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Structure and Mechanism of an Unusual Malonate Decarboxylase and Related Racemases

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The arylmalonate decarboxylase (AMDase, E.C. 4.1.1.76) from *Bordetella bronchiseptica*, catalyses the enantioselective decarboxylation of α-aryl-α-methylmalonates to give optically pure α-arylpropionates (Figure 1).[1] The AMDase is unusual amongst malonate decarboxylases as it does not require biotin or any other co-factors for activity, and does not involve formation of a malonyl thioester-enzyme intermediate.[1d,e] The sequence of the AMDase shows similarity to the Glu and Asp racemases.[2] It is suggested[3,4a] that two Cys residues at the active site of the Glu racemase function in general acid–base catalysis, abstracting the α-proton from the Glu substrate to generate a planar enediolate intermediate which is re-protonated from the opposite face leading to the racemate (Figure 1). The AMDase, on the other hand, possess only one essential Cys residue (Cys188). This led to the suggestion[1e] that the decarboxylation of the substrate 2-methyl-2-phenylmalonate (**2**), catalysed by the AMDase results in an enediolate intermediate, which is protonated on the *si*-face by Cys188 to form (2*R*)-phenylpropionate (**4**; Figure 1).

Whilst the structure of the AMDase is unknown, a number of crystal structures of the related Asp/Glu racemases are available.[4] Despite this, the mechanisms proposed to date for these racemases,[3,4a] and the related AMDase[1e] do not account for how the common putative enediolate intermediate might be stabilised. Indeed the pK_a of the α-proton of a carboxylate anion is >29, which reflects the instability of an endiolate dianion.[5a] Whilst the negative charge of enediolate formed by the AMDase (Figure 1) can be delocalised into the aryl substituent, to some extent, this effect alone could not stabilise the dianion sufficiently to account for the high efficiency of the enzymatic decarboxylation. In the case of the mechanistically related enolase superfamily the enediolate intermediates formed from abstraction of the α-proton of carboxylate substrates are stabilised by coordination to Mg^{2+}.[5] The fact that the AMDase and related Asp/Glu racemases do not require Mg^{2+}, or any other co-factor, which might stabilise the postulated enediolate intermediate, makes them particularly intriguing enzymes from a mechanistic point of view. In addition to this the homochiral carboxylic acid products of the AMDase are also potentially valuable chiral precursors for the synthesis

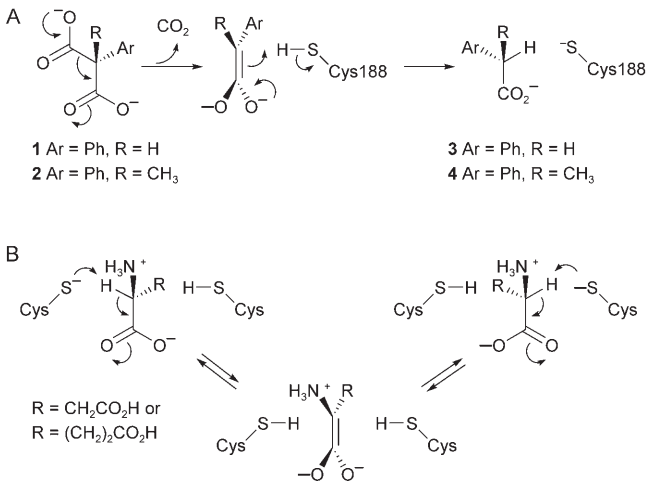

Figure 1. A) The AMDase catalyses decarboxylation of a range of arylmalonates (e.g. Ar = Phenyl, 2-naphthyl or 2-thienyl where R = H or methyl). B) Proposed mechanism for racemisation of Asp and Glu, catalysed by the Asp/Glu-racemase family.

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of pharmaceuticals and other products. Finally the AMDase has also been shown to catalyse an intramolecular aldol-type reaction, whilst site-directed mutagenesis has been used to switch the enantioselectivity of the decarboxylase and also to generate a new racemase activity.[6] This catalytic plasticity therefore makes the AMDase an ideal candidate for the rational redesign and directed evolution of new or improved biocatalysts.

To establish the structure and mechanism of the *B. bronchiseptica* AMDase, the enzyme was overproduced in *E. coli* as a *C*-terminal His_6-fusion protein, purified by Ni-affinity chromatography and shown to possess similar activity to the native enzyme[1d] (Table 1). Following this, the AMDase was crystallised and the structure was solved to 1.5 Å using multiple isomorphous replacement. The structure reveals overall similarity with the crystal structures of Asp/Glu racemases,[4] and is most similar to Asp racemase from *Pyrococcus horikoshii* OT3[4b] (pdf code 1 JFL, Z score 14.6 with a r.m.s.d. of 3.5 Å for 192 Cα atoms). The AMDase is a tightly folded monomer that consists of two four-stranded parallel β-sheets each surrounded by α-helices (Figure 2A). Surprisingly, a single phosphate molecule is tightly bound near the active site Cys188, at the end of a solvent accessible channel located at the end of two β-strands. Notably the phosphate O1/O2 atoms are engaged by six hydrogen bonds of two adjacent oxyanion holes[7] (Figure 2B), which are described as the "dioxyanion hole" from here on in. Kinetic studies also show that phosphate is a weak competitive inhibitor (K_i = 8 mM) for the AMDase-catalysed decarboxylation of 2-methyl-2-phenylmalonate (**2**). This suggests that the phosphate ion mimics the enediolate intermediate, bound to the active site. The importance of the dioxyanion hole was also demonstrated through site-directed mutagenesis. Previously mutagenesis of Thr75 and Ser76 was investigated[6c] and in addition we have subjected Tyr126 to saturation mutagenesis. All of the resulting mutants have been shown to be completely inactive, which supports the idea that the dioxanion hole is essential for catalysis.

Table 1. Kinetic parameters for AMDase and related enzymes[a]

Protein (Microorganism)		K_m [M]	k_{cat} [s^{-1}]	k_{cat}/K_m [$s^{-1}M^{-1}$]
AMDase[b]	**1**	10.7×10^{-3}	316	2.96×10^{4}
B. bronchiseptica KU1201	**2**	26.9×10^{-3}	279	1.04×10^{4}
EAN08019.1	**1**	9.6×10^{-3}	118	1.71×10^{4}
Mesorhizobium sp.BNC1	**2**	22.2×10^{-3}	55.8	0.25×10^{4}
NP_888076.1	**1**	10.0×10^{-3}	12.4	0.12×10^{4}
B. bronchiseptica RB50	**2**	-	-	-
ZP_00801202.1	**1**	123×10^{-3}	0.26	2.1
A. metalliredigenes QYMF	**2**	-	-	-
BAA30082.1	**1**	41.7×10^{-3}	0.09	2.2
P. horikoshii OT3	**2**	-	-	-
MurI (AAF25672)[c]	**1**	3.1×10^{-3}	0.24	77
A. pyrophilus	**2**	-	-	-

[a] Kinetic parameters were determined spectrophotometrically, for decarboxylation of phenylmalonate (**1**) and 2-methyl-2-phenylmalonate (**2**), using BTB as an indicator following methods described previously.[10] [b] Parameters agree closely with Literature data.[1d] [c] Glutamate racemase activity for the MurI from *Aquifex pyrophilus*: $K_m = 0.21\times10^{-3}$ M, $k_{cat} = 3.30\ s^{-1}$, $k_{cat}/K_m = 1.57\times10^{4}\ s^{-1}M^{-1}$.

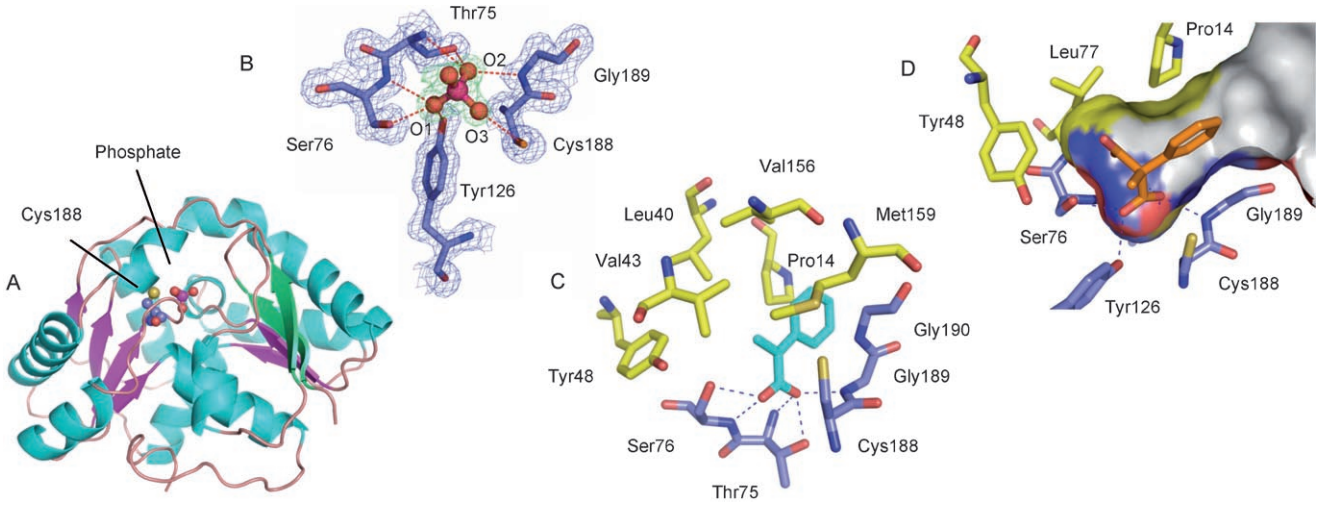


Figure 2. A) Ribbon representation of the AMDase crystal structure with phosphate (pink-red), Cys188 (yellow-blue) and β-sheets near solvent accessible channel (green). B) The AMDase active site showing the hydrogen bonding pattern observed between the O1, O2 and O3 atoms of the phosphate and the dioxyanion hole as well as the active site Cys188 residue. The sigmaA weighted $2F_oF_c$ electron density map is contoured at 1σ and shown in blue mesh, whereas the omit map contoured at 3σ for the bound phosphate is shown as a green mesh. The Cys188 is the only residue that lies outside the allowed region in the Ramachandran plot. The phosphate O1 atom is within hydrogen bonding distance of the side chain of Tyr126 (2.76 Å), and the amide backbone and hydroxy side chain of Ser76 (2.88 and 2.56 Å, respectively). The O2 atom of the phosphate group, on the other hand, is hydrogen-bonded to the amide backbone of Gly189 (2.77 Å) in addition to both the hydroxy group and amide backbone of Thr75 (2.65 and 2.94 Å respectively). Also, the phosphate O3 is within hydrogen bonding distance of the Cys188 (2.97 Å), which presumably stabilises the conformation of the Cys residue. C) Active site model of AMDase structure, with the 2-methyl-2-phenyl-1,1-enediolate intermediate bound. Residues of the smaller binding pocket surrounding the methyl group are shown in yellow and the dioxyanion hole is shown in blue. Tyr126, which also donates one hydrogen bond to the enediolate oxygen atom, is removed for clarity. D) Cross section of the *B. bronchiseptica*. AMDase active site with the substrate 2-methyl-2-phenylmalonate bound in the active site. Solvent accessible surface is shown in grey.

Whilst the true biological substrate for the AMDase is not known, 2-methyl-2-phenylmalonate **2** has been widely used as the model substrate. We therefore positioned the proposed enediolate intermediate derived from the decarboxylation of this substrate into the active site of the structure (Figure 2C), using the coordinates of the O1-P-O2 atoms of the bound phosphate group to position the oxygen atoms of the enediolate into the dioxyanion hole. In the AMDase structure, the active site cavity volume is predominantly hydrophobic in nature and extends above the O1-P-O2 atoms. There is relatively little space available directly above the O1 atom, while in comparison a large cavity extending into the solvent area is available directly above O2. This cavity is ideally shaped for binding of aromatic groups, with the Pro14 side chain positioned approximately 8 Å away from the Gly189–Gly190 amide bond, allowing for ideal van der Waals stacking of a flat aromatic system in between. Furthermore, the aryl side chain is orientated in such a way that it remains co-planar with the enediolate π system, ensuring effective delocalisation of the negative charge which can serve to further stabilise this intermediate. From the available active site volume, it seems the enzyme will be able to accommodate relatively bulky *ortho*, *meta* and *para* groups including a 2-naphthyl substituent, which is in line with the previously determined substrate specificity of this enzyme.[1] The fact that there is little space available above O1 clearly indicates that this is most likely to accommodate the smaller methyl substituent. The energy-minimised model of the proposed intermediate, bound in this way (Figure 2C), positions the Cys188 thiol in close contact (4.16 Å) to the *si*-face of the enolate, which is consistent with a subsequent protonation step leading to the *R*-configured 2-phenylpropionate product **4** (Figure 3).

Previously it was suggested, using ^{13}C-labeling experiments, that decarboxylation of 2-methyl-2-phenylmalonate (**2**) catalysed by the *B. bronchiseptica* AMDase occurs through the loss of the *pro-R* carboxylate with overall inversion of configuration.[9] In a separate series of experiments using a more efficient ^{18}O-labeling strategy, vide infra, we confirmed this initial stereochemical assignment to be correct. Based on this, it is possible to position the substrate 2-methyl-2-phenylmalonate (**2**) in the active site of the AMDase structure. With the location of the oxygen atoms of *pro-S* carboxylate determined by their position relative to the dioxyanion hole and phenyl substituent located in the solvent exposed pocket it is clear that the *pro-R* carboxylate of the substrate would necessarily occupy the opposite side of the active site to the Cys188 residue (Figure 2D, see also Supporting Information). This positions the carboxyl group tightly within the small hydrophobic pocket made up by Leu40, Val43, Val156 and Tyr48, suggesting that hydrophobic destabilisation of the *pro-R* carboxylate of the malonate substrate may be a necessary driving force for decarboxylation. Presumably the neutral carbon dioxide that is lost in the reaction remains bound (or trapped) within this hydrophobic pocket, as the residual electron density is delocalised into the *pro-S* carboxylate of the enediolate intermediate with the build-up of negative charge being stabilised by the dioxyanion hole (Figure 3). Thus the AMDase is able to effect efficient decarboxylation without co-factors or prior activation as an electron-withdrawing thioester, simply by orientating the malonate substrate such that one carboxy group is stabilised by an extensive H-bonding network, whilst the other carboxylate is bound tightly against a small hydrophobic pocket, deprived of any stabilizing electrostatic interactions.

Despite the significant potential of AMDases for applications in biocatalysis, only one such enzyme has been isolated and characterised to date. Accordingly four protein sequences that show moderate to high similarity (30-52% identity) to the AMDase (Table 1, see also Supporting Information) and that contain a Cys residue at the same relative position as the Cys188 in the *B. bronchiseptica* enzyme, were selected from the protein databases. Genes encoding these proteins were synthesised, cloned and overexpressed in *E. coli*. In addition to this, the known glutamate racemase from *Aquifex pyrophilus* (MurI),[4a] which has lower sequence similarity

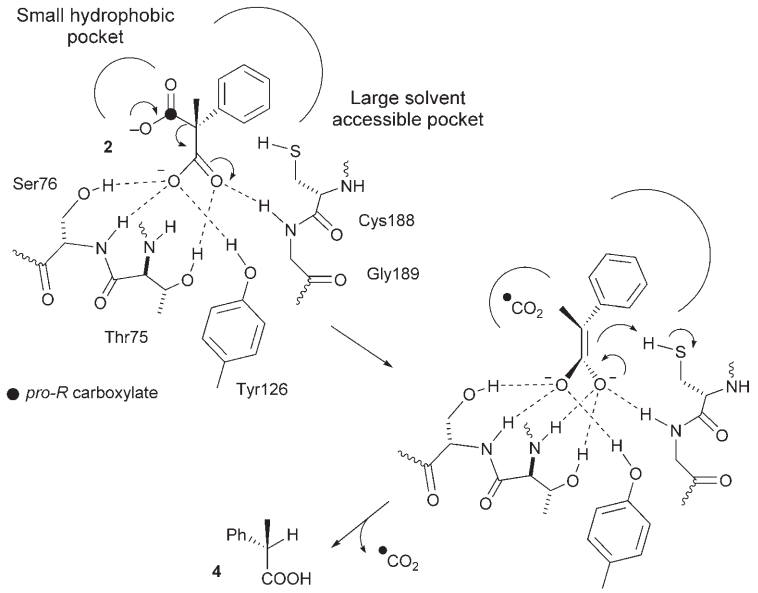
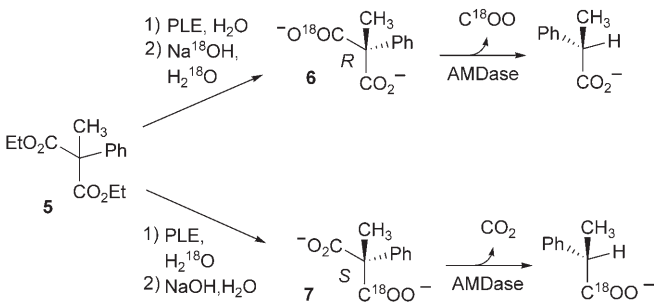


Figure 3. Proposed mechanism of the decarboxylation reaction catalysed by the AMDases. At the pH optimum of the enzyme (8.0), it is predicted that the substrate 2-methyl-2-phenylmalonate will exist predominantly as the dianion.[8] It is therefore more likely that it is the substrate *pro-S*-carboxylate anion rather than the protonated acid that initially binds to the dioxyanion hole.

(20% identity) to the AMDase, was also overproduced and purified. Subsequently, apparent kinetic constants for the AMDase activity of the purified enzymes were established (Table 1). Notably the enzyme from *Mesorhizobium sp.* BNC1, which shows highest similarity (52% identity) to the *B. bronchiseptica* AMDase, showed very efficient decarboxylase activity with both phenylmalonate (**1**) and 2-methyl-2-phenylmalonate (**2**) as substrates.

To probe the stereochemical course of the AMDase from *Mesorhizobium* a stereoselective ^{18}O-labeling approach was developed (Scheme 1). This involved the use of porcine liver



Scheme 1. The ^{18}O-labeling strategy used to determine the stereochemical course of the decarboxylation of 2-methyl-2-phenylmalonic acid catalysed by the AMDases from *Mesorhizobium* sp. BNC1 & *B. bronchiseptica*. PLE = porcine liver esterase.

esterase (PLE) to catalyse the hydrolysis of the diester **5**, which gave the (*R*)-monoester (98% *ee*) in agreement with the literature.[11] The monoester was then hydrolysed with $Na^{18}OH$ in $H_2{}^{18}O$ to give 2-methyl-2-phenylmalonic acid with an ^{18}O label in the *pro-R* carboxylate **6**. Similarly, hydrolysis first with PLE in 98% $H_2{}^{18}O$ followed by saponification with unlabeled NaOH led to the opposite enantiomer **7**. The chiral malonates **6** and **7** were separately incubated with the AMDase from *Mesorhizobium* and *B. bronchiseptica*. The products of the decarboxylation were then methylated with diazomethane and analysed by GC-MS. This showed that decarboxylation catalysed by both enzymes occurs through the loss of the *pro-R* carboxylate with overall inversion of configuration. This along with modeling studies (see Supporting Information) suggests that the mechanisms of the two AMDases are similar.

The other enzymes that were investigated (Table 1) showed low to moderate decarboxylase activity with phenylmalonate (**1**), but no activity with 2-methyl-2-phenylmalonate (**2**). Interestingly the known glutamate racemase from *Aquifex pyrophilus* (MurI) is able to catalyse the decarboxylation of phenylmalonate, albeit with 196-fold lower activity than the native glutamate racemase activity. It is possible to rationalise these results through protein sequence alignments (see Supporting Information). Notably, the active site residues of *B. bronchiseptica* AMDase are almost entirely conserved within the *Mesorhizobium sp.* BNC1 enzyme, with a single active site Cys residue consistent with the AMDase activity. All the other enzymes possess two Cys residues at their putative active site, suggesting that their natural function is as racemases. Furthermore, the dioxyanion hole motifs remain closely conserved across all these enzymes, suggesting that all are likely to form enediolate intermediates. Furthermore, an overlay of the structure of the glutamate racemase (MurI) from *Helicobacter pylori* (PDB code 2 JFX)[4c] and *Enterococcus faecalis* (PDB code 2 JFO)[4c] with the *B. bronchiseptica* AMDase reveals a similar dioxyanion hole structure binding the carboxylate of the D-Glu substrate (Figure 4). Analysis of other racemase structures[4] also shows the presence of similar dioxyanion holes. This suggests that all these enzymes share a common structural motif that serves to stabilise the enediolate intermediates. Surprisingly, given the available structural data,[4] the mechanistic role of those conserved residues that make up the common dioxyanion holes has not been proposed until now. Indeed, whilst single isolated oxyanion holes have been described in many protein structures,[7] the presence of two of these in the form of the dioxyanion hole predicted to stabilise a high-energy enediolate type intermediates has not been described explicitly before, as far as we are aware. However the notable recent structure of diaminopimelate (DAP) epimerase, despite possessing low sequence or overall structural similarity to the AMDase, exhibits some similarity in its active site.[12] In DAP-epimerase, two α-helices orientated towards the substrate carboxy group are also suggested to stabilise the formation of an endiolate intermedi-

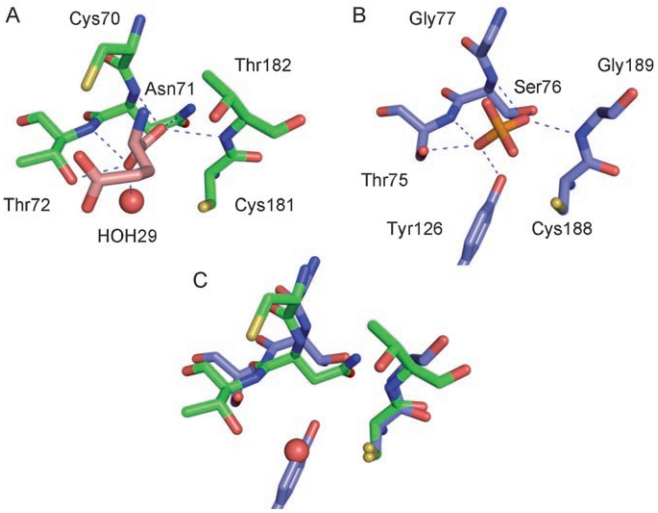


Figure 4. A) The active site of the *H. pylori* Glu-racemase (MurI)[4c] showing how the Glu-carboxylate interacts with the dioxyanion hole. The structural water molecule (2.70 Å), and the amide backbone and hydroxy side chain of Thr72 (3.10 and 2.70 Å, respectively) are all within hydrogen bonding distance of one oxygen atom of the carboxy group. The other oxygen atom is hydrogen-bonded to the backbone amide of Thr182 (2.95 Å) in addition to the side chain and backbone amide groups of Asn71 (3.28 and 2.88 Å, respectively). In the published structure of MurI, the positions of N and O atoms of the amide side chain of Asn71 reversed, with the oxygen atom orientated towards the carboxy group.[4c] However the resolution of this structure is 1.9 Å, which precludes assignment of the amide O and N atoms. B) The active site of the AMDase. C) Overlay of the AMDase and MurI structures.

ate. Such dipole interactions cannot occur in the AMDase, as there are no α-helices correctly orientated relative to the dioxyanion hole.

In summary, the first high-resolution X-ray crystal structure of a co-factor independent aryl malonate decarboxylase (AMDase), which reveals the mechanism of this unusual enzyme, is presented (PDB code 3DG9).[13] Notably, a dioxyanion hole, a hitherto unidentified structural motif,[12] is postulated to be critical in the stabilisation of a putative enediolate intermediate formed during decarboxylation. Sequence alignments and analysis of the structures of the mechanistically related Asp/Glu racemases suggests that these enzymes also possess dioxyanion holes that stabilise similar intermediates. As a result the Asp/Glu racemase also exhibit some AMDase activity. In the mechanistically related enolase superfamily, coordination to Mg^{2+} serves to stabilise enediolate intermediates.[5] The results presented here therefore suggest that nature has evolved at least two distinct solutions to the same mechanistic puzzle, which has been a subject of considerable interest in enzymology.[5] In addition, the structural and mechanistic insight, presented here, provide a firm basis for engineering new decarboxylases, which can provide valuable homochiral carboxylic acids from cheap and accessible malonate precursors.[14] Moreover it may now be possible to design mechanism-based inhibitors that bind to the dioxyanion holes of the Asp/Glu racemases, which and are important targets for the development of new antimicrobial agents.[4c]

Experimental Section

Full experimental details can be found in the Supporting Information.

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[1] a) K. Miyamoto, H. Ohta, *J. Am. Chem. Soc.* **1990**, *112*, 4077–4078; b) K. Miyamoto, H. Ohta, *Biocatalysis* **1991**, *5*, 49–60; c) K. Miyamoto, H. Ohta, *Appl. Microbiol. Biotechnol.* **1992**, *38*, 234–238; d) K. Miyamoto, H. Ohta *Eur. J. Biochem.* **1992**, *210*, 475–481; e) K. Matoishi, M. Ueda, K. Miyamoto, H. Ohta, *J. Mol. Catal. B* **2004**, *27*, 161–168; f) K. Miyamoto, H. Ohta, Y. Osamura, *Bioorg. Med. Chem.* **1994**, *2*, 469–475; g) K. J. Miyamoto, S. Tsuchiya, H. Ohta, *J. Am. Chem. Soc.* **1992**, *114*, 6256–6257.

[2] a) K. A. Gallo, J. R. Knowles, *Biochemistry* **1993**, *32*, 3981–3990; b) K. A. Gallo, M. E. Tanner, J. R. Knowles, *Biochemistry* **1993**, *32*, 3991–3997; c) T. Yamauchi, S. Y. Choi, H. Okada, M. Yohda, H. Kumagai, N. Esaki, K. Soda, *J. Biol. Chem.* **1992**, *267*, 18361–18364.

[3] a) S. Glavas, M. E. Tanner, *Biochemistry* **2001**, *40*, 6199–6204; b) M. E. Tanner, *Acc. Chem. Res.* **2002**, *35*, 237–246.

[4] a) K. Y. Hwang, C. S. Cho, S. S. Kim, H. C. Sung, Y. G. Yu, Y. Cho, *Nat. Struct. Biol.* **1999**, *6*, 422–426; b) L. Liu, K. Iwata, A. Kita, Y. Kawarabayasi, M. Yohda, K. Miki, *J. Mol. Biol.* **2002**, *319*, 479–489; c) T. Lundqvist, S. L. Fisher, G. Kern, R. H. Folmer, Y. Xue, D. T. Newton, T. A. Keating, R. A. Alm, B. L. de Jonge, *Nature* **2007**, *447*, 817–822.

[5] a) E. A. Taylor, D. R. J. Palmer, J. A. Gerlt, *J. Am. Chem. Soc.* **2001**, *123*, 5824–5825; b) L. Song, C. Kalyanaraman, A. A. Fedorov, E. V. Fedorov, M. E. Glasner, S. Brown, H. J. Imker, P. C. Babbitt, S. C. Almo, M. P. Jacobson, J. A Gerlt, *Nat. Chem. Biol.* **2007**, *3*, 486–491; Brown, H. J. Imker, P. C. Babbitt, S. C. Almo, M. P. Jacobson, J. A Gerlt, *Nat. Chem. Biol.* **2007**, *3*, 486–491; c) J. A. Gerlt, P. C. Babbitt, I. Rayment, *Arch. Biochem. Biophys.* **2005**, *433*, 59–70; d) P. C. Babbitt, G. T. Mrachko, M. S. Hasson, G. W. Huisman, R. Kolter, D. Ringe, G. A. Petsko, G. L. Kenyon, . Gerlt J. A. *Science* **1995**, *267*, 1159–1161; e) G. L. Kenyon, J. A. Gerlt, G. A. Petsko, J. W. Kozarich, *Acc. Chem. Res.* **1995**, *28*, 178–186.

[6] a) Y. Terao, K. Miyamoto, H. Ohta, *Chem. Lett.* **2007**, *36*, 420–421; b) Y. Ijima, K. Matoishi, Y. Terao, N. Doi, H. Yanagawa, H. Ohta, *Chem. Commun.* **2005**, 877–879; c) Y. Terao, Y. Ijima, K. Miyamoto, H. Ohta, *J. Mol. Catal. B* **2007**, *45*, 15–20; d) Y. Terao, K. Miyamoto, H. Ohta, *Chem. Commun.* **2006**, *34*, 3600–3602.

[7] J. D. Robertus, J. Kraut, R. A. Alden, J. J. Birktoft, *Biochemistry* **1972**, *11*, 4293–4303.

[8] The first and second pK_a values of 2,2-dimethyl malonic acid are 3.17 and 6.06, respectively. A more electron-withdrawing substituent, such as a phenyl group, is predicted to decrease the pK_a values slightly. See: Brown, H. C.; McDaniel, D. H.; Häfliger, O. in *Determination of Organic Structures by Physical Method*, ed. Braude E. A.; Nachod, F. C. Academic Press, New York, **1955**, p. 567.

[9] K. J. Miyamoto, S. Tsuchiya, H. Ohta, *J. Am. Chem. Soc.* **1992**, *114*, 6256–6257.

[10] a) L. E. Janes, A. C. Lowendahl, R. J. Kazlauskas, *Chem. Eur. J.* **1998**, *4*, 2324–2331; b) A. Banerjee, P. Kaul, R. Sharma, U. C. Banerjee, *J. Biomol. Screening* **2003**, *8*, 559–565.

[11] a) P. Dominguez de Maria, B. Kossmann, N. Potgrave, S. Buchholz, H. Trauthwein, O. May, H. Groeger, *Synlett* **2005**, *11*, 1746–1748; b) S. Wallert, K. Drauz, G. Ian, H. Groeger, P. Dominguez De Maria, C. Bolm, *Green Chem.* **2005**, *7*, 602–605; c) M. Breznik, D. Kikelj, *Tetrahedron Asymmetry* **1997**, *8*, 425–434.

[12] The structure of DAP epimerase shows a carboxy group of an inhibitor bound by a network of five hydrogen bonds at the active site. Minor conformational changes in this structure could result in a similar dioxyanion hole. B. Pillai, M. A. Cherney, C. M. Diaper, A. Sutherland, J. S. Blanchard, J. C. Vederas, M. N. G. James, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 8668–8673; A. Sutherland, J. S. Blanchard, J. C. Vederas, M. N. G. James, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 8668–8673.

[13] The atomic coordinates for *Bordetella bronchiseptica* AMDase have been deposited in the Protein Data Bank (accession number 3DG9).

[14] There are very few non-enzymatic methods available for enantioselective decarboxylation of disubstituted malonic acids: a) M. Amere, M.-C. Lasne, J. Rouden *Org. Lett.* **2007**, *9*, 2621–2624; b) A. Roy, A. Riahi, F. Hénin, J. Muzart, *Eur. J. Org. Chem.* **2002**, 3986–3994; c) H. Brunner, M. A Baur, *Eur. J. Org. Chem.* **2003**, 2854–2862.

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