Enzymatic Substitution of Amides with Thioamides on Peptides

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Sulfur substitution for peptide backbone amide oxygen is a modification present in several RiPPs (Ribosomally synthesized post-translationally modified peptides) and methyl-coenzyme M reductase (MCR), the hallmark enzyme for anaerobic methanogenesis. Despite the significant impact thioamides are expected to exert on the conformational dynamics of a peptide or protein, their biosynthetic installation remains enigmatic. A recent study has shown that ycaO and tfuA genes are responsible for this modification on MCR. Analogous to azoline formation catalyzed by some YcaO homologs, we hypothesized that the YcaO protein installs the thioamide onto its peptidic substrate by the ATP-dependent activation of the amide backbone. In vitro reconstitution studies have revealed that YcaO proteins from methanogens indeed catalyze thioamide substitution on MCR-derived peptides in the presence of ATP and inorganic sulfide and that the TfuA partner protein appears to facilitate the reaction. The YcaO from Methanopyrus kandleri was shown to bind ATP in a manner characteristic to the YcaO family by crystallography study, which supported the ATP-dependent activation mechanism. Substrate tolerance and engagement of the YcaO was investigated by mass spectrometry-based enzymatic assays and fluorescent polarization-based binding studies. With the biochemical capability established, we subsequently surveyed the available bacterial and archaeal genomes for TfuA-associated YcaO-encoding biosynthetic gene clusters, which revealed thioamidated peptides are may be an underrepresented RiPP class.

One Regio- and Stereoisomeric Product in Pd-catalyzed Stereospecific Couplings of Unactivated Alkylboronic Acids

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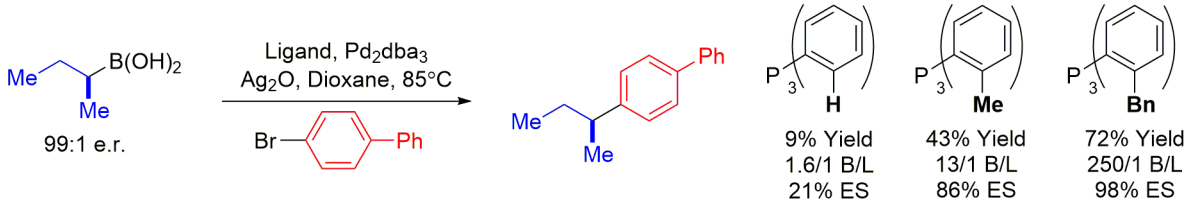
The versatile approach of building block-based synthesis has been more challenging to apply to small molecules rich in Csp^3 carbons and stereocenters. In this context, stereospecific Csp^3 coupling methods must generate products free of contaminating regioisomers and stereoisomers. To suppress the undesired, side-product forming pathways of β-hydride elimination and stereoinvertive transmetalation, phosphine ligands were designed with sterics projecting into the reaction sphere. The novel ligand tris(2-benzylphenyl)phosphine was discovered to give both high regiospecificity (250/1 branched/linear product ratio) and enantiospecificity (98% ES). A further improvement to 100% ES was achieved by tuning ligand electronics. The scope and functional group tolerance have been explored, and the method has also being used for the building block-based synthesis of all four possible stereoisomers of xylarinic acid A.



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