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Supporting Information for

**Characterization of a Thermostable Methylaspartate Ammonia Lyase
from *Carboxythermus hydrogenoformans***

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

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Ct-MAL MKIVDVLCTPGLTGFYFDDQRAIKKGAGHDGFTYTGSTVTEGFTQVRQKGESISVLLVLE 60
Ch-MAL MRIKDVLFVKSSGFYFDDQKAIKSGAVTDGFTYK GKPLTPGFSRVRQGGEAVSIMLFLE 60

Ct-MAL DGQVAHGDCAAVQYSGAGGRDPLFLAKDFIPVIEKEIAPKLI GREITNFKPMAEEFDKMT 120
Ch-MAL NGEIAVGDCVAVQYSGVDGRDPVFLADNFI EVLEEEIKPRLVGYNLVRFREAAARYFTNLT 120
      *

Ct-MAL VN-GNRLHTAIRYGITQAILDAVAKTRKVTMAEVIRDEYNPGA EINAVPVFAQSGDDRYD 179
Ch-MAL DKRGKRYHTALRYGLTQALLDAVAKINRTTMAEVIAE EYGLDLTLNPVPLFAQSGDDRYI 180
      * *

Ct-MAL NVDKMIIKEADVLPHALINNVEEKLGLKGEKLL EYVKWLRDRIIKLRVREDYAPIFHIDV 239
Ch-MAL NADKMILKRVDVLP HGLFN-HPAKTGEEGKNL TEYALWLKQRIKTLG-DHDYLPVFHFDV 238
      *

Ct-MAL YGTIGAAFDVDIKAMADYIQTLAEAAKPFHLRI EGPM DVEDRQKQMEAMRDLRAELDGRG 299
Ch-MAL YGTLGTVFNDNLDRIADYLARLEEKVAPHPLQ IEGPVDLGSKERQIEGLKYLQEKLITLG 298

Ct-MAL VDAELVADEWCNTVEDVKFFTDNKAGHMVQIKTPDLGGVNNIADAICYCKANGMGAYCGG 359
Ch-MAL SKV IIVADEWCNNLSDIKEFVDAGAGGMVQIKSPDLGGVNDIIEAVLYAKEKGTGAYLGG 358
      * * *

Ct-MAL TCNETNRSAEVTNIGMACGARQVLAKPGMGVDEGMMIVKNEMNRVLALVGRRK----- 413
Ch-MAL SCNETDVSAKITVHVGLATGPAQLLVKPGMGVDEGLTIMRNEMMRTLAILQRNKVTFQKKVG 420
      ** *

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

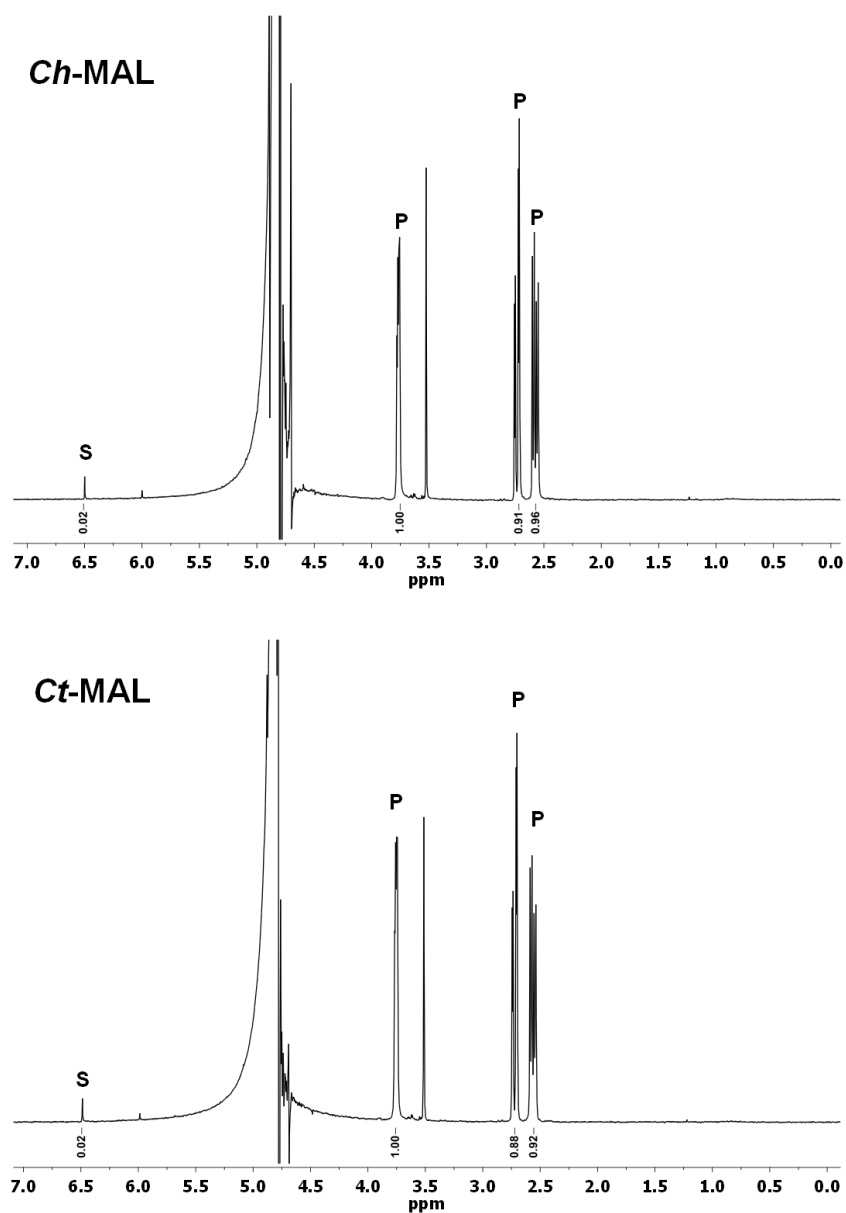


Figure S2. ¹H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed amination of fumarate. The spectra were taken after 7 days of incubation at 22°C. For both *Ch*-MAL and *Ct*-MAL, the ratio of S:P = 1:99, respectively. S, fumarate; P, (*S*)-aspartic acid. The ¹H NMR signals for the enzymatically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

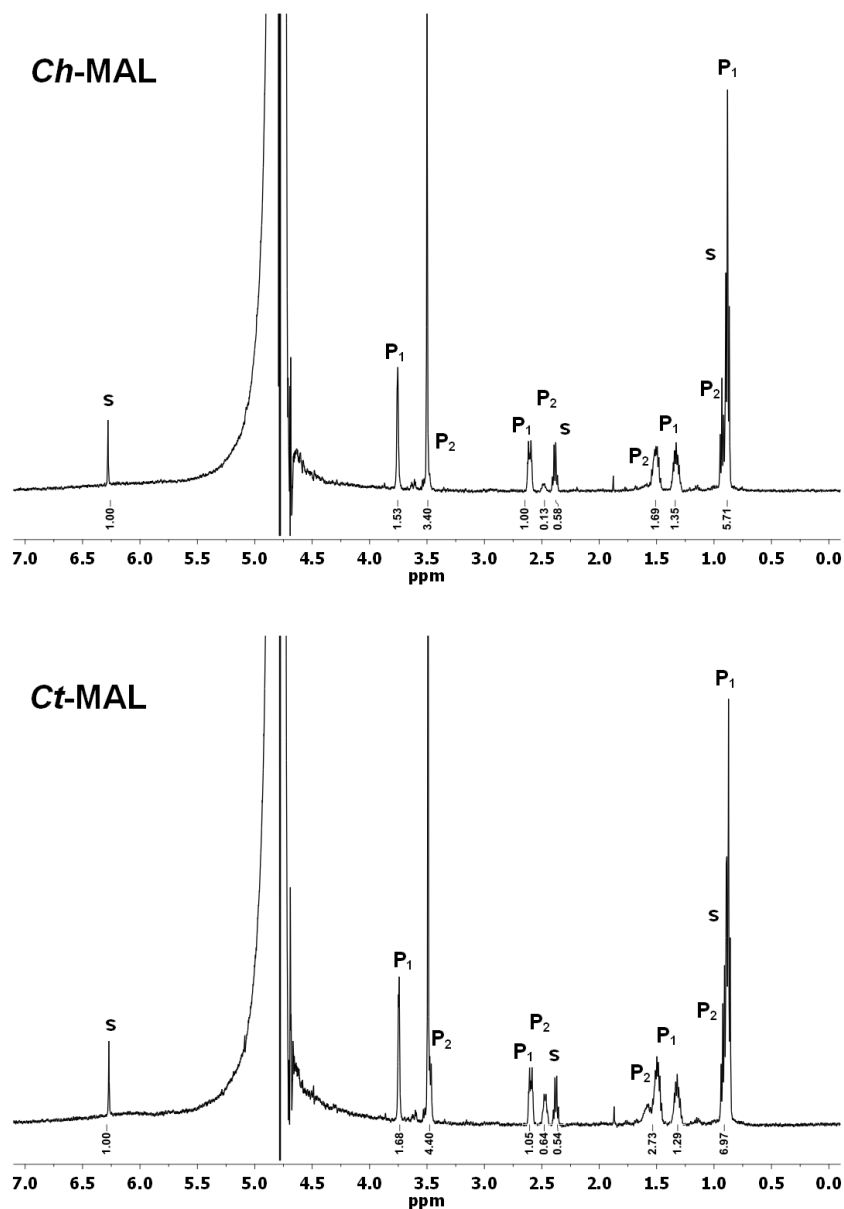


Figure S3. ^1H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed ammonia addition to 2-ethylfumarate. The spectra were taken after 14 days of incubation at 22°C. For *Ch*-MAL and *Ct*-MAL, the ratio of S:P₁:P₂ = 35:60:5 and S:P₁:P₂ = 29:48:23, respectively. S, 2-ethylfumarate; P₁, *threo*-(2*S*,3*S*)-3-ethylaspartate (major diastereoisomer); P₂, *erythro*-(2*S*,3*R*)-3-ethylaspartate (minor diastereoisomer). The ^1H NMR signals for 2-ethylfumarate and (2*S*,3*S*)-3-ethylaspartate are reported elsewhere (Akhtar *et al.* 1987). Impurity (Tris): $\delta = 3.5$ (s).

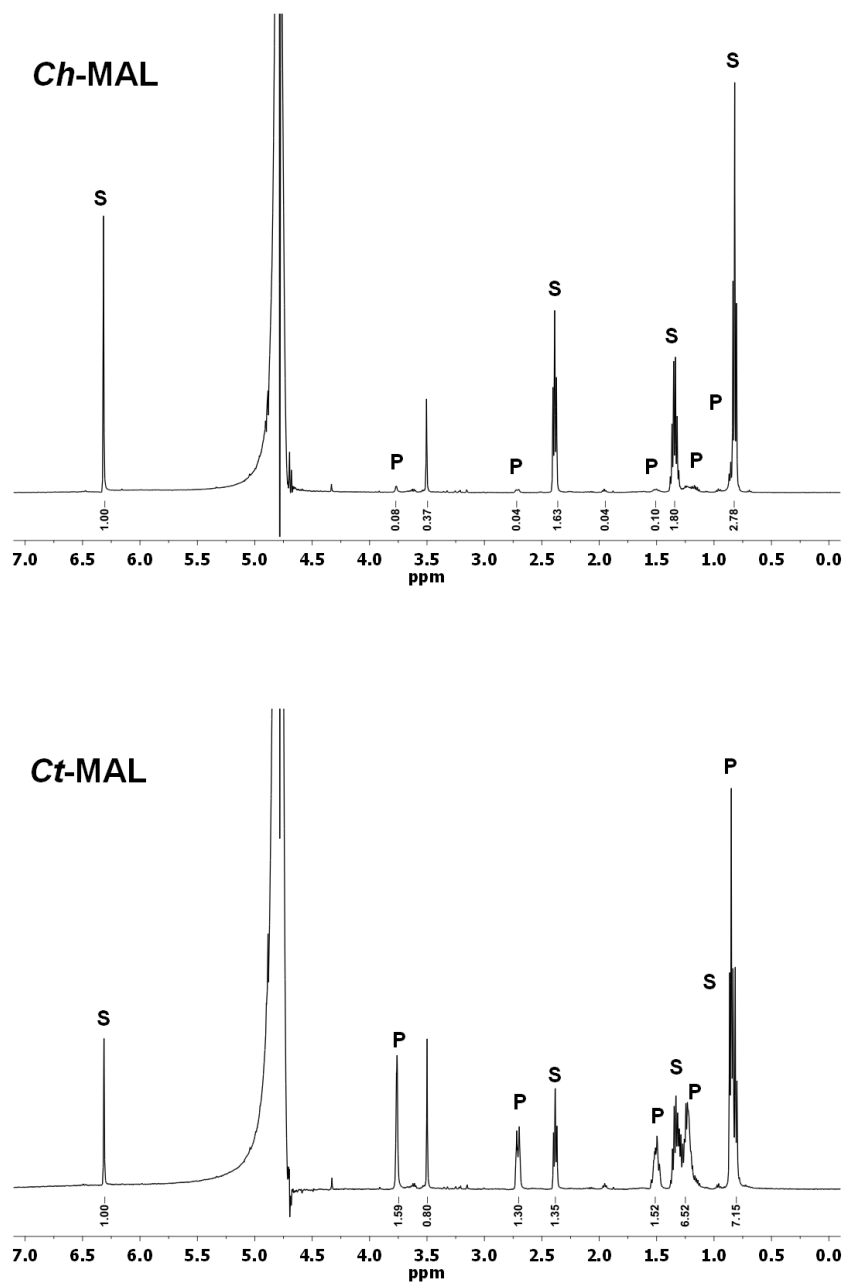


Figure S4. ^1H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed ammonia addition to 2-propylfumarate. The spectra were taken after 14 days of incubation at 22°C. For *Ch*-MAL and *Ct*-MAL, the ratio of S:P = 93:7 and S:P = 39:61, respectively. S, 2-propylfumarate; P, *threo*-(2*S*,3*S*)-3-propylaspartate.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

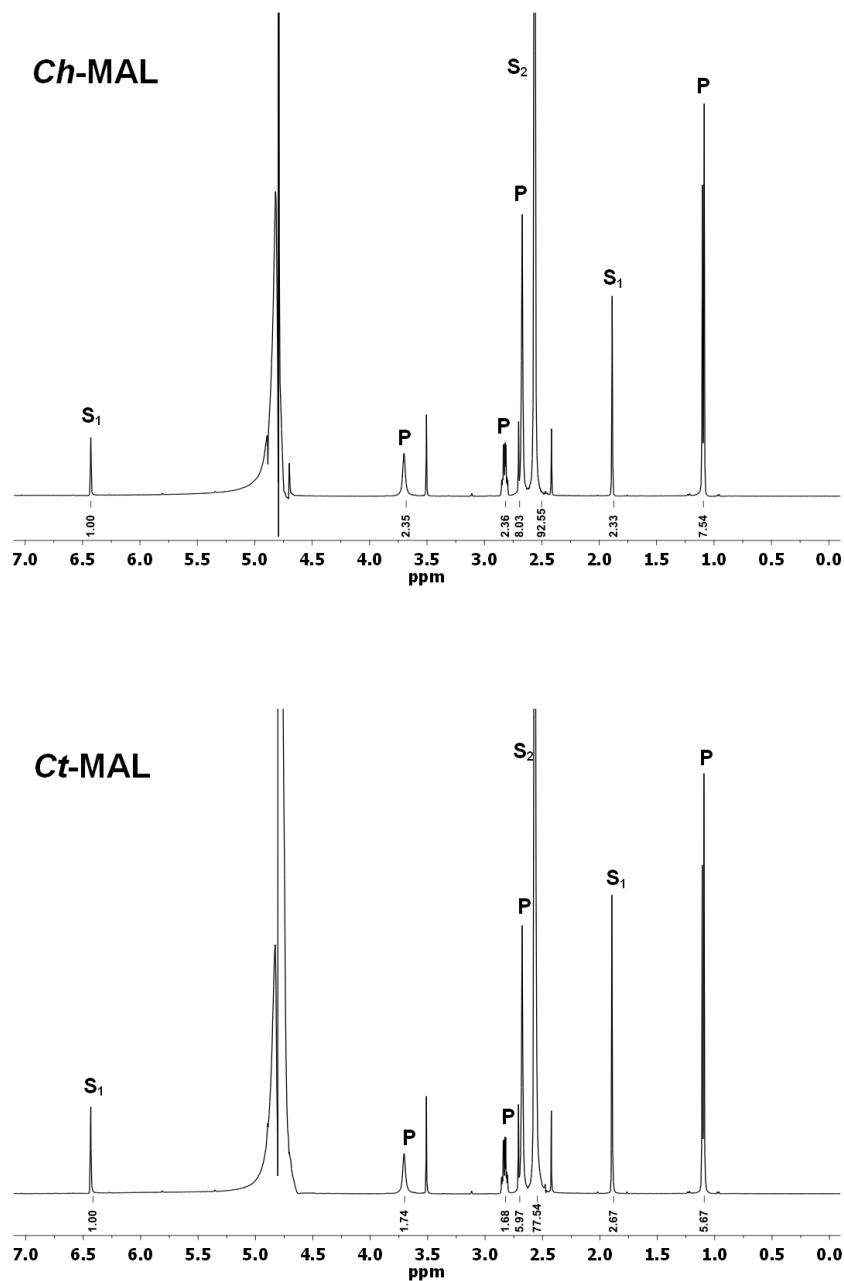


Figure S5. ^1H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed methylamine addition to mesaconate. The spectra were taken after 14 days of incubation at 22°C . For *Ch*-MAL and *Ct*-MAL, the ratio of $\text{S}_1:\text{P} = 30:70$ and $\text{S}_1:\text{P} = 36:64$, respectively. S_1 , mesaconate; S_2 , methylamine; P, *threo*-(2*S*,3*S*)-*N*,3-dimethylaspartate. The ^1H NMR signals for (2*S*,3*S*)-*N*,3-dimethylaspartate are reported elsewhere (Gulzar *et al.* 1997). Impurity (Tris): $\delta = 3.5$ (s).

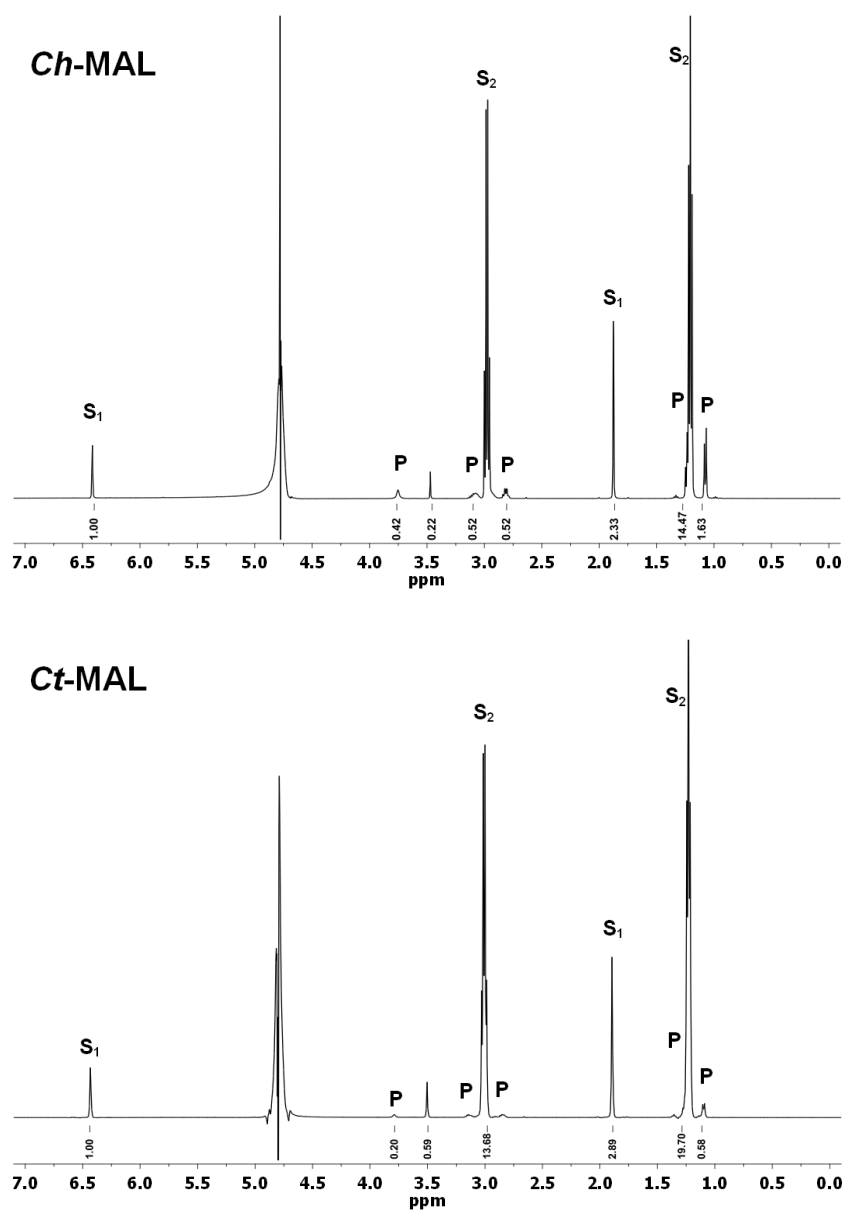


Figure S6. ^1H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed ethylamine addition to mesaconate. The spectra were taken after 14 days of incubation at 22°C . For *Ch*-MAL and *Ct*-MAL, the ratio of $\text{S}_1:\text{P} = 70:30$ and $\text{S}_1:\text{P} = 86:14$, respectively. S_1 , mesaconate; S_2 , ethylamine; P, tentatively identified as 2-ethylamino-3-methylaspartic acid, the enzymatic synthesis of which has not been reported before. Impurity (Tris): $\delta = 3.5$ (s).

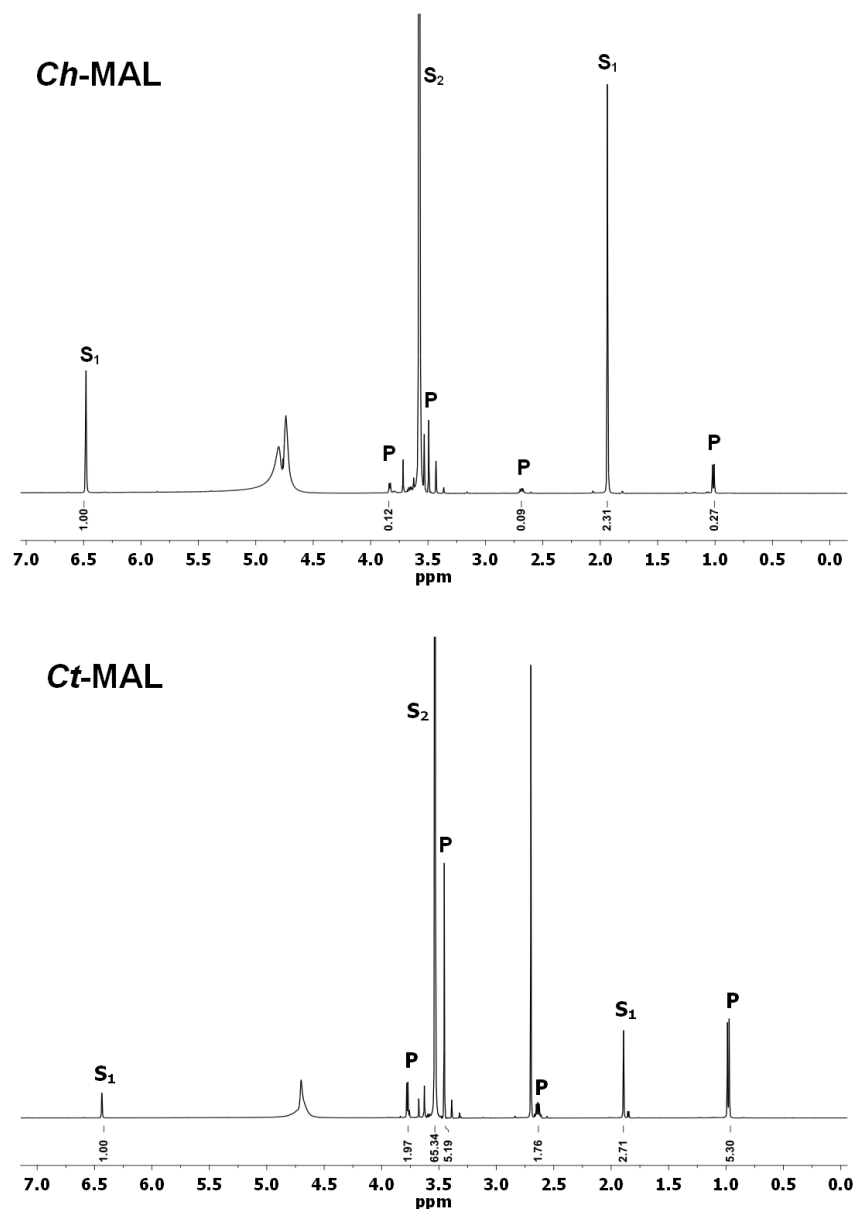


Figure S7. ^1H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed methoxylamine addition to mesaconate. The spectra were taken after 14 days of incubation at 22°C. For *Ch*-MAL and *Ct*-MAL the ratio of $\text{S}_1:\text{P} = 91:9$ and $\text{S}_1:\text{P} = 33:67$, respectively. S_1 , mesaconate; S_2 , methoxylamine; P, *threo*-(2*S*,3*S*)-*N*-methoxy-3-methylaspartate. The ^1H NMR signals for (*2S,3S*)-*N*-methoxy-3-methylaspartate are reported elsewhere (Gulzar *et al.* 1997). Impurities (Tris): $\delta = 3.5$ (s); (DMSO): $\delta = 2.6$ (s).

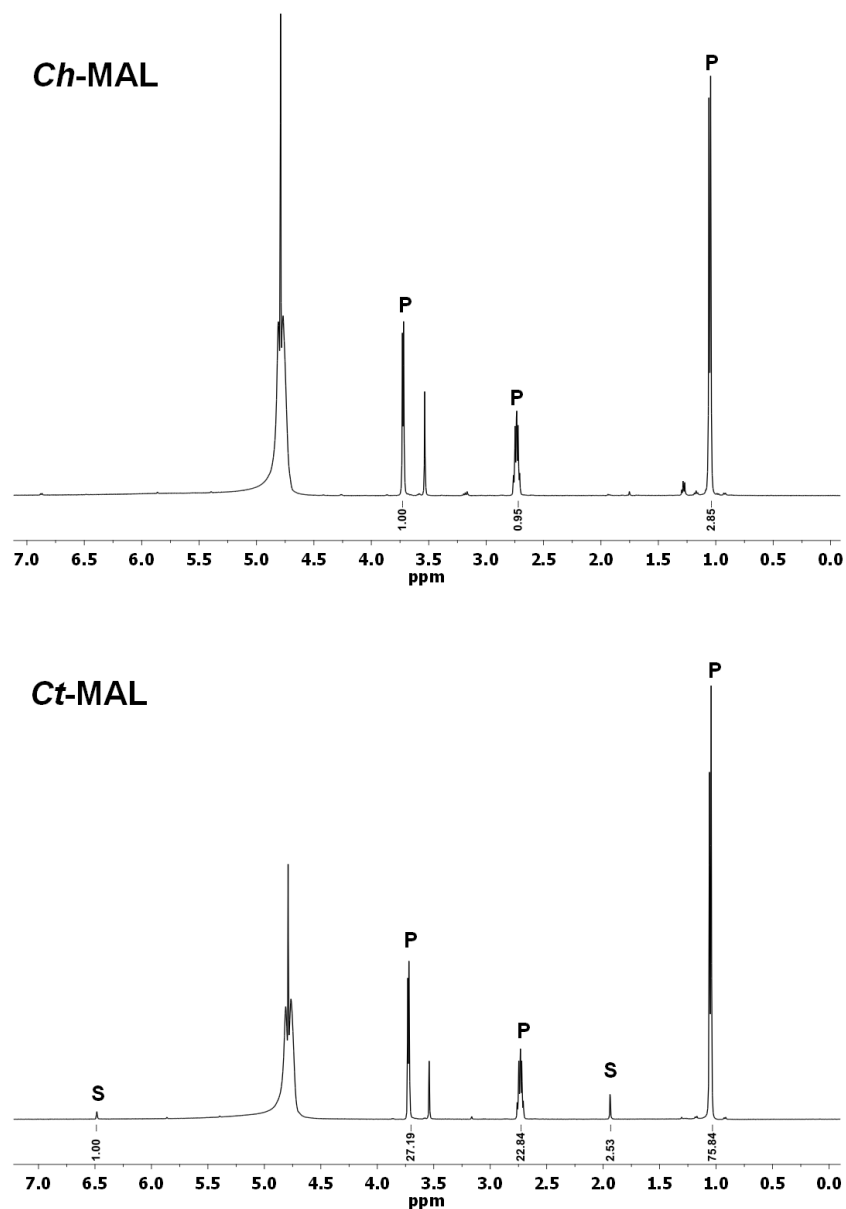


Figure S8. ¹H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed hydroxylamine addition to mesaconate. The spectra were taken after 7 days of incubation at 22°C. For *Ch*-MAL and *Ct*-MAL the ratio of S:P = 1:99 and S:P = 4:96, respectively. S, mesaconate; P, *threo*-(2*S*,3*S*)-*N*-hydroxy-3-methylaspartate.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

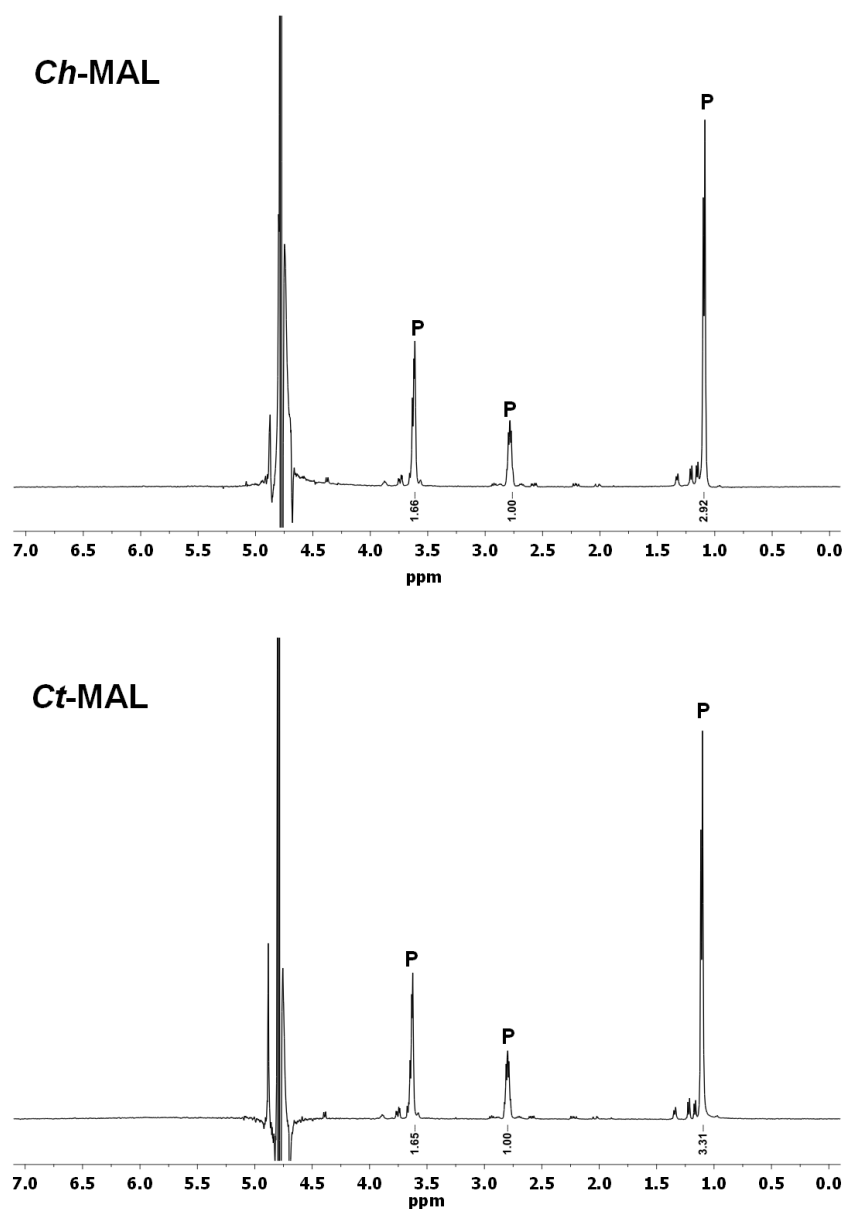


Figure S9. ¹H NMR spectra monitoring the *Ch*-MAL and *Ct*-MAL catalyzed hydrazine addition to mesaconate. The spectra were taken after 7 days of incubation at 22°C. For both *Ch*-MAL and *Ct*-MAL complete conversion of substrate to product was achieved. P, *threo*-(2*S*,3*S*)-2-hydrazino-3-methylaspartate.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

References:

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