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Increased Structural Complexity Leads to Higher Activity: Peptides as Efficient and Versatile Catalysts for Asymmetric Aldol Reactions

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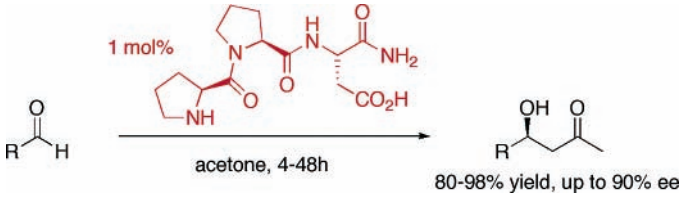
ABSTRACT



Peptides containing a secondary amine and a carboxylic acid in a specific orientation to each other are presented as highly efficient catalysts for asymmetric aldol reactions: (1) their activity is considerably higher compared to that of proline, and (2) the enantioselectivity of the peptidic catalysts can be changed from (*R*)- to (*S*)-selectivity by simple modifications of the secondary structure.

In recent years, the use of secondary amines such as proline to catalyze aldol and aldol-type reactions has become increasingly popular.[1,2] For many substrates good enantioselectivities are achieved; however, often poor activities make the use of large amounts of catalyst necessary. Low reactivity may be difficult to avoid for catalysts as small as proline, because relatively few options exist for structural modifications. Peptides on the contrary offer many sites for functional and structural diversity that can be used to generate optimized catalysts.[3] Thus, peptides can be an ideal compromise between small rigid organocatalysts and enzymes. The first examples of peptidic catalysts for aldol reactions have demonstrated that the purely rational design of efficient catalysts is not straightforward.[4] In this study we employed the combinatorial method of "catalyst-substrate coimmobilization"[5] for the generation and testing of large sets of potential peptidic catalysts. Screening this library identified the most reactive peptidic aldol-organocatalysts that have been developed to date.

The method of catalyst-substrate coimmobilization allows for the identification of catalysts in split-and-mix libraries for essentially any bimolecular reaction.[5] To use this method for the discovery of peptidic catalysts for aldol reactions,

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(2) For recent examples, see: (a) Northrup, A. B.; Mangion, I. K.; Hettche, F.; MacMillan, D. W. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 2152−2154. (b) Tang, Z.; Jiang, F.; Cui, X.; Gong, L.-Z.; Mi, A.-Q.; Jiang, Y.-Z.; Wu, Y.-D. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 5755−5760. (c) Torii, H.; Nakadai, M.; Ishihara, K.; Saito, S.; Yamamoto, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 1983−1986. (d) Halland, N.; Braunton, A.; Bachmann, S.; Marigo, M.; Jørgensen, K. A. *J. Am. Chem. Soc.* **2004**, *126*, 4790−4791.

(3) For reviews see: (a) Miller, S. J. *Acc. Chem. Res.* **2004**, *37*, 601−610. (b) Berkessel, A. *Curr. Opin. Chem. Biol.* **2003**, *7*, 409−419. (c) Jarvo, E. J.; Miller, S. J. *Tetrahedron* **2002**, *58*, 2481−2495.

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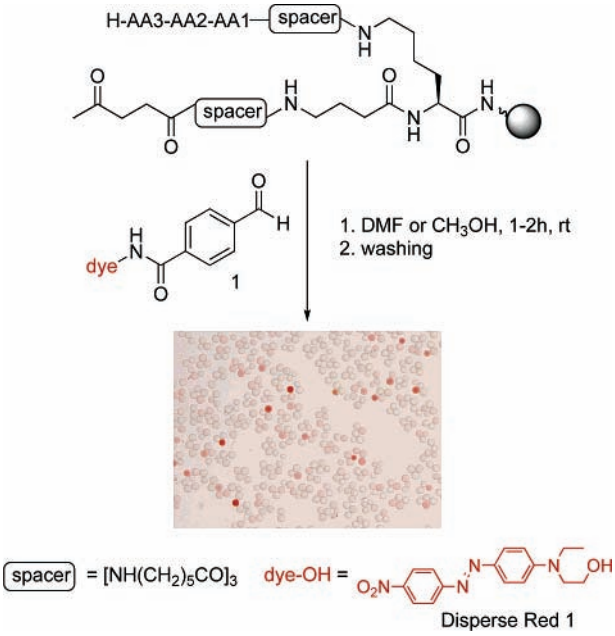
Scheme 1. Substrate-Catalyst Coimmobilization for the Discovery of Peptides as Aldol Catalysts



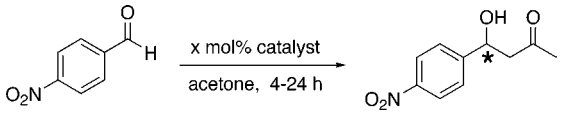
we prepared an encoded[6] split-and-mix library[7] of tripeptides at one end of a bifunctional lysine linker on TentaGel resin and functionalized the other end with a ketone derived from levulinic acid (Scheme 1). Fifteen different D- and L-amino acids were used in each of the three positions; hence the library consisted of maximally $15^3 = 3375$ different tripeptides.[8] The library was then allowed to react with the dye-marked benzaldehyde derivative **1** at room temperature for 1−2 h. After extensive washings that removed any noncovalently bonded dyed compounds, only a few beads (∼1 out of 100) were colored bright red, indicating that the peptides on these beads had been able to mediate the aldol reaction between the resin bound ketone and the dye-marked benzaldehyde derivative.[9] Isolation and analysis of the peptides on several of the red beads revealed two main consensus sequences:

H-L-Pro-D-Ala-D-Asp-NHR (R = resin, **2**: R = H)

H-L-Pro-L-Pro-L-Asp-NHR (R = resin, **3**: R = H)

In common with proline, both peptides contain a secondary amine and a carboxylic acid. However, not all peptides with these two functional groups were selected within the library, only those with the motifs L-Pro-D-Ala and L-Pro-L-Pro that are both indicative of turn elements.[10]

Table 1. Aldol Reaction between *p*-Nitrobenzaldehyde and Acetone Catalyzed by Peptides **2** and **3**



entry	catalyst	mol %	temp (°C)	yield (%)	ee (%)[a]	abs conf[b]
1	**2**	10	rt	73	70	*R*
2	**2**	10	−20	53	81	*R*
3[c]	**3**	1	rt	99	80	*S*
4	**3**	5	−20	98	90	*S*
5[d]	proline	30	rt	68	76	*R*
6	proline	30	−20	30	71	*R*

[a] Determined by chiral phase HPLC analysis. [b] Absolute configuration of the product. [c] Reaction was complete within 4 h. [d] Data taken from ref 11.

We then evaluated the catalytic properties of peptides **2** and **3** in solution-phase aldol reactions after resynthesising them on Rink amide resin. As an initial test reaction the aldol reaction between *p*-nitrobenzaldehyde and acetone was examined, the latter also serving as solvent (Table 1).

These studies revealed that particularly peptide **3** is a highly active catalyst; 1 mol % of **3** is sufficient to obtain the aldol product in 99% yield and 80% ee within 4 h at room temperature. Under the same conditions 30 mol % of proline are necessary to achieve a yield of 68% and an ee of 76%.[11,12] Hence, **3** is more than 30-fold more active, demonstrating that higher structural complexity is a good tradeoff for higher activity. Moreover, the enantioselectivities achieved with the peptidic catalysts can be further raised by 10% ee without a significant loss of reactivity by performing the reaction at −20 °C (entries 2 and 4). We rationalize this result by a stabilization of the conformation of the peptides.

Equally high catalytic activities of **2** and **3** were observed when other aldehydes were used as substrates under otherwise identical conditions (Table 2). Compared to proline,[11] similar yields were observed by using 10 mol % of **2** and only 1 mol % of **3**. Furthermore, even at room temperature the enantioselectivities are either comparable to the ones obtained with proline (entries 3 and 4) or better (entries 1, 2, and 5) with at least one of the two peptides.

Particularly noteworthy is that peptides **2** and **3** generate aldol products with opposite absolute configurations despite the fact that both peptides have L-Pro at the N-termini! Furthermore, for some of the aldol reactions peptide **2** is the better catalyst compared to peptide **3** (entry 4) and for

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(8) AA1 and AA3 = Gly, D-Val, L-Ala, L-Leu, D-Pro, L-Pro, D-Phe, L-Tyr, D-His, L-His, D-Arg, D-Asp, L-Glu, D-Asn, L-Gln; AA2 = Gly, L-Val, D-Ala, D-Leu, D-Pro, L-Pro, L-Phe, D-Tyr, D-His, L-His, L-Arg, L-Asp, D-Glu, L-Asn, D-Gln.

(9) Control experiments revealed that no red beads were observed when the peptide library was reacted with benzaldehyde **1** in (a) the absence of the ketone or (b) when the peptide library was acetylated.

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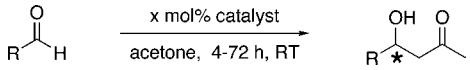
(12) Under the same reaction conditions, the use of 1 mol % of proline at room temperature did not lead to full conversion even after 4 days. At −20 °C, almost no conversion was observed after 4 days with 1 mol % of proline.

Table 2. Aldol Reactions Catalyzed by Peptides **2** and **3**



		10 mol % **2**		1 mol % **3**		30 mol % Pro[c]	
entry	R	yield[a]	ee[b]	yield[a]	ee[b]	yield	ee
1	4-NO_2Ph	73	70 (*R*)	99	80 (*S*)	68	76 (*R*)
2	Ph	58	66 (*R*)	69	78 (*S*)	62	60 (*R*)
3	*c*-Hex	56	83 (*R*)	66	82 (*S*)	63	84 (*R*)
4	*i*-Pr	75	91 (*R*)	79	79 (*S*)	97	96 (*R*)
5	*neo*-Pent	24	70 (*S*)	28	73 (*R*)	22	36 (*S*)

[a] Yields are listed in %; in entries 2, 3, and 5, 30−70% of the aldehydes could be reisolated. [b] The ee was determined by chiral stationary phase HPLC or GC analysis and is listed in %, (*R*) and (*S*) indicate the absolute configuration of the aldol product. [c] Data taken from ref 11.

others peptide **3** is better (entries 1 and 2). This shows that the peptides complement each other and suggests that for a given pair of substrates an ideal peptidic catalyst can be generated.

We then focused our attention on the factors responsible for the observed catalytic activity. Experiments with peptides lacking either the secondary amine or the carboxylic acid showed significantly poorer or no activity at all, indicating that both functional groups are crucial for efficient catalysis. Furthermore, peptides containing these two functional groups at different positions (e.g., H-L-Pro-L-Pro-OH, H-L-Pro-D-Ala-OH) also proved much less active and/or selective demonstrating that the conformation of peptides **2** and **3** and thereby the position of the secondary amine relative to the carboxylic acid is equally crucial for efficient catalysis. This observation further shows that the peptides identified in the combinatorial screening are not only the most active but also the most selective.

Conformational analysis using MacroModel 8.0[13] shed light on the preferred conformations of peptides **2** and **3** and supported our hypothesis of turn-like structures.[10] In the low-energy structures, both peptides adopt turn-like conformations in which the secondary amine of proline is in close proximity to the carboxylic acid of the aspartic acid (Figure 1). Based on the mechanistic studies of proline catalysis[1a,14] where the

Figure 1. Lowest energy structures of H-L-Pro-D-Ala-D-Asp-NH_2 **2** (right) and H-L-Pro-L-Pro-L-Asp-NH_2 **3** (left) as calculated by MacroModel 8.0 and an overlay of the two conformations, **2** in gray and **3** in green (middle).

carboxylic acid is proposed to act as a proton donor, this proximity can be assumed to be crucial for the observed catalysis.

To address the question why peptides **2** and **3** yield opposite enantiomeric aldol products, an overlay of the low-energy structures of peptides **2** and **3** along the atoms of their N-terminal Pro residue was most revealing (Figure 1). Peptide **2** forms a left-handed turn and peptide **3** a right-handed turn, each of which behave almost like mirror images as far as the aspartic acid moieties are concerned. We assume that these oppositely handed turn-conformations are the reason for the formation of enantiomeric aldol products by peptides **2** and **3**.

In conclusion, we have developed tripeptides that are significantly more active than proline as catalysts for asymmetric aldol reactions. Our studies demonstrate that the complexity of tripeptidic catalysts not only is a good trade off for higher activity but also allows for the tuning of the enantioselectivity by simple modifications in the secondary structure.

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Supporting Information Available: Details on the combinatorial screenings; experimental procedures and characterization of **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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