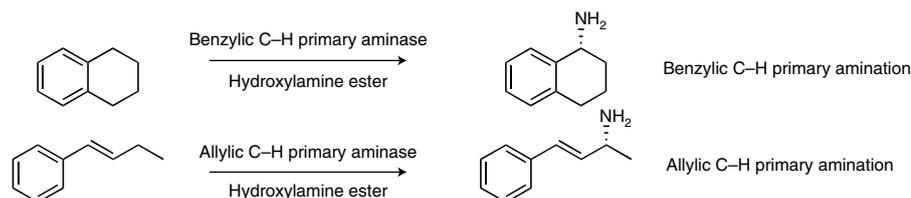


BIOCATALYSIS

Primary amination of C(sp³)-H bonds

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Primary amines play an important role in the biological, chemical and pharmaceutical world. Interestingly, the direct primary amination of C(sp³)-H bonds remained unknown — both in nature and synthetic chemistry.

Now, Zhi-Jun Jia, Shilong Gao and Frances H. Arnold report C(sp³)-H primary aminases as new-to-nature enzymes that can do exactly that. Specifically, the engineered enzymes allow the selective primary amination of tertiary, secondary and primary C-H bonds at benzylic and allylic positions with high regio- and enantioselectivity. A readily available hydroxylamine ester is used as the nitrogen source.

The C(sp³)-H primary aminases are based on cytochrome P450 from *Bacillus megaterium*, which performs C(sp³)-H hydroxylation of fatty acids in nature. The rationale was to identify P450 variants that can use an unprotected iron nitrenoid species for the desired amination reaction.

First, benzylic C-H bonds were targeted for functionalization. A previously engineered P450 variant produced the wanted primary benzylic amine, but with a very low total turnover number (TTN). Based on structural data, residues for site-saturation mutagenesis were chosen

and some rounds of engineering and screening afforded a variant that performed the reaction in an efficient and enantioselective manner. This enzyme allowed the primary C-H amination of several substrates with primary, secondary and tertiary benzylic C-H bonds, and was therefore termed a benzylic C-H primary aminase.

Due to the sensitivity of olefin groups, the selective primary amination of allylic C-H bonds provides an extra challenge compared to benzylic C-H bonds. An intermediate variant from previous screens was taken and successfully evolved into an allylic C-H primary aminase. This enzyme was shown to be capable of selectively functionalizing primary, secondary and tertiary allylic C-H bonds with up to 3930 TTN and 94% enantiomeric excess.

These primary aminases offer new opportunities to introduce amine groups into organic molecules in a stepwise and atom-economic fashion and could be useful for strategies to reformulate the nitrogen metabolism.

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