VIP Asymmetric Catalysis

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Artificial Metalloenzymes for Asymmetric Allylic Alkylation on the Basis of the Biotin–Avidin Technology**

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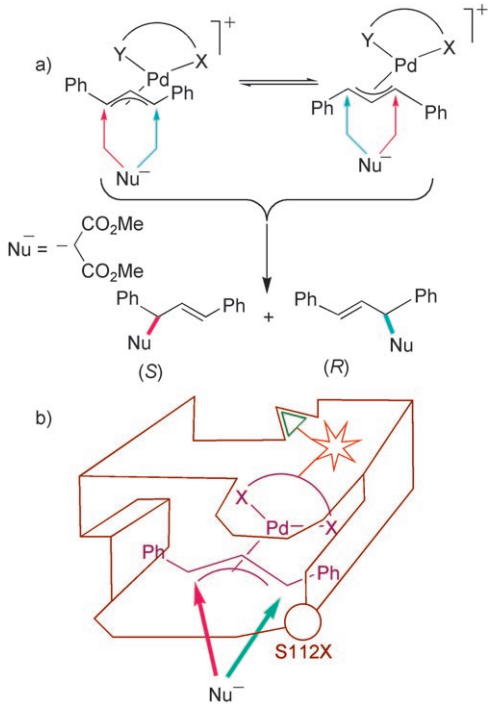
Dedicated to Professor Dieter Seebach on the occasion of his 70th birthday

Palladium-catalyzed C–C bond-forming reactions play a prominent role in the construction of complex organic molecules.[1–4] As this versatile precious metal is absent in natural enzymes, nature has devised very different strategies to create C–C bonds for the construction of complex natural products.[5–7]

The palladium-catalyzed asymmetric allylic alkylation (AAA) is a C–C bond-forming reaction that has attracted much attention.[4,8–13] In this transformation, the enantiodiscrimination event occurs through the external attack of a soft nucleophile on a palladium-bound η^3-allyl moiety (Scheme 1 a).[4,12] The AAA thus bears resemblance to enzymatic reactions, in which the substrate(s) need not necessarily bind to the active site of the enzyme for the reaction to proceed with high stereoselectivity.[7]

In recent years, there has been increasing interest in the creation of artificial metalloenzymes for enantioselective catalysis. For this purpose, an active-catalyst precursor is anchored in a biomolecule, which provides the chiral environment.[14–23] The enantioselective reactions implemented thus far include ester hydrolysis,[24] dihydroxylation,[25] epoxidation,[26,27] sulfoxidation,[28–32] hydrogenation,[33–43] transfer hydrogenation,[44,45] and Diels–Alder reactions.[46–50]

Inspired by the early studies of Wilson and Whitesides,[33] several research groups[34,35,41] have exploited the biotin–avidin technology to ensure the localization of a biotinylated catalytic moiety within (strept)avidin (either avidin or streptavidin). This methodology has allowed the creation of artificial metalloenzymes for enantioselective hydrogenation and transfer-hydrogenation reactions.[33–45]



Scheme 1. The postulated enantiodiscrimination event in the AAA in a) a homogeneous catalytic system and b) an artificial metalloenzyme. The host protein (streptavidin, brown) displays high affinity for the anchor (biotin, green triangle). The introduction of an amino acid spacer (red star) combined with a chelating ligand (purple) allows the chemical optimization of both the activity and the selectivity of the catalyst. Each spacer places the palladium moiety in a distinct chiral environment. Site-directed mutagenesis at position S112X enables the genetic fine-tuning of the host protein to afford an enantioselective allylic alkylase based on the biotin–avidin technology.

Herein we describe our efforts to create artificial metalloenzymes for the AAA. We show that a combination of chemical and genetic optimization allows the identification of $[Pd(\eta^3\text{-allyl})(\text{biotin–spacer–ligand})]^+ \subset$(strept)avidin catalysts that afford both *R* and *S* alkylation products (in up to 90 and 82 % *ee*, respectively; Scheme 1).

With the aim of identifying the most promising chelating ligand, we evaluated five different biotinylated scaffolds in the presence of (strept)avidin (Scheme 2). Little, if any, conversion was detected (Table 1, entry 1): Most of the starting 1,3-diphenylallylacetate was hydrolyzed to the corresponding 1,3-diphenylallyl alcohol. We therefore screened various surfactants that are commonly used in aqueous

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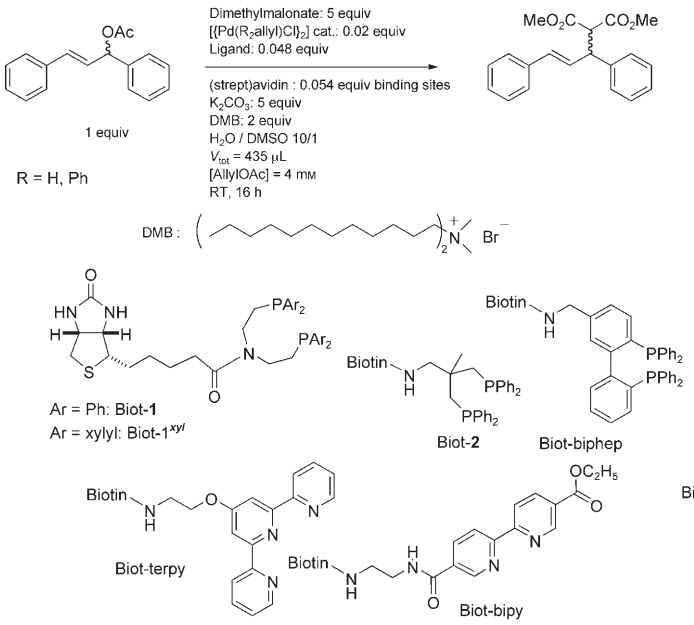
Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.



Scheme 2. Reaction conditions and biotinylated ligands used for the AAA catalyzed by artificial metalloenzymes.

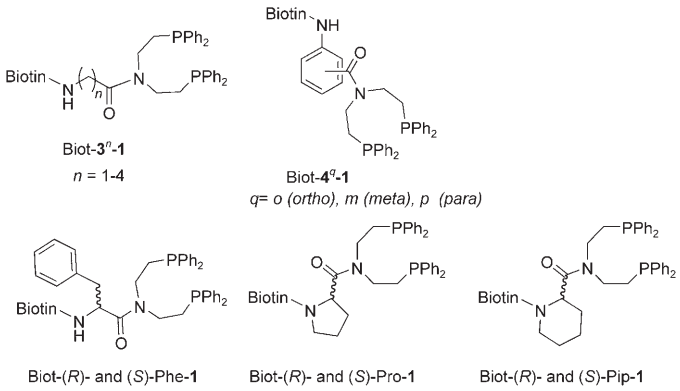
Table 1: Identification of the best ligand scaffold for the AAA.[a]

Entry	Ligand	Protein	*ee* [%]	Conversion [%][b]
1	Biot–**1**	WT Sav	14	< 5[c]
2	Biot–**1**	WT Sav	29	21
3	Biot–$\mathbf{1}^{xyl}$	WT Sav	18	23
4	Biot–terpy	WT Sav	23	< 5
5	Biot–**1**	WT Avi	10	50
6	Biot–$\mathbf{1}^{xyl}$	WT Avi	6	17

[a] Reaction conditions: The catalyst precursor was prepared in situ from [PdCl(η^3-allyl)] (0.02 equiv) and the biotinylated ligand (0.048 equiv) in DMSO, and an aqueous solution of (strept)avidin (binding sites: 0.054 equiv), K_2CO_3 (5 equiv), DMB (2 equiv), $H_2C(CO_2Me)$ (5 equiv), and the substrate (1 equiv, final concentration: 4 mM) was added. Final volume: 435 μL, DMSO/H_2O = 1:10. The reaction mixture was stirred for 16 h at room temperature. [b] No starting material was detected; only hydrolysis and alkylation products were identified. [c] No surfactant was added. WT = wild-type, Sav = streptavidin, Avi = avidin.

organometallic catalysis for their effect on the reaction.[51–54] When didodecyldimethylammonium bromide (DMB; 40 equiv relative to (strept)avidin) was added, a significant increase in yield was observed with Biot–**1**, despite the appearance of a suspension (Table 1 entry 2). Gel electrophoresis revealed that (strept)avidin remained largely tetrameric and active toward biotin binding (see the Supporting Information). Despite the addition of DMB, ligand scaffolds other than Biot–**1** afforded mostly the hydrolysis product (Scheme 2, Table 1 (only the ligands with which conversion was observed are listed)). It thus appears that the nature of the biotinylated ligand plays a key role in determining the activity of the resulting artificial metalloenzyme. Having identified Biot–**1** as the most promising ligand scaffold, we addressed the effect of the diarylphosphine groups by substituting the phenyl groups in Biot–**1** with xylyl groups (Biot–$\mathbf{1}^{xyl}$). As no significant improvement was detected (compare entries 2 and 5 in Table 1 with entries 3 and 6), we focused on ligand **1** for the chemogenetic optimization.

As we have shown previously for artificial hydrogenases, the introduction of an amino acid spacer between the biotin anchor and the diphosphine moiety allows rapid optimization of the performance of the artificial metalloenzmye. Each spacer places the metal moiety in a distinct chiral environment, which may have a significant effect on both the activity and the selectivity. A total of 13 biotinylated complexes of the type [Pd(η^3-allyl)(Biot–spacer–**1**)]$^+$ (see Scheme 3) and [Pd-



Scheme 3. Chemical diversity of the biotinylated ligands obtained by the introduction of an amino acid spacer between the biotin anchor and the diphosphine moiety (Biot–spacer–**1**).

(η^3-allyl)(Biot–**1**)]$^+$ were combined with 22 (strept)avidin isoforms and screened in the AAA.[55] The results of this chemogenetic optimization are displayed as a fingerprint in Figure 1, and selected results are collected in Table 2.[56] From these data, several trends emerge:

a) The presence of (strept)avidin leads to an acceleration of the alkylation reaction relative to that with the protein-free biotinylated catalysts and thus prevents extensive hydrolysis of the substrate. Some of the enantiomerically pure ligands afforded enantiomerically enriched alkylation products, albeit in moderate yield, in the absence of the host protein (up to 46% *ee* and 31% conversion for Biot–(*S*)-Pip–**1**; Table 2, entry 17). The incorporation of the palladium complexes within (strept)avidin led to a significant improvement in both activity and selectivity.
b) Conformationally constrained spacers that place either one (in Biot–Pip–**1** and Biot–Pro–**1**) or two non-carbonyl carbon atoms (in Biot–$\mathbf{4}^{o}$–**1**) between the biotin unit and the ligand conferred the highest activity and selectivity to the catalyst.
c) The catalytic runs performed with [Pd(η^3-Ph_2allyl)(Biot–$\mathbf{4}^{o}$–**1**)]$^+$⊂S112X Sav stand out clearly in terms of both activity and selectivity. With this ligand, the *R* alkylation product was favored for most streptavidin mutants (Table 2, entries 1–4; up to 90% *ee* (*R*) and 95% conversion was observed with [Pd(η^3-Ph_2allyl)(Biot–$\mathbf{4}^{o}$–**1**)]$^+$⊂S112A). However, [Pd(η^3-Ph_2allyl)(Biot–$\mathbf{4}^{o}$–

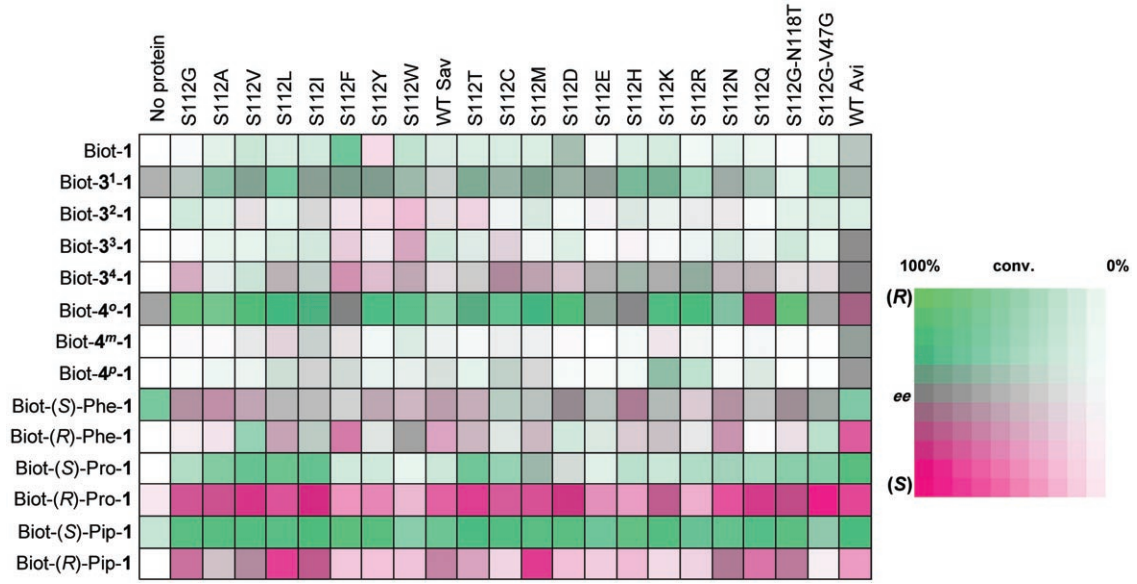


Figure 1. Fingerprint display of the results of the chemogenetic optimization of the AAA.

Table 2: Selected results of the experiments with different catalysts.[a]

Entry	Ligand	Protein	*ee* [%]	Conversion [%][b]
1	Biot–**4ᵒ**–**1**	S112A	90	95
2	Biot–**4ᵒ**–**1**	S112A	93[c]	20[c]
3	Biot–**4ᵒ**–**1**	S112G	88	95
4	Biot–**4ᵒ**–**1**	S112G-N118T	87	96
5	Biot–**4ᵒ**–**1**	S112Q	−31	96
6	Biot–(*R*)-Phe–**1**	–	−36	5
7	Biot–(*R*)-Phe–**1**	WT Avi	−65	77
8	Biot–(*S*)-Pro–**1**	–	–	< 5
9	Biot–(*S*)-Pro–**1**	S112A	75	81
10	Biot–(*S*)-Pro–**1**	S112F	81	74
11	Biot–(*S*)-Pro–**1**	S112Y	80	87
12	Biot–(*R*)-Pro–**1**	–	−55	12
13	Biot–(*R*)-Pro–**1**	S112G	−54	96
14	Biot–(*R*)-Pro–**1**	S112G-V47G	−82	92
15	Biot–(*R*)-Pro–**1**	S112T	−68	98
16	Biot–(*R*)-Pro–**1**	WT Avi	−72	89
17	Biot–(*S*)-Pip–**1**	–	46	31
18	Biot–(*S*)-Pip–**1**	S112H	73	99
19	Biot–(*R*)-Pip–**1**	–	–	< 5
20	Biot–(*R*)-Pip–**1**	S112L	−74	65
21	Biot–(*R*)-Pip–**1**	S112M	−73	88

[a] Reaction conditions: The catalyst precursor was prepared in situ from [PdCl(η^3-Ph_2allyl)] (0.02 equiv) and the biotinylated ligand (0.048 equiv) in DMSO, and an aqueous solution of (strept)avidin (binding sites: 0.054 equiv), K_2CO_3 (5 equiv), DMB (2 equiv), $H_2C(CO_2Me)$ (5 equiv), and the substrate (1 equiv, final concentration: 4 mM) was added. Final volume: 435 μL, DMSO/H_2O = 1:10. The reaction mixture was stirred for 16 h at room temperature. A list of replicates is presented in the Supporting Information. [b] No starting material was detected; only hydrolysis and alkylation products were identified. [c] No DMB was added.

1)]$^+$⊂S112Q Sav afforded the *S* alkylation product (31% *ee*, 96% conversion; Table 2, entry 5).

d) Both pyrrolidine- and piperidine-based ligands, Biot–Pro–**1** and Biot–Pip–**1**, performed similarly to afford either the *R* or the *S* reduction product, depending on the absolute configuration of the spacer. The best *S* selectivity was observed in the presence of a double mutant of streptavidin, with [Pd(η^3-Ph_2allyl)(Biot–(*R*)-Pro–**1**)]$^+$⊂S112G-V47G Sav (82% *ee*, 92% conversion; Table 2, entry 14).

e) In the absence of DMB, slightly improved enantioselectivity was observed with [Pd(η^3-Ph_2allyl)(Biot–**4ᵒ**–**1**)]$^+$⊂S112 A at the cost of a significant erosion in the conversion (93% *ee* (*R*), 20% yield versus 90% *ee*, 95% yield; Table 2, entries 1 and 2). This result further suggests that, despite the appearance of a suspension, the structure of the artificial metalloenzyme is little affected by the presence of chaotropic agents (surfactant and base; see also the Supporting Information).

These general trends emphasize the power of the chemical optimization of artificial metalloenzymes, whereas genetic optimization can be regarded as a fine-tuning process. The presence of a well-defined chiral environment around the palladium moiety can be deduced from the appearance of an induced CD signal upon the incorporation of the complex in (strept)avidin.[23] The introduction of a spacer (Biot–**1** versus Biot–**4ᵒ**–**1**) and/or mutation at position S112 (WT Sav versus S112A or S112Q) influences the resulting induced CD band (Figure 2). Thus, chemogenetic optimization is a powerful tool that allows the systematic variation of the chiral environment around the palladium moiety.

In contrast to other reactions in which artificial metalloenzymes have been implemented, such as the hydrogenation of alkenes and the transfer hydrogenation of ketones, the AAA is a catalytic transformation that has no equivalent in enzymatic catalysis. This study thus extends significantly the potential of artificial biotin–avidin-type metalloenzymes. We are currently investigating the AAA of more challenging substrates on the basis of the biotin–avidin technology, as well

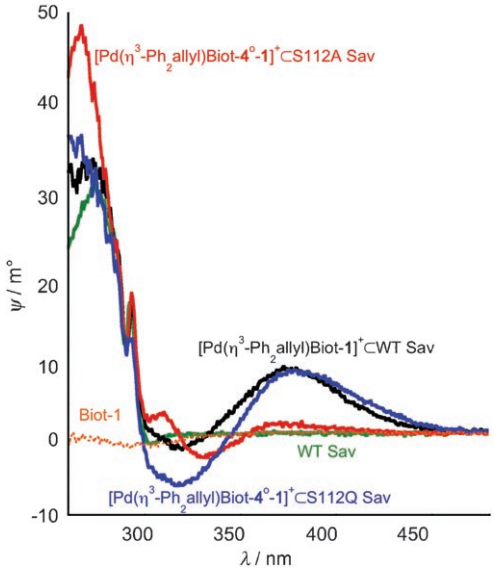


Figure 2. Circular dichroism spectra of Biot–**1** (orange dotted line), WT Sav (green line), [Pd(η³-Ph_2allyl)(Biot–**1**)]⁺⊂WT Sav (black line), [Pd(η³-Ph_2allyl)(Biot–**4°–1**)]⁺⊂S112A Sav (red line), and [Pd(η³-Ph_2allyl)(Biot–**4°–1**)]⁺⊂S112Q Sav (blue line). The spectra reveal the change in the chiral environment around the palladium moiety upon the introduction of a complex with an achiral spacer into WT Sav or Sav with different mutations at position S112X ([artificial metalloenzyme] = 33 μM).

as the mechanistic (by NMR and CD spectroscopy) and structural aspects (by X-ray crystallography) of the reaction.

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[55] S112P Sav was not included in this study, as it is poorly expressed and difficult to purify to homogeneity by iminobiotin-affinity chromatography.
[56] A comprehensive list of all catalytic runs is provided in the Supporting Information. The screening was performed with commercially available $[\{Pd(\eta^3\text{-allyl})Cl\}_2]$. Following the identification of the best biotinylated-ligand–protein combinations, the catalytic runs were reproduced with $[\{Pd(\eta^3\text{-}Ph_2\text{allyl})Cl\}_2]$, which, in most cases, promoted better conversion and afforded the products with slightly improved selectivities (see the Supporting Information)..

