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Alteration of the Diastereoselectivity of 3-Methylaspartate Ammonia Lyase by Using Structure-Based Mutagenesis

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Supporting Information

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Supporting Information

for

Alteration of the Diastereoselectivity of 3-Methylaspartate Ammonia Lyase by Structure-Based Mutagenesis

Hans Raj, Barbara Weiner, Vinod Puthan Veetil, Carlos R. Reis, Wim J. Quax, Dick B. Janssen, Ben L. Feringa, and Gerrit J. Poelarends*

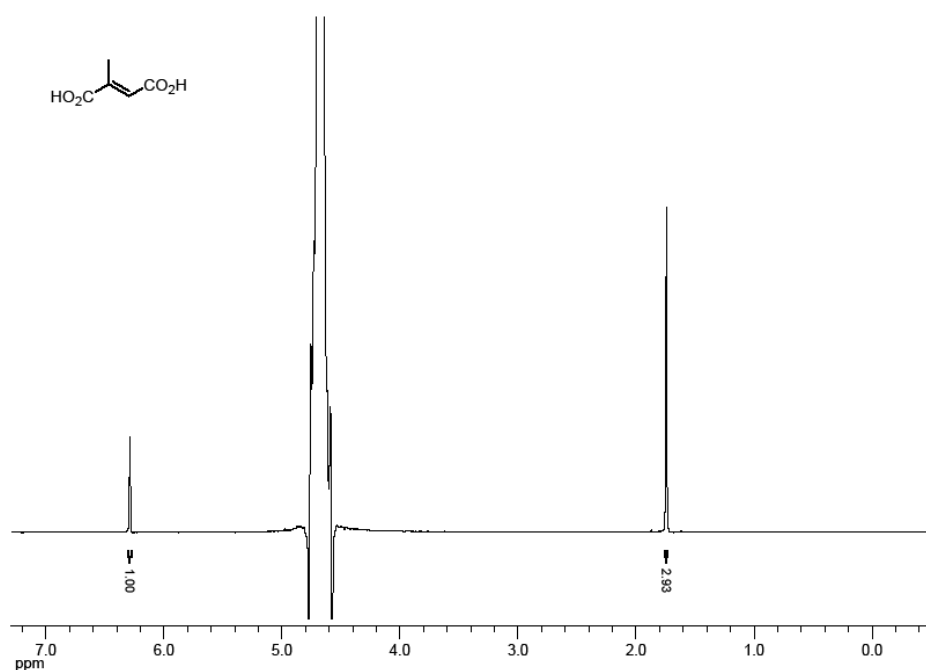


Figure S1. ¹H NMR spectrum of mesaconate (**1**). $\delta = 1.73$ (s, 3H; CH₃), 6.28 (s, 1H; CH).

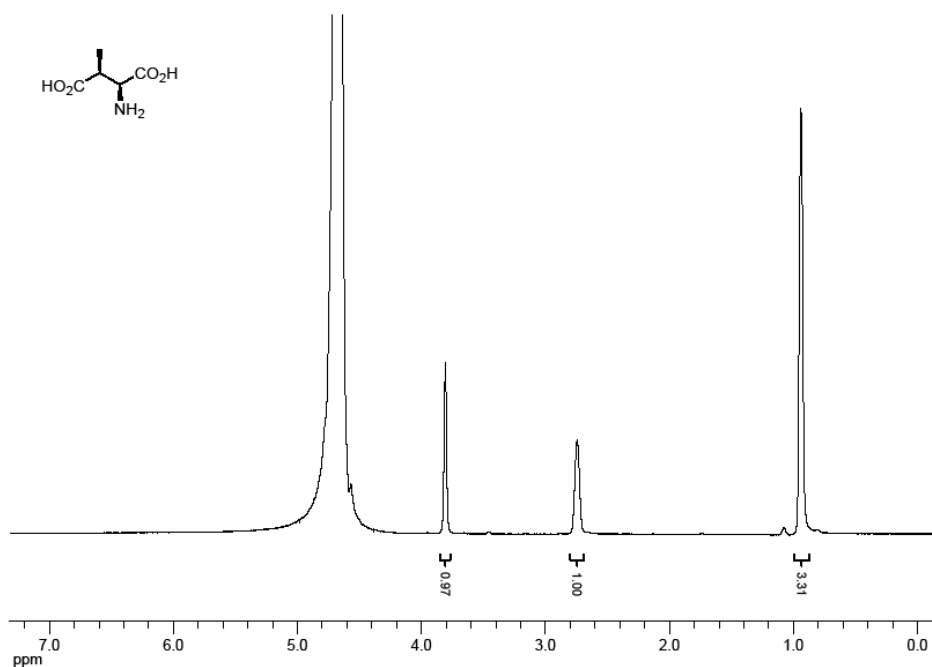


Figure S2. ^1H NMR spectrum of the material used as substrate **2**, which is a 1:1 mixture of the enantiomers (2*S*,3*S*)- and (2*R*,3*R*)-3-methylaspartic acid. The (2*R*,3*R*)-enantiomer is not a substrate nor an inhibitor of MAL. $\delta = 0.94$ (s, 3H; CH_3), 2.74 (s, 1H; CH), 3.81 (s, 1H; CH).

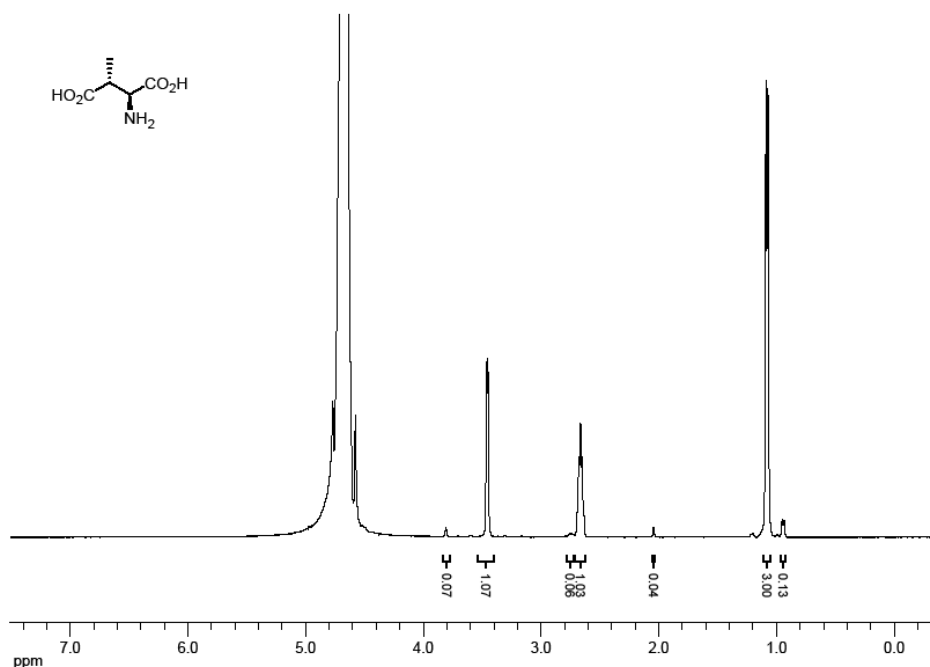


Figure S3. ^1H NMR spectrum of (2*S*,3*R*)-3-methylaspartic acid (**3**). $\delta = 1.08$ (d, $^3J = 7.0$ Hz, 3H; CH_3), 2.63-2.70 (m, 1H; CH), 3.46 (d, $^3J = 4.0$ Hz, 1H; CH). Contaminant **2** (5-6%): $\delta = 0.94$ (d), 2.04 (s), 2.74 (m), 3.81 (s).

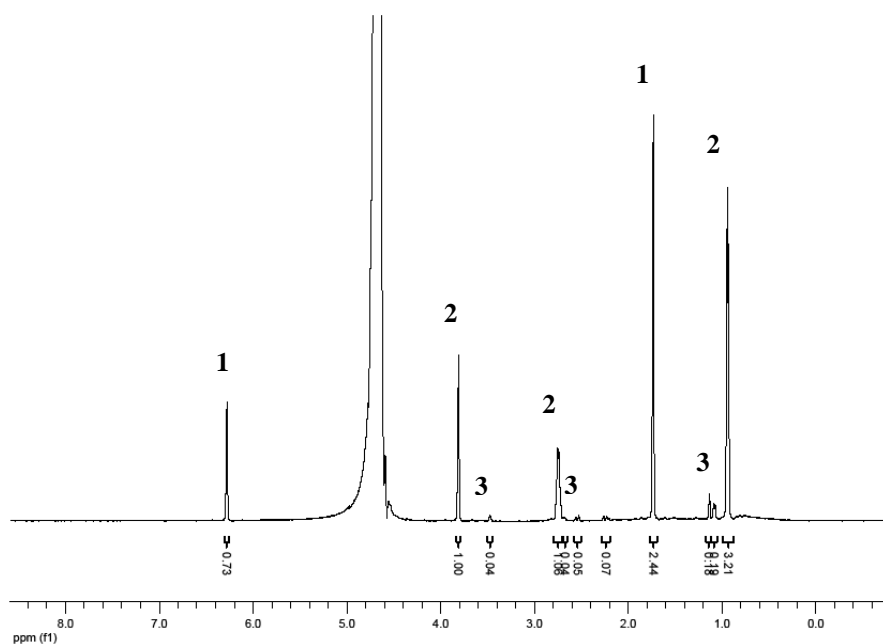


Figure S4. ^1H NMR spectrum identifying the products of the MAL-catalyzed deamination of **2**. The spectrum was taken after a 13 day-incubation period. Ratio **1** : **2** : **3** = 41 : 57 : 2. $\delta = 0.93$ (d, $^3J = 6.5$ Hz, 3H; CH_3), 1.08 (d, $^3J = 7.5$ Hz, 3H; CH_3), 1.74 (s, 3H; CH_3), 2.71-2.78 (m, 2H; CH), 3.46-3.48 (m, 1H; CH), 3.81 (s, 1H; CH), 6.28 (s, 1H; CH_3). Impurity $\delta = 1.13$ (s), 2.24 (d), 2.54 (d).

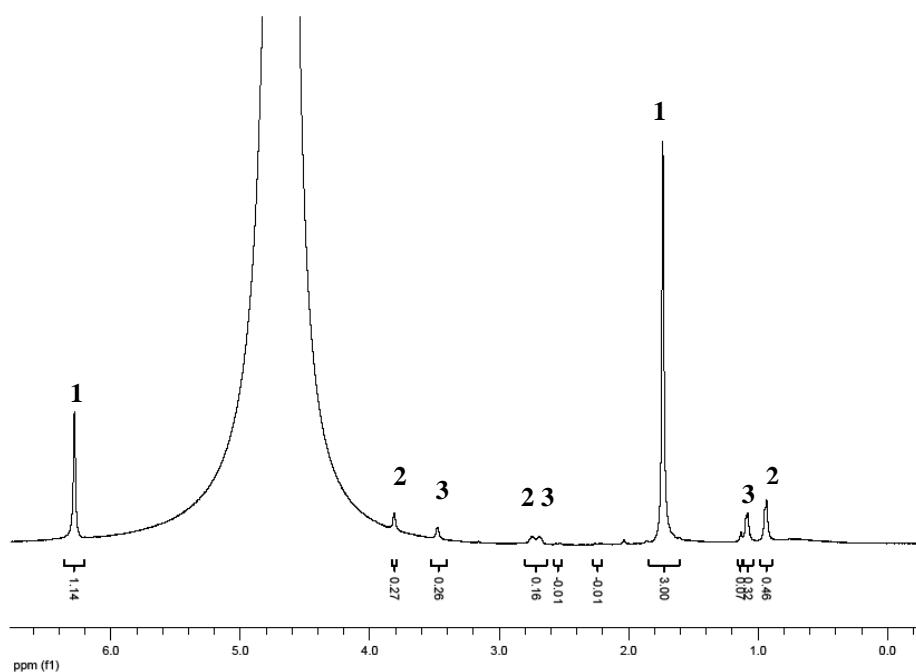


Figure S5. ^1H NMR spectrum identifying the products of the MAL-catalyzed deamination of **3**. The spectrum was taken after a 13 day-incubation period. Ratio **1** : **2** : **3** = 79 : 12 : 9. $\delta = 0.93$ (d, $^3J = 6.5$ Hz, 3H; CH_3), 1.08 (d, $^3J = 7.5$ Hz, 3H; CH_3), 1.74 (s, 3H; CH_3), 2.65-2.78 (m, 2H; CH), 3.47 (s, 1H; CH), 3.81 (s, 1H; CH), 6.28 (s, 1H; CH_3). Impurity $\delta = 1.13$ (s), 2.24 (d), 2.54 (d).

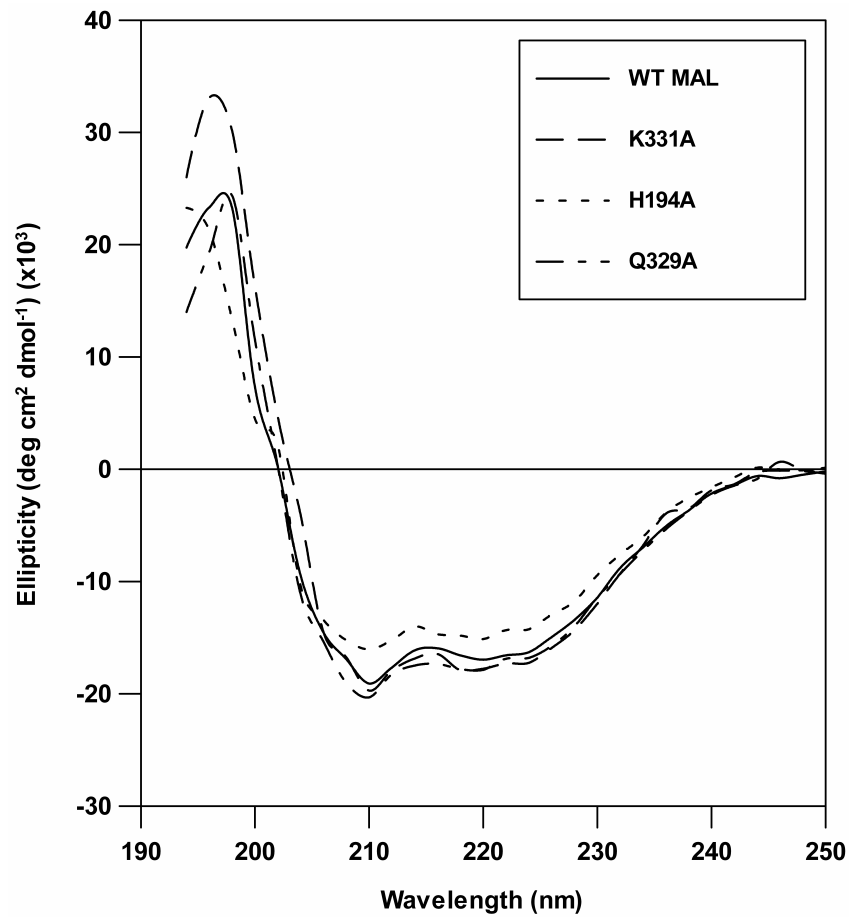


Figure S6. Superimposed far-UV CD spectra of wild-type and mutant enzymes. The CD spectra of all the mutants were comparable to that of wild-type MAL, but for clarity only the CD spectra of the K331A, H194A, and Q329A mutants are shown. Spectra were measured in 10 mM Tris buffer (pH 8.0), containing 2 mM MgCl₂ and 0.1 mM KCl, at a protein concentration of approximately 3.2 μ M.