Chemical optimization of artificial metalloenzymes based on the biotin-avidin technology: (*S*)-selective and solvent-tolerant hydrogenation catalysts *via* the introduction of chiral amino acid spacers†

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Incorporation of biotinylated-[rhodium(diphosphine)]$^+$ complexes, with enantiopure amino acid spacers, in streptavidin affords solvent-tolerant and selective artificial metalloenzymes: up to 91% ee (*S*) in the hydrogenation of *N*-protected dehydroamino acids.

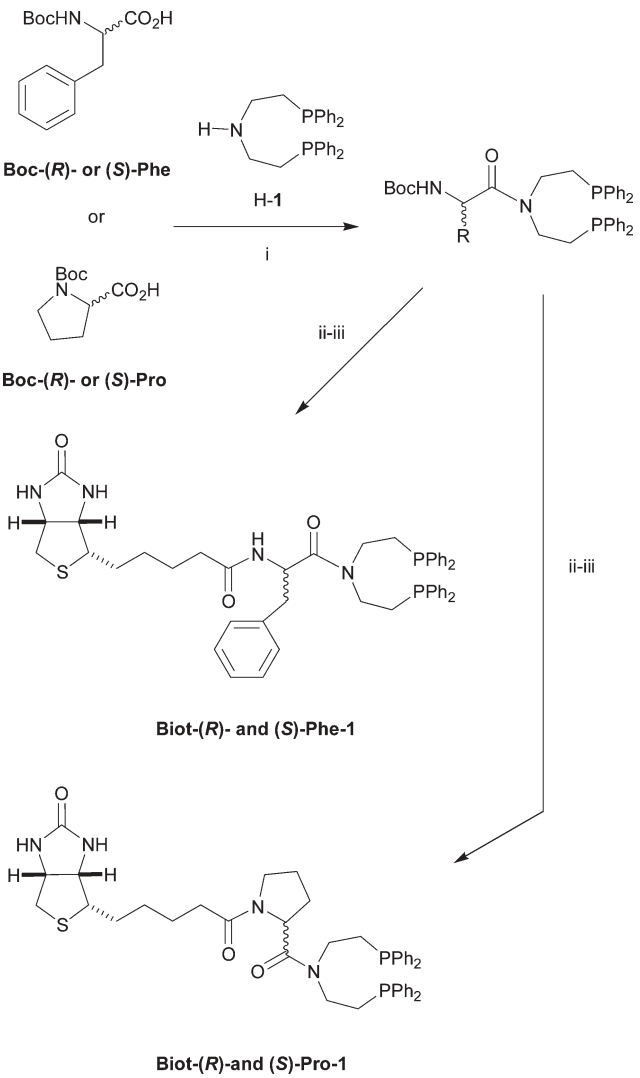
Traditionally, enantioselective catalysis has been subdivided into three areas: homogeneous-, heterogeneous- and enzymatic-catalysis.[1,2] In recent years, two additional areas have witnessed a "rebirth": organocatalysis[3–8] and artificial metalloenzymes.[9–12]

In this later area, an achiral catalyst precursor is incorporated into a protein to produce an enantioselective hybrid catalyst with properties reminiscent both of enzymes and of homogeneous catalysts. Both covalent and supramolecular anchoring strategies have been pursued successfully to yield sulfoxidation, ester hydrolysis and hydrogenation artificial metalloenzymes.[9,13–16] Inspired by Whitesides' early report, we have recently exploited the biotin-avidin technology to produce artificial hydrogenases.[10,12,17–20] Relying on both chemical- and genetic-optimization strategies (*e.g.* chemogenetic), we produced both (*R*)- and (*S*)-selective catalysts for the hydrogenation of *N*-protected dehydroamino acids.[21] Herein, we report on our efforts to improve both on the stability and on the selectivity of artificial metalloenzymes based on the biotin-avidin technology.

Having demonstrated that the introduction of a short achiral amino acid spacer between the biotin anchor and the rhodium-diphosphine moiety has, in some cases, a positive influence on both the activity and the selectivity of hybrid catalysts, we proceeded to incorporate enantiopure amino acid spacers.

For initial studies, we focused on phenylalanine ((*R*)- or (*S*)-Phe) and proline ((*R*)- or (*S*)-Pro) as spacers. As the biotin-binding pocket in streptavidin is lined with four tryptophane residues, we speculated that an aromatic side chain on the spacer may lock, *via* π–π interactions, the catalyst in a privileged position. In the same spirit, proline was selected for its restricted degrees of freedom imposed by the pyrrolidine ring.

The synthesis of Biot-(*R*)-Phe-1, Biot-(*S*)-Phe-1, Biot-(*S*)-Pro-1 and Biot-(*R*)-Pro-1 is outlined in Scheme 1. Starting from the commercially available *N*-Boc-protected amino acids, the two pairs of epimeric ligands can be prepared in three steps in 34–58% overall yield.



Scheme 1 Synthesis of biotinylated ligands bearing an enantiopure amino acid spacer. Reagents: (i) chlorodimethoxytriazine, NMO, CH_3CN, RT, 48 h; (ii) trifluoroacetic acid, anisole, CH_2Cl_2, RT, 3 h; (iii) (+)-biotin-OC_6F_5, $N(i\text{-}Pr)_2Et$, DMF, RT, 48 h.

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† Electronic supplementary information (ESI) available: Full experimental details of the synthesis of ligands and hydrogenation procedures. See http://dx.doi.org/10.1039/b509015f

Table 1 Hydrogenation experiments[a]

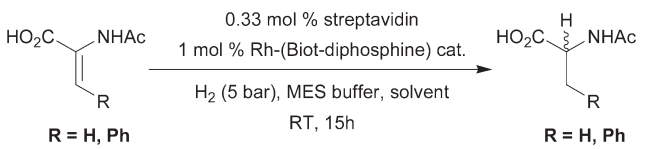
		N-AcAla		*N*-AcPhe	
Entry	Ligand	ee (%)	Conversion (%)	ee (%)	Conversion (%)
1	Biot-(*R*)-Phe-1	66 (*R*)	100	64 (*R*)	100
2	Biot-(*S*)-Phe-1	73 (*S*)	100	64 (*S*)	88
3	Biot-(*S*)-Pro-1	23 (*R*)	100	23 (*R*)	100
4	Biot-(*R*)-Pro-1	86 (*S*)	100	91 (*S*)	100
5	Biot-(*R*)-Pro-1/45% dmso	87 (*S*)	100	86 (*S*)	94
6	Biot-(*R*)-Pro-1/biphasic EtOAc	83 (*S*)	90	87 (*S*)	85
7	Biot-1	94 (*R*)	100	93 (*R*)	85
8	Biot-1/45% dmso	16 (*R*)	71	24 (*R*)	9
9	Biot-1/biphasic EtOAc	30 (*R*)	56	31 (*R*)	5

[a] Both substrates (260 μl, 50 eq. each/Rh cat., 24 mM) were dissolved in 0.38 M MES, added to a solution containing $[Rh(COD)(L)]BF_4$ (100 μl, 1 eq., 0.62 mM in dmso) and streptavidin (tetrameric, 100 μl, 0.33 eq./Rh cat., 0.21 mM in H_2O) in a 3 mL Pyrex tube. The final volume was adjusted with solvent (640 μl, water, dmso or EtOAc) to 1100 μL and the mixture hydrogenated at 5 bar H_2 for 15 h.

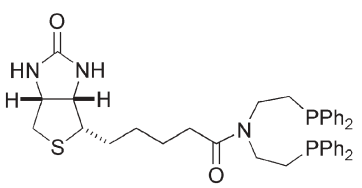
The new ligands were tested in the rhodium catalyzed hydrogenation of both α-acetamidoacrylic and α-acetamidocinnamic acids in the presence of streptavidin to yield *N*-acetamidoalanine (*N*-AcAla) and *N*-acetamidophenylalanine (*N*-AcPhe) respectively (Scheme 2). For comparison purposes, catalytic runs were performed with the biotinylated ligand devoid of spacer Biot-1 (Scheme 3).[18] The results are summarized in Table 1.‡ Introduction of a phenylalanine spacer, yields the reduction products in up to 73% ee (Table 1, entries 1, 2). Interestingly, depending on the absolute configuration of the Phe-spacer, both *R* and *S* products are obtained with nearly identical but opposite enantioselectivity.

Introduction of an (*S*)-proline spacer, produces (*R*)-*N*-AcAla and (*R*)-*N*-AcPhe with very modest enantioselectivity (entry 3). In the presence of the (*R*)-proline spacer however, good enantioselectivities and conversions are obtained for both (*S*)-*N*-AcAla (86% ee) and (*S*)-*N*-AcPhe (91% ee). In contrast to the phenylalanine spacer where both combinations are equally effective in terms of activity and selectivity, the ligand with a proline spacer yields clear matched- and mismatched-combinations.

These results suggest that the chiral environment, and possibly the position of the catalyst within the streptavidin binding site, changes dramatically upon inverting the configuration of the spacer. This emphasizes the importance of second coordination sphere interactions between the spacer and the host protein in positioning the rhodium moiety within the biotin binding pocket.



Scheme 2 Hydrogenation of both α-acetamidoacrylic (R = H) and α-acetamidocinnamic acids (R = Ph).



Scheme 3 Structure of Biot-1.

In order to test the stability and robustness of the (*S*)-selective artificial metalloenzyme, we tested $[Rh(COD)(Biot\text{-}(R)\text{-}Pro\text{-}1)]^+ \subset$streptavidin in the presence of increasing amounts of dimethyl sulfoxide (dmso). Increasing the organic solvent content from 9% (used so far for all screening experiments) to 45%, only a very modest erosion in activity and selectivity is observed (Table 1, entry 5). Very similar results are obtained under biphasic reaction conditions using ethylacetate as organic phase (Table 1, entry 6). In strong contrast, the (*R*)-selective catalyst $[Rh(COD)(Biot\text{-}1)]^+ \subset$streptavidin, performs poorly in the presence of increasing amounts of organic (water miscible or non-miscible) solvents (Table 1, entries 7–9).

These experiments demonstrate that incorporation of (*R*)-Pro as a spacer yields an (*S*)-selective hydrogenation catalyst with enhanced stability towards organic solvents (both miscible and non-miscible). Due to the poor solubility of olefin substrates in water, coupled to the straightforward incorporation of enantiopure amino acid spacers between biotin and the ligand, these findings thus significantly broaden the scope of artificial metalloenzymes based on the biotin-avidin technology.

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Notes and references

‡ In the absence of streptavidin but otherwise identical reaction conditions, all catalyst precursors afford quantitatively the reduction products in very low ee (ee < 10% in all cases).

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