Table S1. Mutagenic primers used in the creation of BphI variants. Site-directed mutagenesis to replace Tyr-290 was carried out according to the megaprimer method. The other mutations were performed according to a modified Quikchange (Stratagene) method which uses non-overlapping primers.

Mutation		Sequence 5' → 3'
R16A	FOR	GACATGACGCTGGCCGACGGCATGCAC
	REV	GTGCATGCCGTCGGCCAGCGTCATGTC
R16K	FOR	GACATGACGCTGAAGGACGGCATGCAC
	REV	GTGCATGCCGTCCTTCAGCGTCATGTC
H20A	FOR	GCTGCGCGACGGCATGGCCCCCAAGCGCCACCAG
	REV	CTGGTGGCGCTTGGGGGCCATGCCGTCGCGCAGC
H20S	FOR	GCTGCGCGACGGCATG TCCCCCAAGCGCCACCAG
	REV	CTGGTGGCGCTTGGGGGACATGCCGTCGCGCAGC
L87A	FOR	CAGTGCCGCGCTCTTGCCCGGCATCGGCACC
	REV	GCAAGAGCGCGGCACTGACCTTGGCCTGCTTC
L89A	FOR	CTGCTCGCGCCCGGCATCGGCACCGTCGAAC
	REV	GCCGGGCGCGAGCAGGGCACTGACCTTGGC
Y290F	FOR	AAAGCTGGAAAAGACCCCGGC
	REV	
Y290S	FOR	AAAGCTGGAAGAGACCCCGGC
	REV	

SUPPLEMENTARY FIGURE LEGENDS

Fig S1. Active site of DmpG showing the proposed alternative conformation of Tyr-291. The substrate, shown with carbon atoms in cyan, was modeled into the active site of DmpG by superimposing the pyruvyl moiety of (4*S*)-HOPA on the experimentally observed oxalate. Overlap in van der Waals radii was observed between C4 of the substrate and the hydroxyl oxygen of Tyr-291 (~2.6 Å; carbon atoms of Tyr-291 shown in grey). A clockwise rotation of 14.5º about the Cα of Tyr-291 (carbon atoms in green) afforded by a space in the active site, would relieve this steric clash. Distance measurements between the hydroxyl oxygens of the two different conformations of Tyr-291 and the C4 of the modeled HOPA are shown in red. Numbers in brackets correspond to the residue numbers in BphI. Image was generated using PyMOL

Fig S2. Multiple sequence alignment of aldolases which are orthologs of BphI and associate with a dehydrogenase. The position of residues which were studied are shown in black arrows and the numbers correspond to residue numbers in BphI. DmpG (P51016) from *Pseudomonas putida* CF600 is 54% identical, MhpE *Escherichria coli* (P51020) is 56% identical, NahM from *Psuedomonas putida* (P51017) is 74% identical and XylK (P51019) from *Psuedomonas putida* is 55% identical. Uniprot accession numbers are shown in brackets. This alignment was generated using ClustalX (*17*) and visualized using ESPript (*18*).

Fig S3. Circular dichroism spectroscopy of BphI and R16A and R16K variants. Far-UV circular dichroism spectra were recorded on a Jasco J-815 spectropolarimeter using a 1 mm path-length quartz cuvette. All protein samples were set to a final concentration of 1 μM in 20 mM HEPES, pH 8.0. Measurements were made in continuous scanning mode at a scanning speed of 50 $nm \cdot min^{-1}$ with a time constant of 1 s and bandwidth of 1 nm at 25 °C

Fig S4. Active site of DmpG with bound pyruvate. Model was generated by superimposing pyruvate on the experimentally determined structure containing the pyruvate enolate analogue, oxalate. The Cδ of Leu-87 is 3.6 Å from the C3 carbon of pyruvate. Numbers in brackets correspond to the residue numbers in BphI. Images were generated using PyMOL.

Fig S1.

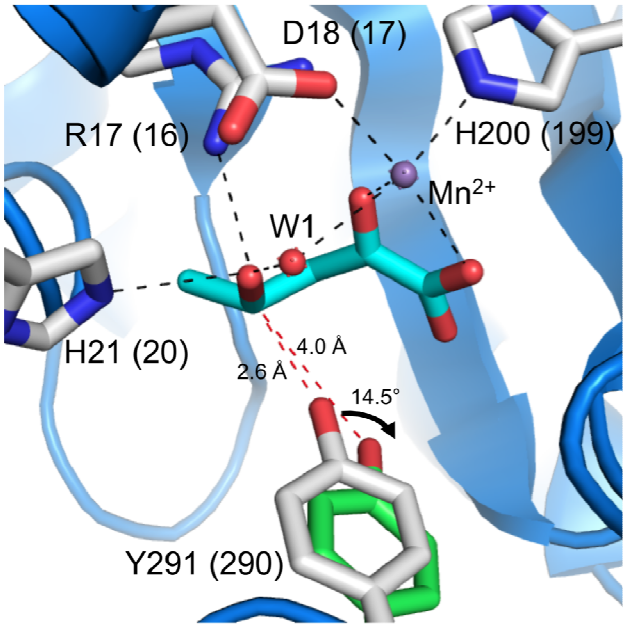


Fig S2.

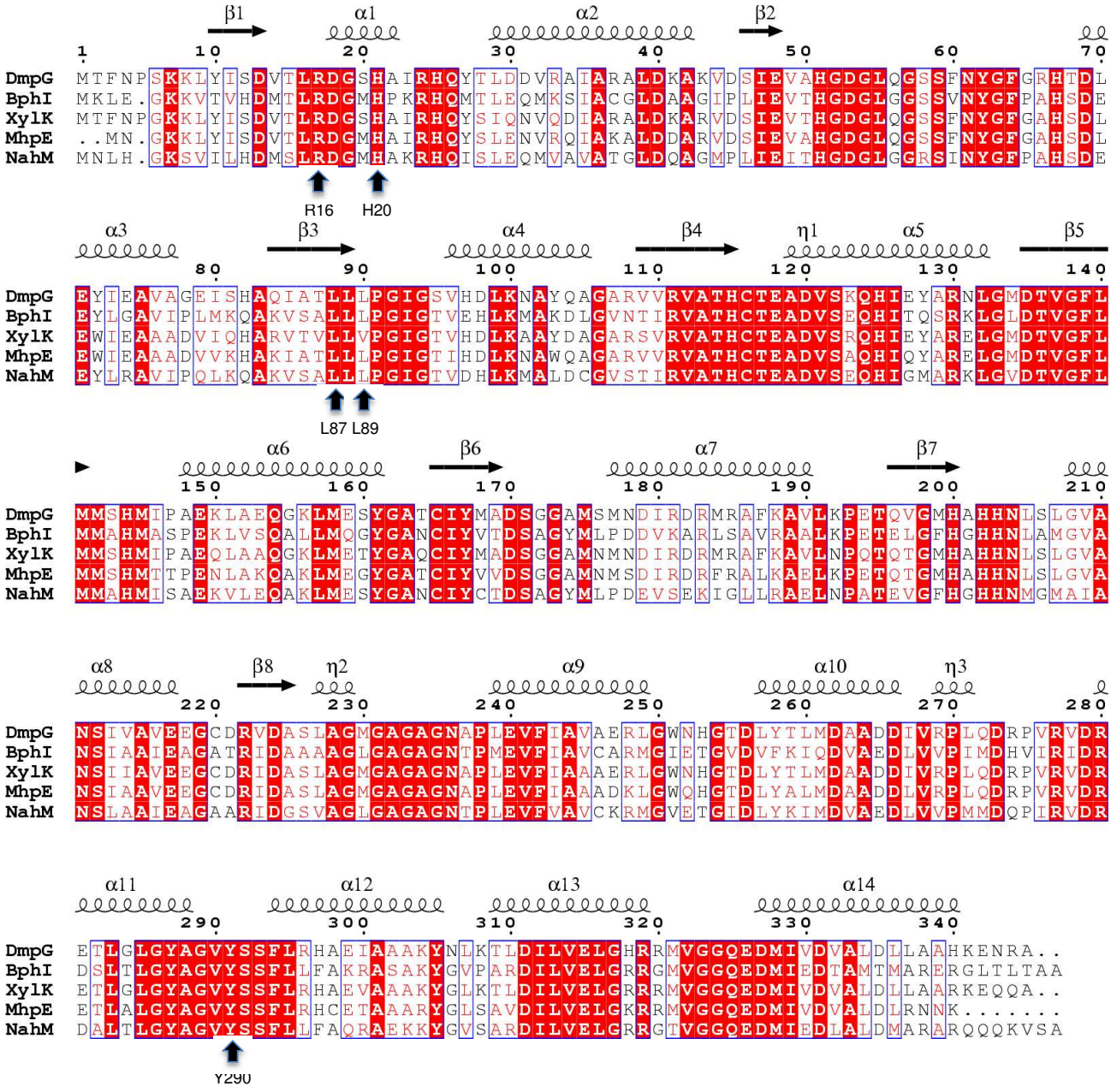


Fig S3.

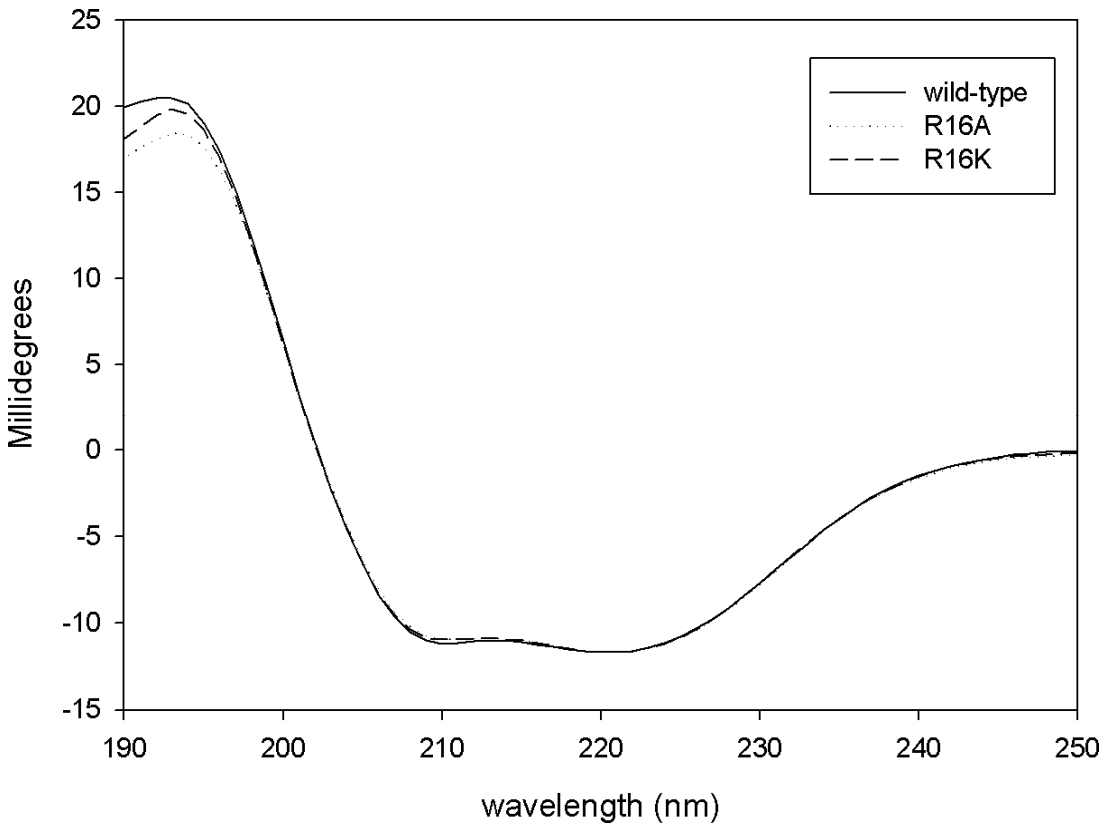
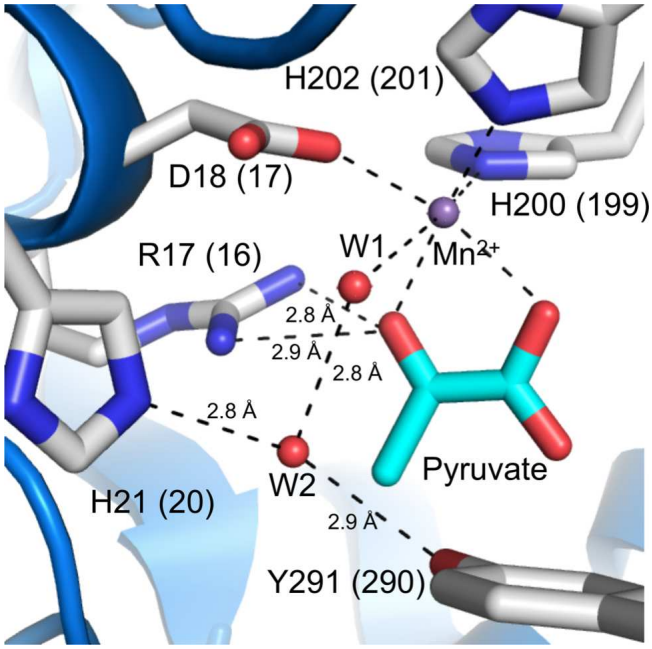

25
20
15
10
5
0
-5
-10
-15
Millidegrees
190
200
210
220
230
240
250
wavelength (nm)
wild-type
R16A
R16K

Fig S4.


H202 (201)
D18 (17)
H200 (199)
R17 (16)
W1
Mn2+
2.8 Å
2.9 Å
2.8 Å
2.8 Å
Pyruvate
W2
H21 (20)
2.9 Å
Y291 (290)