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Supporting Information for

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Figures S1, S2, S3, S4, S5, S6, S7, S8 and S9, and the corresponding legends, are provided below.

Figure S1. Amino acid sequence alignment of the MAL proteins from *Clostridium tetanomorphum* (*Ct*-MAL) and *Carboxydothermus hydrogenoformans* Z-2901 (*Ch*-MAL). Identical residues are shaded in gray. The ten active site residues of *Ct*-MAL are indicated by an asterisk.

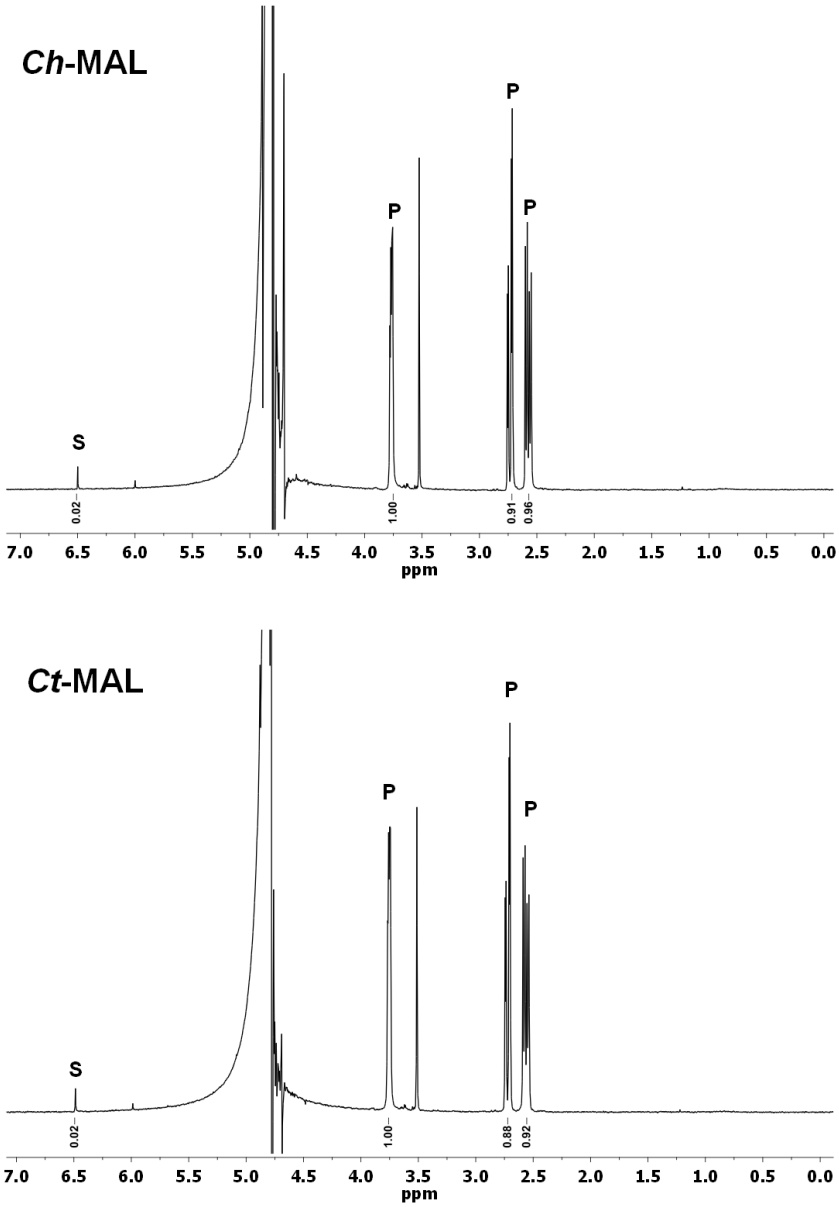

Figure S2. ^{1}H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed amination of fumarate. The spectra were taken after 7 days of incubation at 22°C. For both *Ch*-MAL and *Ct*-MAL, the ratio of S:P = 1:99, respectively. S, fumarate; P, (*S*)-aspartic acid. The ^{1}H NMR signals for the enzymatically generated (*S*)-aspartic acid are identical to those found with an authentic standard. Impurity (Tris): δ = 3.5 (s).

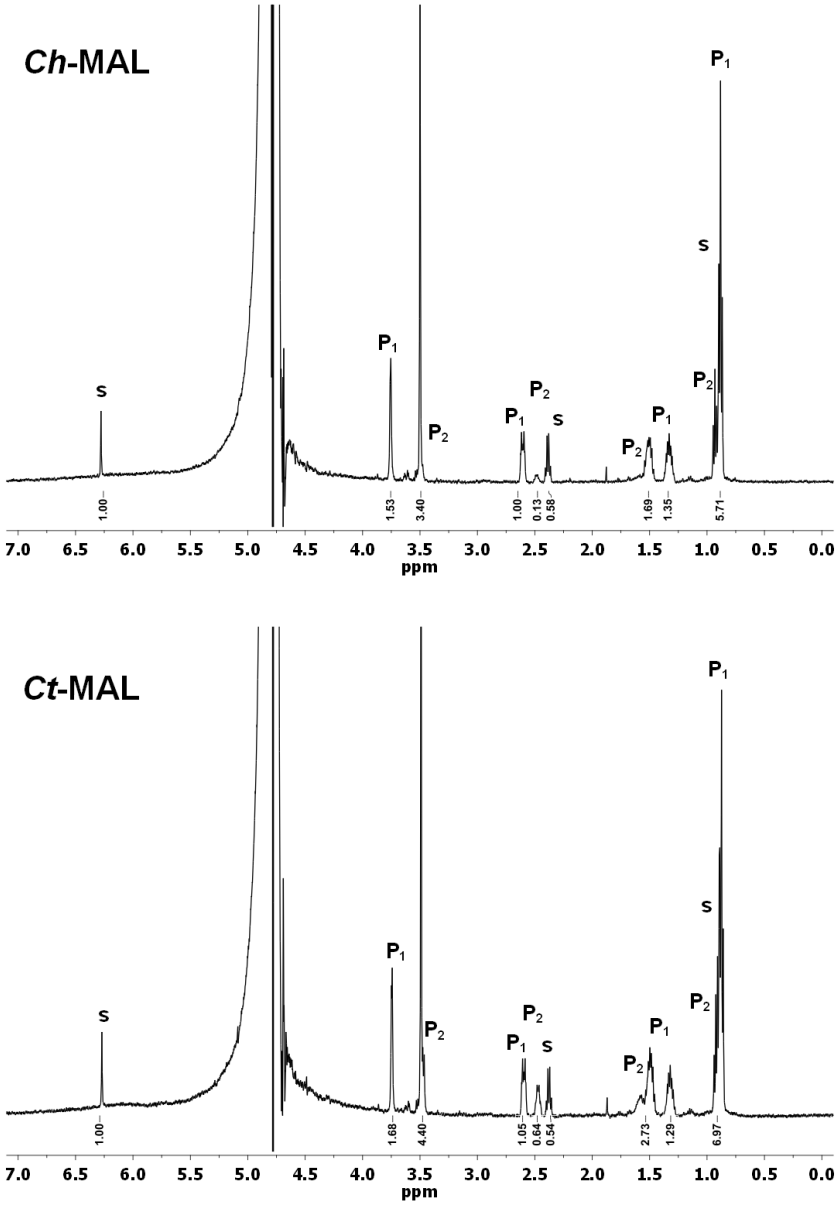

Figure S3. ^{1}H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed ammonia addition to 2-ethylfumarate. The spectra were taken after 14 days of incubation at 22°C. For *Ch*-MAL and *Ct*-MAL, the ratio of S:P_1:P_2 = 35:60:5 and S:P_1:P_2 = 29:48:23, respectively. S, 2-ethylfumarate; P_1, *threo*-(2*S*,3*S*)-3-ethylaspartate (major diastereoisomer); P_2, *erythro*-(2*S*,3*R*)-3-ethylaspartate (minor diastereoisomer). The ^{1}H NMR signals for 2-ethylfumarate and (2*S*,3*S*)-3-ethylaspartate are reported elsewhere (Akhtar *et al.* 1987). Impurity (Tris): δ = 3.5 (s).

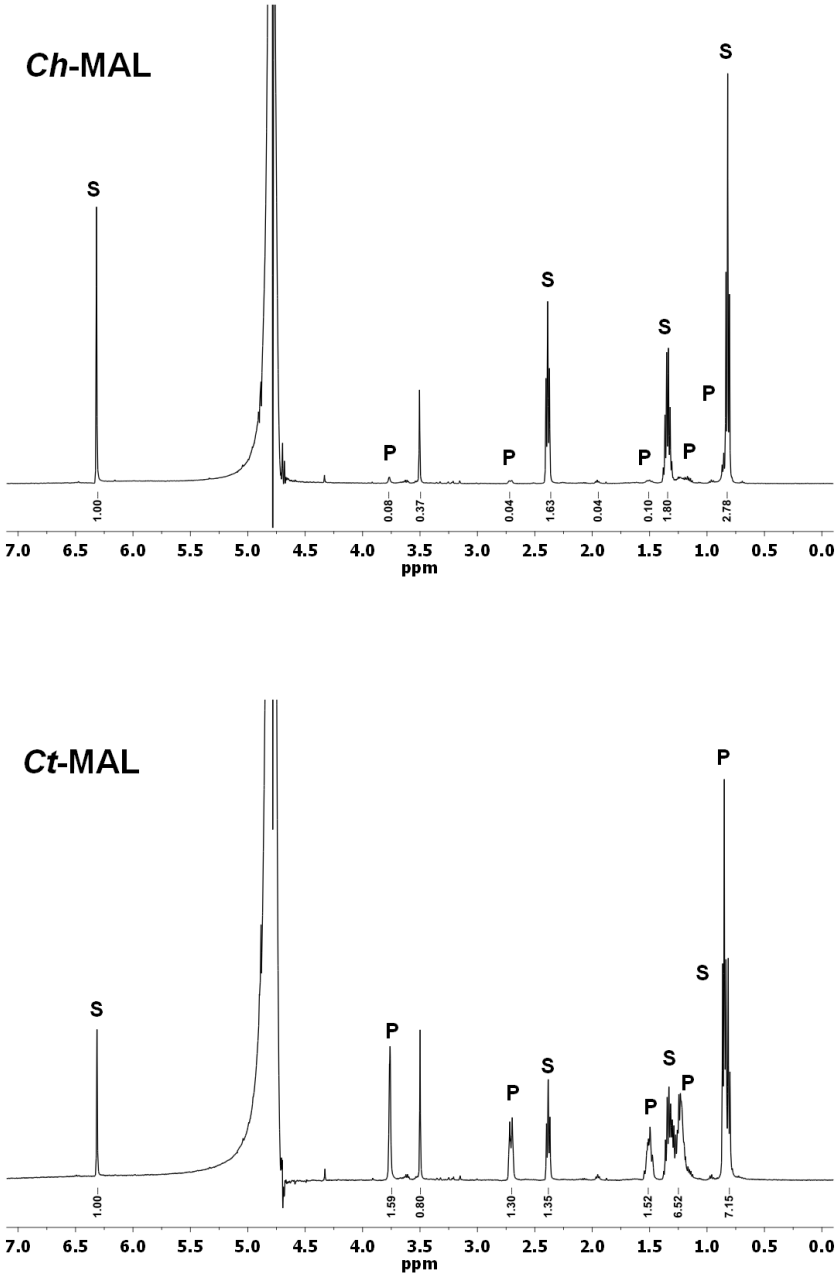

Figure S4. ^{1}H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed ammonia addition to 2-propylfumarate. The spectra were taken after 14 days of incubation at 22°C. For *Ch*-MAL and *Ct*-MAL, the ratio of S:P = 93:7 and S:P = 39:61, respectively. S, 2-propylfumarate; P, *threo*-(2*S*,3*S*)-3-propylaspartate. ^{1}H NMR consistent with literature data (Akhtar *et al.* 1987). Impurity (Tris): δ = 3.5 (s).

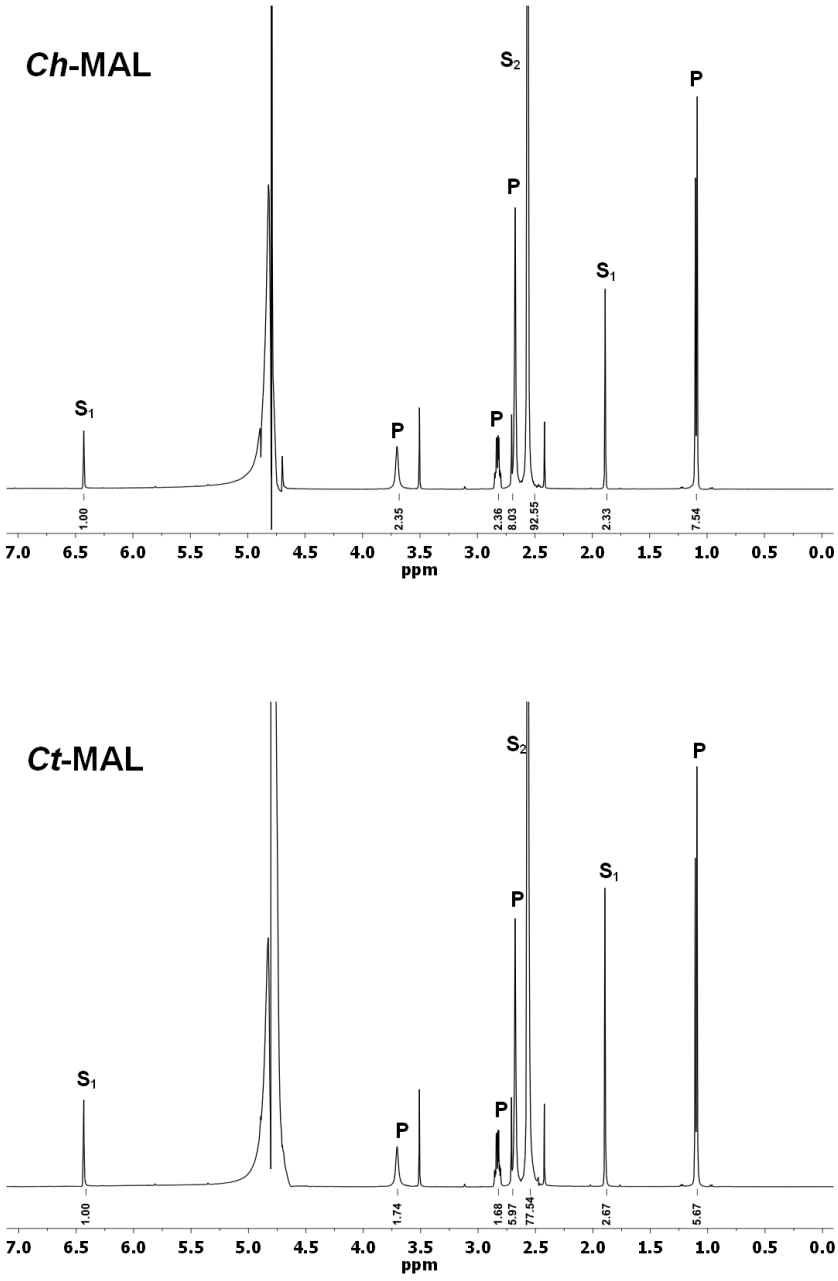

Figure S5. ^{1}H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed methylamine addition to mesaconate. The spectra were taken after 14 days of incubation at 22°C. For *Ch*-MAL and *Ct*-MAL, the ratio of S_1:P = 30:70 and S_1:P = 36:64, respectively. S_1, mesaconate; S_2, methylamine; P, *threo*-(2*S*,3*S*)-*N*,3-dimethylaspartate. The ^{1}H NMR signals for (2*S*,3*S*)-*N*,3-dimethylaspartate are reported elsewhere (Gulzar *et al.* 1997). Impurity (Tris): δ = 3.5 (s).

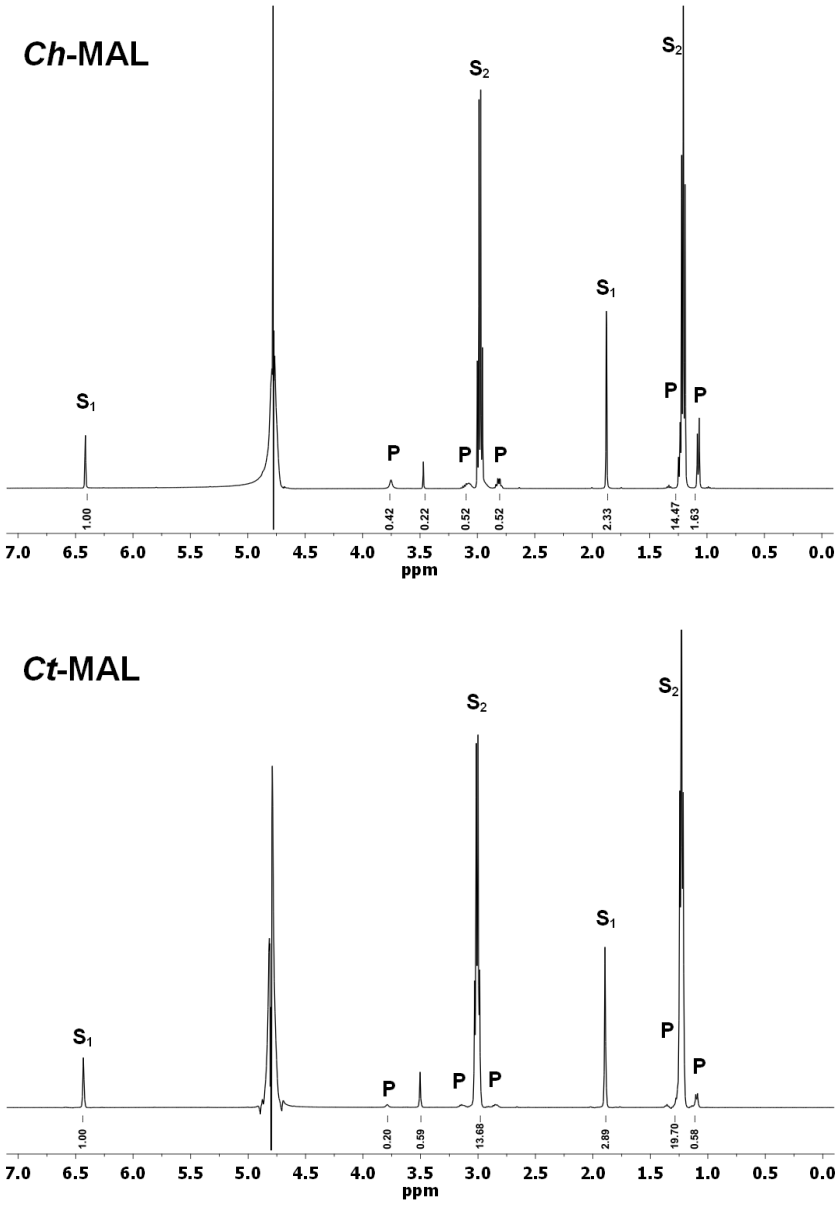

Figure S6. ^{1}H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed ethylamine addition to mesaconate. The spectra were taken after 14 days of incubation at 22°C. For *Ch*-MAL and *Ct*-MAL, the ratio of S_1:P = 70:30 and S_1:P = 86:14, respectively. S_1, mesaconate; S_2, ethylamine; P, tentatively identified as 2-ethylamino-3-methylaspartic acid, the enzymatic synthesis of which has not been reported before. Impurity (Tris): δ = 3.5 (s).

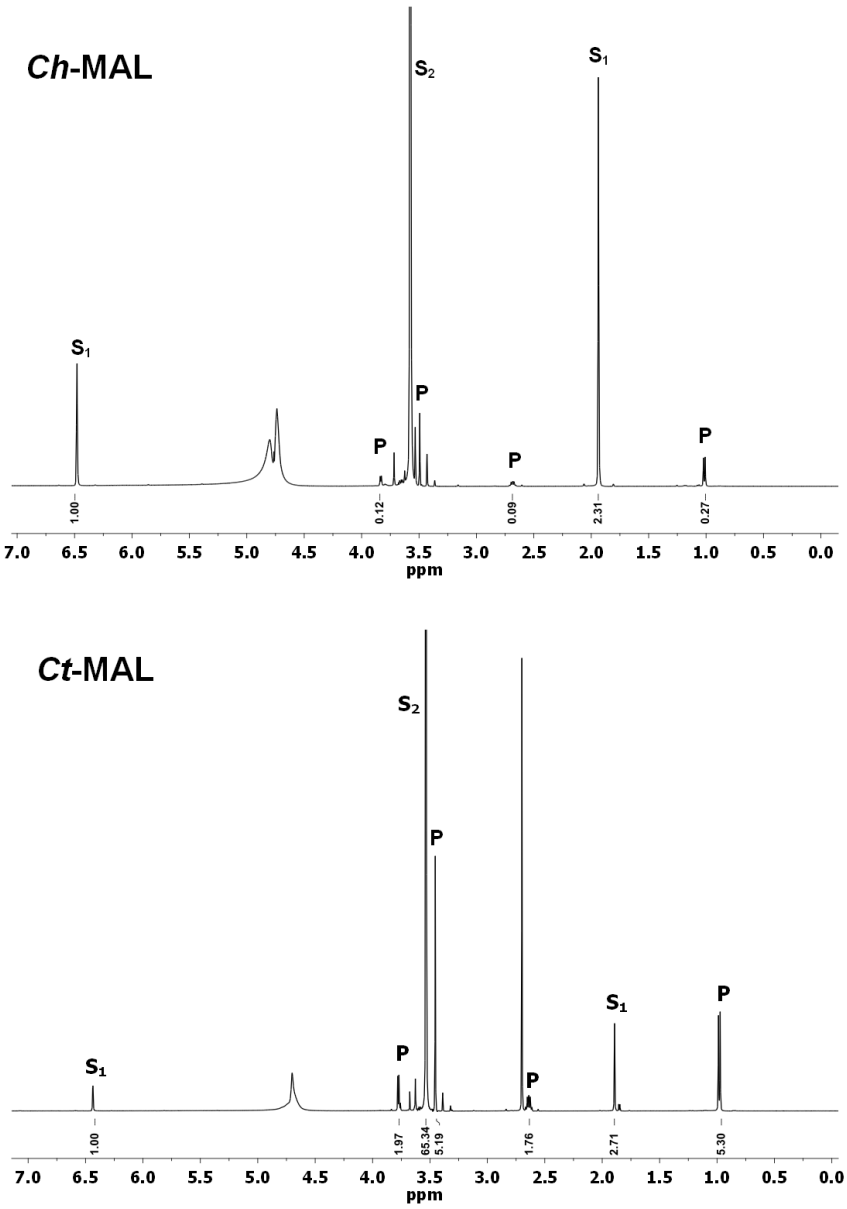

Figure S7. ^{1}H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed methoxylamine addition to mesaconate. The spectra were taken after 14 days of incubation at 22°C. For *Ch*-MAL and *Ct*-MAL the ratio of S_1:P = 91:9 and S_1:P = 33:67, respectively. S_1, mesaconate; S_2, methoxylamine; P, *threo*-(2*S*,3*S*)-*N*-methoxy-3-methylaspartate. The ^{1}H NMR signals for (2*S*,3*S*)-*N*-methoxy-3-methylaspartate are reported elsewhere (Gulzar *et al.* 1997). Impurities (Tris): δ = 3.5 (s); (DMSO): δ = 2.6 (s).

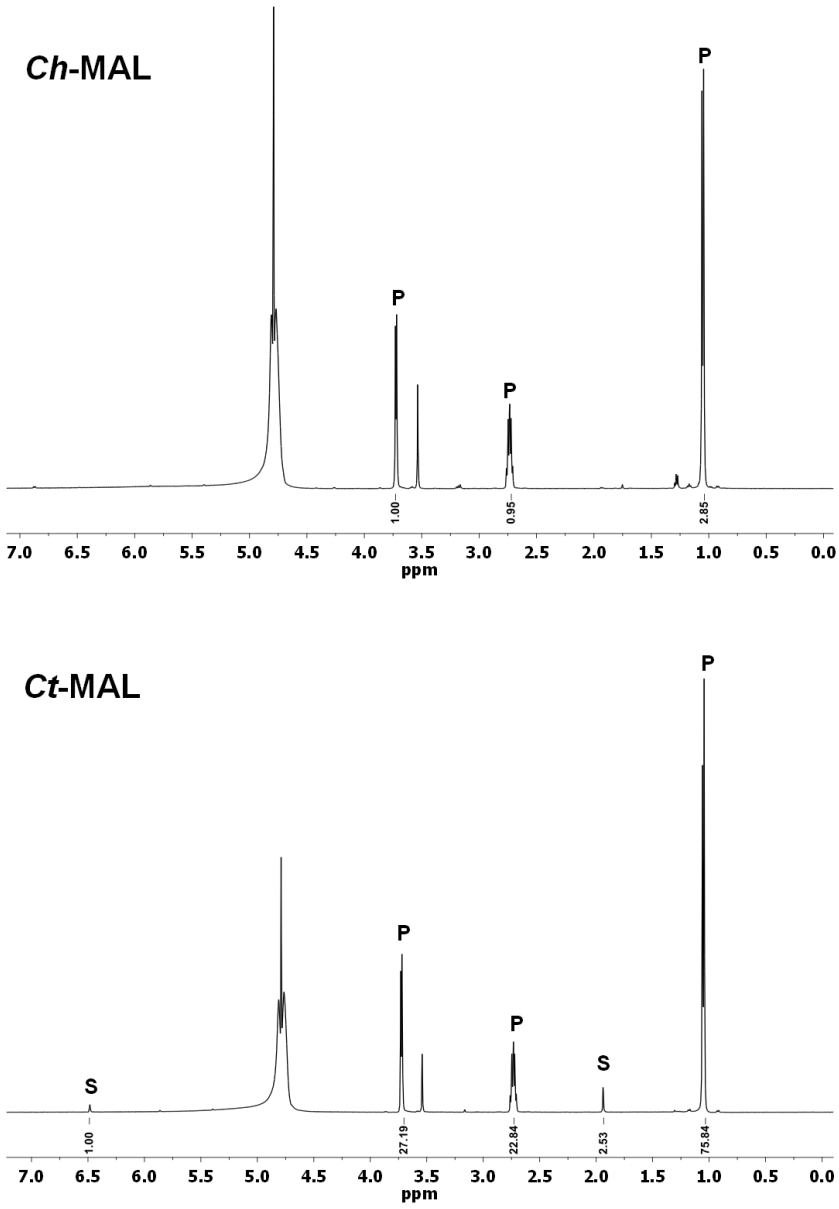

Figure S8. ^{1}H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed hydroxylamine addition to mesaconate. The spectra were taken after 7 days of incubation at 22°C. For *Ch*-MAL and *Ct*-MAL the ratio of S:P = 1:99 and S:P = 4:96, respectively. S, mesaconate; P, *threo*-(2*S*,3*S*)-*N*-hydroxy-3-methylaspartate. The ^{1}H NMR signals for (2*S*,3*S*)-*N*-hydroxy-3-methylaspartate are reported elsewhere (Gulzar *et al.* 1997). Impurity (Tris): δ = 3.5 (s).

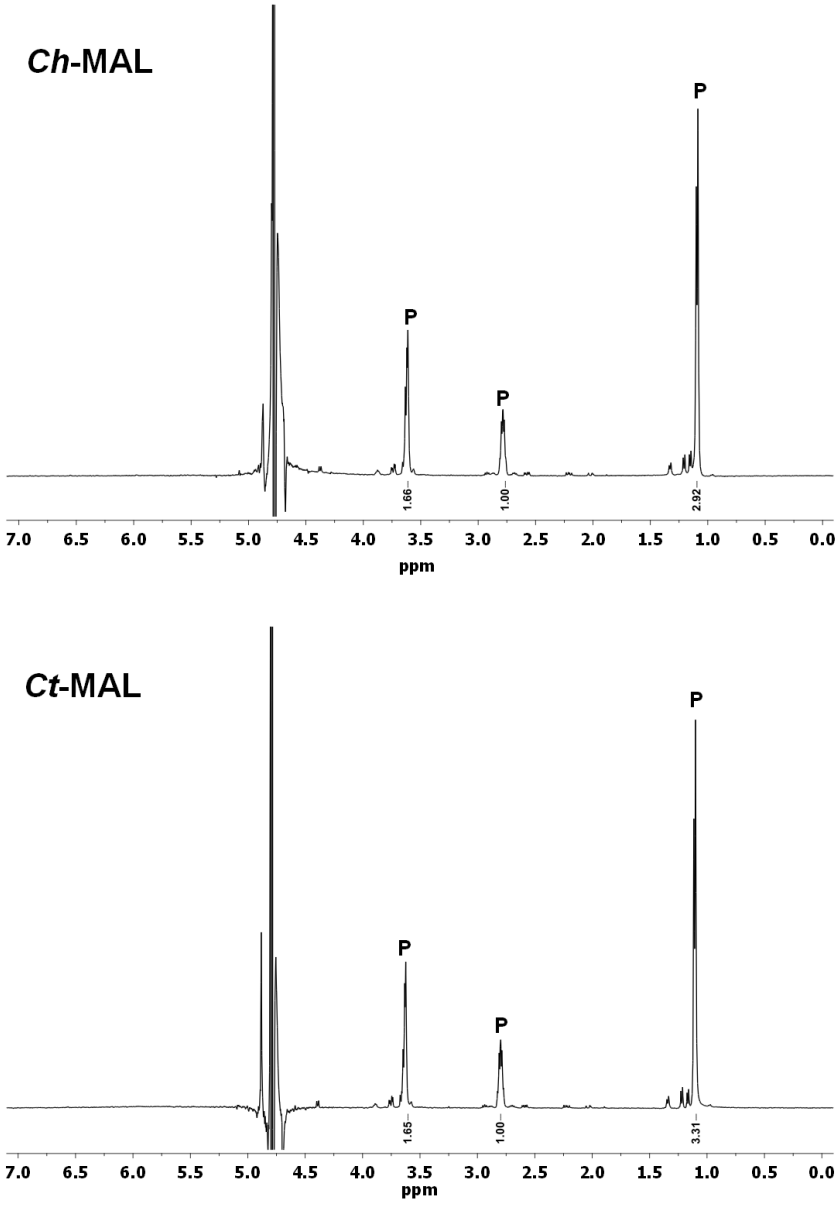

Figure S9. ^{1}H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed hydrazine addition to mesaconate. The spectra were taken after 7 days of incubation at 22°C. For both *Ch*-MAL and *Ct*-MAL complete conversion of substrate to product was achieved. P, *threo*-(2*S*,3*S*)-2-hydrazino-3-methylaspartate. The ^{1}H NMR signals for (2*S*,3*S*)-2-hydrazino-3-methylaspartate are reported elsewhere (Gulzar *et al.* 1997).

References:

Akhtar M, Botting NP, Cohen MA, Gani D (1987) Enantiospecific synthesis of 3-substituted aspartic acids via enzymatic amination of substituted fumaric acids. Tetrahedron 43: 5899-5908

Gulzar MS, Akhtar M, Gani D (1997) Preparation of *N*-substituted aspartic acids via enantiospecific conjugate addition of *N*-nucleophiles to fumaric acids using methylaspartase: synthetic utility and mechanistic implications. J Chem Soc Perkin Trans 1: 649-655