Supporting Information for

Campylobacter jejuni KDO8P Synthase, its Inhibition by KDO8P Oxime, and Control of the Residence Time of Slow-binding Inhibition

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Table	S1.	Primer	sequences.
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Name	Primer sequence
	Gateway cloning ^a
Forward	5' - GGGG <u>ACA AGT TTG TAC AAA AAA GCA GGC T</u> TC GAA AAC CTG TAT TTT CAG GGC ATG AAA AAA ATG ATA CTC ATT GCT GGT CC - 3'
Reverse	5' - GGG G <u>AC CAC TTT GTA CAA GAA AGC TGG GT</u> C <i>TTA</i> <i>TTT GTT TTC CTT TAT GAT ATT TTG TAT TTC</i> - 3'
	Stop codon removal ^b
Forward	5' - CATAAAGGAAAACAAA <u>TAA</u> CATCACCATCACCATCAC - 3'
Reverse	5' - GTGATGGTGATGGTGATGTT <u>ATT</u> TGTTTTCCTTTATG - 3'
^a Underline = at	tB restriction site, bold = TEV protease cleavage site, italics =

KDO8PS gene

^b Underline = stop codon, italics = KDO8PS gene

atgaaaaaatgatactcattgctggtccttgcgtgatagaaagcaaagatttgatttttM K K M I L I A G P C V I E S K D L I F aaagttgctgaacagttaaaaaattttaatgaaaatccaaatatagaattttatttcaaa K V A E Q L K N F N E N P N I E F Y F Κ tcaaqttttgataaqqccaatcqcacaaqtattaattcttttcqaqqtcctqqtcttgaa S S F D K A N R T S I N S F R G P G L E gaaggattaaaaattttacaaagcgtaaaagatgaatttggtatgaaaatcttaaccgat E G L K I L Q S V K D E F G M K I L T D atacacgaaagcaatcaagcaaaccctgtaagtgaagtagctgatgtcttgcaaattcct I H E S N Q A N P V S E V A D V L Q I Ρ gcttttttatgtcgtcaaaccgatttacttgtagccgcagcaaaaactaaggcaaaaatt A F L C R Q T D L L V A A A K T K A K Т aatatcaaaaaaqqacaatttttaaacccaaqcqatatcaaatacaqcqttaaaaaaqtt N I K K G Q F L N P S D I K Y S V K K V $\verb|ctacaaacccgtggtatagaagatgaaggctatgaagctgctcaaagaaatggtgttttt||$ L Q T R G I E D E G Y E A A Q R N G V F gtagctgaaagaggggctagctttggctatggaaatttagtagtagatatgcgttcttta V A E R G A S F G Y G N L V V D M R S L gttatcatgcgtgaatttgctccagttatttttgatgctacccatagcgtacaaatgcca V I M R E F A P V I F D A T H S V Q M Ρ ggggctgcaggtggaagtagcggagggaaaagcgaatttgtagaacctttagcaagagcg G A A G G S S G G K S E F V E P L A R Α gcagcagccgtaggcatagatggctttttctttgaaacacatattaatccttgcqaqqct A A A V G I D G F F F E T H I N P C E A ttatgcgatggacctaatatgcttaatcttacacgccttaaaaattgcgttaatacatta L C D G P N M L N L T R L K N C V N T L ctagaaatacaaaatatcataaaggaaaacaaataa LEIQNIIKENK-

Figure S1. Sequence of wild type *C. jejuni* KDO8PS (KDO8PS_{wt}).

The *C*-terminal hexa-histidine tag was removed by introducing a stop codon, TAA (green text) before the His₆-tag sequence.

atgcatcatcatcatcatcacatcacaagtttgtacaaaaaagcaggcttcgaaaacctg M H H H H H H I T S L Y K K A G F E N T. tattttcagggcatgaaaaaatgatactcattgctggtccttgcgtgatagaaagcaaa YFQGMKKMILIAGPCVIESK gatttgatttttaaagttgctgaacagttaaaaaattttaatgaaaatccaaatatagaaD L I F K V A E Q L K N F N E N P N I E ttttatttcaaatcaaqttttqataaqqccaatcqcacaaqtattaattcttttcqaqqt FYFKSSFDKANRTSINSFRG $\verb|cctggtcttgaagaaggattaaaaattttacaaagcgtaaaagatgaatttggtatgaaa||$ P G L E E G L K I L Q S V K D E F G M K atcttaaccgatatacacgaaagcaatcaagcaaaccctgtaagtgaagtagctgatgtc I L T D I H E S N Q A N P V S E V A D V ttgcaaattcctgcttttttatgtcgtcaaaccgatttacttgtagccgcagcaaaaact L Q I P A F L C R Q T D L L V A A A K Т aaggcaaaaattaatatcaaaaaaggacaatttttaaacccaagcgatatcaaatacagc K A K I N I K K G Q F L N P S D I K Y S gttaaaaaagttctacaaacccgtggtatagaagatgaaggctatgaagctgctcaaaga V K K V L Q T R G I E D E G Y E A A Q R aatggtgtttttgtagctgaaagaggggctagctttggctatggaaatttagtagtagat N G V F V A E R G A S F G Y G N L V V D atgcgttctttagttatcatgcgtgaatttgctccagttatttttgatgctacccatagc M R S L V I M R E F A P V I F D A T H S gtacaaatgccaggggctgcaggtggaagtagcggagggaaaagcgaatttgtagaacctV Q M P G A A G G S S G G K S E F V E P ttagcaagagcggcagcagccgtaggcatagatggctttttctttgaaacacatattaatL A R A A A A V G I D G F F F E T H I N $\verb|ccttgcgaggctttatgcgatggacctaatatgcttaatcttacacgccttaaaaattgc||$ P C E A L C D G P N M L N L T R L K N C gttaatacattactagaaatacaaaatatcataaaggaaaacaaataa V N T L L E I Q N I I K E N K

Figure S2. Sequence of His₆-tagged *C. jejuni* KDO8PS (KDO8PS_{H6}). The *N*-terminal hexahistidine tag is shown in blue text. TEV protease recognizes the ENLYFQG recognition site (red text) and cleaves at the Gln↑Gly (Q↑G) bond (underlined).



Figure S3. 13% SDS-PAGE gel of KDO8PSwt.

Lanes: 1) Molecular weight standards ladder; 2) cell lysate; 3) 0 - 60% (NH₄)₂SO₄ fractionation supernatant; 4) 60 - 80% (NH₄)₂SO₄ fractionation pellet; 5) Phenyl Sepharose fraction; 6) Q-Sepharose anion exchange purification; 7, 8, 9) Size exclusion chromatography.



Figure S4. 13% SDS-PAGE gel of KDO8PS_{H6}.

Lanes: 1) Molecular weight standards ladder; 2) lysate; 3) column flow-through; 4) buffer A wash; 5) 10 % buffer B wash; 6) Pure fraction eluted at 80 % buffer B.

Table S2. Dynafit models of the steady state kinetic mechanisms.

A = Mn²⁺, B = PEP, C = A5P, P = KDO8P, Q = P_i. All concentration units are μ M and time units are s⁻¹. Association rate constants have units of μ M⁻¹ s⁻¹.

```
"Random A" kinetic mechanism
[task]
     task = fit
     data = rates
     approximation = king-altman
model random A, ordered B, C
[reaction]
A + B + C ==> APQ
[mechanism]
reaction A + B + C = APQ
E + A
       <==> EA
                               k1
                                     k-1
                          :
EA + B
       <==> EAB
                               k2
                                      k-2
                          :
EAB + C <==> EABC
                               k3
                                      k-3
                          :
EABC
        ==> E + APQ
                               k4
                          :
E + B
        <--> EB
                                k5
                                      k-5
                          :
EB + A
       <--> EAB
                               k6
                                      k-6
                          :
                               k7
                                      k-7
EB + C <--> EBC
                         :
EBC + A <--> EABC
                          :
                               k8
                                      k-8
[constants]
     = 10
k1
k-1
     = 3162 ?
k2
     = 0.1
k-2
     = 5.2 ?
kЗ
     = 10
     = 435 ?
k-3
     = 2.5 ?
k4
     = 0.1
k5
k-5
     = (k5 k-2 k-1 k6) / (k2 k1 k-6)
k6
     = 1
     = 6.5 ?
k-6
k7
     = 1000
   = (k7 k-3 k-6 k8) / (k6 k-8 k3)
k-7
     = 1000
k8
k-8
     = 4910 ?
Ordered kinetic mechanism
[task]
     task = fit
     data = rates
     approximation = king-altman
model sequential ordered steady-state ter ter
[reaction]
A + B + C ==> APQ
```

```
[mechanism]
reaction A + B + C ==> APQ
               :
                          k1 k-1
k2 k-2
 E + A <==> EA
 EA + B <==> EAB
                      :
EAB + C <==> EABC
                           k3
                                k-3
                      :
EABC ==> E + APQ
                      :
                           k4
[constants]
k1 = 10
k-1 = 1323.89 ?
k2 = 1
k-2 = 574.843 ?
k3 = 1000
k-3 = 21038.1?
k4 = 2.37612 ?
```

able S3. Microscopic rate constants for the steady state "random A" sequential ter ter kinetic mechanism for KD08PSwt
ther two rounds of initial scans and optimization, the five best sets of initial estimates were optimized (Figure 2.5). The initial estimates of the
ssociation rate constants (k_1, k_2) were fixed, while the dissociation rate constants (k_1, k_2), and k_{cat} (k_4) were optimized. A = Mn ²⁺ , B = PEP,
= A5P

	Kandom A	Kandom A	Kandom A	Kandom A	Random A	Random A
	195	146	367	373	234	212
<i>k</i> ₁ (µM ⁻¹ s ⁻¹)	10	10	10	10	10	
$k_{-1}(s^{-1})$	$(0.03 \pm 9.90) \times 10^{5}$	$(0.012 \pm 127) \times 10^4$	$(0.012 \pm 130) \times 10^4$	$(0.3 \pm 252) \times 10^4$	$(0.01 \pm 134) \times 10^4$	$(0.1 \pm 47) \times 10^2$
<i>k</i> ₂ (µM ⁻¹ s ⁻¹)	0.1	0.1	0.1	0.1	0.1	0.1
$k_{-2}(s^{-1})$	5 ± 439	7 ± 1016	7 ± 1029	5 ± 1198	7 ± 1079	7 ± 14
<i>k</i> ₃ (µM ⁻¹ s ⁻¹)	10	10	10	10	1000	10
$k_{-3}(s^{-1})$	$(0.4 \pm 3.9) \times 10^3$	$(0.4 \pm 8.5) \times 10^3$	$(0.4 \pm 8.6) \times 103$	$(0.04 \pm 1.10) \times 10^4$	$(0.4 \pm 9.1) \times 10^5$	419 ± 163
k_4 (s ⁻¹)	2.5 ± 0.5	2.5 ± 1.1	2.5 ± 1.2	2.5 ± 1.4	2.5 ± 1.2	2.5 ± 0.1
k₅ (μM ⁻¹ s ⁻¹)	0.1	0.1	0.1	0.1	0.1	0.01
$k_{-5}(s^{-1})^{a}$	252	1.9	2	142	1.3	0.0089
k ₆ (μϺ ⁻¹ S ⁻¹)	, -	÷	0.1	0.1	-	-
$k_{-6}(s^{-1})$	7 ± 8797	$(0.04 \pm 21) \times 10^3$	4 ± 2100	1 ± 2332	$(0.04 \pm 22) \times 10^3$	45 ± 159
<i>k</i> ₇ (µM ⁻¹ s ⁻¹)	1000	10	10	1000	1000	10
$(k_{-7} (s^{-1}))^{b}$	5.8×10^4	4.4×10^{3}	4.4×10^{3}	9.3×10^4	4.5×10^{5}	4.9×10^{3}
k ₈ (μϺ ⁻¹ s ⁻¹)	1000	1000	1000	1000	1000	1000
k_{-8} (s ⁻¹)	$(0.05 \pm 2) \times 10^{5}$	(0.4 ± 5) × 10 ⁵	$(0.4 \pm 5) \times 10^5$	$(0.5 \pm 6) \times 10^5$	$(0.4 \pm 5) \times 10^5$	$(3.8 \pm 4.4) \times 10^3$
		Derived kin	letic constants			
$k_{\text{cat}}(\mathbf{S}^{-1})$	2.5 ± 0.5	2.5 ± 1.1	2.5 ± 1.2	2.5 ± 1.4	2.5 ± 1.2	2.5 ± 0.1
(- M4) V. M.M			10 001 ± 1 2) × 105		(0 03 T 3 E) ~ 102	0 ± /1 E × 105/
$(= k_{-1}/k_{1})$	01 × (8.8 ± 00.0)			201 × (C.7 I CO.0)	201 × (C.7 I CO.0)	(_UI × C.I) I O
K _d ,B (µM)	52 ± 4388	(0.07±10) × 10 ³	$(0.07\pm10) \times 10^3$	(0.05± 12) × 10 ³	$(0.07\pm11) \times 10^3$	70 ± 137
$(= k_{-2}/k_2)$						
K _d , C (µM)	44 ± 395	42 ± 848	42 ± 862	43± 1097	42± 908	42 ±16
(k-=/k=) (nM)	2515	19	20	1425	13	0.89
(k_{-6}/k_{6}) (µM)	$(0.1\pm 88) \times 10^2$	$(0.4\pm 207) \times 10^2$	$(0.1\pm 233) \times 10^2$	$(0.4\pm 220) \times 10^2$	(0.4± 218) × 10 ²	45 ± 159
k-7/k7) (µM)	58	444	444) 93	452	488
k-s/ks) (µM)	5 ± 212	4 ± 460	4 ± 468	5 ± 578	4 ± 490	3.8 ± 4.4

^b Redundant parameter, calculated as $k_{-7} = (k_7 k_3 k_{-6} k_8) / (k_6 k_8 k_3)$. The standard error was not calculated.

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From the 4050 sets of initial conditions (see main text), the top 500 were fully optimized, and the top 100 results yielded the same six sets of optimized parameters multiple times. Thus, each solution set represents multiple optimizations to the same final parameters.

on set		G	0		L	S
nber	#1	#2	#3	#4	£#	9#
M ⁻¹ s ⁻¹)	1000	10	Ļ	Ļ	٢	Ł
(s ⁻¹)	$(2.5 \pm 0.8) \times 10^5$	$(2.4 \pm 0.8) \times 10^3$	170 ± 70	80 ± 42	70 ± 40	70 ± 40
M ⁻¹ s ⁻¹)	0.1	0.1	0.1	—	10	1000
(s ⁻¹)	1 ± 4	1.2 ± 4.0	3 ± 5	660 ± 520	7 ± 6	7 ± 6
M ⁻¹ s ⁻¹)	0.1	1000	1000	1000		.
(s ⁻¹)	2 ± 1	(4.8± 1.1) × 10 ⁴	(4.7 ±1.1) × 10 ⁴	$(2.1\pm1.2) \times 10^4$	15 ± 12	15 ± 12
(s ⁻¹)	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
	Der	ived kinetic constant	S ^a			
(s ⁻¹)	2.5 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
(Ml) (ss	$(3 \pm 0.1) \times 10^{-3}$	0.3 ± 0.01	3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
ss) (JM)	25 ± 1	25 ± 1	25 ± 1	2.0 ± 0.1	2.00 ± 0.01	$(2 \pm 0.1) \times 10^{-3}$
ss) (JM)	49 ± 10	48 ± 11	47 ± 11	21 ± 12	17 ± 12	17 ± 12
s) (JMM)	250 ± 80	240 ± 80	170 ± 70	17 ± 12	140 ± 40	70 ± 40
(Ml) (ss	10 ± 40	10 ± 40	30 ± 50	580 ± 520	930 ± 820	970 ± 870
(Mn) (si	20 ± 9	24 ± 11	60 ± 11	5090 ± 12	67560 ± 12	686310 ± 12

I ne derived kinetic constants are calculated as:⁴¹ $k_{cat} = k_4$, $K_{M,Mn}(ss) = k_4/k_1$, $K_{M,PEP}(ss) = k_4/k_2$, $K_{M,A5P}(ss) = (k_4 + k_3)/k_3$, $K_{I,Mn}(ss) = k_{-1}/k_1$, $K_{I,PEP}(ss) = k_{-2}/k_2$, $K_{I,A5P}(ss) = k_{-2}/k_3 + k_{-3}/k_3$, $K_{I,Mn}(ss) = k_{-1}/k_1$, $K_{I,PEP}(ss) = k_{-2}/k_2$, $K_{I,A5P}(ss) = k_{-2}/k_3 + k_{-3}/k_3$, $K_{I,Mn}(ss) = k_{-1}/k_1$, $K_{I,PEP}(ss) = k_{-2}/k_2$, $K_{I,A5P}(ss) = k_{-2}/k_3 + k_{-3}/k_3$, $K_{I,Mn}(ss) = k_{-1}/k_1$, $K_{I,PEP}(ss) = k_{-2}/k_2$, $K_{I,A5P}(ss) = k_{-2}/k_3$, $K_{I,Mn}(ss) = k_{-1}/k_1$, $K_{I,PEP}(ss) = k_{-2}/k_3$, $K_{I,A5P}(ss) = k_{-2$

Table S5. Purification of wild type KDO8PS.

The purification involved ammonium sulphate precipitation, hydrophobic interaction chromatography on Phenyl-Sepharose, anion exchange chromatography on Q-Sepharose, and size exclusion chromatography on Superose 12.

Fraction	Protein (mg)	Specific activity (s ⁻¹) ^a	Activity Yield (%) ^b	Purification (fold)
Cell lysate	166	1.1	100	1
60 % ammonium sulphate fractionation	50	2.1	58	2
80 % ammonium sulphate fractionation	34	3.1	58	3
Hydrophobic Chromatography	18	5	49	5
Anion Exchange Chromatography	13	4	28	4
Size exclusion Chromatography	5	2.1 °	6	2

^a Specific activity was measured with the Malachite green/ammonium molybdate assay and the concentration of the enzyme was measured with the Pierce™ BCA Protein Assay Kit (Thermo Scientific).

- ^b Activity yield = specific activity × protein × 100%
- ^c The specific activity after size exclusion chromatography decreased 2-fold lower relative to the preceding steps, presumably due to the length of the purification protocol.

Table S6. Oligomeric structure of KDO8PS.

Protein standards and KDO8PS molecular weights and elution volumes on a Superose 12 10/300 GL size exclusion column.

Protein ID	Molecular Weight (kDa)	Ve/V0
Blue dextran 200	2000	1.00
Ferritin	440	1.27
catalase	232	1.43
Aldolase	158	1.47
KDO8PS _{wt}	123 ± 3	1.51
KDO8PS _{H6}	120 ± 13	1.52
Ovalbumin	45	1.64
Ribonuclease	13.7	1.94

 KDOBPS MILLIAGPOVIESKDLIFKVABOLKNENENIEFYFKSSFDKANRTSINSFRGFGLEGGLKILQ KDOBPS MITSNYTTRYPLIAGEOVIESLEMIKSIATKLOPTANDERLDFYFKASSFDKANRTSINSFRGHGLESYKKALR MITSNYTTRYPLIAGEOVIESLEMIKSIATKLOPTANDERLDFYFKASSFDKANRSSIHSFRGHGLESYKKALR MITSNYTTRYPRYTIAGEORIESELLKVGEEIKKLSEKFEVFYKSSFDKANRSSIHSFRGHGLESYKKALR MISCHNADDLEPVLIGGCNNULLSRDLAKTCHTLALREELKGELEIVMRYFEKPRTTVGMGLINDPHMDNSF KDOBPS SVKDEF GMKLITDTHESNQANPVSEVADVLQIPATLCRQTDLLVAAKTAKINIKGOF I [3]GDINVADDLEPVLFGGNNULLSRDLARTLALREELKGELEIVMRYFEKPRTTVGMGLINDPHMDNSF KDOBPS SVKDEF GMKLITDTHESNQANPVSEVADVLQIPATLCRQTDLLLVAAKTAKINIKGOF NINSKOPS SVKEEF GLKITTDTHESNQAPVSEVADVDULOLPATLARTKAKINIKGOF NINSKOPS SVKEEF GLKITTDTHESNQAPVSEVADVDULOLPATLARAKTGRAVNVKGOF NINSKOPS SELKOFF GUKITTDVHESYQASVAAKTADILOLPATLARAKTGRAVNVKGOF NINDGRS SUKDEF GLKITTDTHESNQAPLODALLOLORPAKTAKINIKKOF NINDGRS SILKOFF GVKITTDVHESYQASVAAKTADILOLPATLARAKTAKINIKKOF NINDGRS SILKOFF GVKITTDVHESYQASVAAKTADILOLPATLARAKTAKINIKKOF NINDGRS SILKOFF GVKITTDVHESYQASVAAKTADILOLPATLARAKTAKINIKKOF NINDGRS SILKOFF GVKITTDVHESYQASVAKTADILOLORATTESQTHALANTNIKKOF NINDGRS BILKOFF GVKITTDVHESYQASVAKTADINIKKOF RUDOBFS BILKOFF GVKITKOFK NINDGRS BILKOFF RUDOBFS <	64 74 68 82 117	137 147 141 155 207	209 221 219 279	
KDO8PS KDO8PS WUS KDO8PS DO8PS AHPS (Phe) KDO8PS KDO8PS AHPS (Phe) AHPS (Phe) APA	 MILIAGPCVIESKDLIFKVAEQLKNFNENPNIEFYFKSSFDKANRTSINSFRGFGLEEGLKILG MKTSNTKTPKPVLIAGPCVIESLENLRSIAIKLQPLANNERLDFYFKASFDKANRTSLESYRGFGLEKGLEMLG MEKFLVIAGPCAIESEELLLKVGEEIKRLSEKFR-eVEFVFKSSFDKANRSSIHSFRGHGLEYGVKALF [8] GDINVANDLFFVLFGGMNVLESRDLAMRICEHYVTVTQKLGIPYVFKASFDKANRSSIHSYRGFGLEEGMKIFY [43] HKILKGNDDRLLVVIGPCSIHDPVAAKEYATRLLALREELKGELEIVMRVYFEKPRTTVGWKGLINDPHMDNSF 	 65 SVKDEF GMKILTDIHESNQANPVSEVADVLQIPAFLCRQTDLLVAAAKTKAKINIKKGQF LNPSDIKYSVKK 75 TIKDEF GYKILTDVHESYQASVAAKVADILQIPAFLCRQTDLIVEVSQTNAIVNIKKGQF MNPKDMQYSVLK 69 KVKEEF GLKITTDIHESWQAEPVAEVADIIQIPAFLCRQTDLLLAAAKTGRAVNVKKGQF LAPWDTKNVVEKI 83 ELKQTF GVKIITDVHEPSQAQPVADVVDVIQLPAFLARQTDLVEAMAKTGAVINVKKPQF VSPGQMGNIVDKH 118 QINDGL[13] GLPAAGEFLDMITPQYLADLMSWGAIGARTTESQVHRELASGLSCPVGFKNGTD[4] VAIDAINAAGAPH 	 138 lqtrGIEDEGYEAAQ-RNGVFVAERGASFGYGNLVVDMRSLVIMREFAP VIFDATHSVQMPGAAGGSSGGKSB 148 lktrdssigsptyetal-kngvwlcergssfgyGNLVVDMRSLKIMREFAP VIFDATHSVQMPGGANGKSSGDSS 142KFGGAKEIYLTERGTTFGYNNLVVDFRSLFIMKQWAK VIYDATHSVQLPGGLGDKSGGMRB 156KFGGGNEKVILCDRGANFGYDNLVVDMLGFSIMKKVSGNSPVIFDVTHALQCRDFFGAASGGRRS 208CFLSVTKWGHSAIVnTSGNGDCHIILRGGKepNYSAKHVAEVKEGLNKAG [4] VMIDFSHANSSKQFKKQN 	 210 FVEPLARAAAVGIDGFFFETHINPCEALCDGPNMLNLTRLKNCVNTLLEIQNIIKENK 222 FPFILPRAAAVGIDGLFAETHIDPKNALSDGANMLKPDELEHLVTDMLKIQNLF 23 FIFPLIRAAVAVGCDGVFMETHPEPEKALSDASTQLPLSQLEGIIEAILEIREVASKYYETIPVK 203 FIFPLIRAAVAVGCDGVFMETHPEPEKALSDASTQLPLSQLEGIIEAILEIREVASKYYETIPVK 203 FIFPLIRAAVAVGLAGLFIEAHPDPEHAKCDGPSALPLAKLEPELKQMKAIDDLVKGFEELDTSK 210 QVAELARAGMAVGLAGLFIEAHPDPEHAKCDGPSALPLAKLEPELKQMKAIDDLVKGFEELDTSK 220 QVAELARAGMAVGLAGLFIEAHPDPEHAKCDGPSALPLAKLEPELKQMKAIDDLVKGFEELDTSK 280 DVCADVCQQIAGGeKAIIGVMVESHLVEGNQSLESGEPLAYGKSITDACIGWEDTDALLR-QLANAVK[4] 350
<pre>>> jejuni Pylori >> coli F >> coli F</pre>	<pre>: jejuni KD08PS I. pylori KD08PS I. aeolicus KD08PS I. coli KD08PS I. coli DAHPS (Phe)</pre>	<pre>: jejuni KD08FS I. pylori KD08FS I. aeolicus KD08FS I. coli KD08FS I. coli DAHPS (Phe)</pre>	<pre>: jejuni KD08PS I. pylori KD08PS I. aeolicus KD08PS : coli KD08PS : coli DAHPS (Phe)</pre>	<pre>C: jejuni KD08PS T: pylori KD08PS L. aeolicus KD08PS T: coli KD08PS C: coli DAHPS(Phe)</pre>

Figure S5. Amino acid sequence alignment of KD08PSs and DAHPS.

Amino acid sequences of metal-dependent and -independent KDO8PSs, plus E. coli DAHPS(Phe). The metal-dependent KDO8PSs were from *C. jejuni, Helicobacter pylori, Aquifex aeolicus,* and the metal-independent KDO8PS was from *E. coli.* The conserved metal-binding residues are highlighted in cyan. The green-highlighted K is presumed general acid catalyst in THI breakdown. Numbers in brackets (e.g., [13]) indicate the number of amino acids left out of the alignment figure to save space. The Genbank accession numbers are: *C. jejuni* KDO8PS – EFV08406.1, *H. pylori* KDO8PS – AAD05587.1, *A. aeolicus* KDO8PS – O66496.1; *E. coli* KDO8PS – P0A715.1, *E. coli* DAHPS(Phe) – P0AB92.



Figure S6. Kinetic parameters for KDO8PS_{H6.}

Kinetic constants for KDO8PS_{H6} with rapid equilibrium sequential ordered ter ter kinetic mechanism. Initial velocity vs. [substrate] plots for: (a) Mn^{2+} , (b) PEP, and (c) A5P fitted to equation **1**, **1a**. The inset graphs are Hanes plots, [S]/(v_0 /[E]₀) vs. [S].



Figure S7. KDO8P oxime mode of inhibition of with respect to PEP.

 $KDO8PS_{wt}$ activity was measured in the presence of 500 μ M KDO8P oxime and increasing concentrations of PEP. Increasing [PEP] outcompeted KDO8P oxime, as the activity of the inhibited KDO8PS_{wt} (red dots) returned to the control activity (no KDO8P oxime, blue dot) at higher [PEP].



Figure S8. Fast-binding inhibition of KDO8PS_{H6} by KDO8P oxime.

 $K_i = 10 \pm 2 \mu M$, with 9% residual rate.



Figure S9. Rate of onset of slow-binding inhibition (k_{obs}) of KDO8PS_{wt} as a function of [KDO8P oxime].

Residual rate ($v_{0,t}/v_{0,0}$) versus incubation time for varying [KDO8P oxime] was fitted to equation **7** to derive k_{obs} at each inhibitor concentration. The k_{obs} values were used directly in equation **8** (Figure 8) to fit $k_{on,conc}$ and $k_{off,conc}$. There was enzyme inactivation when [I] = 0, presumably due to enzyme denaturation, characterized by $k_{denaturation} = 0.011 h^{-1}$. In contrast there was no continued loss of activity at high [I] and long incubation times, implying that the inhibitor stabilized the enzyme. Therefore, it was not clear whether $k_{denaturation}$ should be subtracted from k_{obs} . Subtracting $k_{denaturation}$ and fitting the new k_{obs} values to equation **8** yielded $k_{off,conc} = (0.9 \pm 5.2) \times 10^{-4} h^{-1}$, or $t_R = 80$ days to ∞ . The corresponding analysis for KDO8PSH6, where the treatment of $k_{denaturation}$ was more clearcut, yielded $k_{off,conc} = 0.016 h^{-1}$, in good agreement with the more conservative treatment of the wild type data, where $k_{off,conc} = 0.014 h^{-1}$ (Figures S10 – S12). This supported the more conservative treatment of the KDO8PS_{wt} data.



Figure S10. Rate of onset of slow-binding inhibition (k_{obs}) of KDO8PS_{H6} as a function of [KDO8P oxime].

For the His₆-tagged enzyme, residual rate ($v_{0,t}/v_{0,0}$) versus incubation time for varying [KDO8P oxime] was fitted to equation **7** to derive k_{obs} at each inhibitor concentration. Unlike KDO8PS_{wt}, the rate of inactivation when [I] = 0, $k_{denaturation}$, was significant, and was subtracted from k_{obs} for other inhibitor concentrations.



Figure S11. Rate of onset of slow-binding inhibition (k_{obs}) of KDO8PS_{H6} as a function of [KDO8P oxime].

The k_{obs} values from Figure S10 were fitted versus [KDO8P oxime] (eq. 8). $k_{on,conc} = 0.10 \pm 0.01 h^{-1}$, $k_{off,conc} = 0.016 \pm 0.002 h^{-1}$, with the Hill coefficient fixed at 1.8 (see Figure S12).



Figure S12. Concentration dependence of slow-binding inhibition of $KDO8PS_{H6}$ after 46 h preincubation.

Initial velocities were fitted to equation **9**. $IC_{50} = 590 \pm 110 \mu M$, $n = 1.8 \pm 0.5$. The Hill coefficient was lower than for wild type enzyme, 3.5, but still exhibited cooperativity. Inhibitor concentrations are those in the preincubation mixture, before dilution into the assay mixture.