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Discovery of New Peptide-Based Catalysts for the Direct Asymmetric Aldol Reaction

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Abstract—A series of oligo-peptide based catalysts were prepared using Fmoc solid-phase peptide synthesis. It was found that peptides with N-terminal proline residues catalyzed an aldol reaction yielding enantiomeric enriched product. Peptide H-Pro-Glu-Leu-Phe-OH catalyzed the reaction with good activity and moderate enantioselectivity (66% *ee*). Furthermore, it was shown that an acidic side chain and/or *C*-termini are essential to catalysis.
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The aldol reaction is one of the most powerful carbon–carbon bond forming reactions. The addition of a nucleophilic ketone donor to an electrophilic aldehyde acceptor in the presence of a chiral catalyst gives access to enantiomeric rich aldol products.[1]

The direct asymmetric Aldol reaction is particularly attractive because the energetically demanding activation of the ketone moiety to a reactive enolate-type species is not necessary. Inspired by Nature's aldolase enzymes researchers have concentrated on two classes of chiral aldolase catalysts: Type I and type II aldolase mimics.[1] Type I mimics consists most often of amino acid catalysts, which activate the donor via enamine formation and the acceptor through a hydrogen bond with an acid functionality.[2] Proline is the catalyst of choice for a wide range of reactions such as Robinson annulations,[3] intermolecular aldol condensations,[4] Mannich reactions,[5] Michael additions,[6] cyclodehydrations[7] and α-amination of aldehydes.[8] Type I aldolase catalytic antibodies were obtained from designed haptens using either a cofactor strategy[9] or by reactive immunisation against 1,3-diketone haptens.[10] Key to the function of these catalysts is an active site primary amine functional group buried in a hydrophobic pocket with a highly perturbed pK_a amine. Type II aldolase mimics consists of bimetallic catalysts containing a Lewis acidic metal for aldehyde activation and a Brønsted base for enolate generation to form an active complex.[11]

In this letter we report the discovery of unprotected peptide-based catalysts for the direct asymmetric aldol reaction. Peptides—being composed of the same building blocks (amino acid subunits) as enzymes—have the potential to create a chiral binding environment in which the donor is activated and reacts with the incoming acceptor in such a way that enantiomerically enriched aldol products could result. Side-chain protected peptides have been reported to act as catalysts for kinetic resolutions of alcohols,[12] asymmetric conjugate additions of azides[13] and asymmetric phosphorylations.[14] Catalytic peptides bearing a catalytic lysine residue have been isolated by phage display selection for binding against 1,3-diketones.[15] By contrast, the peptides described here are based on proline-type catalysis.

Design Principles and Synthesis

We prepared two classes of peptides following two different designs. The first class was based on a primary amine as catalytic group, as seen in type I aldolases and catalytic antibodies. This primary amine was placed in β-position to a quaternary ammonium group, which would lower it's pK_a down to pK_a = 7 as seen in type I aldolases,[10b] and provide hydrophobic groups that might enable substrate binding interactions (Class **I–a**). Alternatively, we also looked at the possibility to use the

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peptide's free N-terminus as a catalytic group (Class **I–b**) (Fig. 1).

The second class was based on the chemical proline-based catalysts, and comprised peptides with either a secondary amine (Class **II–a**) or proline (Class **II–b**) at the N-terminus. These peptides incorporated at least one free carboxyl function such as to compensate for the proline's own carboxyl group, which was used for the peptide linkage. Proline's free carboxyl group has been shown to be essential for aldol-type catalysis.[4a] The carboxylic function in our peptides was placed in close proximity to the catalytic amine either directly as the closest substituent or spatially by intercalating the β-turn inducing aminoisobutyric acid residue next to the proline (Fig. 1).[16]

Class **I–a** peptides were obtained by reacting the Fmoc deprotected peptidyl-resin with a large excess of alkylation agent (R^1X, Scheme 1). Class **I–b** and **II–b** were readily available using standard Fmoc SPPS. Class **II–a** was obtained via $CsOH{\cdot}H_2O$ promoted mono-*N*-alkylation.[17] Chloroacetylation followed by nucleophilic displacement with amines[18] (e.g., R^2R^3NH) gave also access to class **II–a** peptides. The peptide products were cleaved from the solid support using TFA/TIS/H_2O (95:2.5:2.5). Most peptides were sufficiently pure (>95%) as judged by HPLC and ^1H NMR analysis to be used directly for screening. The remaining peptides were further purified by preparative RP-HPLC purification before use.

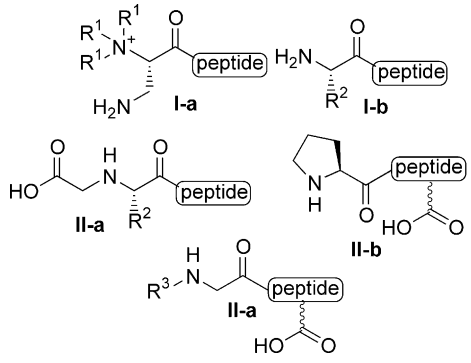
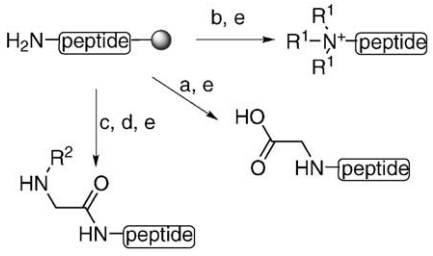


Figure 1. Design of potential catalysts for the direct asymmetric aldol reaction. R^1 represents a hydrophobic group, R^2 any amino acid side-chain and R^3 aliphatic or aromatic functionality.



Scheme 1. Synthesis of N-terminal modified peptides: (a) $CsOH{\cdot}H_2O$, $BrCH_2COOC(CH_3)_3$, DMF; (b) R^1X, DMF, DIEA; (c) $(ClCH_2CO)_2O$, DMF/collidine (8:2); (d) R^2NH_2, DIEA, DMF; (e) TFA/TIS/H_2O (95:2.5:2.5).

The peptides were first assayed for retroaldolization catalysis in aqueous buffer based using individual stereoisomers of the fluorogenic aldol **1**, which undergoes retroaldolization to form aldehyde **2** and subsequently umbelliferone **3** by β-elimination.[19] There was no activity with any of the peptides under the conditions of this assay (100 μM fluorogenic aldolase substrates ((*R*,*R*)-**1**, (*S*,*S*)-**1**, (*R*,*S*)-**1** and (*S*,*R*)-**1**), 20 μM phosphate buffer (pH = 8.00) and 10, 100 or 500 μM catalysts). Proline itself also did not show any measurable activity under these conditions (Fig. 2).

We next turned our attention to the much studied condensation between acetone and *p*-nitrobenzaldehyde **4**, which has been used broadly to test proline catalysis.[4a,6] The peptides, used as trifluoroacetate salts were stirred in DMSO/acetone (4:1) with one equivalent of *N*-methyl morpholine (NMM, which it itself did not catalyze the reaction[20]) as buffer, and after 15 min **4** was added. The reaction was run for 18 h and followed by isocratic RP-HPLC. Any enantiomeric excess of the aldol product **5** was determined by chiral-phase HPLC (Fig. 3).

Results and Discussion

Peptides containing either a primary amino group (Class **I**) or an acyclic secondary amine (Class **II–a**) did not catalyze the reaction (entries 1, 2 and 3, Table 1). Only N-terminal prolyl-peptides bearing an additional carboxyl group showed activity, showing that catalytic functionality of proline's carboxyl group could indeed be restored by a more remote carboxyl group, such as in the simple dipeptide H-Pro-Gly-OH (entry 7), which also showed a moderate yet significant enantiomeric excess in the reaction. Surprisingly, the dipeptide H-Pro-Leu-OH (entry 5) was catalytically inactive, suggesting a non-productive interaction of the hydrophobic side chain on catalysis. Presentation of the carboxyl group as a side chain in the dipeptide H-Pro-Asp-NH_2 (entry 6) was ineffective both in terms of catalysis and in terms of enantioselectivity.

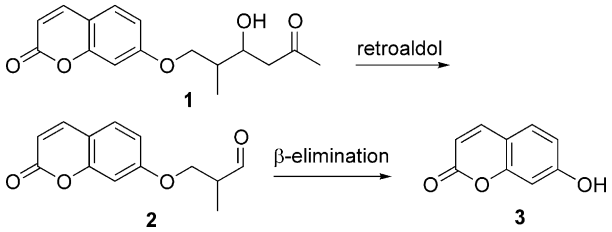


Figure 2. Principle of fluorogenic aldolase assay.

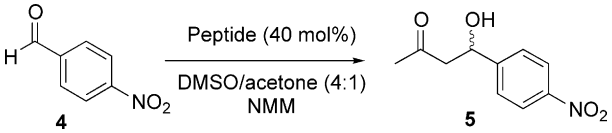


Figure 3. Direct asymmetric aldol condensation between acetone and *p*-nitrobenzaldehyde.

Table 1. Direct asymmetric aldol reaction between acetone and *p*-nitrobenzaldehyde catalyzed by simple peptides and derivatives

Entry	Class	Peptide	Conversion[a]	*ee*[b]
1	I-a	5 Peptides, R^1 = Me	<5%	—
2	I-b	3 Peptides, R^2 = Ser, Phe or Leu	<5%	—
3	II-a	2 Peptides, R^2 = Pro, or $R^3 = C_6H_{11}$	<5%	—
4	II-b	H-Pro-Leu-NH_2	<5%	—
5	II-b	H-Pro-Leu-OH	<5%	—
6	II-b	H-Pro-Asp-NH_2	39%	<5%
7	II-b	H-Pro-Gly-OH	99%	46%
8	II-b	H-Pro-Glu-Leu-Phe-OH	96%	66%
9	II-b	H-Pro-Aib-Glu-Phe-OH	94%	37%
10	II-b	H-Pro-Asp-Leu-Phe-OH	95%	50%
11	II-b	H-Pro-Aib-Asp-Phe-OH	97%	12%

[a]Determined by analytical RP-HPLC, 254 nm, on a Vydac peptide & protein column.
[b]Determined by analytical chiral HPLC, 254 nm, on a Chiralpak AS column. The major enantiomer was assigned to be (*R*) by comparison with the literature. See also ref 6.

Remote attachment of a carboxyl group via the β-turn inducer amino-isobutyric acid afforded a catalytically active peptide, but without improvement of enantioselectivity. The β-turn induction turned out to be non-essential. Indeed, appendage with the hydrophobic pair Leu-Phe gave the best results and restored both activity and enantioselectivity to levels comparable to that of proline itself, as seen with H-Pro-Asp-Leu-Phe-OH (entry 10) and H-Pro-Glu-Leu-Phe-OH (entry 8).

In summary, this is the first report of peptides with N-terminal proline residues as asymmetric aldol reaction catalysts. The catalytic activity and enantioselectivity of proline could be matched by appendage of simple amino acids presenting a free carboxyl function as co-catalyst. Considered that most modifications on proline, in particular amidation of it's carboxyl side chain,[4a] are incompatible with catalysis, our discovery opens the way for the preparation of a large family of proline-based aldol catalysts by standard combinatorial peptide synthesis.

Acknowledgements

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