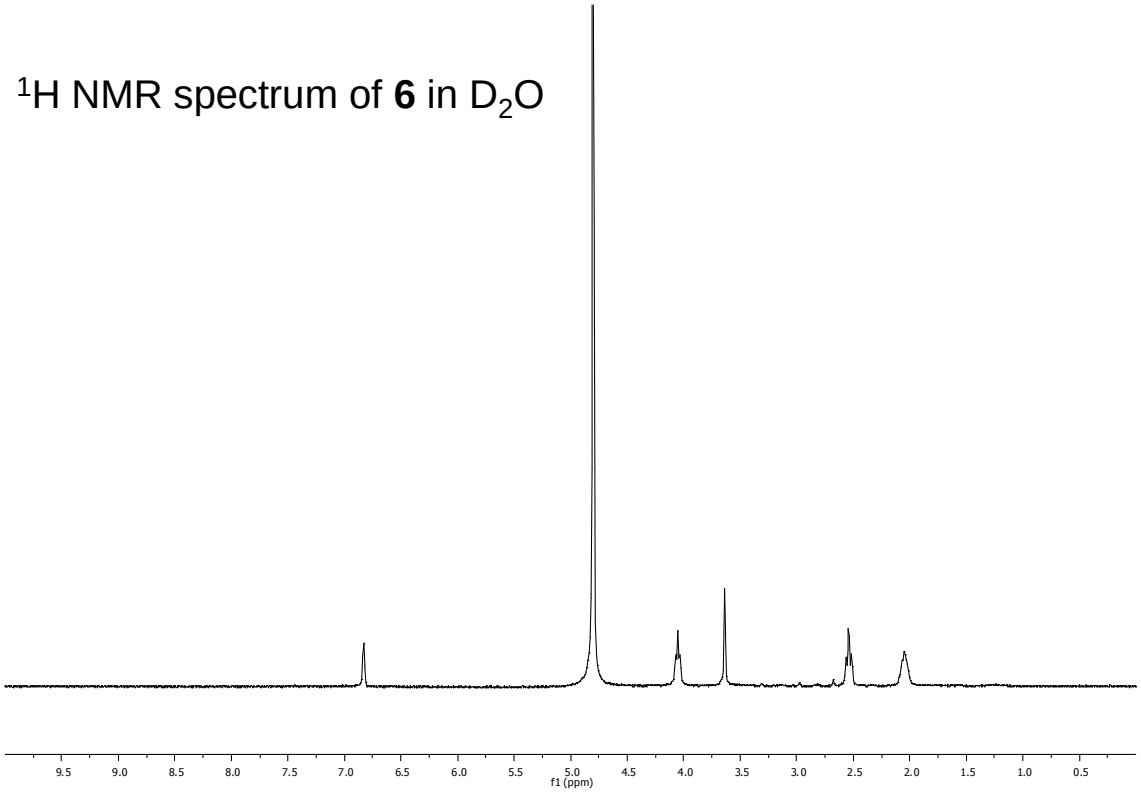
3,3'-(4,5-diamino-1,2-phenylene)bis(oxy)dipropanoic acid● xHCl, 5. To a solution of tin (II) chloride (83 mg, 0.370 mmol) in concentrated HCl (1 mL) was added **9b** (19.5 mg, 0.057 mmol) in one portion. The reaction mixture was then heated to 50 °C and stirred in air for 2.5 h. Concentration of the solution *in vacuo* gave a brown oil that was then coevaporated with methanol (3 x 5 mL) to give a brown solid. The resulting solid was dissolved in water (30 mL), and hydrogen sulfide was bubbled through the solution to precipitate the tin as an insoluble brown sulfide. The mixture was then filtered through celite with water (2 x 15 mL), and the filtrate was collected. Further treatment of the filtrate with hydrogen sulfide followed by another filtration through celite was performed to insure complete removal of the tin from the product. Concentration of the solution *in vacuo* yielded the hydrochloride salt, **5,** as a purple solid (10.2 mg, 0.029 mmol, 50.4%). mp: > 230 °C; ^{1}H NMR (300 MHz, D_2O) δ 6.83 (s, 2H), 4.05 (t, J = 5.9 Hz, 4H), 3.64 (s, partial exchange with solvent), 2.54 (t, J = 7.0 Hz, 4H), 2.05 (s, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 171.60, 147.96, 119.80, 110.08, 65.73, 33.66; MS-DART (*m/z*): $[2M + Sn]^{2+}$ was observed. The molecular ion for **5** was elusive. However, the mass of the corresponding Sn compound was found to be 339 *m/z*.

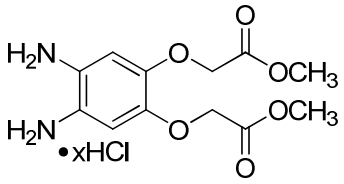




6

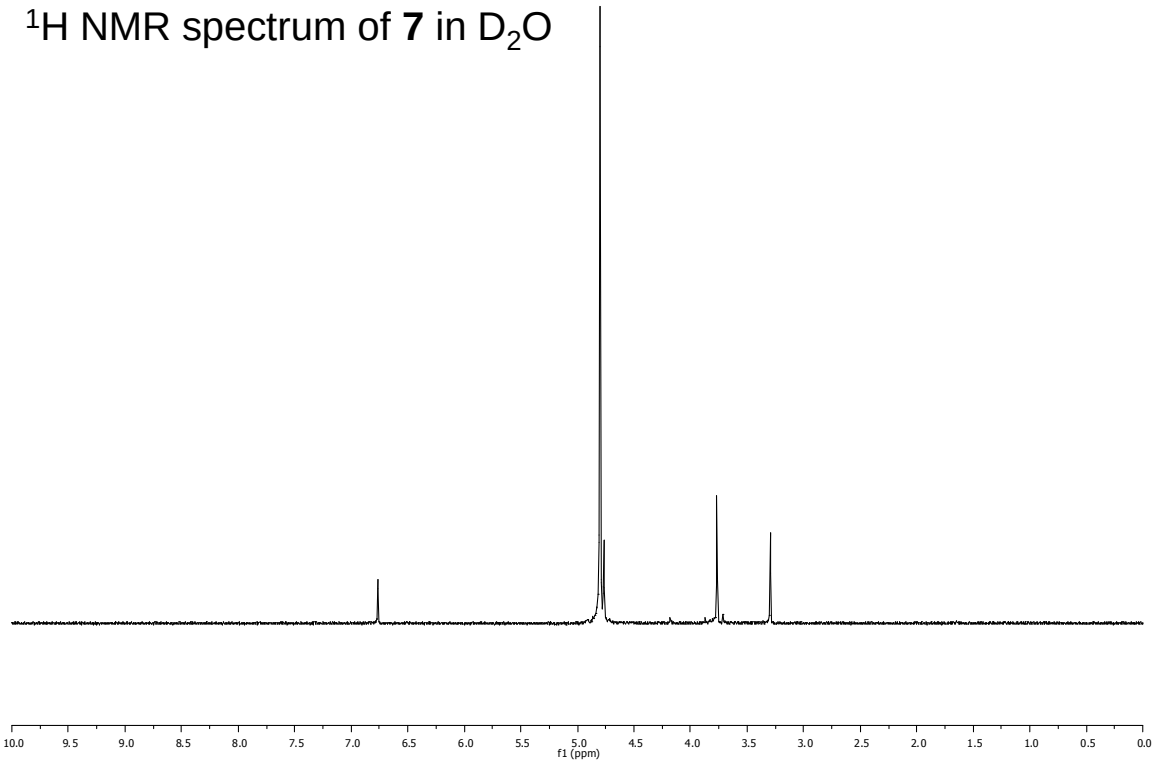
4,4'-(4,5-diamino-1,2-phenylene)bis(oxy)dibutanoic acid ● xHCl, 6. To a solution of tin (II) chloride (0.108 g, 0.478 mmol) in concentrated HCl (3 mL) was added **9c** (0.028 g, 0.072 mmol) in one portion. The reaction was then heated to 50 °C and stirred in air for 2.5 h. Concentration of the reaction *in vacuo* gave a brown oil that was then coevaporated with methanol (3 x 10 mL) to yield a brown solid. The resulting solid was dissolved in water (20 mL), and hydrogen sulfide was bubbled through the solution to precipitate the tin as an insoluble brown sulfide. The mixture was then filtered through celite with water (2 x 15 mL), and the filtrate was collected. Further treatment of the filtrate with hydrogen sulfide followed by another filtration through celite was performed to insure complete removal of the tin from the product. Concentration of the solution *in vacuo* yielded the hydrochloride salt, **6,** as a purple solid (0.011 g, 0.030 mmol, 40.8%). mp: > 230 °C; ^{1}H NMR (300 MHz, D_2O) δ 6.81 (s, 2H), 4.24 (m, 4H), 3.68 (s, partial exchange with solvent), 2.76 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 173.92, 148.37, 119.05, 109.31, 68.84, 29.89, 24.14; HRMS-DART (*m/z*): $[M+H]^+$ calculated for $C_{14}H_{20}N_2O_6$ 313.13996; found, 313.13888.





7

Dimethyl 2,2'-(4,5-diamino-1,2-phenylene)bis(oxy)diacetate ● xHCl, 7. Under inert atmosphere, **4** (75.6 mg, 0.230 mmol) was dissolved in 20 mL dry methanol. The resulting solution was cooled to 0 °C, and thionyl chloride (0.034 mL, 0.459 mmol) was added dropwise. The reaction was allowed to reflux for 1 h and was then concentrated *in vacuo* to yield **7** as a purple solid (76.2 mg, 0.213 mmol, 93%). mp: 105 °C; ^{1}H NMR (300 MHz, D_2O) δ 6.77 (s, 2H), 4.77 (s, partially under solvent), 3.77 (s, 6H), 3.30 (s, 4H); ^{13}C NMR (101 MHz, D_2O) δ 171.59, 144.30, 123.17, 108.88, 66.66, 52.81; HRMS-ESI (*m/z*): $[M+H]^+$ calculated for $C_{12}H_{17}N_2O_6$ 285.10866; found, 285.10983.



(*R*)-3-(1,2-dihydroxyethyl)-2-methylquinoxaline-5-carboxylic acid and (*R*)-2-(1,2-dihydroxyethyl)-3-methylquinoxaline-5-carboxylic acid (DPD-BAQ), reaction with DPD followed by ^{1}H NMR. The known compound, **DPD,** was synthesized according to a previously reported procedure.[1] Briefly, (*S*)-4,5-cyclohexylidenedioxy-2,3-pentadione, **Pro-DPD**, (1.1 mg, 4.7 mmol) was fully dissolved in D_2O (1.1 mL), and D_2SO_4 (1.1 μL) was then added to the solution. Deprotection was then allowed to proceed for 3 h. Tag **2** (1 molar equiv, 1.2 mg, 4.7 mmol) was then added to the **DPD** solution, and the reaction was monitored by ^{1}H NMR for 1 h. At this time, a second molar equiv of **2** (1.2 mg, 4.7 mmol) was added to the reaction mixture, and monitoring by ^{1}H NMR was continued for another hour. After 2 h, a third molar equiv of **2** (1.2 mg, 4.7 mmol) was added to the reaction mixture, and monitoring by ^{1}H NMR was continued for another 0.5 h. After a total time of 2.5 h, conversion of **DPD** to **DPD-BAQ** was not complete, and the experiment was abandoned due to the sluggishness of the reaction. ^{1}H NMR (300 MHz, D_2O) characterized as a mixture of regioisomeric products. See Supporting Information Figure S1a for chemical shift assignments.

(*R*)-ethyl 3-(1,2-dihydroxyethyl)-2-methylquinoxaline-5-carboxylate and (*R*)-ethyl 2-(1,2-dihydroxyethyl)-3-methylquinoxaline-5-carboxylate (DPD-EBAQ), reaction with DPD followed by ^{1}H NMR. Known compound, **DPD,** was synthesized according to a previously reported procedure.[1] Briefly, **Pro-DPD** (1 mg, 4.7 mmol) was fully dissolved in D_2O (1 mL), and D_2SO_4 (1 μL) was then added to the solution. Deprotection was then allowed to proceed for 3 h. Tag **3** (1 molar equiv, 1.2 mg, 4.7 mmol) was then added to the **DPD** solution, and the reaction was monitored by ^{1}H NMR for 1 h. At this time, a second molar equiv of **3** (1.2 mg, 4.7 mmol) was added to the reaction mixture, and monitoring by ^{1}H NMR was continued for another 1 h. After a total time of 2 h, **DPD** had completely converted to **DPD-EBAQ** as judged by ^{1}H NMR. ^{1}H NMR (300 MHz, D_2O) characterized as a mixture of regioisomeric products. See Supporting Information Figure S2a for chemical shift assignments.

(*R*)-dimethyl 2,2'-(2-(1,2-dihydroxyethyl)-3-methylquinoxaline-6,7-diyl)bis(oxy)diacetate (DPD-M1CQ), Reaction with DPD Followed by ^{1}H NMR. The known compound, **DPD,** was synthesize according to a previously reported procedure.[1] Briefly, **Pro-DPD** (1.1 mg, 4.7 mmol) was dissolved fully in D_2O (1.1 mL), and D_2SO_4 (1.1 μL) was then added to the solution. Deprotection was then allowed to proceed for 3 h. Tag **7** (1 molar equiv, 1.9 mg, 4.7 mmol) was then added to the **DPD** solution, and the reaction was monitored by ^{1}H NMR for 0.5 h. At this time, a second molar equiv of **7** (1.9 mg, 4.7 mmol) was added to the reaction mixture, and monitoring by ^{1}H NMR was continued for another 0.5 h. After a total time of 1 h, **DPD** had completely converted to **DPD-M1CQ** as judged by ^{1}H NMR. ^{1}H NMR (300 MHz, D_2O) δ 7.58 (s, 1H), 7.36 (s, 1H), 5.28 (t, J = 5.5 Hz, 1H), 5.05 (d, J = 6.0 Hz, 4H), 3.95 – 3.71 (m, 2H, peak partially overlapping with the methyl ester protons from both **7** and **DPD-MICQ**), 3.79 (s, 3H, peak overlapping with protons on **DPD-M1CQ**), 2.85 (s, 3H).

Comparison of the Ionization Efficiencies for the Quinoxalines Derived from Reaction of Diamine Tags 2-7 with Synthetic DPD To generate each quinoxaline for study, an ~25 μL solution of the tagging reagent (14 mM in H_2O) was mixed in a 1:1 v/v ratio with a solution of **DPD** (4.7 mM in H_2O, pH = 1.8) to give a solution containing a 3:1 mixture of the diamine tag to **DPD**. After the reactions were allowed to proceed for 2 h at ambient temperature, each of the 6 resulting solutions were diluted in water to a final concentration of 10 μM. Determination of the relative ionization efficiency for each was then accomplished via LC-MS/MS analysis utilizing the appropriate SRM detection parameters. See Figure 2 in the manuscript for results.

Determination of the Approximate Linear Range and Limit of Detection for DPD-M1CQ To determine the ability to detect **DPD-M1CQ** via LC-MS/MS, an ~200 μL solution of the tagging reagent (14 mM in H_2O) was mixed in a 1:1 ratio with a solution of **DPD** (4.7 mM in H_2O, pH = 1.8) to give a solution containing a 3:1 mixture of the diamine tag to **DPD**. After reaction was allowed to proceed for 30 min at ambient temperature, a 1:100 dilution of the sample was performed to give a 23.5 μM solution of **DPD-M1CQ** in H_2O. Further half log-dilutions of the molecule in water gave samples with a series of concentration ranging from 74 pM to 7.4 mM. For this experiment, samples were stored at -80 ºC until no more than 5 h before LC-MS/MS analysis. The total ion counts measured via each of the appropriate SRMs was determined by integrating the peak for **DPD-M1CQ** in each mass chromatogram. The results of these data are summarized in Supporting Information Figure S4 and S5 and discussed in the manuscript.

Validation of Selectivity for Tag 7 in Complex Media Three aliquots of sterile LB (200 μL) were mixed with a solution of tag **7** (14 mM in 20 μL H_2O). After thorough mixing, the solutions were allowed to incubate for 1 h at ambient temperature to mimic the conditions used for the reaction of **DPD** with **7**. To determine whether reaction of **7** with components in the media had occurred, the samples were analyzed via LC-MS/MS, and control samples containing only LB were also analyzed. During these experiments, the SRMs 381-201, 381-202, and 381-231 that were projected to be optimal for the detection of **DPD** were monitored. The results of these experiments are discussed in the text of the manuscript and shown in Supporting Information Figure S6. No signal was observed above background in any samples containing only LB in the SRMs used to detect either **DPD-M1CQ** or **(^{13}C)DPD-M1CQ**.

Comparison of Internal Standards (^{13}C)DPD-M1CQ and (^{13}C)DPD To determine the relative efficacy of these internal standards, the [**DPD**] from a culture of *V. harveyi* BB120 grown for 9 h was measured in duplicate relative to either **(^{13}C)DPD-M1CQ** or **(^{13}C)DPD**. The samples were prepared as described in the General Procedure for the Determination of the [**DPD**] entry in the methods section, except that a solution of **(^{13}C)DPD-M1CQ** (341 μM in H_2O) was substituted as the internal standard for two of the samples. The initial **(^{13}C)DPD-M1CQ** solution (2.35 mM in H_2O) was produced by mixing a solution of **7** (14 mM in H_2O) with **(^{13}C)DPD** (4.7 mM in H_2O) in a 1:1 v/v ratio and allowing the reaction to proceed for 1 h at ambient temperature. The data from these analyses are presented in Supporting Information Table S2 and discussed in the text of the manuscript.

Data Used to Determine the Ratio of Signal Intensities for Each SRM of DPD-M1CQ and (^{13}C)DPD-M1CQ The analyses of these data are presented in the Supporting Information Discussion and Supporting Information Table S3 and are also graphically summarized in Supporting Information Figure S7.

Data Used to Determine Signal Intensity Ratios for DPD-M1CQ and (^{13}C)DPD-M1CQ SRMs

Signal Ratios for DPD-M1CQ SRMs in *E. coli* WT Cultures

Time (h)	Observed Signal (ion counts)				Signal Normalized to SRM 381-363			
	381-201	381-202	381-231	381-363	381-201	381-202	381-231	381-363
1.50	1.28×10^5	2.42×10^5	2.89×10^5	5.98×10^5	21.4	40.5	48.3	100
2.00	3.01×10^5	5.92×10^5	6.09×10^5	1.37×10^6	22.0	43.2	44.5	100
2.50	5.12×10^5	9.70×10^5	9.94×10^5	2.23×10^6	22.9	43.4	44.5	100
3.00	9.81×10^5	1.93×10^6	1.91×10^6	4.35×10^6	22.5	44.3	43.8	100
3.75	1.28×10^6	2.54×10^6	2.47×10^6	5.72×10^6	22.3	44.4	43.1	100
4.50	2.45×10^5	4.63×10^5	5.21×10^5	1.10×10^6	22.3	42.2	47.5	100
1.50	2.23×10^5	4.09×10^5	4.92×10^5	9.81×10^5	22.7	41.6	50.1	100
2.00	4.75×10^5	9.14×10^5	9.89×10^5	2.08×10^6	22.9	44.0	47.6	100
2.50	9.73×10^5	1.91×10^6	1.88×10^6	4.26×10^6	22.9	45.0	44.2	100
3.00	1.38×10^6	2.76×10^6	2.68×10^6	6.28×10^6	22.0	43.9	42.7	100
3.75	1.94×10^6	3.88×10^6	3.68×10^6	8.70×10^6	22.3	44.6	42.3	100
				Average	22.1	42.9	46.7	100

Signal Ratios for (^{13}C)DPD-M1CQ SRMs in *E. coli luxS*$^-$ Cultures

Time (h)	Observed Signal (ion counts)					Signal Normalized to 382-364				
	382-201	382-202	382-203	382-232	382-364	382-201	382-202	382-203	382-232	382-364
1.50	5.30×10^4	8.58×10^5	1.72×10^6	1.64×10^6	4.04×10^6	1.3	21.2	42.5	40.7	100
2.00	5.90×10^4	9.24×10^5	1.84×10^6	1.74×10^6	4.30×10^6	1.4	21.5	42.9	40.5	100
2.50	6.10×10^4	9.00×10^5	1.80×10^6	1.73×10^6	4.21×10^6	1.4	21.4	42.7	41.0	100
3.75	2.59×10^4	4.95×10^5	9.83×10^5	9.41×10^5	2.32×10^6	1.1	21.3	42.3	40.5	100
4.50	5.88×10^4	9.56×10^5	1.90×10^6	1.81×10^6	4.47×10^6	1.3	21.4	42.6	40.4	100
1.50	5.20×10^4	8.51×10^5	1.69×10^6	1.59×10^6	3.89×10^6	1.3	21.9	43.5	40.9	100
2.00	5.28×10^4	8.34×10^5	1.65×10^6	1.55×10^6	3.81×10^6	1.4	21.9	43.2	40.7	100
2.50	5.23×10^4	8.60×10^5	1.69×10^6	1.60×10^6	3.89×10^6	1.3	22.1	43.4	41.2	100
3.00	3.70×10^4	6.96×10^5	1.40×10^6	1.32×10^6	3.20×10^6	1.2	21.8	43.8	41.1	100
3.75	4.92×10^4	8.94×10^5	1.78×10^6	1.66×10^6	4.10×10^6	1.2	21.8	43.3	40.6	100
4.50	3.53×10^4	7.18×10^5	1.41×10^6	1.34×10^6	3.29×10^6	1.1	21.9	42.9	40.7	100
					Average	1.3	21.6	43.0	40.8	100

Determination of the [DPD] in *E. coli* Wild Type Strain BW25113 and *luxS*$^-$ Strain JW2662-1

A culture of both strains was grown overnight, and then used to make two 2% (v/v) inocula of each in fresh LB (25 mL) in 125 mL glass Erlenmeyer flasks as described in the Bacterial Strains and Growth Conditions entry in the methods section of this document. At the time intervals indicated in the table below, the cell density was determined by measuring the OD_{600} for 1 mL aliquots of each culture, and separate 300 μL aliquots were used for measurement of the [**DPD**]. Samples were prepared and analyzed as described in the general procedure for the Determination of the [**DPD**] entry in the methods section of this article. Only one of the cultures for the *luxS*$^-$ strain was fully analyzed as no **DPD** was detected, although several of the samples from random times in the second culture were used to confirm the lack of signal. The data collected for and the type of correction applied to each sample is summarized in the data tables below.

[DPD] Data for *E. coli luxS*$^-$ Strain JW2662-1, Duplicate Culture 1

Time (h)	Observed Signal for DPD-M1CQ (ion counts)	Observed Signal for (^{13}C)DPD-M1CQ (ion counts)	Correction Type*	Corrected Signal for DPD-M1CQ (ion counts)	Corrected Signal for (^{13}C)PD-M1CQ (ion counts)	[DPD] (μM)†
0.00	9.64×10^3	1.16×10^6	1	3.49×10^2		0.003
0.50	7.27×10^3	1.02×10^6	1	-8.49×10^2		-0.008
1.00	7.12×10^3	1.02×10^6	1	-1.06×10^3		-0.010
1.50	6.26×10^3	1.02×10^6	1	-1.94×10^3		-0.019
2.00	3.61×10^3	4.70×10^5	1	-1.47×10^2		-0.003
2.50	1.48×10^4	1.58×10^6	1	2.14×10^3		0.014
3.00	4.94×10^3	6.62×10^5	1	-3.59×10^2		-0.005
4.00	5.45×10^3	9.23×10^5	1	-1.94×10^3		-0.021
4.50	8.38×10^3	1.16×10^6	1	-8.84×10^2		-0.008
5.00	1.14×10^4	1.44×10^7	1	-1.03×10^5		-0.072
5.75	7.48×10^3	1.17×10^6	1	-1.89×10^3		-0.016
6.50	4.31×10^3	9.21×10^5	1	-3.06×10^3		-0.033
7.25	6.33×10^2	3.65×10^5	1	-2.29×10^3		-0.063

*Correction Type 1: Corr. **DPD-M1CQ** Signal = Obs. **DPD-M1CQ** signal – 0.008 x Obs. **(^{13}C)DPD-M1CQ** signal
Correction Type 2: Corr. **(^{13}C)DPD-M1CQ** Signal = Obs. **(^{13}C)DPD-M1CQ** signal – 0.144 x Obs. **DPD-M1CQ** signal
†The use of three decimal places should not be taken to imply that all three are significant. Instead, these data are used to indicate the lack of signal observed in the *luxS*$^-$ strain.

[DPD] Data for *E. coli luxS*$^-$ Strain JW2662-1, Duplicate Culture 2

Time (h)	Observed Signal for DPD-M1CQ (ion counts)	Observed Signal for (^{13}C)DPD-M1CQ (ion counts)	Correction Type*	Corrected Signal for DPD-M1CQ (ion counts)	Corrected Signal for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)†
3.00	1.01×10^3	1.12×10^5	1	1.08×10^2		0.010
3.50	1.09×10^3	1.66×10^5	1	-2.37×10^2		-0.014
4.00	4.23×10^3	3.58×10^5	1	1.37×10^3		0.038

*Correction Type 1: Corr. **DPD-M1CQ** Signal = Obs. **DPD-M1CQ** signal – 0.008 x Obs. **(^{13}C)DPD-M1CQ** signal
Correction Type 2: Corr. **(^{13}C)DPD-M1CQ** Signal = Obs. **(^{13}C)DPD-M1CQ** signal – 0.144 x Obs. **DPD-M1CQ** signal
†The use of three decimal places should not be taken to imply that all three are significant. Instead, these data are used to indicate the lack of signal observed in the *luxS*$^-$ strain.

[DPD] Data for *E. coli* WT Strain BW25113, Duplicate Culture 1

Time (h)	Observed Signal for DPD-M1CQ (ion counts)	Observed Signal for (^{13}C)DPD-M1CQ (ion counts)	Correction Type*	Corrected Signal for DPD-M1CQ (ion counts)	Corrected Signal for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)
0.00	5.55×10^3	3.72×10^5	1	2.58×10^3		0.1
0.50	9.70×10^3	3.64×10^5	1	6.78×10^3		0.2
1.00	2.84×10^4	3.47×10^5	1	2.56×10^4		0.7
1.50	7.11×10^4	3.38×10^5	None			2.1
2.00	1.67×10^5	3.94×10^5	2		3.70×10^5	4.5
2.50	5.59×10^5	7.03×10^5	2		6.23×10^5	9.0
3.00	6.40×10^5	5.33×10^5	2		4.41×10^5	14.5
3.50	9.06×10^5	5.69×10^5	2		4.39×10^5	20.7
4.00	7.55×10^5	5.78×10^5	2		4.69×10^5	16.1
4.50	2.26×10^4	3.76×10^5	1	1.96×10^4		0.5
5.00	1.24×10^4	2.87×10^5	1	1.02×10^4		0.4
5.75	2.99×10^4	4.22×10^5	1	2.66×10^4		0.6
6.50	2.14×10^4	3.93×10^5	1	1.82×10^4		0.5
7.25	1.23×10^4	3.16×10^5	1	9.78×10^3		0.3

*Correction Type 1: Corr. **DPD-M1CQ** Signal = Obs. **DPD-M1CQ** signal – 0.008 x Obs. **(^{13}C)DPD-M1CQ** signal
Correction Type 2: Corr. **(^{13}C)DPD-M1CQ** Signal = Obs. **(^{13}C)DPD-M1CQ** signal – 0.144 x Obs. **DPD-M1CQ** signal

[DPD] Data for *E. coli* WT Strain BW25113, Duplicate Culture 2

Time (h)	Observed Signal for DPD-M1CQ (ion counts)	Observed Signal for (^{13}C)DPD-M1CQ (ion counts)	Correction Type*	Corrected Signal for DPD-M1CQ (ion counts)	Corrected Signal for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)
0.00	1.47×10^4	5.28×10^5	1	1.05×10^4		0.2
0.50	1.72×10^4	5.82×10^5	1	1.25×10^4		0.2
1.00	5.41×10^4	6.21×10^5	1	4.91×10^4		0.8
1.50	1.87×10^5	8.43×10^5	None			2.2
2.00	3.91×10^5	8.97×10^5	2		8.41×10^5	4.7
2.50	7.17×10^5	9.06×10^5	2		8.03×10^5	8.9
3.00	9.45×10^5	8.07×10^5	2		6.71×10^5	14.1
3.50	1.33×10^6	8.43×10^5	2		6.51×10^5	20.5
4.00	8.60×10^5	6.86×10^5	None		5.62×10^5	15.3
4.50	2.48×10^4	4.87×10^5	1	2.09×10^4		0.4
5.00	3.08×10^4	6.08×10^5	1	2.60×10^4		0.4
5.75	2.74×10^4	4.06×10^5	1	2.41×10^4		0.6
6.50	1.76×10^4	3.10×10^5	1	1.51×10^4		0.5
7.25	7.41×10^3	2.23×10^5	1	5.63×10^3		0.3

*Correction Type 1: Corr. **DPD-M1CQ** Signal = Obs. **DPD-M1CQ** signal – 0.008 x Obs. **(^{13}C)DPD-M1CQ** signal
Correction Type 2: Corr. **(^{13}C)DPD-M1CQ** Signal = Obs. **(^{13}C)DPD-M1CQ** signal – 0.144 x Obs. **DPD-M1CQ** signal

E. coli [DPD] Data Used to Construct Figure 4A

Time (h)	[DPD] in WT Replicate 1 (μM)	[DPD] in WT Replicate 2 (μM)	Average [DPD] in WT (μM)	Range	[DPD] in *luxS*$^-$ (μM)[†]
0.00	0.1	0.2	0.1	± 0.1	0.003
0.50	0.2	0.2	0.2	± 0.0	-0.008
1.00	0.7	0.8	0.8	± 0.0	-0.010
1.50	2.1	2.2	2.2	± 0.1	-0.019
2.00	4.5	4.7	4.6	± 0.1	-0.003
2.50	9.0	8.9	9.0	± 0.0	0.014
3.00	14.5	14.1	14.3	± 0.2	-0.005
3.50	20.7	20.5	20.6	± 0.1	-0.014
4.00	16.1	15.3	15.7	± 0.4	-0.021
4.50	0.5	0.4	0.5	± 0.0	-0.008
5.00	0.4	0.4	0.4	± 0.0	-0.072
5.75	0.6	0.6	0.6	± 0.0	-0.016
6.50	0.5	0.5	0.5	± 0.0	-0.033
7.25	0.3	0.3	0.3	± 0.0	-0.063

[†]The use of three decimal places should not be taken to imply that all three are significant. Instead, these data are used to indicate the lack of signal observed in the *luxS*$^-$ strain.

E. coli Cell Growth Data Used to Construct Figure 4A

Time (h)	Cell Growth of WT Replicate 1 (OD_{600})	Cell Growth of WT Replicate 2 (OD_{600})	Cell Growth of *luxS*$^-$ Replicate 1 (OD_{600})	Average Cell Growth (OD_{600})
0.00	0.048	0.098	0.029	0.058
0.50	0.073	0.074	0.060	0.069
1.00	0.142	0.140	0.123	0.135
1.50	0.367	0.374	0.304	0.348
2.00	0.705	0.684	0.581	0.657
2.50	1.257	1.246	1.000	1.168
3.00	1.863	1.939	1.495	1.766
3.50	2.419	2.468	1.868	2.252
4.00	2.638	2.648	2.346	2.544
4.50	3.023	3.146	2.768	2.979
5.00	3.567	3.343	3.234	3.381
5.75	4.034	4.058	4.176	4.089
6.50	4.522	5.020	4.682	4.741
7.25	5.194	5.882	5.033	5.370

Determination of the [DPD] in *V. harveyi* Wild Type Strain BB120 and *luxS*$^-$ Strain MM30

A culture of both strains was grown overnight, and then used to make two 2% (v/v) inocula of each in fresh LM (25 mL) in 125 mL glass Erlenmeyer flasks as described in the Bacterial Strains and Growth Conditions entry in the methods section of this document. At the time intervals indicated in the table below, the cell density was determined by measuring the OD_{600} for 1 mL aliquots of each culture, and separate 300 μL aliquots were used for measurement of the [**DPD**]. Samples were prepared and analyzed as described in the General Procedure for the Determination of the [**DPD**] entry in the methods section of this article. Only one of the cultures for the *luxS*$^-$ strain was fully analyzed as no **DPD** was detected, although several of the samples from random times in the second culture were used to confirm the lack of signal. The data collected for and the type of correction applied to each sample is summarized in the data tables below.

[DPD] Data for *V. harveyi luxS*$^-$ Strain MM30, Duplicate Culture 1

Time (h)	Observed Signal for DPD-M1CQ (ion counts)	Observed Signal for (^{13}C)DPD-M1CQ (ion counts)	Correction Type*	Corrected Signal for DPD-M1CQ (ion counts)	Corrected Signal for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)†
0.00	1.37×10^4	1.02×10^6	1	5.47×10^3		0.054
0.50	3.07×10^3	5.48×10^5	1	-1.32×10^3		-0.024
1.00	4.85×10^3	6.08×10^5	1	-1.46×10^1		0.000
1.50	1.76×10^3	5.05×10^5	1	-2.28×10^3		-0.045
2.00	2.86×10^3	5.32×10^5	1	-1.40×10^3		-0.026
2.75	1.59×10^3	4.89×10^5	1	-2.33×10^3		-0.048
3.50	2.66×10^3	4.96×10^5	1	-1.31×10^3		-0.026
4.25	1.88×10^3	3.91×10^5	1	-1.25×10^3		-0.032
5.00	1.81×10^3	3.16×10^5	1	-7.11×10^2		-0.023
5.75	3.64×10^3	3.39×10^5	1	9.23×10^2		0.027
6.50	4.63×10^3	3.75×10^5	1	1.63×10^3		0.043
7.25	7.01×10^3	4.89×10^5	1	3.10×10^3		0.063
8.00	5.14×10^3	4.43×10^5	1	1.59×10^3		0.036
10.00	2.23×10^3	2.95×10^5	1	-1.28×10^2		-0.004
12.00	2.39×10^3	3.26×10^5	1	-2.16×10^2		-0.007

*Correction Type 1: Corr. **DPD-M1CQ** Signal = Obs. **DPD-M1CQ** signal – 0.008 x Obs. **(^{13}C)DPD-M1CQ** signal
Correction Type 2: Corr. **(^{13}C)DPD-M1CQ** Signal = Obs. **(^{13}C)DPD-M1CQ** signal – 0.144 x Obs. **DPD-M1CQ** signal
†The use of three decimal places should not be taken to imply that all three are significant. Instead, these data are used to indicate the lack of signal observed in the *luxS*$^-$ strain.

[DPD] Data for *V. harveyi luxS*$^-$ Strain MM30, Duplicate Culture 2

Time (h)	Observed Signal for DPD-M1CQ (ion counts)	Observed Signal for (^{13}C)DPD-M1CQ (ion counts)	Correction Type*	Corrected Signal for DPD-M1CQ (ion counts)	Corrected Signal for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)†
5.75	2.71×10^3	2.83×10^5	1	4.41×10^2		0.0156
6.5	1.84×10^3	1.81×10^5	1	3.95×10^2		0.0218
7.25	2.06×10^3	1.63×10^5	1	7.54×10^2		0.0463

*Correction Type 1: Corr. **DPD-M1CQ** Signal = Obs. **DPD-M1CQ** signal – 0.008 x Obs. **(^{13}C)DPD-M1CQ** signal
Correction Type 2: Corr. **(^{13}C)DPD-M1CQ** Signal = Obs. **(^{13}C)DPD-M1CQ** signal – 0.144 x Obs. **DPD-M1CQ** signal
†The use of three decimal places should not be taken to imply that all three are significant.

[DPD] Data for *V. harveyi* WT Strain BB120, Experiment 1, Duplicate Culture 1

Time (h)	Observed Signal for DPD-M1CQ (ion counts)	Observed Signal for (^{13}C)DPD-M1CQ (ion counts)	Correction Type*	Corrected Signal for DPD-M1CQ (ion counts)	Corrected Signal for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)
0.00	3.82×10^3	5.20×10^5	1	-3.45×10^2		0.0
0.50	9.05×10^3	6.23×10^5	1	4.06×10^3		0.1
1.00	2.84×10^4	3.53×10^5	1	2.55×10^4		0.7
1.50	3.92×10^4	6.39×10^5	1	3.41×10^4		0.5
2.00	8.92×10^4	5.93×10^5	1	8.45×10^4		1.4
2.75	1.01×10^5	2.62×10^5	2		2.47×10^5	4.1
3.50	5.80×10^5	8.26×10^5	2		7.42×10^5	7.8
4.25	6.41×10^5	6.26×10^5	2		5.33×10^5	12.0
5.00	5.87×10^5	4.44×10^5	2		3.59×10^5	16.3
5.75	9.58×10^5	6.21×10^5	2		4.83×10^5	19.8
6.50	8.61×10^5	4.99×10^5	2		3.75×10^5	23.0
7.25	7.95×10^5	4.77×10^5	2		3.63×10^5	22.0
8.00	9.24×10^5	6.30×10^5	2		4.97×10^5	18.6
8.75	5.95×10^5	5.01×10^5	2		4.16×10^5	14.3
10.00	2.28×10^5	2.82×10^5	2		2.49×10^5	9.1
12.00	1.77×10^5	3.22×10^5	2		2.97×10^5	6.0

*Correction Type 1: Corr. **DPD-M1CQ** Signal = Obs. **DPD-M1CQ** signal – 0.008 x Obs. **(^{13}C)DPD-M1CQ** signal
Correction Type 2: Corr. **(^{13}C)DPD-M1CQ** Signal = Obs. **(^{13}C)DPD-M1CQ** signal – 0.144 x Obs. **DPD-M1CQ** signal

[DPD] Data for *V. harveyi* WT Strain BB120, Experiment 1, Duplicate Culture 2

Time (h)	Observed Signal for DPD-M1CQ (ion counts)	Observed Signal for (^{13}C)DPD-M1CQ (ion counts)	Correction Type*	Corrected Signal for DPD-M1CQ (ion counts)	Corrected Signal for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)
0.00	$1.67E \times 10^4$	1.06×10^6	1	8.23×10^3		0.1
0.50	1.46×10^4	8.40×10^5	1	7.92×10^3		0.1
1.00	1.56×10^4	6.45×10^5	1	1.04×10^4		0.2
1.50	3.14×10^4	4.93×10^5	1	2.75×10^4		0.6
2.00	5.04×10^4	3.34×10^5	1	4.77×10^4		1.4
2.75	6.80×10^4	1.78×10^5	2		1.68×10^5	4.0
3.50	7.31×10^4	1.03×10^5	2		9.23×10^4	7.9
4.25	5.76×10^5	5.35×10^5	2		4.53×10^5	12.7
5.00	6.35×10^5	4.43×10^5	2		3.51×10^5	18.1
5.75	3.72×10^5	2.26×10^5	2		1.72×10^5	21.6
6.50	1.35×10^6	8.14×10^5	2		6.20×10^5	21.8
7.25	1.79×10^6	1.14×10^6	2		8.78×10^5	20.4
8.00	1.33×10^6	9.87×10^5	2		7.95×10^5	16.8
8.75	7.94×10^5	7.38×10^5	2		6.24×10^5	12.7
10.00	8.14×10^5	1.10×10^6	2		9.80×10^5	8.3
12.00	4.75×10^5	9.81×10^5	2		9.13×10^5	5.2

*Correction Type 1: Corr. **DPD-M1CQ** Signal = Obs. **DPD-M1CQ** signal – 0.008 x Obs. **(^{13}C)DPD-M1CQ** signal
Correction Type 2: Corr. **(^{13}C)DPD-M1CQ** Signal = Obs. **(^{13}C)DPD-M1CQ** signal – 0.144 x Obs. **DPD-M1CQ** signal

[DPD] Data for *V. harveyi* WT Strain BB120, Experiment 2, Duplicate Culture 1

Time (h)	Observed Signal for DPD-M1CQ (ion counts)	Observed Signal for (^{13}C)DPD-M1CQ (ion counts)	Correction Type*	Corrected Signal for DPD-M1CQ (ion counts)	Corrected Signal for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)
0.00	6.65 x10^3	5.19 x105	1	2.50 x103		0.0
0.50	6.87 x10^3	5.03 x105	1	2.84 x103		0.1
1.00	4.88 x10^3	3.63 x105	1	1.98 x103		0.1
1.50	9.70 x10^4	1.15 x10^6	1	8.79 x10^4		0.8
2.00	1.15 x10^5	6.75 x10^5	1	1.10 x10^5		1.6
2.75	3.46 x10^5	8.20 x10^5	2		7.70 x10^5	4.5
3.50	6.90 x10^5	9.27 x10^5	2		8.28 x10^5	8.3
4.25	1.42 x10^6	1.30 x10^6	2		1.09 x10^6	13.0
5.00	1.77 x10^6	1.28 x10^6	2		1.02 x10^6	17.4
5.75	1.84 x10^6	1.19 x10^6	2		9.21 x10^5	20.0
6.50	2.54 x10^6	1.57 x10^6	2		1.21 x10^6	21.1
7.25	3.77 x10^5	2.14 x10^5	2		1.59 x10^5	23.7
8.00	5.29 x10^5	3.65 x10^5	2		2.88 x10^5	18.4
10.00	3.70 x10^5	3.65 x10^5	2		3.12 x10^5	11.9
12.00	5.79 x10^5	8.88 x10^5	2		8.05 x10^5	7.2

*Correction Type 1: Corr. **DPD-M1CQ** Signal = Obs. **DPD-M1CQ** signal – 0.008 x Obs. **(^{13}C)DPD-M1CQ** signal
Correction Type 2 = Corr. **(^{13}C)DPD-M1CQ** Signal = Obs. **(^{13}C)DPD-M1CQ** signal – 0.144 x Obs. **DPD-M1CQ** signal

[DPD] Data for *V. harveyi* WT Strain BB120, Experiment 2, Duplicate Culture 2

Time (h)	Observed Signal for DPD-M1CQ (ion counts)	Observed Signal for (^{13}C)DPD-M1CQ (ion counts)	Correction Type*	Corrected Signal for DPD-M1CQ (ion counts)	Corrected Signal for (^{13}C)DPD-M1CQ (ion counts)	[DPD] (μM)
0.00	7.12 x10^3	4.29 x10^5	1	3.69 x10^3		0.1
0.50	4.09 x10^3	1.88 x10^5	1	2.59 x10^3		0.1
1.00	3.85 x10^3	2.21 x10^5	1	2.08 x10^3		0.1
1.50	1.90 x10^4	3.14 x10^5	1	1.65 x10^4		0.5
2.00	2.76 x10^4	2.18 x10^5	1	2.58 x10^4		1.2
2.75	9.95 x10^4	2.39 x10^5	2		2.25 x10^5	4.4
3.50	2.57 x10^5	3.38 x10^5	2		3.00 x10^5	8.6
4.25	3.32 x10^5	3.10 x10^5	2		2.63 x10^5	12.6
5.00	4.51 x10^5	3.26 x10^5	2		2.61 x10^5	17.3
5.75	5.29 x10^5	3.27 x10^5	2		2.51 x10^5	21.1
6.50	8.13 x10^5	5.43 x10^5	2		4.26 x10^5	19.1
7.25	7.50 x10^5	4.75 x10^5	2		3.67 x10^5	20.4
8.00	4.54 x10^5	3.07 x10^5	2		2.42 x10^5	18.8
10.00	3.96 x10^5	3.98 x10^5	2		3.41 x10^5	11.6
12.00	3.51 x10^5	4.73 x10^5	2		4.23 x10^5	8.3

*Correction Type 1: Corr. **DPD-M1CQ** Signal = Obs. **DPD-M1CQ** signal – 0.008 x Obs. **(^{13}C)DPD-M1CQ** signal
Correction Type 2: Corr. **(^{13}C)DPD-M1CQ** Signal = Obs. **(^{13}C)DPD-M1CQ** signal – 0.144 x Obs. **DPD-M1CQ** signal

V. harveyi [DPD] Data Used to Construct Figure 4B

Time (h)	[DPD] in WT Replicate 1 (μM)	[DPD] in WT Replicate 2 (μM)	Average [DPD] in WT (μM)	Range	[DPD] in *luxS*$^-$ (μM)[†]
0.00	0.0	0.1	0.1	± 0.0	0.054
0.50	0.1	0.1	0.1	± 0.0	-0.024
1.00	0.1	0.1	0.1	± 0.0	0.000
1.50	0.8	0.5	0.6	± 0.1	-0.045
2.00	1.6	1.2	1.4	± 0.2	-0.026
2.75	4.5	4.4	4.5	± 0.0	-0.048
3.50	8.3	8.6	8.4	± 0.1	-0.026
4.25	13.0	12.6	12.8	± 0.2	-0.032
5.00	17.4	17.3	17.3	± 0.0	-0.023
5.75	20.0	21.1	20.5	± 0.5	0.027
6.50	21.1	19.1	20.1	± 1.0	0.043
7.25	23.7	20.4	22.1	± 1.6	0.063
8.00	18.4	18.8	18.6	± 0.2	0.036
10.00	11.9	11.6	11.7	± 0.1	-0.004
12.00	7.2	8.3	7.7	± 0.6	-0.007

[†]The use of three decimal places should not be taken to imply that all three are significant. Instead, these data are used to indicate the lack of signal observed in the *luxS*$^-$ strain.

V. harveyi Cell Growth Data Used to Construct Figure 4B

Time (h)	Cell Growth of WT Replicate 1 (OD_{600})	Cell Growth of WT Replicate 2 (OD_{600})	Cell Growth of *luxS*$^-$ Replicate 1 (OD_{600})	Average Cell Growth (OD_{600})
0.00	-0.009	0.000	-0.011	-0.007
0.50	0.000	0.000	0.000	0.000
1.00	0.034	0.040	0.039	0.038
1.50	0.111	0.120	0.118	0.116
2.00	0.279	0.296	0.294	0.290
2.75	0.830	0.858	0.815	0.834
3.50	1.737	1.800	1.312	1.616
4.25	3.084	3.032	2.344	2.820
5.00	3.132	3.372	3.332	3.279
5.75	3.840	3.972	4.140	3.984
6.50	4.880	4.980	4.896	4.919
7.25	5.828	5.584	5.580	5.664
8.00	6.604	6.476	5.984	6.355
10.00	7.476	7.420	7.264	7.387
12.00	8.436	8.232	7.960	8.209

References

(1) Semmelhack, M. F.; Campagna, S. R.; Federle, M. J.; Bassler, B. L. *Org. Lett.* **2005**, *7*, 569-572.
(2) De Keersmaecker, S. C. J.; Varszegi, C.; van Boxel, N.; Habel, L. W.; Metzger, K.; Daniels, R.; Marchal, K.; De Vos, D.; Vanderleyden, J. *J. Biol. Chem.* **2005**, *280*, 19563-19568.
(3) White, A. W.; Almassy, R.; Calvert, A. H.; Curtin, N. J.; Griffin, R. J.; Hostomsky, Z.; Maegley, K.; Newell, D. R.; Srinivasan, S.; Golding, B. T. *J. Med. Chem.* **2000**, *43*, 4084-4097.
(4) Kasina, S.; Fritzberg, A. R.; Johnson, D. L.; Eshima, D. *J. Med. Chem.* **1986**, *29*, 1933-1940.
(5) Cere, V.; Sandri, E.; Dalmonte, D. *Tetrahedron* **1972**, *28*, 3271-3276.
(6) Gansow, O. A.; Kausar, A. R.; Triplett, K. B. *J. Heterocycl. Chem.* **1981**, *18*, 297-302.
(7) Tyman, J. H. P.; Grundy, J.; Brown, G. R. *J. Chem. Soc. Perkin Trans. I* **1981**, 336-343.