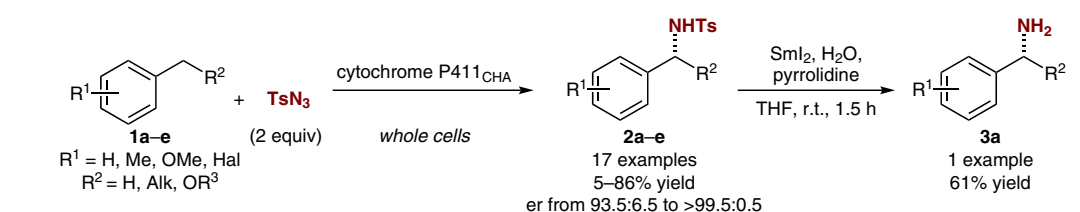
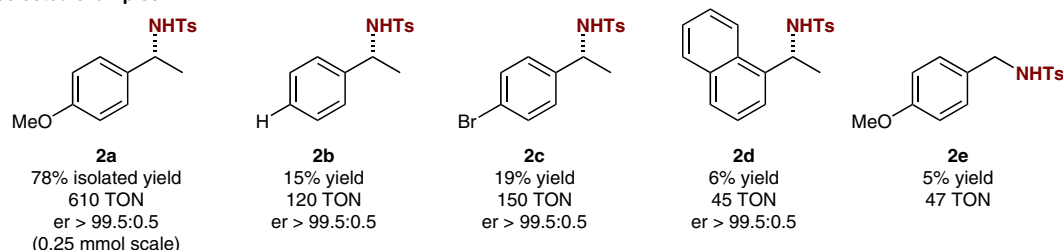


Directed Evolution toward an Iron-Heme Enzyme for Asymmetric C–H Amination



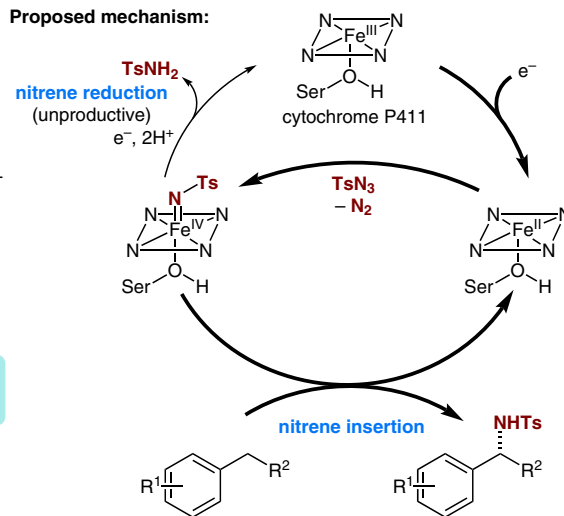
Selected examples:



Directed evolution for C–H amination:

variant	yield	er	TON
P-4	11 ± 1%	43:57	310
P-4 A82L	51 ± 3%	88.5:11.5	1000
P-4 A82L A78V	66 ± 2%	90:10	1200
P-4 A82L A78V F263L	66 ± 2%	>99.5:0.5	NA
P-4 A82L A78V F263L E267D (P411 _{CHA})	66 ± 3%	>99.5:0.5	1000

Proposed mechanism:



Significance: Arnold and co-workers report the directed evolution from iron-heme P450_{BM3} to P411_{CHA} for the highly enantioselective intermolecular amination of benzylic C–H bonds with up to 1300 catalytic turnovers. The authors suggest that the reaction proceeds through a commonly accepted iron nitrenoid intermediate, which undergoes nitrene insertion to afford valuable benzyl amines in up to 87% yield and >99.5:0.5 er.

Comment: The authors discovered that P-4, a P450_{BM3} variant with 17 mutations from the wild-type, catalyzes the benzylic C–H amination of 4-ethylanisole, albeit with low enantioselectivity. Through sequential rounds of site-selective mutagenesis, P-411_{CHA} was found to dramatically improve the yield and enantioselectivity of the reaction for a wide range of electronically-differentiated substrates. X-ray crystallography showed that all of the beneficial mutations lie within the active site of the enzyme.

SYNFACTS Contributors: Benjamin List, Jennifer L. Kennemur
Synfacts 2017, 13(09), 0981 Published online: 18.08.2017

DOI: 10.1055/s-0036-1590761; Reg-No.: B06417SF