

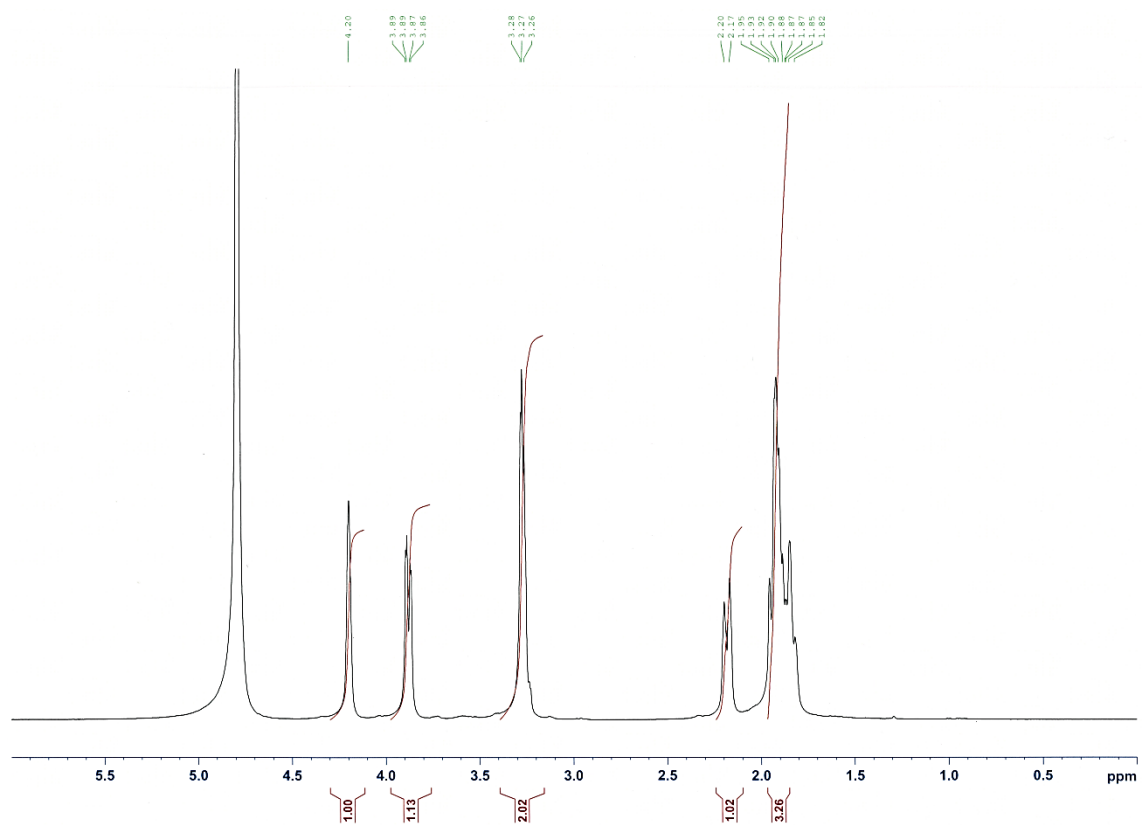
TABLE S1. Substrate specificity analysis of six Pip4H enzymes of filamentous fungi.

Substrate	Pip4H					
	<i>Fo</i>	<i>Fg</i>	<i>Cg</i>	<i>Ao</i>	<i>Pr</i>	<i>An</i>
L-Pip	+ ^a	+	+	+	+	+
D-Pip	-	-	-	-	-	+
L-Pro	+	-	+	-	-	+
D-Pro	-	-	-	-	-	+
L-Leu	+	+	+	+	+	-
D-Leu	-	-	-	-	-	-
L-AABA	+	-	-	-	-	+
D-AABA	-	-	-	-	-	-
<i>cis</i> -5-L-HyPip	+	+	+	+	+	+

a, novel peaks of amino acid products with the molecular weights heavier than their original substrates by 16 Da detected in the LC-MS analysis

FIGURE S1.

A



B

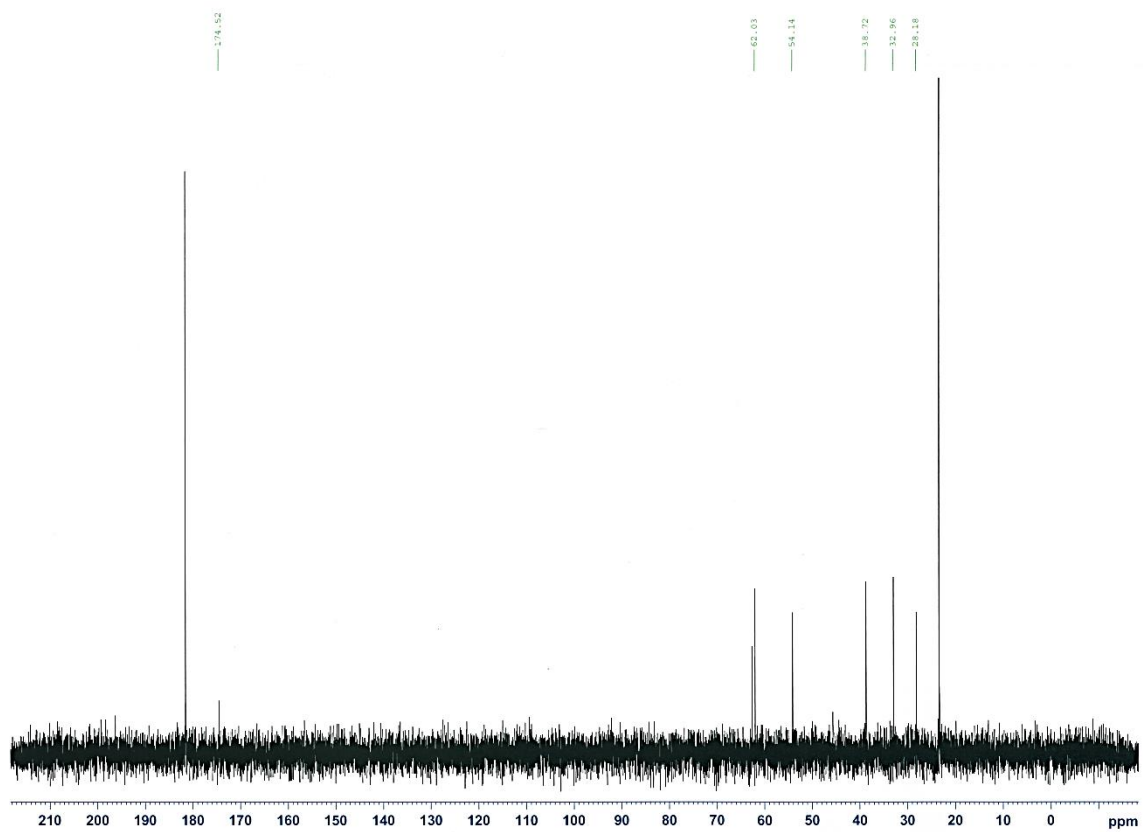
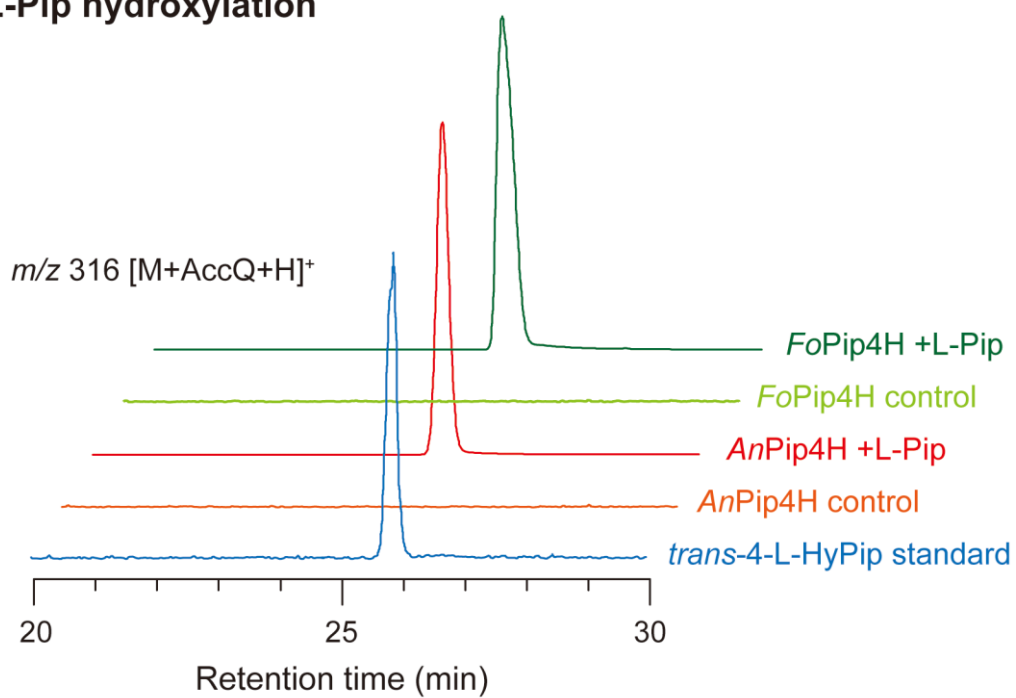


FIGURE S1. NMR analysis of *trans*-4-L-HyPip produced in the reaction of *Fusarium oxysporum* c8D. ^1H NMR (A) and ^{13}C NMR (B) were recorded on an Avance 500 (BrukerBioSpin, MA, USA). D_2O solution was used for the analysis. Chemical shifts are reported in parts per million (ppm, δ). Coupling constants (J values) are given in Hz, and peak multiplicities are denoted by s (singlet), d (doublet), dd (doublet of doublets), and m (multiplet).

FIGURE S2.

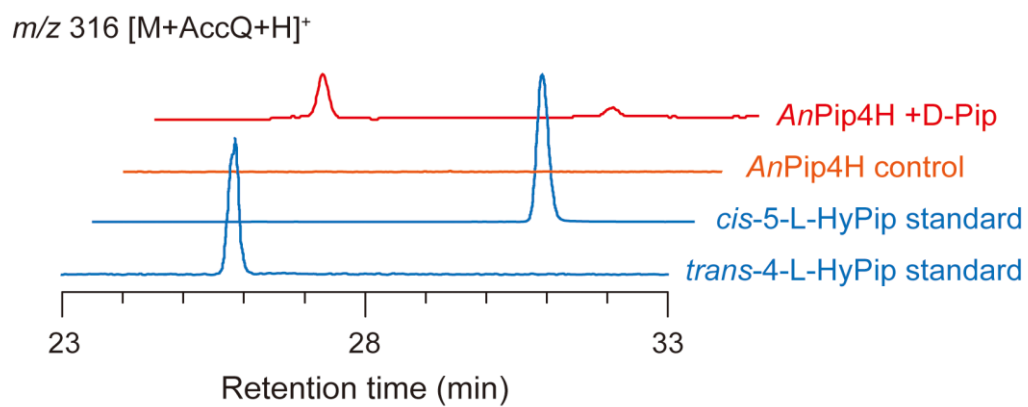
A

L-Pip hydroxylation



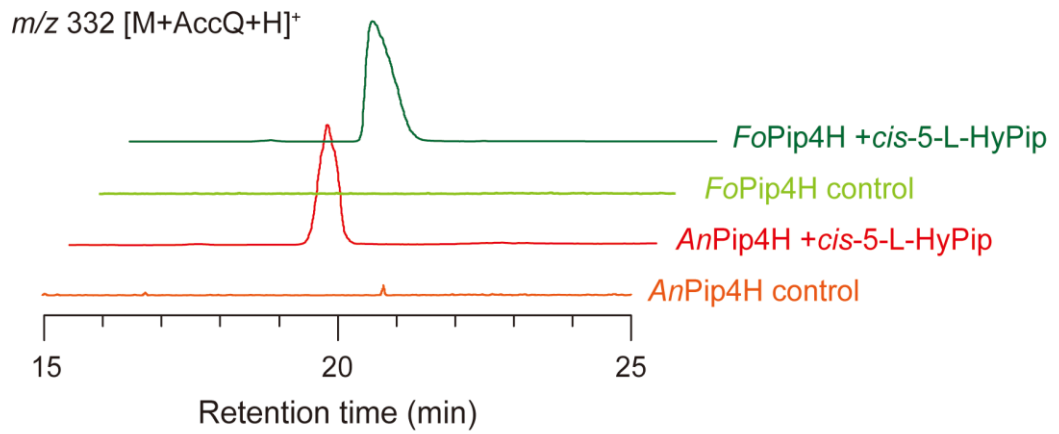
B

D-Pip hydroxylation



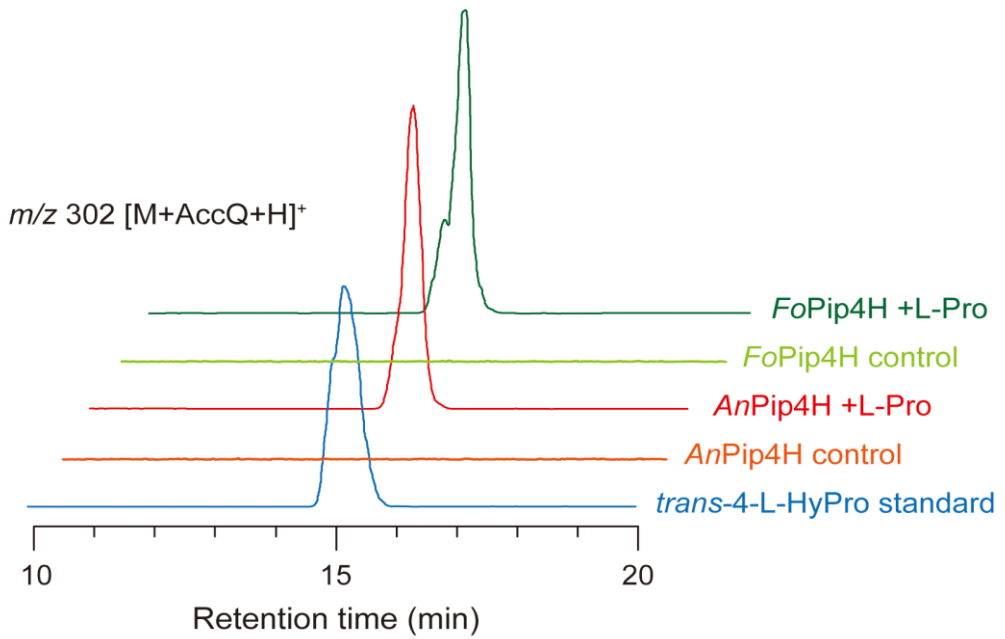
C

***cis*-5-L-HyPip hydroxylation**



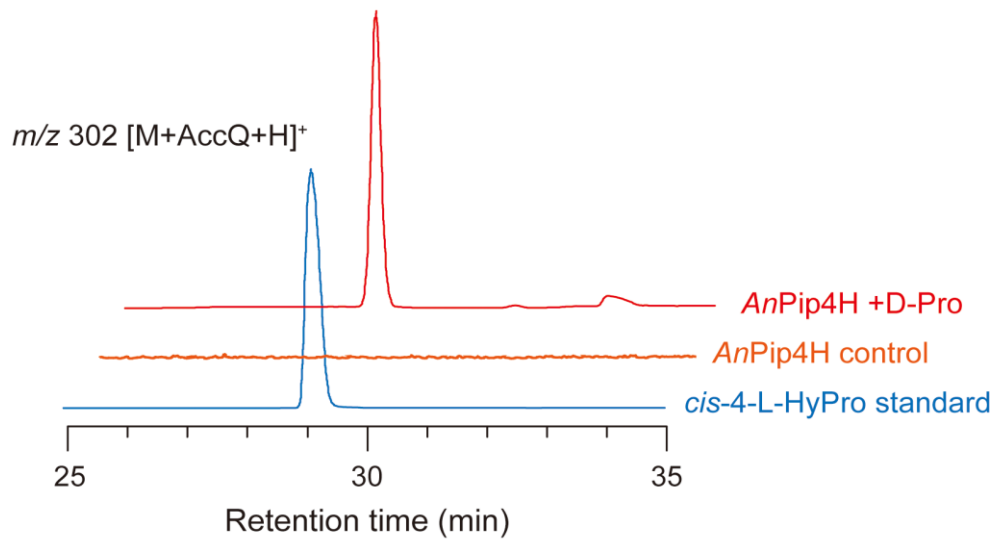
D

L-Pro hydroxylation



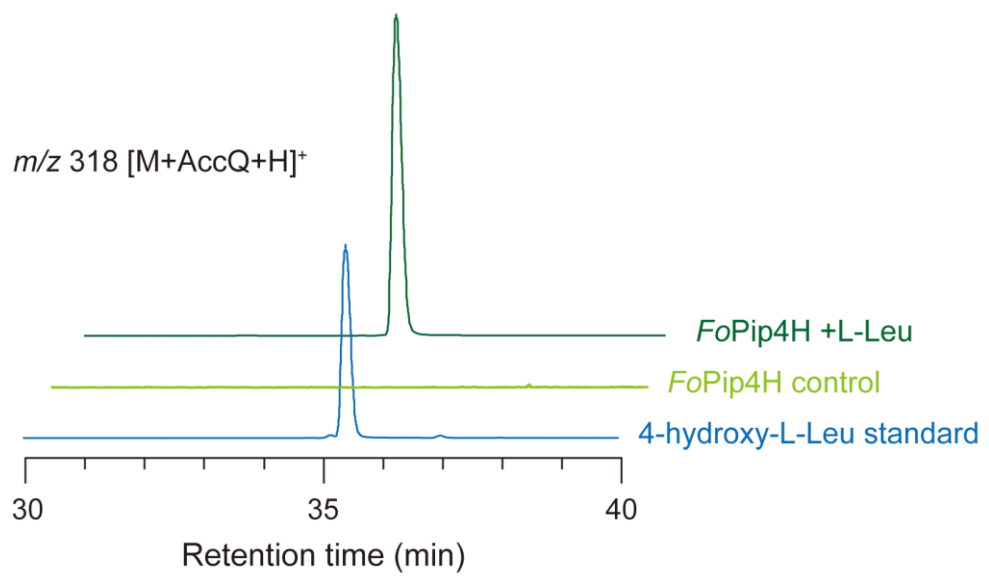
E

D-Pro hydroxylation



F

L-Leu hydroxylation



G

L-AABA hydroxylation

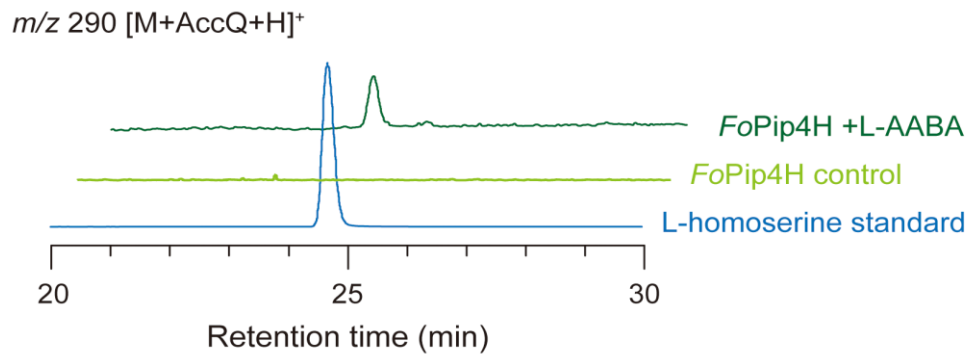


Figure S2. HPLC/ESI-MS analysis of AccQ-Tag derivatized amino acids in the reaction mixtures of *FoPip4H* and *AnPip4H* with various substrates. In each figure, a selected-ion monitoring (SIM) chromatogram of the reaction mixture with a substrate, the reaction mixture without substrate, and, if any, a standard reagent of product were shown. (A) Hydroxylation of L-Pip with *FoPip4H* and *AnPip4H*. Reaction mixtures and a standard reagent of *trans*-4-L-HyPip were compared in SIM chromatogram at m/z 316. (B) Hydroxylation of D-Pip with *AnPip4H*. Reaction mixtures and standard reagents of *cis*-5-L-HyPip and *trans*-4-L-HyPip were compared in SIM chromatogram at m/z 316. (C) Hydroxylation of *cis*-5-L-HyPip with *FoPip4H* and *AnPip4H*. Reaction mixtures were compared in SIM chromatogram at m/z 332. (D) Hydroxylation of L-Pro with *FoPip4H* and *AnPip4H*. Reaction mixtures and a standard reagent of *trans*-4-L-HyPro were compared in SIM chromatogram at m/z 302. (E) Hydroxylation of D-Pro with *AnPip4H*. Reaction mixtures and a standard reagent of *cis*-3-L-HyPro were compared in SIM chromatogram at m/z 302. (F) Hydroxylation of L-Leu with *FoPip4H*. Reaction mixtures and a standard reagent of 4-hydroxy-L-Leu were compared in SIM chromatogram at m/z 318. (G) Hydroxylation of L-AABA with *FoPip4H*. Reaction mixtures and a standard reagent of L-homoserine were compared in SIM chromatogram at m/z 290.