Small peptides as modular catalysts for the direct asymmetric aldol reaction: ancient peptides with aldolase enzyme activity†

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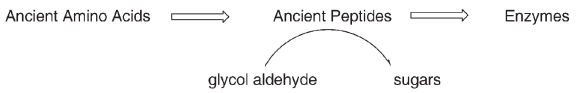
Simple peptides and their analogues having a primary amino group as the catalytic residue mediate the direct asymmetric intermolecular aldol reaction with high stereoselectivity and furnish the corresponding aldol products with up to 99% ee; this intrinsic ability of highly modular peptides may explain the initial molecular evolution of aldolase enzymes.

The asymmetric aldol reaction is an ancient process,[1] which enzymes have catalyzed for billions of years.[2] Two types of aldolase enzymes have evolved: Type I utilize a catalytic primary amine residue and Type II use a zinc co-factor for the generation of a chiral enamine intermediate or a zinc enolate as the reactive nucleophiles, respectively.[2]

The asymmetric aldol reaction is also a fundamental reaction in organic synthesis.[3] The ability to prepare enolates selectively has inspired chemists to develop several catalytic asymmetric aldol reactions.[4] For example, several chiral transition metal complexes have been utilized as catalysts for the asymmetric aldol reaction.[5] In addition, aldolase enzymes are also employed as biocatalysts for the asymmetric aldol reaction.[6]

In recent years, the utilization of secondary amines such as proline to catalyze the direct asymmetric aldol reaction has gained increased attention.[7–9] Furthermore, the enantioselective aldol reaction between unmodified ketones and aldehydes is catalyzed by peptides containing a secondary amine at the *N*-terminus.[10] Peptides have the advantage of allowing for employment of combinatorial chemistry as well as functional and structural diversity.[11] However, peptides with a primary amine at the *N*-terminus are considered not to catalyze the asymmetric aldol reaction, which significantly limits the structural diversity. Thus, it would be highly desirable to allow for the employment of primary amino acid derived peptides and their combinations in the design of peptide catalysts.

Recently, we found that primary amino acids can catalyze the intermolecular aldol reaction with high enantioselectivity.[12] Moreover, ancient amino acids catalyze the asymmetric neogenesis of sugars and form peptides under prebiotic conditions.[1,13,14] Based on these discoveries, we became interested in whether peptides with a primary amine would be able to catalyze the asymmetric aldol reaction. Moreover, in a prebiotic scenario, the initial evolution of Type I aldolase enzymes may have been *via* the ancient amino acids polymerization to peptides, which could have catalyzed the asymmetric formation of sugars (Equation 1).



(1)

Herein, we present the unprecedented use of peptides and their analogues with a catalytic primary amine residue as catalysts for the asymmetric aldol reaction. Furthermore, ancient peptides have an intrinsic ability to catalyze the asymmetric formation of sugars under prebiotic conditions, which may explain the initial evolution of aldolase enzymes.

We initially prepared a small library of dipeptides based on the simple amino acids alanine and valine. Next, we screened the peptides' (30 mol%) ability to catalyze the asymmetric aldol reaction between *p*-nitrobenzaldehyde (0.5 mmol) and cyclohexanone **1a** (1.5 mmol) to form **2a** in wet DMSO (2.0 mL, 90 μL H_2O) (Table 1).

To our delight, all the peptides in Table 1 catalyzed the asymmetric formation of the desired aldol adduct **2a** in high yield

Table 1 Examples of screened peptides as catalysts for the direct asymmetric intermolecular aldol reaction between **1a** and *p*-nitrobenzaldehyde

1a **2a**: R = *p*-$NO_2C_6H_4$

Entry	Catalyst	Time (h)	Yield (%)[a]	Dr[b]	Ee (%)[c]
1	L-ala-L-ala	24	73	8 : 1	91
2	L-ala-L-ala	95	84[d]	3 : 1[d]	93[d]
3	L-ala-L-ala	72	70[e]	4 : 1[e]	89[e]
4	L-ala-L-phe	24	70	2 : 1	93
5	L-val-L-val	24	75	1 : 1	94
6	L-val-L-phe	24	72	2 : 1	96
7	L-ala-L-gly	48	46	4 : 1	81
8	L-val-L-phe	48	82[d]	4 : 1[d]	98[d]
9	L-ala-L-val	41	87	1 : 1	87
10	L-val-L-ala	48	92	1 : 1	98
11	L-ser-L-ala	24	55[f]	2 : 1	92
12	L-ala-L-ala-L-ala	48	90	1 : 2	81

[a] Isolated yield after silica-gel column chromatography. [b] *anti : syn* ratio as determined by NMR analyses. [c] Determined by chiral-phase HPLC analyses. [d] Reaction performed at 6 °C. [e] 10 mol% peptide catalyst. [f] 20 equiv. H_2O.

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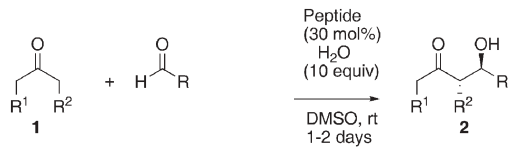
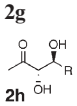
with 1 : 1–10 : 1 dr and 81–98% ee. Importantly, the addition of water increased the efficiency and the stereoselectivity of the reaction.[15] Furthermore, decreasing the temperature of the reaction improved the steretoselectivity. For example, ala-ala catalyzed the asymmetric formation of **2a** in 84% yield with 3 : 1 dr and 93% ee at 6 °C. The amount of catalyst could also be decreased to 10 mol%. The highest ee of **2a** was accomplished when val-ala was used as the catalyst. In addition, tripeptides catalyzed the asymmetric aldol reactions. For example, ala-ala-ala catalyzed the asymmetric assembly of **2a** in 90% yield with 1 : 2 dr and 81% ee. Interestingly, the diastereoselectivity was changed from *anti* to *syn* as compared to the dipeptide catalyzed reactions. We next investigated the dipeptide catalyzed direct intermolecular aldol reaction between a set of different biomimetic donor ketones **1** and acceptor aldehydes (Table 2).

The peptide-mediated reactions were efficient and furnished the desired aldol products **2a–h** in good yield with up to 99% ee. In particular, the dipeptide-catalyzed reactions with cyclic ketones **1a–b** proceeded with high chemo-, diastereo- and enantioselectivity. In comparison, pro-pro catalyzed the formation of aldol product **2b** in 45% yield with 2 : 1 dr and < 5% ee. The peptide-mediated asymmetric aldol reactions with **1c** as the donor were regiospecific and exclusively furnished *vic*-diol **2h**. Moreover, the peptides were more efficient and assembled the aldol products in higher yield as compared to simple alanine. The peptides complemented each other, which suggests that for a given pair of substrates an ideal peptide catalyst can be generated. Peptide analogues prepared from primary amino acids and amino alcohols did also catalyze the reaction. For example, L-val-D-phealaninol

Table 2 Peptide- and peptide analogues-catalyzed direct intermolecular asymmetric aldol reactions

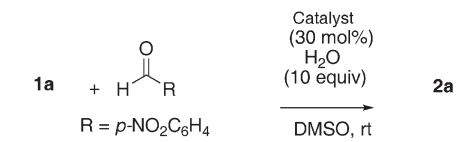


1 **2**

Entry	Peptide	Ketone	R	Prod.	Yield (%)[a]	Dr[b]	Ee (%)[c]
1	L-ala-L-ala	**1a**	4-$NO_2C_6H_4$	**2a**	73	8 : 1	91
2	L-ala-L-ala	 **1b**	4-$NO_2C_6H_4$	**2b**	70	3 : 1	99
3	L-ala-L-phe	**1b**	4-$NO_2C_6H_4$	**2b**	88	5 : 1	99
4	L-val-D-phealaninol	**1b**	4-$NO_2C_6H_4$	**2b**	80	4 : 1	99
5	L-ala-L-ala	**1b**	4-CNC_6H_4	**2c**	65[d]	13 : 1	99
6	L-ala-L-ala	**1b**	4-BrC_6H_4	**2d**	55[d]	2 : 1	92
7	L-ala-L-phe	**1a**	4-BrC_6H_4	**2e**	88[d]	2 : 1	99
8	L-ala-L-ala	**1a**	4-BrC_6H_4	**2e**	86[d]	2 : 1	96
9	L-ala-L-ala	**1a**	4-ClC_6H_4	**2f**	67[d]	2 : 1	98
10	L-ala-L-ala	**1b**	*i*-Pr	**2g**	50[d]	2 : 1	97
11	L-ala-L-ala	 **1c**	4-$NO_2C_6H_4$	 **2h**	93	1 : 1	75

[a] Isolated yield after silica-gel column chromatography. [b] *anti : syn* ratio as determined by NMR analyses. [c] Determined by chiral-phase HPLC analyses. [d] 5 equiv. water was used.

Table 3 Peptide-catalyzed direct asymmetric aldol reactions



Entry	Catalyst	Time (h)	Yield (%)[a]	Dr[b]	Ee (%)[c]
1	L-ala-L-ala	24	73	8 : 1	91
2	L-ala-D-ala	48	88	4 : 1	77
3	L-ala-L-gly	48	46	4 : 1	81
4		72	16	1 : 1	12
5	L-ala-L-ala-OEt	73	55	2 : 1	80

[a] Isolated yield after silica-gel column chromatography. [b] *anti : syn* ratio as determined by NMR analyses. [c] Determined by chiral-phase HPLC analyses.

mediated the formation of aldol adduct **2b** in 80% yield with 4 : 1 dr and 99% ee. The ability of primary amino acid derived amides to catalyze the asymmetric aldol reaction adds an additional dimension in the diversification, modularity and generation of novel catalyst libraries.

Proton transfer from the carboxylic group and hydrogen-bond activation, which is facilitated by the presence of water,[15] were important to achieve a high asymmetric induction (Table 3). In addition, the stereochemistry of the dipeptide's second stereocenter affects the enantioselectivity of the reaction. For example, changing L-ala-L-ala to L-ala-D-ala decreased the ee of **2a** from 91 to 77% and the absence of an alkyl group at the second stereocenter did also decrease the ee by 10%.

The stereochemistry of the β-hydroxy group of the aldol adducts **2** derived by the primary amine peptide and primary amino acid derived amide catalysis was *R* as determined by chiral-phase HPLC analysis, optical rotation and comparison with the literature.[9,12] The relative stereochemistry of the cyclic aldol products **2** was *anti* as determined by NMR spectroscopy and comparison with the literature.[9,12] Based on the absolute and relative configuration of aldol products **2**, we propose that the L-amino acid derived dipeptides catalyzed the direct asymmetric aldol reactions between ketones and aldehydes *via* the plausible six-membered chair-like transition states **I** and **II**, where the *Re*-face of the catalytically generated chiral enamine is approached by the *Si*-face of the acceptor aldehyde (Fig. 1).[16]

Importantly, we also found that the ancient dipeptides had an intrinsic ability to catalyze the asymmetric formation of sugars under prebiotic conditions (Equation 2). For example, L-ala-L-ala catalyzed the asymmetric formation of D-erythrose and D-threose

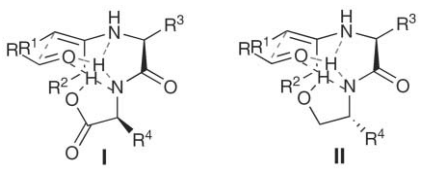
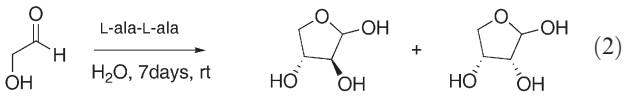


Fig. 1 Plausible transition states **I** and **II** for the dipeptide- and amino acid-amide-catalyzed direct asymmetric aldol reaction.

in a 2 : 1 ratio and 15% ee *via* dimerization of glycol aldehyde. Moreover, the asymmetric induction was three-times higher when the ancient catalyst was ala-ala as compared to alanine.[1b]



(2)

Thus, increased structural complexity gave higher enantioselectivity. In a prebiotic scenario, amino acids formed on Earth or brought to it by comets and meteorites condensed nonenzymatically to form peptide oligomers.[13,17] Next, the peptides catalyzed the asymmetric formation of sugars that could form the backbones of ancient RNA.[18]

In summary, we have demonstrated for the first time that peptides and their analogues with a primary amino acid at the *N*-terminus can be employed as highly stereoselective catalysts for the direct asymmetric intermolecular aldol reaction. This will dramatically expand the structural diversity and modularity in the design and combinatorial construction of novel organocatalysts. A peptide library based on the simple amino acids alanine and valine catalyzed the asymmetric formation of the desired aldol products in high yield and up to 99% ee. The peptide-catalyzed reactions are accelerated by water, and are inexpensive, operationally simple and environmentally benign. We also found that ancient peptides have an intrinsic ability to catalyze the asymmetric neogenesis of sugars under prebiotic conditions, which may explain the initial molecular evolution of aldolase enzymes. Further expansion of peptide catalysis in organocatalytic asymmetric C–C bond-forming reactions, combinatorial catalyst preparation and density functional theory calculations are ongoing.

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