Catalyst Screening

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Screening Rhodium Metallopeptide Libraries "On Bead": Asymmetric Cyclopropanation and a Solution to the Enantiomer Problem**

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Peptides are, in many ways, ideal ligands for stereoselective catalysis. Metalloenzymes use polypeptides to bind transition-metal centers and to control chemo-, diastereo-, and enantioselectivity. For chemists, the allure of peptides is equally strong: peptides are modular, functional-group-rich structures that can be easily synthesized in parallel by automated methods. Efficient screening of ligand diversity is especially valuable because rational design of chiral ligands remains a daunting challenge. Furthermore, any single ligand may not be optimal for a new reaction of interest, and so generating ligand diversity quickly allows solutions for new selectivity problems. Herein, we describe an on-bead screen of rhodium(II) metallopeptides to discover new catalysts for asymmetric reactions of diazo compounds.[1]

Peptides and peptide-like architectures have become important catalysts in organocatalytic applications.[2] A wide-range of reactions, including aldol and related enolate couplings,[3] oxidation,[4] and conjugate additions[5] have proven amenable to peptide catalysis. However, the use of polypeptides in transition-metal catalysis remains limited, in part because of the difficulty in creating well-defined metal-binding sites with natural side chains.[6] Amino acids with unnatural side chains, such as phosphino[7] or pyridyl[8] groups, are one solution to this problem. Alternatively, complete transition-metal complexes have been bound to larger protein structures.[9] We recently described[10] asymmetric Si–H insertion with chelating[11] bis-carboxylate nona-peptides as ligands for rhodium catalysis.[12]

In initial studies, we synthesized and tested about 40 bis-peptide complexes $\{Rh_2(peptide)_2\}$ over many weeks, arriving at an efficient catalyst that delivered over 90% *ee*. Although this was more efficient than traditional multistep ligand synthesis, we wanted to increase the ease and speed of optimization. The synthesis of each bis-peptide catalyst required preparative HPLC purification of both the peptide and final metallopeptide, which is a significant commitment of time and materials. To expand the utility of rhodium metallopeptide catalysts, we decided to develop a new method for preliminary peptide screening. Among the challenges we hoped to address with an on-bead library was the "enantiomer problem:" Chirality derived from natural sources is typically available in only one enantiomeric form, requiring new approaches to access the opposite enantiomer.[13]

We chose to examine rhodium(II) metallopeptide catalysts for asymmetric cyclopropanation reactions. Cyclopropanation of styrene with α-diazophenylacetate was conducted with bis-peptide complexes from our previous silane-insertion library. We continued to employ 2,2,2-trifluoroethanol (TFE) as solvent, because it provided a good combination of metallopeptide solubility and clean diazo reaction. We identified a catalyst, Rh_2(**L16**)$_2$-A, affording the cyclopropane (1*S*,2*R*)-**2b** in 93% *ee* (Figure 1, see Supporting Information for details). However, we had absolutely no starting point for developing catalysts that would afford the opposite enantiomer, so a high-throughput approach seemed necessary.

An on-bead screen brings a number of challenges. Foremost, in previous studies bis-peptide complexes were significantly more selective than mono-peptide complexes [Rh_2(peptide)$(OAc)_2$]. However, it is not possible to build bis-peptide complexes on solid support. Therefore, we decided to screen mono-peptide complexes "on bead", identify the best

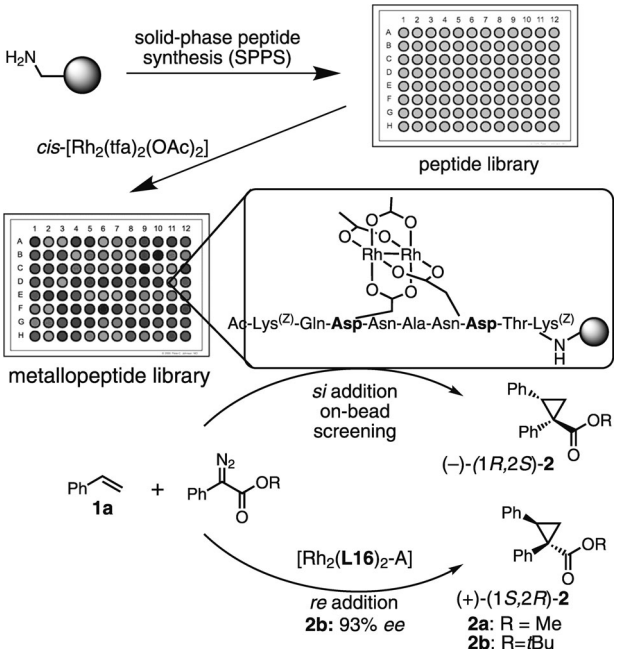

Figure 1. Screening peptide ligands for cyclopropanation. Conditions: catalyst (ca. 0.15 μmol), diazo (6 μmol), and styrene (60 μmol) in CF_3CH_2OH. For structural formula of $Rh_2(L_{16})_2$-A, see Figure 3. All ligands are linked to resin at the C-terminus and acetylated at the N-terminus. Lysine side chain amines are capped with the benzyloxycarbonyl (Z) group; tfa = trifluoroacetate.

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candidates, and then synthesize the corresponding bis-peptide complexes in solution. This approach assumes that sequences optimized as mono-peptide complexes will generally be more selective as bis-peptide complexes, a reasonable but unproven assumption. This approach also accepts that some possible catalysts might be passed over in the service of expediency.

We verified that rhodium metallopeptides could be synthesized on solid support with selectivities similar to a solution-phase catalyst.[7a] Adapting our solution-phase method,[10,14] an on-resin peptide was metalated with *cis*-$[Rh_2(tfa)_2(OAc)_2]$ and *N*,*N*-diisopropylethylamine in TFE. Among several different resins with varying loading levels, Novasyn TGR (a polystyrene-polyethylene glycol resin, 0.17–0.29 mmol g^{-1}) afforded catalyst for silane insertion that gave 81% *ee*, comparable to that obtained for the same catalyst in solution[12a] (see Supporting Information for details). The on-bead catalyst was reused three times without any diminution in *ee* value. Other resins examined, especially those with higher loading, produced inferior results.

Our library design focused on sequences with rhodium-binding carboxylates (aspartate, D) in *i* and *i*+4 positions. This spacing induces helical order,[15] and proved optimal in previous studies.[10] There is tremendous sequence diversity available in even these short nona-peptides. We chose to synthesize libraries in 96-well-plate format. We created a library with variation focused at the residues immediately neighboring the rhodium center. We included a diverse group of 6–10 amino acids at the variable positions, covering a range of steric demand and polarity (Table 1). The choice to include significant diversity meant synthesizing only a small portion of the theoretical library. In addition, we included a few methionine (which offer potential ligand–rhodium interactions) residues. As designed, library 1 contains 14400 theoretical members, of which we synthesized 94 unique sequences. Because we severely restricted the number of methionine residues, the synthetic library was heavily biased for the 1620-member subset of the theoretical library that excludes methionine (and proline in position *i*+1). Our synthetic library contained 80 members of this subset, 5% of theoretical sequences. Control sequences on Rink amide resin were found to be over 90% pure by MALDI-TOF mass spectrometry and HPLC. Following synthesis, the library was screened against methyl α-phenyldiazoacetate (6-μmol scale, 1 mg). The entire process of peptide synthesis, metalation, cyclopropanation, and chiral analysis took about one week for a single 96-well plate.

Our library screen identified a number of metallopeptides that furnished, in moderate *ee*, the "opposite" enantiomer, (−)-(1*R*,2*S*)-**2a**. The catalysts yielding enantioselectivity greater than 25% are shown in Table 2 (see Supporting Information for details). To visualize broad trends, we prepared "scatter plots" of *ee* values for each amino acid at a given position. The scatter plot of the *i*+3 position (Figure 2) shows a predominance of asparagine (Asn; N) in the best sequences, though the preference is not absolute. The *i*−1 position (Supporting Information) showed a predominance of glycine (Gly; G) among high-*ee* value catalysts.

Table 1: Amino acid variation incorporated in library 1.

i−2	*i*−1	*i*	*i*+1	*i*+2	*i*+3	*i*+4	*i*+5	*i*+6
K^Z	P	D	A	A	P	D	W	K^Z
M[a]	W		I	M[a]	W		P	
	Y		S		Y		A	
	N		W		N		I	
	S		M[a]		S		N	
	T		P[a]		T		M[a]	
	L				L			
	I				I			
	G				G			
	M[a]				M[a]			

[a] This amino acid was constrained to 2% at this position. "K^Z" signifies a lysine residue with the side-chain amine protected as a benzyloxycarbonyl (Z) carbamate.

Table 2: Sequences from library 1 that gave over 25% *ee* for the formation of the *si* addition product, (−)-**2a**.

Ligand	*i*−2	*i*−1	*i*	*i*+1	*i*+2	*i*+3	*i*+4	*i*+5	*i*+6	*ee* [%]
L1.03	K^Z	G	D	I	A	G	D	I	K^Z	27
L1.06	K^Z	G	D	W	A	N	D	I	K^Z	33
L1.07	K^Z	G	D	I	A	N	D	W	K^Z	46
L1.09	K^Z	G	D	A	A	P	D	N	K^Z	27
L1.12	K^Z	G	D	I	A	Y	D	P	K^Z	33
L1.13	K^Z	I	D	P	A	G	D	P	K^Z	29
L1.15	K^Z	I	D	W	A	N	D	M	K^Z	44
L1.27	K^Z	L	D	A	A	P	D	P	K^Z	31
L1.42	K^Z	P	D	P	A	L	D	A	K^Z	38
L1.47	K^Z	P	D	A	A	N	D	W	K^Z	28
L1.68	K^Z	T	D	W	A	N	D	I	K^Z	36
L1.78	K^Z	W	D	A	M	N	D	A	K^Z	39

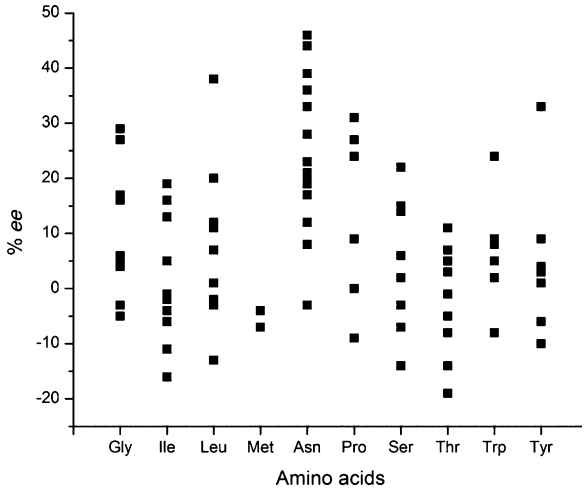


Figure 2. Scatter plot of enantioselectivity for amino acids in position *i*+3 of Library 1. Positive values indicate the *si* addition product, (−)-**2a**.

Table 3: Amino acid variation incorporated in library 2.

i−2	*i*−1	*i*	*i*+1	*i*+2	*i*+3	*i*+4	*i*+5	*i*+6
K^Z	G	D	I	A	N	D	W	K^Z
	A		W		Q[a]		I	
	W		L		M[a]		M	
	Q		N				Y	
			T				L	
							T	
							F	

[a] This amino acid was constrained to 10% at this position.

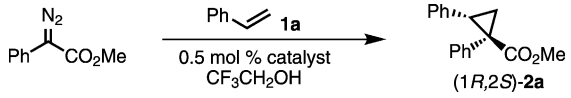
Scatter plots for other positions did not exhibit clear-cut "winning" residues (see Supporting Information for details).

We created a second 96-well-plate library (**L2.xx**, Table 3), taken from a more focused theoretical set of 420 sequences and incorporating trends observed in the first library. Scatter plot analysis of the second library did not identify new residues that consistently out-performed the others (see Supporting Information for details). We did identify 10 sequences that gave greater than 45% *ee* as mono-peptide catalysts on bead. Previous studies had indicated that significant improvements in enantioselectivity were often observed upon moving to bis-peptide catalysts, and so we deemed it likely that optimal peptides identified in library 2 could form the basis for selective bis-peptide catalysts.

"Winning" peptide sequences from library 2 were synthesized, producing two isomeric metallopeptides (denoted $[Rh_2(L)_2$-A] and $[Rh_2(L)_2$-B]) as a result of parallel and antiparallel orientations of the peptide ligands, which were separated and tested independently (Table 4, entries 1–4).[16] There was no direct correlation between on-resin, mono-peptide, and bis-peptide solution catalysts, but several bis-peptide catalysts outperformed the corresponding mono-peptide catalysts. The highest *ee* value was obtained with the catalyst $[Rh_2($**L2.47**$)_2$-A], which provided 83% *ee* for styrene cyclopropanation with methyl α-diazophenylacetate (at −35 °C), and 92% *ee* for *tert*-butyl α-diazophenylacetate (at −50 °C). We also synthesized bis-peptide catalysts from "poor" sequences from library 2 (Table 4 entries 5—8). The average *ee* value of these "poor" sequences as bis-peptide catalysts was far lower than that of the "winning" sequences as bis-peptide catalysts, and no "poor" sequence reached the level of *ee* values seen with the best sequence, **L2.47**. One isomer of the catalyst derived from **L2.12** did achieve significant *ee* value (Table 4, entry 5), demonstrating that some reasonable candidates are not picked up by this approach. However, the design of **L2.12** (a member of library 2) would not have been possible without the information gleaned from library 1 that informed our design of library 2, such as the importance of the $i+3$ asparagine residue.

We looked at a variety of olefin substrates with $[Rh_2($**L2.47**$)_2$-A]. We also examined catalyst $[Rh_2($**L16**$)_2$-A] to access the "normal" (+)-enantiomer (Table 5, Figure 3).[17] High *ee* (>90%) was observed for a variety of olefins, including styrene derivatives and ethyl vinyl ether. Our routine conditions involve 0.25 mol% catalyst, and we ran the reaction in Table 5 entry 2 at lower loading (0.1 mol%) with comparable yield and selectivity. In addition, we found that the metallopeptide catalysts were effective for

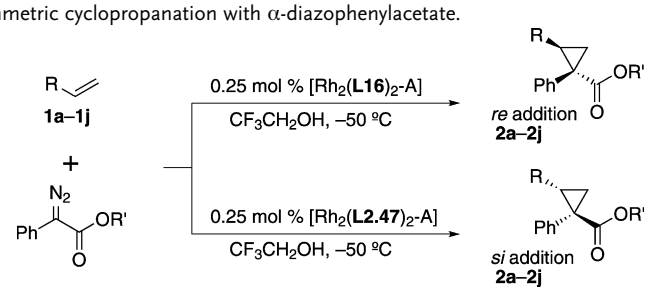
Table 4: Enantioselectivity of soluble catalysts derived from the best on-bead sequences.

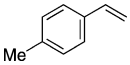
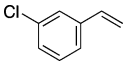
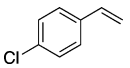
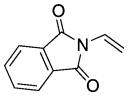
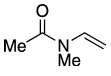


Entry	Ligand	Sequence	*ee* [%]			
			$[Rh_2($**L**$)(OAc)_2]$ on-bead, RT	$[Rh_2($**L**$)(OAc)_2]$ −35 °C	$[Rh_2($**L**$)_2$-A] −35 °C	$[Rh_2($**L**$)_2$-B] −35 °C
1	**L2.47**	KQDNANDTK	54	50	**83**	63
2	**L2.13**	KGDLANDIK	46	47	61	14
3	**L2.63**	KGDLANDWK	46	44	68	43
4	**L2.85**	KGDNANDYK	42	58	49	49
5	**L2.12**	KADLANDIK	20	9	15	75
6	**L2.89**	KGDTANDYK	17	13	28	22
7	**L2.09**	KQDWAQDFK	6	18	3	−27
8	**L2.95**	KADNAQDYK	3	5	13	−4

[a] Best value highlighted.

Table 5: Asymmetric cyclopropanation with α-diazophenylacetate.



Entry	Alkene	R′	Prod.	$[Rh_2($**L16**$)_2$-A] *re* prod. *ee*[a] [%]	yield[b] [%]	$[Rh_2($**L2.47**$)_2$-A] *si* prod. *ee*[a] [%]	yield[b] [%]
1		Me	**2a**	75[c]	95[c]	68[c]	84[c]
2		*t*Bu	**2b**	93	96	92	92
3		*t*Bu	**2c**	90	87	92	94
4		*t*Bu	**2d**	94	88	95	80
5		*t*Bu	**2e**	90	92	97	94
6		*t*Bu	**2f**	90	64	94	67
7		*t*Bu	**2g**	92	90	95	76
8		*t*Bu	**2h**	55[d]	87[d]	80[d]	93[d]
9		*t*Bu	**2i**	87[e]	51[e]	85[e]	42[e]
10		*t*Bu	**2j**	90	98	93	99

[a] The absolute configuration of **2a** was established by comparison to published data; that of other products is assumed by analogy. [b] Yields of isolated pure material. [c] Reaction at room temperature. [d] Reaction at −25 °C. [e] Reaction at 8 °C in hexafluoroisopropanol:CH_2Cl_2 (1:22).

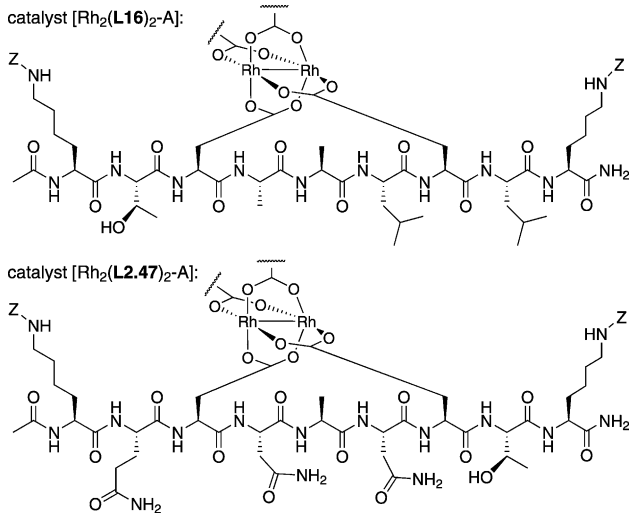


Figure 3. Optimized ligand structures. **L16** = $K^ZTDAALDLK^Z$; **L2.47** = $K^ZQDNANDTK^Z$.

cyclopropanation of *N*-vinyl compounds (Table 5, entries 9,10). Despite their potential value as β-amino acids or as intermediates for further elaboration, rhodium catalysts have been little studied for cyclopropanation of *N*-vinyl compounds, and then mostly for racemic reactions.[18] One report describes enantioselective cyclopropanations with diazoketones,[19] and one paper reported that only moderate *ee* (not over 55%) could be achieved for diazoacetate derivatives with common catalysts for enantioselective cyclopropanations.[20] The two metallopeptide catalysts are also highly diastereoselective, providing only the *trans*-product with respect to the ester group.

In conclusion, the strategy described herein facilitates the search for new metallopeptide catalysts. At the onset of this study, despite synthesizing around 40 peptides for our previous report, we had no lead sequence to begin the search for catalysts providing "opposite" enantiomer products. In two rounds of screening, the library approach identified sequence trends that favor formation of the "opposite" enantiomer and delivered a selective catalyst. The first ("normal") class of sequences, providing *re*-face addition products, is characterized by bulk (i.e. leucine, isoleucine, phenylalanine) at the $i-1$ and $i+3$ positions. In contrast, the "enantiomeric" sequences, providing *si*-face addition, are characterized by polar carboxamide side chains (glutamine and asparagine) at these same positions. These results allow us to infer that the mechanism of stereoinduction may be different for the two sequence classes identified. Of particular note, the rhodium-catalyzed cyclopropanation of *N*-vinyl species affords β-amino-acid derivatives with high *ee* values. This work demonstrates the value of peptides as asymmetric ligands and provides an example of the benefits of modular ligands in general.

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[16] The "A" and "B" designations are based on HPLC run time. We are unable to determine the tertiary structure of the metallopeptide catalysts.
[17] Interestingly, catalyst **L16** is a counter-example to the approach herein. We subsequently found that the corresponding monopeptide complex of **L16** is a poor cyclopropanation catalyst (*ee* < 5 %).
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