Bacterial tetrabromopyrrole debrominase shares a reductive dehalogenation strategy with human thyroid deiodinase

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General Methods

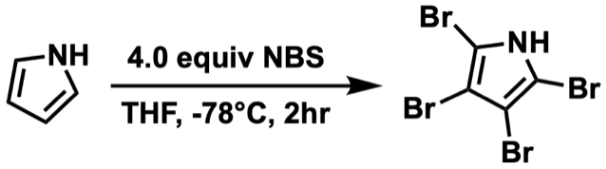
Materials

All materials were purchased from Fisher Scientific, Alfa Aesar, or Sigma-Aldrich. NBS and NCS were purified by recrystallization prior to use with DI water and glacial acetic acid, respectively. Preparative column chromatography was carried out on a Teledyne ISCO CombiFlash® Rf+ Lumen™ flash chromatography system using celite for sample loading and silica gel 60 (EMD, 40-63µm) for the stationary phase. ^{1}H NMR spectra were recorded in $CDCl_3$ (residual solvent referenced to 7.26 ppm) on a Jeol 500 MHz NMR spectrometer. LCMS analysis was conducted on an Agilent 6530 Accurate-Mass Q-TOF MS (MassHunter software, Agilent) equipped with a dual electrospray ionization (ESI) source and an Agilent 1260 LC system (ChemStation software, Agilent) with diode array detector.

Bacterial growth selection

E. coli transformed with plasmids were grown in the presence of corresponding antibiotic: pET28 (kanamycin, 50 mg/L) and pCDFDuet (spectinomycin, 50 mg/L).

Synthetic Methods



2,3,4,5-tetrabromopyrrole: This compound was synthesized using a protocol from previous work.[1] A flame dried 250 mL round bottom flask under Ar was charged with freshly distilled pyrrole (0.377 g, 5.62 mmol, 1.0 equiv) and 20 mL dry THF. The reaction was cooled to -78°C and a solution of NBS (4.00 g, 22.47 mmol, 4.0 equiv) in 65 mL dry THF was added dropwise via addition funnel over 30 minutes. Upon addition of NBS, the reaction was warmed to -10°C for 2 hours. The reaction was then warmed to room temperature and concentrated in vacuo. The crude mixture was resuspended in EtOAc (30 mL) and H_2O (25 mL) and the aqueous layer was extracted with EtOAc (3 x 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo to afford a white crystalline powder that slowly turned gray. Purification by flash chromatography (linear gradient 0-10% EtOAc in hexanes) and concentration under a stream of argon furnished 2,3,4,5-tetrabromopyrrole as an off-white crystalline solid (249.7 mg, 0.652 mmol, 12%). HRMS (ESI) m/z calc'd for C_4HNBr_4 $[M-H]^-$: 381.6729, found 381.6715.

2,3,4,5-tetrachloropyrrole: A flame dried 50 mL round bottom flask under Ar was charged with freshly distilled pyrrole (0.100 g, 1.49 mmol, 1.0 equiv) and 10 mL dry THF. NCS (1.19 g, 8.94 mmol, 6.0 equiv) was directly added to the flask and the reaction was stirred at room temperature for 2 hours. Upon completion, hexanes cooled to 0°C was added to the reaction and the resulting solution was filtered. The filtrate was concentrated in vacuo to afford a white solid that slowly turned brown. The crude material was taken forward without further purification (119.6 mg). HRMS (ESI) m/z calc'd for C_4HNCl_4 $[M-H]^-$: 203.8761, found 203.8768.

Table S1. Primers used in this study

Bmp8 M27A For	CAATTAGAGCCTGTATTTCGCGGAGCGGAACAAAATTTGGGCTTTTT
Bmp8 M27A Rev	AAAAAGCCCAAATTTTGTTCCGCTCCGCGAAATACAGGCTCTAATTG
Bmp8 F55A For	CGGTAGCGTTTGGTGGTTTGGCCAAATGTATCGATGCATTCA
Bmp8 F55A Rev	TGAATGCATCGATACATTTGGCCAAACCACCAAACGCTACCG
Bmp8 Y84F For	GCTGCATGTTTTGCAAAAGTCATTTCTCTCATATTGCGACCCGAACACACGTCAA
Bmp8 Y84F Rev	ACTTTTGCAAAACATGCAGCCCGCAGCTGAGCTACTGATCATTGCAATCGCCCAT
Bmp8 H88V For	GCTGCATGTATTGCAAAAGTGTTTTCTCTCATATTGCGACCC
Bmp8 H88V Rev	GGGTCGCAATATGAGAGAAAACACTTTTGCAATACATGCAGC
Bmp8 L166A For	GCTGCAATTATCGCAATTTGCGGTTTTGCAAATCGCTGGAATGCTG
Bmp8 L166A Rev	CAGCATTCCAGCGATTTGCAAAACCGCAAATTGCGATAATTGCAGC
Bmp8 N170A For	CGGTTTTCTAAATCGCTGGGCTGCTGCTATGGATAGTCAG
Bmp8 N170A Rev	CTGACTATCCATAGCAGCAGCCCAGCGATTTAGAAAACCG
Bmp8 E178Q For	GAATGCTGCTATGGATAGTCAGATACAGGCCGCTCCTAGAG
Bmp8 E178Q Rev	CTCTAGGAGCGGCCTGTATCTGACTATCCATAGCAGCATTC
PLCMD For	ATGCCATCATATGCCTTTAGTTACCCCTTTATCTC
PLCMD Rev	ATGGCATCTCGAGTTAAGACTGATGTTTTCCAACTTCC
pET28 Up	CATATGGCTGCCGCGCGGCACC
pET28 Down	CT CGAGCACCACCACCACCACCACTGAG

Table S2. Data collection and refinement statistics

	Apo Bmp8	Bmp8 C82A Tribromopyrrole Complex
Accession code	6OHI	6OHJ
Data collection		
Space group	$P6_122$	$P6_122$
Cell dimensions		
a, b, c (Å)	65.5, 65.5, 288.7	65.8, 65.8, 288.9
α, β, γ (°)	90.0, 90.0, 120.0	90.0, 90.0, 120.0
Resolution (Å)	144.4-2.3 (2.312-2.272)	57.0-3.2 (3.249-3.194)
R_{sym}*	14.5 (152.2)	17.9 (146.7)
R_{pim}	3.2 (31.7)	4.6 (35.6)
$I / \sigma I$	17.1 (2.2)	14.0 (2.4)
Completeness (%)	97.8 (100)	98.5 (100)
Redundancy	21.9 (23.7)	16.2 (17.5)
$CC_{1/2}$	0.99 (0.80)	0.99 (0.90)
Refinement		
Resolution (Å)	56.7-2.27	57.0-3.19
No. reflections	17455	6716
R_{work} / R_{free} (%)	19.8/23.5	23.2/28.2
No. atoms		
Protein	2896	2906
Water	59	2
B-factors (Å^2)		
Protein	45	95
Water	44	82
Tribromopyrrole		207
R.m.s. deviations		
Bond lengths (Å)	0.005	0.003
Bond angles (°)	1.0	0.5

1. Highest resolution shell is shown in parenthesis.
2. R-factor = $\Sigma(|F_{obs}|-k|F_{calc}|)/\Sigma |F_{obs}|$ and R-free is the R value for a test set of reflections consisting of a random 5% of the diffraction data not used in refinement.

* Due to the high redundancy of data, R_{sym} is a poor indication or quality. Instead, $CC_{1/2}$ and R_{pim} were used.

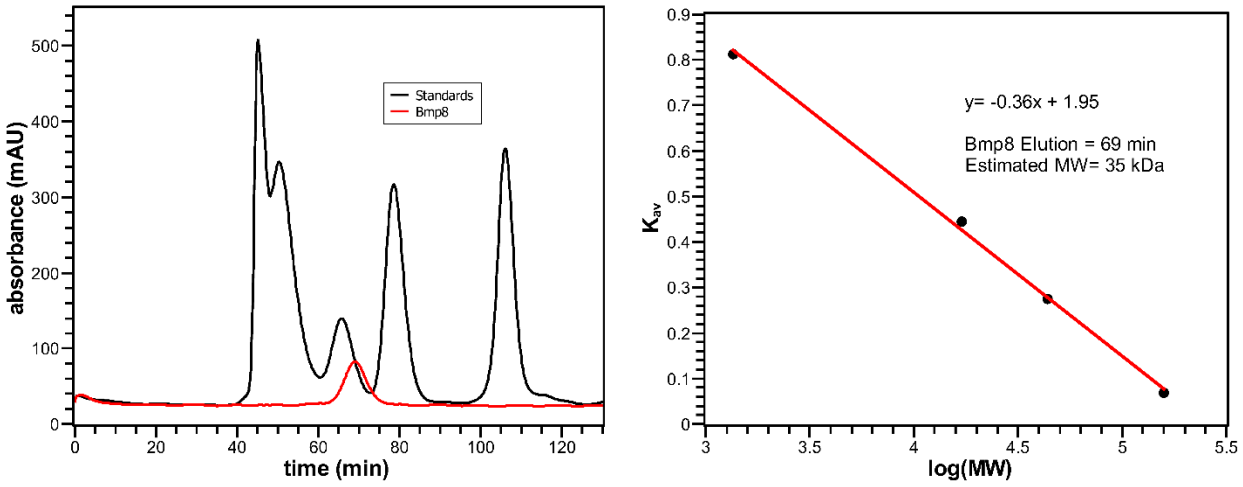


Figure S1. Gel filtration was used to estimate the size of Bmp8 in solution. A standard curve was constructed with BioRad Gel Filtration Standard (#1511901). Bmp8 eluted at 69 mins and was estimated to be ~35 kDa in size. The molecular weight of the His-tag cut Bmp8 is 21,567 Da. The calculated complex is 1.7 monomers in size, and most likely a dimer.

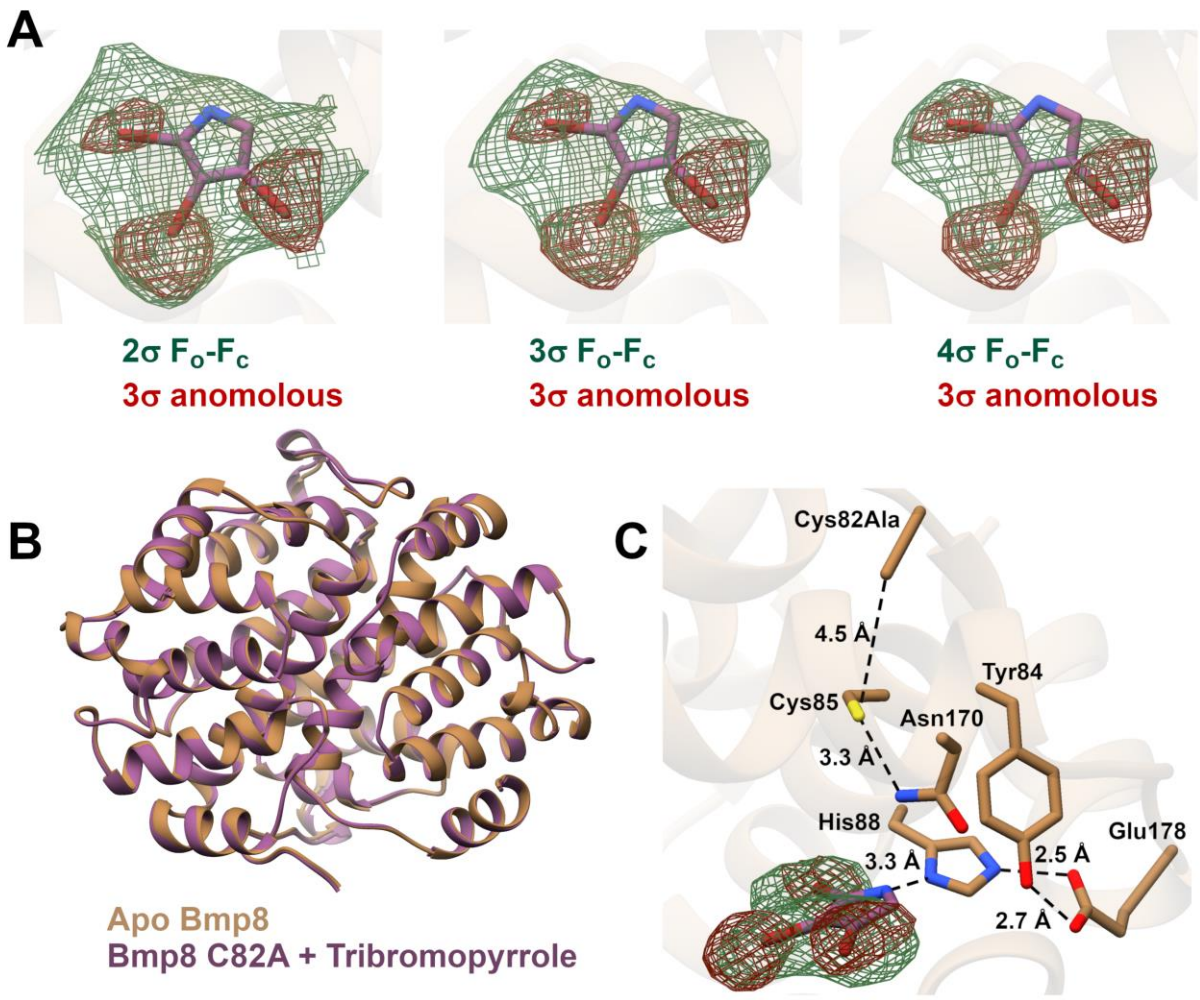


Figure S2. (A) Unhindered view of F_o-F_c and anomalous maps of the Bmp8 C82A + tribromopyrrole structure refined without tribromopyrrole present. (B) Overlay with Apo Bmp8 and Bmp8 C82A + tribromopyrrole indicates that there are no notable structural movements in the presence of product. (C) Active site of Bmp8 C82A + tribromopyrrole structure with distances indicated.

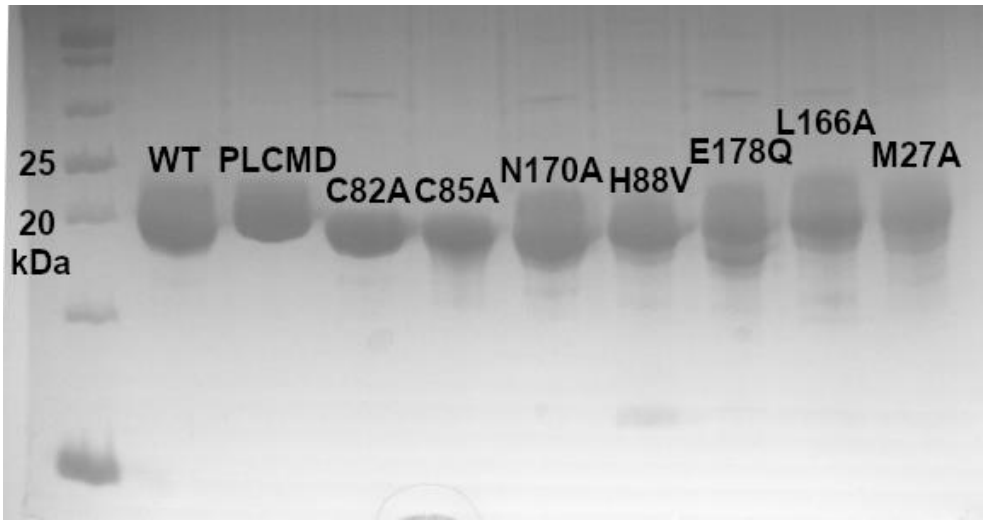
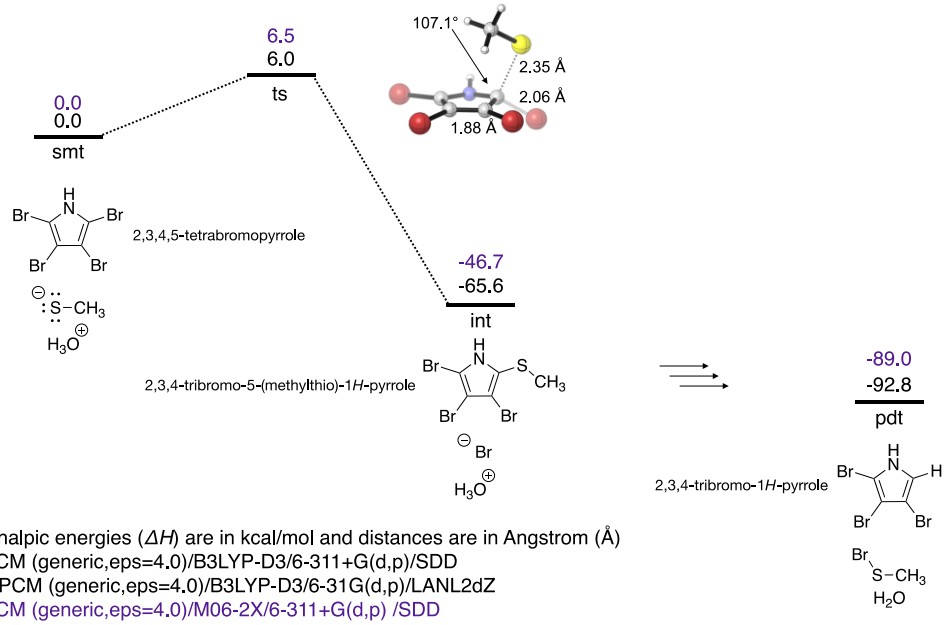


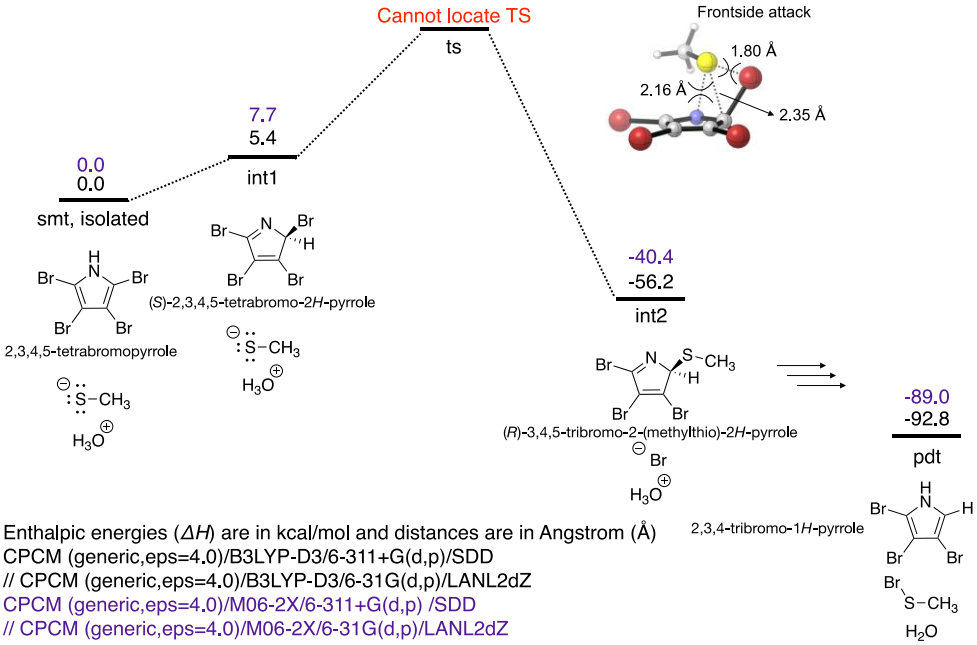
Figure S3. SDS-PAGE gel of proteins purified in this work. Fisher BioReagents EZ-Run Rec Protein Ladder was used as the marker.

Attack on sp^2-hybridized C5 (S_NAr)

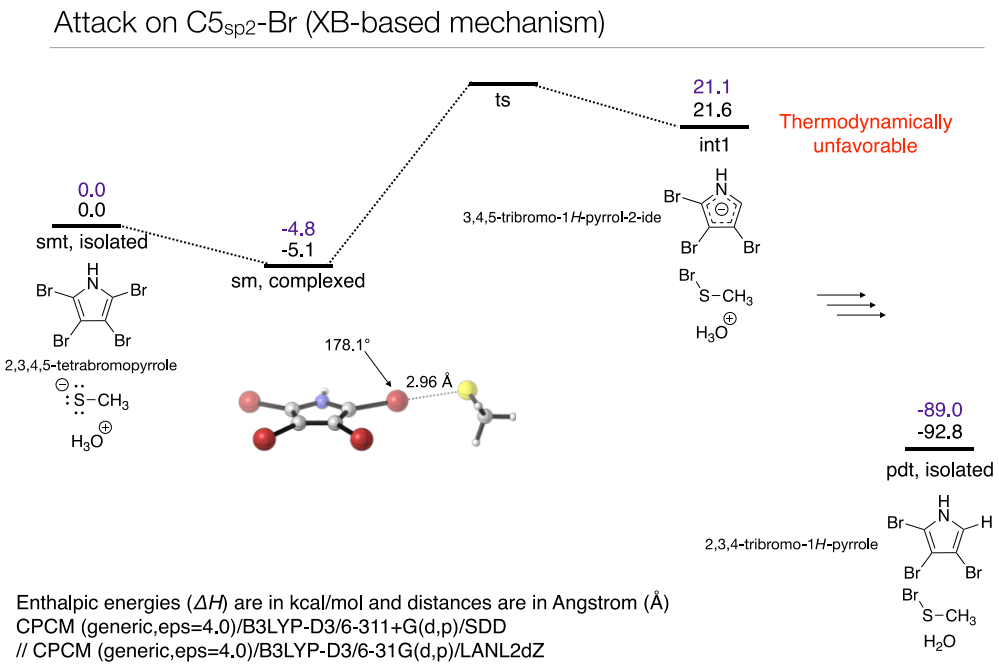


Enthalpic energies (*ΔH*) are in kcal/mol and distances are in Angstrom (Å)
CPCM (generic,eps=4.0)/B3LYP-D3/6-311+G(d,p)/SDD
// CPCM (generic,eps=4.0)/B3LYP-D3/6-31G(d,p)/LANL2dZ
CPCM (generic,eps=4.0)/M06-2X/6-311+G(d,p) /SDD
// CPCM (generic,eps=4.0)/M06-2X/6-31G(d,p)/LANL2dZ

Attack on sp^3-hybridized C5 (S_N2)



Enthalpic energies (*ΔH*) are in kcal/mol and distances are in Angstrom (Å)
CPCM (generic,eps=4.0)/B3LYP-D3/6-311+G(d,p)/SDD
// CPCM (generic,eps=4.0)/B3LYP-D3/6-31G(d,p)/LANL2dZ
CPCM (generic,eps=4.0)/M06-2X/6-311+G(d,p) /SDD
// CPCM (generic,eps=4.0)/M06-2X/6-31G(d,p)/LANL2dZ



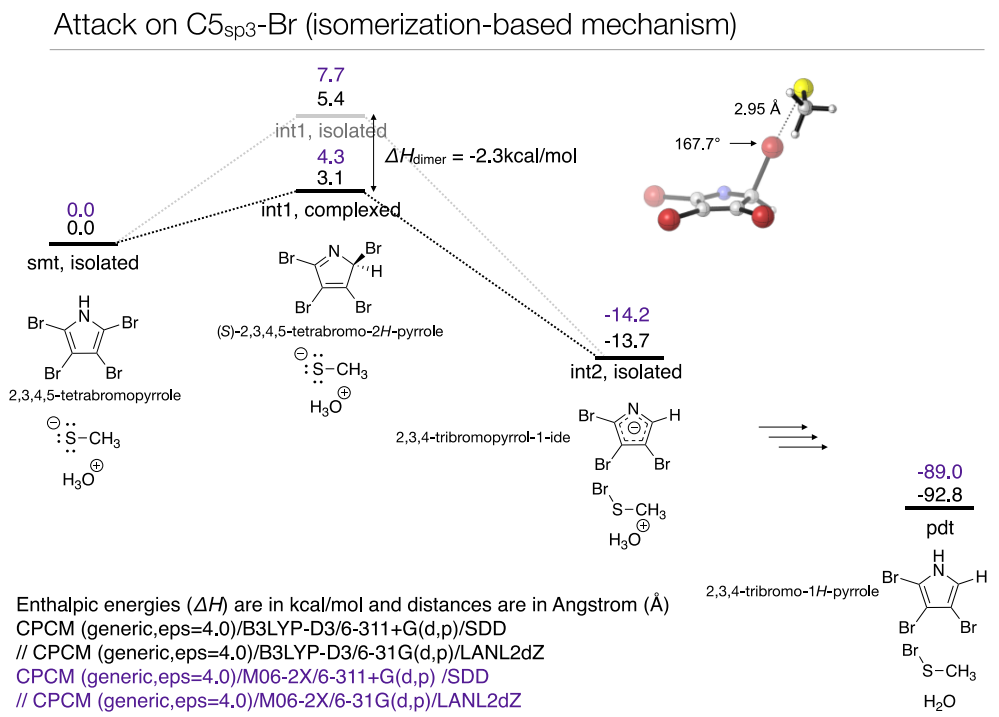


Figure S4. Calculated energetics for all possible debromination mechanisms of 2,3,4,5-tetrabromopyrrole.

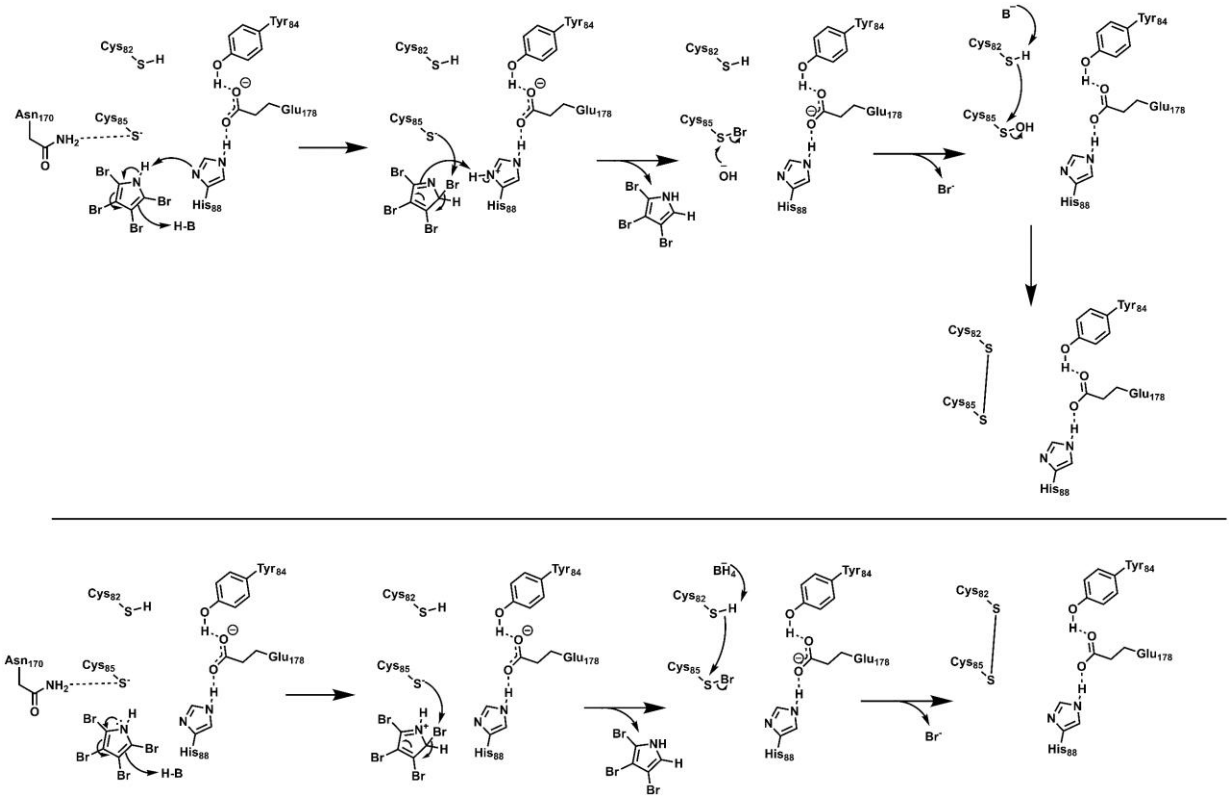


Figure S5. Alternative Bmp8 mechanisms.

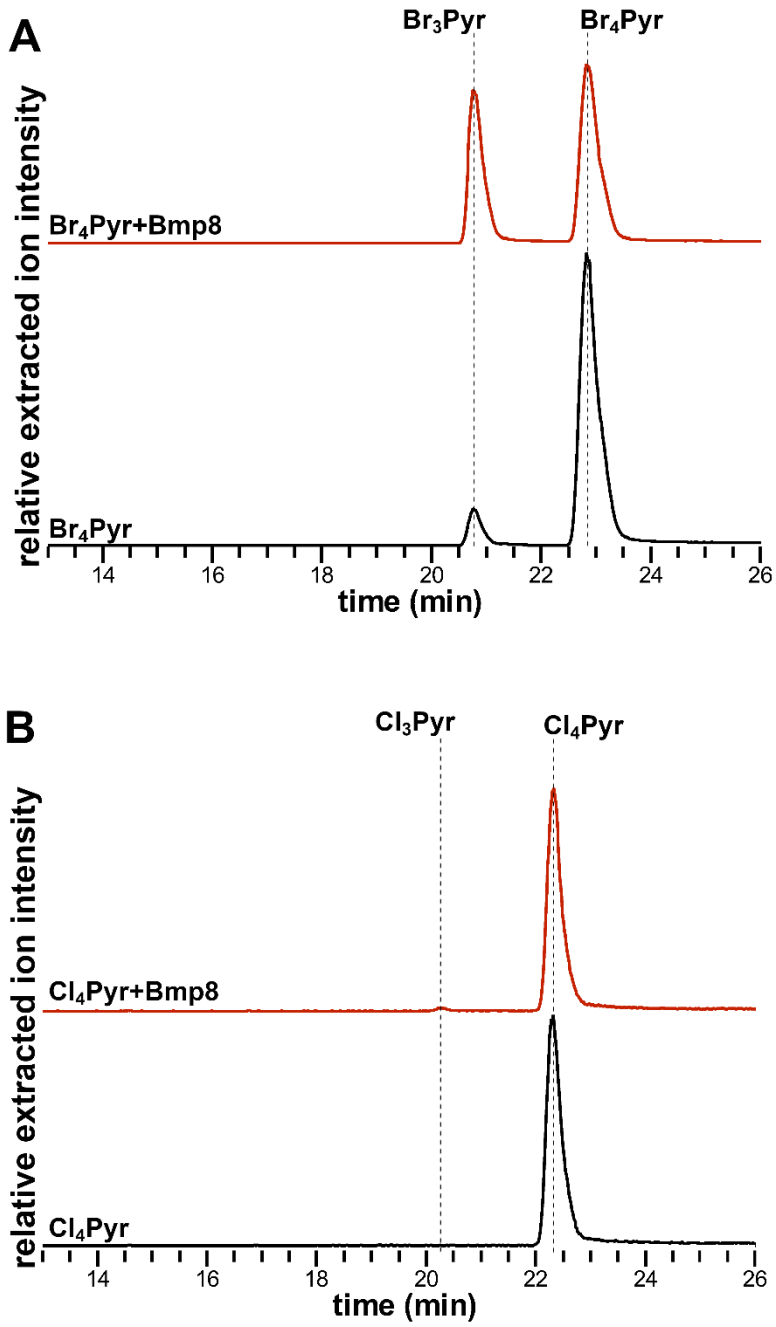


Figure S6. LCMS analysis was used to evaluate Bmp8's relative ability to dehalogenate A) tetrabromopyrrole (Br_4Pyr) compared to B) tetrachloropyrrole (Cl_4Pyr). Traces represent the combined extracted ion chromatograms for the expected major isotopic $[M\text{-}H]^-$ masses of A) Br_4Pyr (381.7 *m/z*) and Br_3Pyr (301.8 *m/z*) or B) Cl_4Pyr (203.9 *m/z*) and Cl_3Pyr (167.9 *m/z*).

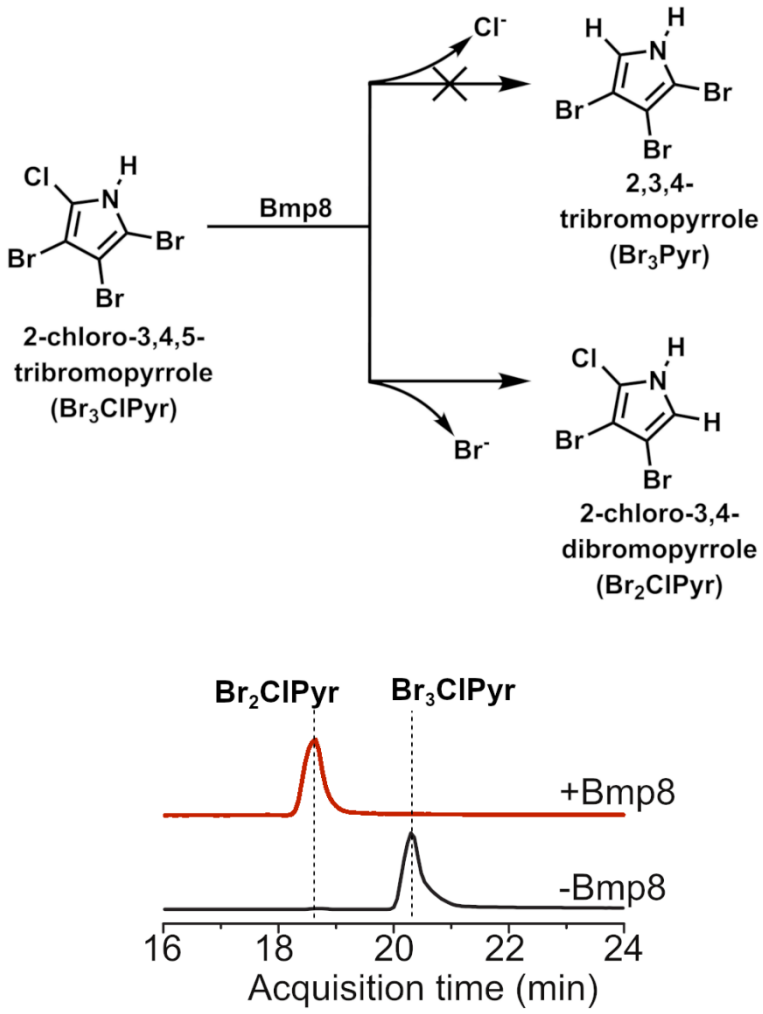
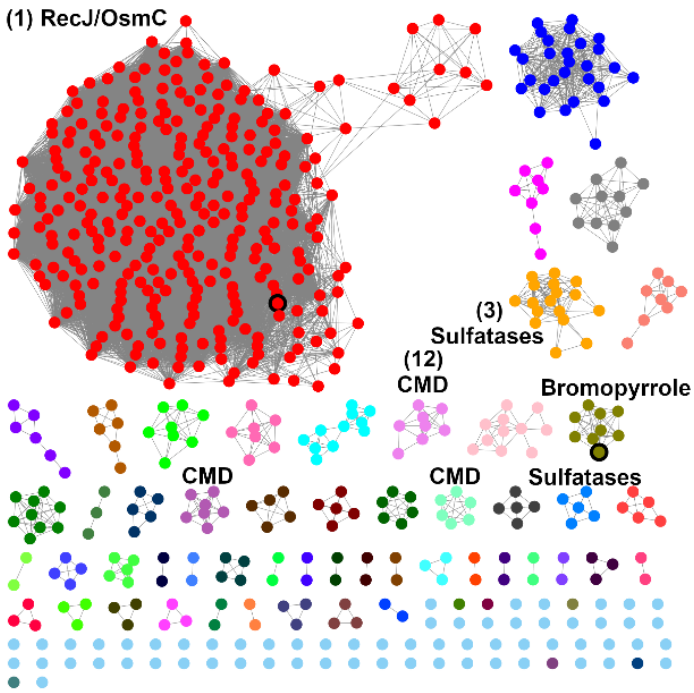
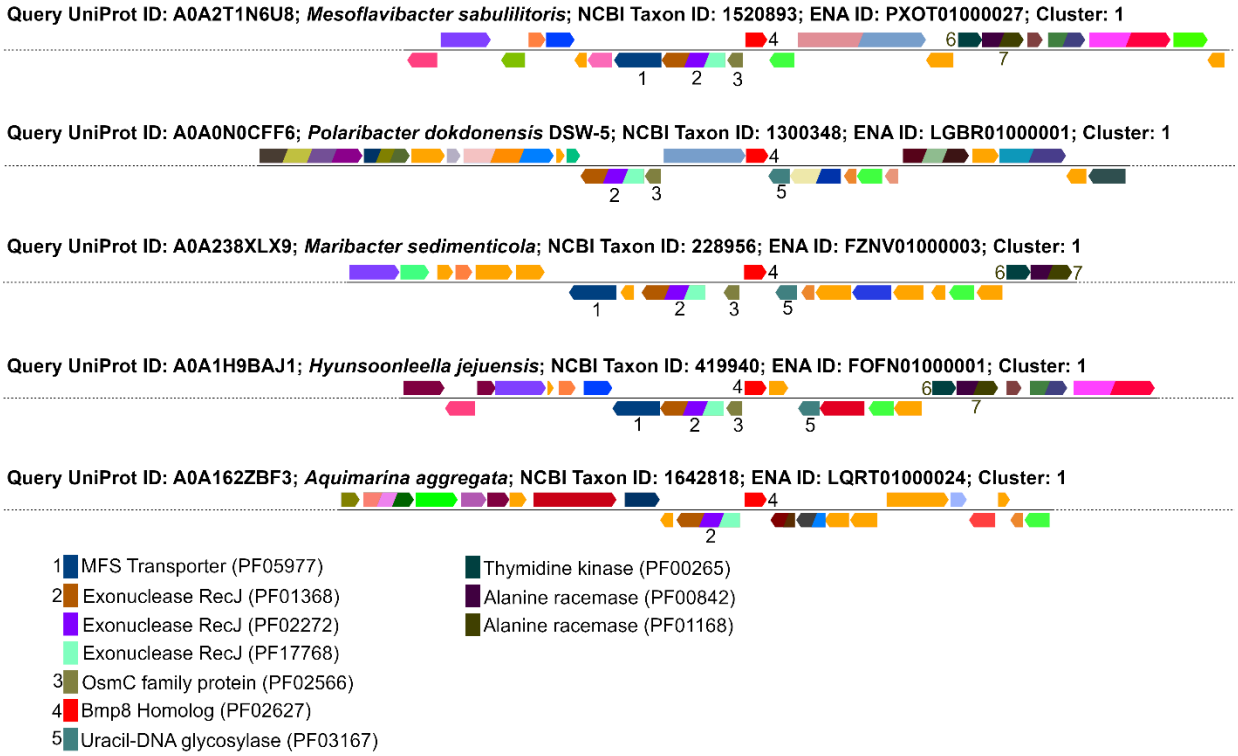


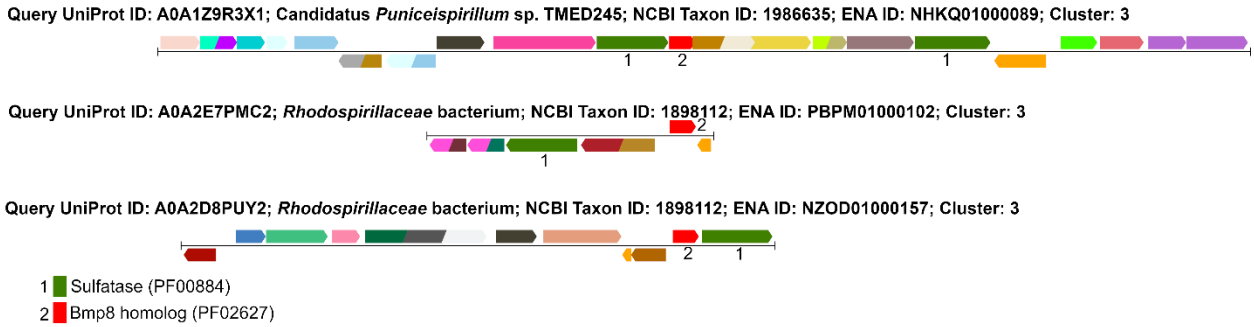
Figure S7. Bmp8 was tested for activity with 2-chloro-3,4,5-tribromopyrrole. Analysis by LCMS indicated that Bmp8 was able to debrominate the substrate, but no significant amount of dechlorinated product was detected. Traces represent the combined extracted ion chromatograms for the expected $[M-H]^-$ masses of Br_3ClPyr (333.7 *m/z*), Br_2ClPyr (255.8 *m/z*), and Br_3Pyr (302.8 *m/z*).



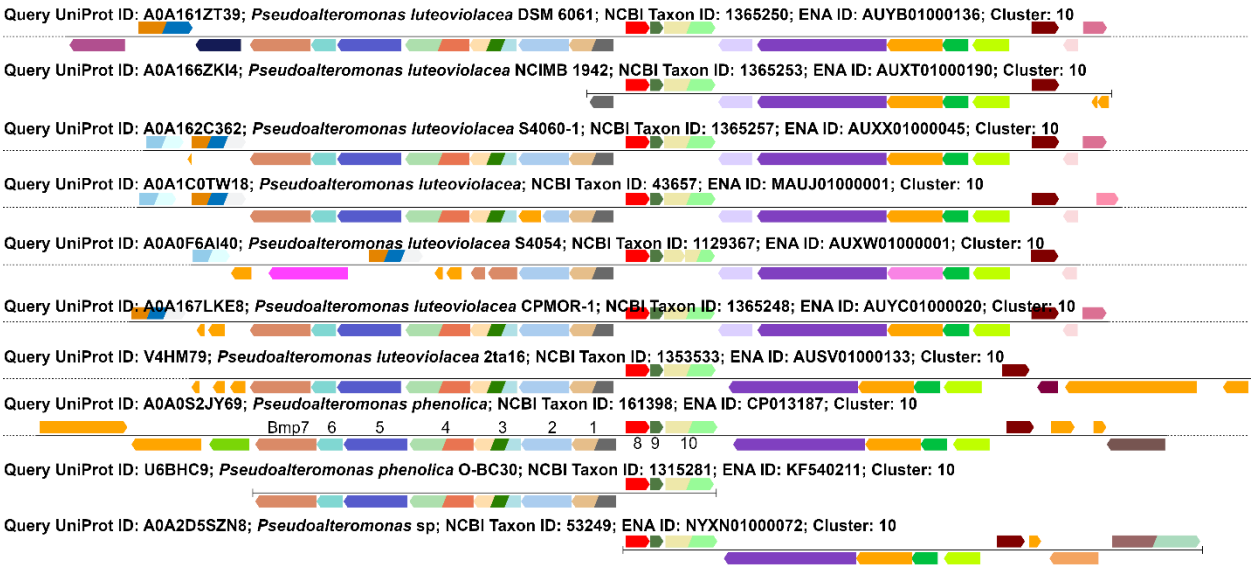
Cluster 1 (RecJ/OsmC) Examples



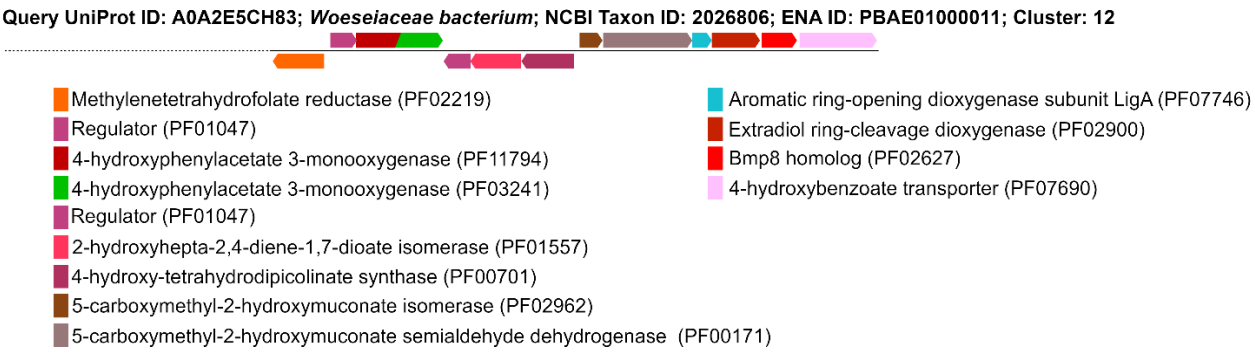
Cluster 3 (Sulfatase) Examples



Cluster 10 (Bromopyrrole)



Cluster 12 (CMD) Example



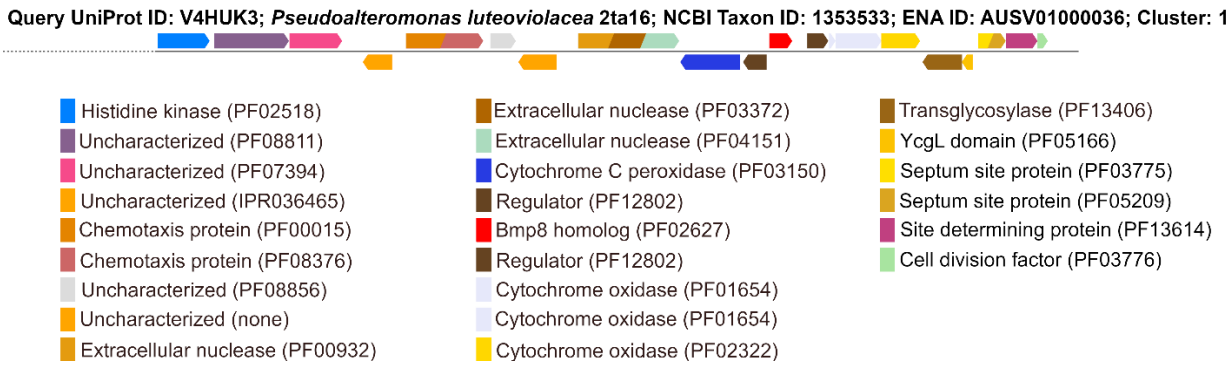


Figure S8. Example biosynthetic genes clusters of different Bmp8 homologs. Annotations and PFAMs were obtained from the genome neighborhood network gene cluster diagrams.[2]

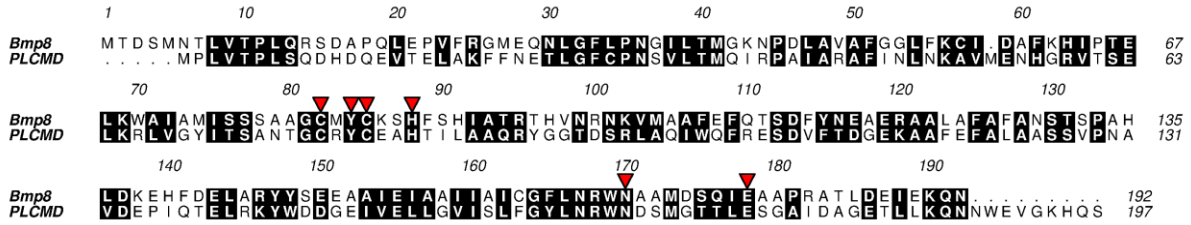


Figure S9. Sequence alignment of Bmp8 and PLCMD. Proposed catalytic residues are indicated.

Cartesian coordinates, energies, and frequencies

1. For all possible debromination mechanisms of 2,3,4,5-tetrabromopyrrole substrate.

Starting Materials

2,3,4,5-tetrabromopyrrole

C	1.11575900	-0.90031800	0.00001300
C	0.71343300	0.41735400	0.00007400
C	-0.71342500	0.41745100	0.00017400
C	-1.11589300	-0.90017900	0.00015400
N	-0.00010200	-1.69535200	-0.00007000
Br	2.87524200	-1.67030500	0.00000300
Br	1.86635300	1.96110400	-0.00005100
Br	-1.86617600	1.96140400	0.00002800
H	-0.00018800	-2.70525100	0.00088100
Br	-2.87537100	-1.67029200	-0.00006200

Thiolate

C	1.13752600	-0.00017000	-0.00003400
S	-0.71668900	0.00002500	0.00000800
H	1.54737200	0.59208500	-0.82923300
H	1.54753200	0.42268200	0.92706100
H	1.54696800	-1.01414100	-0.09774200

Hydronium ion

O	0.00000000	0.00000000	0.00000000
H	0.00000000	0.97267900	0.00000000
H	-0.84236500	-0.48634000	0.00000000
H	0.84236500	-0.48634000	0.00000000

Intermediates

2,3,4-tribromo-5-(methylthio)-1*H*-pyrrole

C	-1.11490400	-0.84482200	-0.08056700
C	-0.49007900	0.38691500	-0.03396500
C	0.90174700	0.13675900	-0.15863400
C	1.09407100	-1.23287300	-0.26886700
N	-0.16170900	-1.80927100	-0.21604400
Br	-2.98405000	-1.28968400	-0.00227900
Br	-1.35793600	2.09873800	0.15524300
Br	2.31068900	1.45782200	-0.14435600
H	-0.33711700	-2.80271900	-0.27620100
S	2.54554800	-2.19967500	-0.46425800
C	3.22705000	-2.15044100	1.24453900
H	4.15307100	-2.72949100	1.22212000
H	3.44748500	-1.12016400	1.52822200
H	2.52786100	-2.60177300	1.94995800

(*S*)-2,3,4,5-tetrabromo-2*H*-pyrrole

C	-1.07488200	-0.88693100	0.33926200
C	-0.55495100	0.48068500	0.11979900
C	0.76485000	0.40299400	0.37370400
C	1.05721900	-1.01745900	0.77437900
N	-0.19868900	-1.74935200	0.69885900
Br	-2.89283700	-1.32206000	0.10616900
Br	-1.55356900	1.96508100	-0.40733800
Br	2.04123200	1.75351800	0.30316800
Br	2.36899800	-1.84000200	-0.46773200
H	1.50357300	-1.10906000	1.76576500

(*R*)-3,4,5-tribromo-2-(methylthio)-2*H*-pyrrole

C	1.11672300	0.75879600	-0.40371800
C	0.26528600	-0.42428900	-0.17355300
C	-0.98804600	-0.01828900	-0.45261100
C	-0.92741700	1.43768800	-0.84800500
N	0.49464800	1.80896000	-0.78570200
Br	2.98974900	0.72089100	-0.14301900
Br	0.84436200	-2.10367300	0.40571400
Br	-2.56160000	-1.00559600	-0.32271600
H	-1.29224700	1.59283900	-1.86923200
S	-1.96588500	2.54228700	0.19310400
C	-1.37418200	2.04220100	1.84959300
H	-1.84904600	2.71796700	2.56306000
H	-1.66908300	1.01398900	2.06933200
H	-0.29003700	2.15246900	1.91762300

2,3,4-tribromopyrrol-1-ide

C	-1.09152400	-0.89398100	-0.00001900
C	-0.00418400	-0.02160600	0.00003300
C	1.11642900	-0.88565000	0.00002800
C	0.62762100	-2.18812400	0.00024300
N	-0.74146600	-2.18314000	-0.00013200
H	1.19407800	-3.11117100	0.00038700
Br	-3.00607600	-0.36562800	-0.00008500
Br	-0.00330100	1.93113100	0.00004100
Br	3.01240900	-0.35609400	0.00001000

3,4,5-tribromo-1*H*-pyrrol-2-ide

C	-1.09805200	-0.84059100	0.00000000
C	0.02810500	-0.04304400	0.00002900
C	1.11616000	-0.95066500	0.00006800
C	0.71003400	-2.27613200	0.00016200
N	-0.68419200	-2.14686400	0.00001500
Br	-3.00423400	-0.33318500	-0.00010700
Br	0.01821000	1.92679800	-0.00001600
Br	3.03110500	-0.37601000	0.00007500
H	-1.32597500	-2.92544600	-0.00000900

Bromide ion

Br	0.00000000	0.00000000	0.00000000

Methyl hypobromothioite

S	1.11339000	-0.73515000	-0.00000200
C	2.06727900	0.81580000	0.00002300
H	1.87267000	1.40055900	0.90001900
H	3.11334800	0.48844200	-0.00086100
H	1.87164900	1.40146000	-0.89923400
Br	-1.05930200	0.10220400	-0.00000100

Transition states

S_NAR at C5 position

C	-0.69057900	-0.30535600	3.02838200
S	-1.98661000	-1.26598000	2.15818100
C	1.22208500	-0.88856400	-0.12110100
C	0.94782500	0.45185600	-0.11762200
C	-0.47570200	0.59080100	-0.16375000
C	-1.02792200	-0.68494800	-0.11737700
N	0.02430600	-1.58267100	-0.23870800
Br	2.91139200	-1.82863200	-0.22113800
Br	2.24941900	1.88745400	-0.13907200
Br	-1.47311900	2.24900000	-0.08800700
H	-0.36796800	0.52513100	2.38938500
H	-1.08032600	0.11877500	3.96005800
H	-0.08268400	-2.52150900	0.11968100
Br	-2.74190700	-1.18948500	-1.19881100
H	0.18988100	-0.91257000	3.26572900

Complexes

2,3,4,5-tetrabromopyrrole and thiolate

C	0.00000000	0.00000000	0.00000000
S	0.00000000	0.00000000	1.84657558
C	7.04351348	0.00000000	2.90868038
C	7.22816262	0.31354069	1.57966547
C	5.92932687	0.40790201	1.00074749
C	5.00246666	0.15130191	1.98618397
N	5.69718115	-0.09554708	3.14326848
Br	8.33225608	-0.28669470	4.32029383
Br	8.93362544	0.57060127	0.69612865
Br	5.52306684	0.82412431	-0.85030275
H	0.51388644	-0.88082502	-0.40094850
H	-1.02661349	-0.00673229	-0.38468016
H	5.27237199	-0.31539681	4.03265139
Br	2.96094362	0.09334663	1.96877066
H	0.50206617	0.88786634	-0.40013072

(*S*)-2,3,4,5-tetrabromo-2*H*-pyrrole and thiolate

C	4.49859100	-0.48167100	1.30202800
S	4.56865500	1.34615400	1.03873000
C	-1.56071200	0.98398400	-0.43719700
C	-1.31102400	-0.42710700	-0.14511000
C	-0.11065700	-0.69255100	-0.70973100
C	0.41245400	0.57547200	-1.30481400
N	-0.63985000	1.57106000	-1.11312700
Br	-3.12920600	1.88540800	0.14691400
Br	-2.41332600	-1.57561400	0.84518900
Br	0.84851900	-2.29483300	-0.67633600
H	4.45184700	-0.73143500	2.36826500
H	3.60749000	-0.90425200	0.82044600
H	5.37730000	-0.98152400	0.87841100
Br	1.99995600	1.12342800	-0.39468500
H	0.66390000	0.48892400	-2.36380300

Products

2,3,4-tribromo-1*H*-pyrrole

C	0.65387200	-2.15499900	0.00026200
C	1.12405500	-0.86084300	0.00010000
C	0.00237900	0.01644400	0.00016200
C	-1.12164900	-0.78380600	0.00006800
N	-0.71777600	-2.09122200	-0.00021000
Br	2.93514100	-0.34532800	-0.00006600
Br	0.03115200	1.89167700	0.00003100
H	-1.34354800	-2.88293400	0.00039400
Br	-2.93106000	-0.30878600	-0.00004500
H	1.18288300	-3.09399100	0.00033900

Water

O	0.00000000	0.00000000	0.11988500
H	0.00000000	0.75748300	-0.47954100
H	0.00000000	-0.75748300	-0.47954100

Table S3. Energies from geometry optimization and single point calculations using B3LYP-D3 for all possible debromination mechanisms of 2,3,4,5-tetrabromopyrrole substrate.

	Geometry optimizations				**Single point calc.**
Structures	**ZPVE**	**E**	**H**	**G**	**H**
Starting Material					
2,3,4,5-tetrabromopyrrole	-260.3939	-260.3521	-260.3411	-260.3921	-261.2504
Thiolate	-438.1987	-438.1620	-438.1580	-438.1860	-438.2080
Hydronium ion	-76.7961	-76.7630	-76.7592	-76.7804	-76.7850
Intermediates					
2,3,4-tribromo-5-(methylthio)-1*H*-pyrrole	-685.3516	-685.2708	-685.2579	-685.3128	-685.9906
(*S*)-2,3,4,5-tetrabromo-2*H*-pyrrole	-260.3831	-260.3420	-260.3314	-260.3821	-261.2418
(*R*)-3,4,5-tribromo-2-(methylthio)-2*H*-pyrrole	-685.3334	-685.2534	-685.2409	-685.2950	-685.9759
2,3,4-tribromopyrrol-1-ide	-247.3475	-247.3089	-247.2996	-247.3458	-248.006
3,4,5-tribromo-1*H*-pyrrol-2-ide	-247.2839	-247.2456	-247.2361	-247.2828	-247.9502
Bromide ion	-13.3209	-13.3209	-13.3186	-13.3371	-13.5721
Methyl hypobromothioite	-451.2642	-451.2254	-451.2199	-451.2542	-451.4738
Transition States					
S_NAR at C5 position	-698.5994	-698.5214	-698.5063	-698.5692	-699.4489
Complexes					
2,3,4,5-tetrabromopyrrole and thiolate	-698.6042	-698.5254	-698.5092	-698.5746	-699.4666
(*S*)-2,3,4,5-tetrabromo-2*H*-pyrrole and thiolate	-698.5832	-698.5042	-698.4894	-698.5542	-699.4534
Products					
2,3,4-tribromo-1*H*-pyrrole	-247.8440	-247.7917	-247.7824	-247.8285	-248.4783
Water	-76.4250	-76.4036	-76.3999	-76.4213	-76.4392

ZPVE = zero-point vibrational energy; E = electronic energy; H = enthalpy; G = Gibbs free energy.

Table S4. Energies from geometry optimization and single point calculations using M06-2X for all possible debromination mechanisms of 2,3,4,5-tetrabromopyrrole substrate.

	Geometry optimizations				Single point calc.
Structures	**ZPVE**	**E**	**H**	**G**	**H**
Starting Material					
2,3,4,5-tetrabromopyrrole	-260.1224	-260.0795	-260.0687	-260.1194	-260.9783
Thiolate	-438.1322	-438.0953	-438.0913	-438.1189	-438.1422
Hydronium ion	-76.7593	-76.7261	-76.7223	-76.7435	-76.7478
Intermediates					
2,3,4-tribromo-5-(methylthio)-1*H*-pyrrole	-685.0644	-684.9820	-684.9693	-685.0238	-685.7047
(*S*)-2,3,4,5-tetrabromo-2*H*-pyrrole	-260.1069	-260.0646	-260.0541	-260.1046	-260.9660
(*R*)-3,4,5-tribromo-2-(methylthio)-2*H*-pyrrole	-685.0432	-684.9617	-684.9493	-685.0034	-685.6892
2,3,4-tribromopyrrol-1-ide	-247.1276	-247.0876	-247.0786	-247.1243	-247.7842
3,4,5-tribromo-1*H*-pyrrol-2-ide	-247.0629	-247.0233	-247.0140	-247.0600	-247.7279
Bromide ion	-13.2702	-13.2702	-13.2678	-13.2863	-13.4956
Methyl hypobromothioite	-451.1451	-451.1057	-451.1003	-451.1344	-451.3589
Transition States					
S_NAR at C5 position	-698.2429	-698.1631	-698.1481	-698.2089	-699.1102
Complexes					
2,3,4,5-tetrabromopyrrole and thiolate	-698.2636	-698.1826	-698.1669	-698.2309	-699.1282
(*S*)-2,3,4,5-tetrabromo-2*H*-pyrrole and thiolate	-698.2415	-698.1611	-698.1468	-698.2074	-699.1136
Products					
2,3,4-tribromo-1*H*-pyrrole	-247.6165	-247.5632	-247.5540	-247.5998	-248.2498
Water	-76.3895	-76.3678	-76.3640	-76.3854	-76.4014

ZPVE = zero-point vibrational energy; E = electronic energy; H = enthalpy; G = Gibbs free energy.

2. For dehalogenation of other substrates

Starting Materials

2,3,4,5-tetrachloropyrrole

C	1.11816700	-0.77433900	-0.00009700
C	0.71448400	0.54448500	0.00008800
C	-0.71450400	0.54449000	0.00009600
C	-1.11816100	-0.77433000	0.00000000
N	0.00000200	-1.56837800	0.00021900
H	0.00000300	-2.57813600	0.00148500
Cl	1.74008000	1.93184900	0.00002800
Cl	2.69703900	-1.45198800	-0.00011500
Cl	-1.74009600	1.93186600	-0.00002700
Cl	-2.69701900	-1.45202500	-0.00009400

2,3,4-tribromo-5-chloro-1*H*-pyrrole

C	-0.94710100	1.57574800	-0.00003200
C	-1.06834200	0.20199000	0.00005100
C	0.25376000	-0.33657600	0.00021000
C	1.12336100	0.73170500	0.00018300
N	0.38949100	1.89021200	0.00000700
H	0.76900900	2.82712700	0.00108200
Cl	-2.14620700	2.80606900	-0.00000300
Br	-2.71670000	-0.78070500	-0.00005500
Br	0.73367600	-2.19505800	0.00003300
Br	3.03502400	0.78150700	-0.00007900

Intermediates

(*S*)-2,3,4,5-tetrachloro-2*H*-pyrrole

C	-1.06854200	-0.79940400	0.18994300
C	-0.58562700	0.59381700	0.03156700
C	0.74243600	0.53480700	0.24455700
C	1.07758000	-0.90319100	0.57436900
N	-0.16522200	-1.65992000	0.47244600
H	1.48148900	-0.99930600	1.58565300
Cl	-1.55548400	1.95624400	-0.34412500
Cl	-2.73359100	-1.22352400	0.00428500
Cl	2.32628800	-1.59319500	-0.54361100
Cl	1.88513900	1.80533200	0.22842700

(*S*)-3,4,5-tribromo-2-chloro-2*H*-pyrrole

C	-1.09874800	0.81862700	-0.21534500
C	-0.16082500	-0.32025100	-0.07262700
C	1.06372600	0.21191400	-0.22657400
C	0.88416000	1.68772500	-0.50410000
N	-0.55341600	1.94998900	-0.43607700
H	1.25329600	1.95620500	-1.49773600
Cl	1.76257500	2.73496800	0.67874800
Br	-3.02461600	0.62301700	-0.07755900
Br	-0.63707500	-2.14669600	0.25892100
Br	2.76246100	-0.66171200	-0.20640700

Methyl hypochlorothioite

S	0.48918100	-0.71932200	-0.00002000
C	1.61532300	0.71218800	-0.00005500
H	1.48101400	1.31521100	0.89905200
H	2.61889500	0.27403700	0.00107800
H	1.48226300	1.31474100	-0.89961700
Cl	-1.35888300	0.25482600	0.00000800

3,4,5-trichloro-1*H*-pyrrol-2-ide

C	-1.09662200	-0.57269100	-0.00002500
C	-0.00367600	0.29630900	0.00001400

C	1.12054600	-0.56497600	0.00001500
C	0.62416900	-1.86624300	0.00021400
N	-0.74341600	-1.86669500	-0.00012500
H	1.19358700	-2.78813100	0.00035300
Cl	2.81049500	-0.07134500	0.00000900
Cl	-0.00311300	2.04697700	0.00001900
Cl	-2.79892200	-0.08736200	-0.00007400

Complexes

2,3,4,5-tetrachloropyrrole and thiolate

C	-4.87792500	0.94197600	-0.08837300
S	-4.99043600	-0.90376100	0.01200700
C	2.14513600	-0.96522100	-0.00568300
C	2.06113000	0.41086400	-0.00103100
C	0.67138400	0.74237300	0.01543700
C	-0.03634700	-0.44257400	0.02028800
N	0.87093800	-1.47248200	0.00734200
H	-5.41046800	1.33513400	-0.96382600
H	-5.31021300	1.42479600	0.79744000
H	0.63014900	-2.45311500	0.00709500
H	-3.83540200	1.27579100	-0.16392100
Cl	-1.74199600	-0.73985900	0.03610600
Cl	-0.00067200	2.33565800	0.02655100
Cl	3.38743300	1.52034600	-0.01296600
Cl	3.52559300	-1.99494300	-0.02405200

(*S*)-2,3,4,5-tetrachloro-2*H*-pyrrole and thiolate

C	-4.20675900	0.78963200	1.08223800
S	-4.41544300	-0.95683200	0.51512200
C	1.86355300	-1.00720500	-0.20496400
C	1.77793900	0.43905400	0.05276600
C	0.66625200	0.84761000	-0.59633600
C	0.04110200	-0.35812000	-1.24523500
N	0.92598500	-1.48893300	-0.93269800
H	-4.13850700	0.85694500	2.17504200
H	-3.28989500	1.22773100	0.66871100
H	-5.04587400	1.42128700	0.76542600
H	-0.03167800	-0.24185800	-2.33044300
Cl	-1.56068400	-0.73181900	-0.61895200
Cl	0.00525600	2.42706300	-0.68405000
Cl	2.87318600	1.36901000	1.00242900
Cl	3.14216100	-1.99355300	0.44630200

2,3,4-tribromo-5-chloro-1*H*-pyrrole and thiolate

C	-5.66809300	0.29876800	0.00000100
S	-5.60325500	-1.55140200	0.00000100
C	1.47587000	-1.07965500	-0.00000100
C	1.30533600	0.28665000	0.00000000
C	-0.10001800	0.52688100	-0.00000100
C	-0.73277900	-0.69919200	-0.00000100
N	0.23877700	-1.66934700	-0.00000100
H	-6.19089000	0.68727600	0.88342500
H	-4.66069700	0.73356200	0.00000800
H	0.05891700	-2.66342100	-0.00000100
H	-6.19087900	0.68727700	-0.88342800
Br	3.08317800	-2.14133700	0.00000100
Br	2.71015300	1.61279500	0.00000000
Br	-0.98382200	2.24481600	0.00000000
Cl	-2.41473500	-1.11806700	-0.00000200

(*S*)-3,4,5-tribromo-2-chloro-2*H*-pyrrole and thiolate

C	-4.54814000	0.10118500	1.15640900
S	-4.74067800	-1.72792300	0.97003500
C	1.44435700	-1.00091600	-0.44476400
C	1.06183800	0.39018500	-0.15530300

C	-0.15865400	0.54471600	-0.70797600
C	-0.54931400	-0.76669900	-1.33525000
N	0.58262800	-1.67923500	-1.10644600
H	-4.46442400	0.39321900	2.21074400
H	-3.64706400	0.46455600	0.64496300
H	-5.40394500	0.64274300	0.73404000
H	-0.73476900	-0.67364000	-2.40884200
Cl	-1.97465800	-1.46760700	-0.57714500
Br	-1.26623400	2.04166300	-0.67809200
Br	2.06563100	1.63838900	0.81249300
Br	3.08892600	-1.73968000	0.14492600

Products

2,3,4-trichloro-1*H*-pyrrole

C	0.65133200	-1.84691400	0.00020100
C	1.12941000	-0.55563500	0.00008100
C	0.01134200	0.33017700	0.00013800
C	-1.11758600	-0.46600600	0.00006000
N	-0.71998300	-1.77497700	-0.00028500
H	-1.34929900	-2.56382200	0.00030700
H	1.17505100	-2.78877100	0.00027500
Cl	0.05073700	2.05717300	0.00000800
Cl	2.79947700	-0.08990900	-0.00007100
Cl	-2.78155800	-0.02563400	-0.0000230

Table S5. Energies from geometry optimization and single point calculations using B3LYP-D3 for dehalogenation of other substrates.

	Geometry optimizations				**Single point calc.**
Structures	**ZPVE**	**E**	**H**	**G**	**H**
Starting Material					
2,3,4,5-tetrachloropyrrole	-2048.5481	-2048.5032	-2048.4934	-2048.5388	-2048.6590
2,3,4-tribromo-5-chloro-1*H*-pyrrole	-707.4433	-707.4007	-707.3901	-707.4396	-708.1030
Intermediates					
(*S*)-2,3,4,5-tetrachloro-2*H*-pyrrole	-2048.5338	-2048.4896	-2048.4801	-2048.5252	-2048.6471
(*S*)-3,4,5-tribromo-2-chloro-2*H*-pyrrole	-707.4119	-707.3701	-707.3597	-707.4090	-708.0896
Methyl hypochlorothioite	-898.2969	-898.2576	-898.2523	-898.2851	-898.3178
3,4,5-trichloro-1*H*-pyrrol-2-ide	-1588.4604	-1588.4195	-1588.4112	-1588.4529	-1588.5609
Complexes					
2,3,4,5-tetrachloropyrrole and thiolate	-2486.7542	-2486.6722	-2486.6572	-2486.7185	-2486.8702
(*S*)-2,3,4,5-tetrachloro-2*H*-pyrrole and thiolate	-2486.7387	-2486.6573	-2486.6427	-2486.7047	-2486.8562
2,3,4-tribromo-5-chloro-1*H*-pyrrole and thiolate	-1145.6314	-1145.5515	-1145.5358	-1145.5999	-1146.3138
(*S*)-3,4,5-tribromo-2-chloro-2*H*-pyrrole and thiolate	-1145.6105	-1145.5311	-1145.5165	-1145.5785	-1146.2989
Products					
2,3,4-trichloro-1*H*-pyrrole	-1589.0347	-1589.0347	-1589.0347	-1589.0347	-1589.0347

ZPVE = zero-point vibrational energy; E = electronic energy; H = enthalpy; G = Gibbs free energy.

3. For dehalogenation of Dios enzyme.

Starting Material

2,6-diiodobenzene-1,4-diol

C	1.20274700	1.81677100	0.00000400
C	-0.00517400	2.51842900	0.00000000
C	-1.21271600	1.81411600	0.00000000
C	-1.19978400	0.42210500	0.00000400
C	-0.00595200	-0.31743100	0.00000200
C	1.18824400	0.42625900	0.00000400
O	-0.07797300	-1.67042200	0.00000000
O	0.06464300	3.88127900	-0.00001500
I	-3.07256400	-0.61801100	0.00000000
I	3.07896000	-0.60262400	0.00000000
H	2.13242600	2.37171500	0.00001200
H	-2.15538800	2.35030100	0.00000200
H	0.81275300	-2.05343900	-0.00003900
H	-0.82634900	4.25669400	0.00006900

Selenol

Se	-1.33577800	-0.49170900	-0.00000500
C	-2.03248100	1.35023200	-0.00000200
H	-1.71071500	1.87246800	-0.89996900
H	-3.12021600	1.23702500	-0.00062800
H	-1.71161700	1.87159700	0.90077100
I	1.21045100	0.06859700	0.00000000

Complex

2,6-diiodobenzene-1,4-diol and selenol

C	-2.85894600	1.73947300	-0.01661900
C	-1.77171300	2.61349900	-0.04812800
C	-0.47107400	2.09497300	-0.06135800
C	-0.24702400	0.72226900	-0.04334500
C	-1.32413100	-0.17876700	-0.01138900
C	-2.61540500	0.36753900	0.00095400
O	-1.05364000	-1.51458400	0.00514400
O	-2.04319600	3.95803500	-0.06495800
I	1.87761500	-0.02698400	-0.06581200
I	-4.34909100	-0.95634500	0.04922200
H	-3.86543500	2.13897900	-0.00676400
H	0.37715700	2.77572300	-0.08636400
H	-1.88380000	-2.01329700	0.02862300
H	-1.20986000	4.44736300	-0.08903400
Se	4.94481100	-0.84428500	-0.07057700
C	5.51276500	0.97064200	0.51728200
H	6.60476200	1.02047100	0.55970800
H	5.15546000	1.73032300	-0.18290000
H	5.11426900	1.19716800	1.5097510

Table S6. Energies from geometry optimization and single point calculations using B3LYP-D3 for Dios enzyme.

	Geometry optimizations				Single point calc.
Structures	**ZPVE**	**E**	**H**	**G**	**H**
Starting Material					
2,6-diiodobenzene-1,4-diol	-404.2599	-404.1723	-404.1613	-404.2109	-404.3372
Selenol	-2439.3853	-2439.3494	-2439.3453	-2439.3734	-2441.5512
Complex					
2,6-diiodobenzene-1,4-diol and selenol	-2843.2158	-2843.0918	-2843.0754	-2843.1411	-2845.8992

ZPVE = zero-point vibrational energy; E = electronic energy; H = enthalpy; G = Gibbs free energy.

Supplementary References

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