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Asymmetric Michael Reaction Catalyzed by Mimicked Peptides

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Abstract Peptides mimicked from active site of promiscuous aldo-ketoreductase were synthesized and tested as asymmetry catalysts in the Michael adduct reaction of aldehydes or ketones with nitroolefins to furnish the corresponding γ-nitroaldehydes, γ-nitroketones with up to 93 % yield, 99:1 dr and 71 % ee at room temperature and on eco-friendly solvents. Aspartic acid residue as second amino acid produced greater enantioselectivity.

Keywords Michael adduct · Asymmetry · Peptides · Aldo-ketoreductase · Promiscuous

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1 Introduction

The asymmetric Michael reaction is a well known fundamental method for the enantioselective construction of carbon–carbon bonds in organic synthesis [1]. With the recent increase in demand for optically active pharmaceutical compounds, much progress has been made in the development of asymmetric organocatalysts, which provides for the preparation of Michael adducts with high enantiomeric purity [2]. In the past decade, a lot of research has been conducted with asymmetric organocatalysts due to it being environmentally benign (heavy metal free), low cost, and stability in moisture and air [3, 4]. Since the pioneering work of List et al. in the application of the amino acid proline as a catalyst for the aldol reaction [5], a number of short peptides have been synthesized and employed as asymmetric organocatalysts in various organic transformations [6–8]. Peptides are structurally diverse and have multifunctional groups, and are therefore able to create strong hydrogen bonds with nitroolefins, which provide the necessary conditions to enhance stereoselectivity [9–11]. Design and synthesis of the artificial enzyme that is able to catalyze various asymmetric organic reactions have long been dream of chemists. Therefore, peptides, which have an enzyme-like structure, are ideal asymmetric artificial enzymes [12]. Type I aldolase, which works through an enamine has inspired synthetic chemists to delve into the possibility of miniaturizing these enzymes into short peptides [13]. The active site of aldo-ketoreductase (AKR) is comprised of tetrad amino acids residues, Asp47, Tyr52, Lys77 and His110, which makes it similar to aldolase. Several peptides were envisaged to able to asymmetrically catalyze Michael reactions via covalent and non-covalent interactions between catalysts and substrates (Fig. 1). Peptides described in Fig. 1 were derived from the AKR active sites.

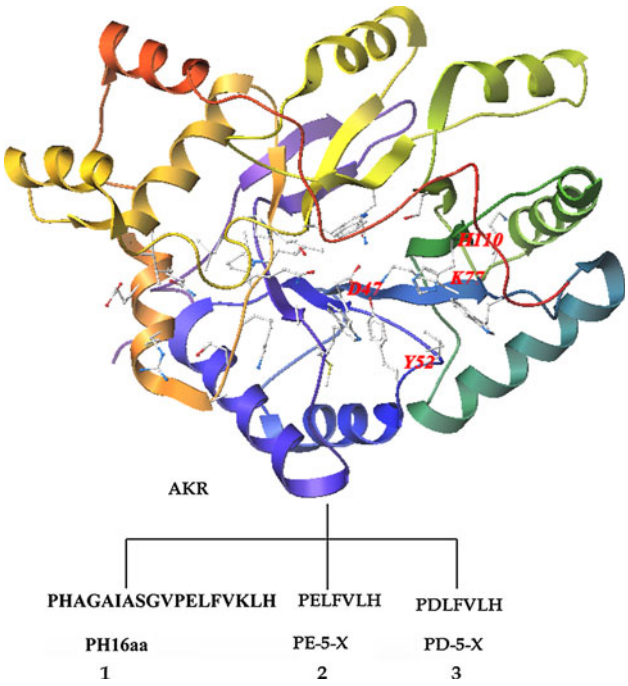


Fig. 1 Mimicked peptides based on AKR as asymmetry catalyst

2 Experimental

2.1 General Information

All chemicals were purchased and used without further purification. Analytical thin layer chromatography (TLC) was performed using Merck 60 F_{254} precoated silica gel plate (0.2 mm thickness). Flash chromatography was performed using Merck silica gel 60 (70–230 mesh). Fourier transform infrared spectroscopy (FT-IR); Perkin Elmer Spectrum 100 was used for the identification of functional groups. NMR data was recorded on 500 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR (JEOL JNM ECA) spectrometer. The relative and absolute configurations (dr) of the Michael adducts were determined through comparison with ^{1}H NMR spectroscopic analysis and HPLC with chiral columns. Mass spectra (MS) were measured with a spectrometer (DIMS QP5050A SHIMADZU). Liquid Chromatography–Mass Spectrometry (LC–MS, Agilent). Optical rotations were measured on a JASCO P-2000 Polarimeter. Enantioselectivity was determined by HPLC (Waters 1525 Binary Pump and UV-Water 2489) analysis employing a Daicel ChiralPak OD-H and AD-H columns (4.6 mm × 250 mm).

2.2 General Procedure for Asymmetric Michael Reaction

A catalyst (5 mol%) was added to a mixture of aldehydes 2 eq and iPrOH:H_2O (0.5:0.5 ml). After stirring at ambient temperature for 20 min, nitrostyrene (1 eq) was introduced. The reaction mixture was stirred at the same temperature for 15–72 h and concentrated with rotary evaporator under reduced pressure, followed by precipitation by EtOAc. The precipitate was filtrated for reuse, and the EtOAc layer was concentrated. The residue was purified through column chromatography on silica gel by using ethylacetate:*n*-hexane (1:3) as an eluent to produce the corresponding Michael adducts for further analysis.

2.3 Catalyst Preparation

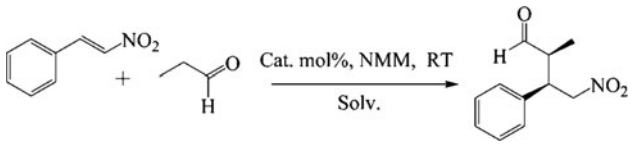
All peptides were used for this work (Fig. 1). Synthesis and purification of peptides were performed according to the Fmoc solid-phase strategy. Briefly, the peptide was manually synthesized from an Fmoc-Rink-Amide-Am-Resin, using a 3-fold molar excess of amino acid derivatives with HCTU/DIPEA as a coupling reagent and with double coupling cycles. At the end of the synthesis, the peptides were separated from the cocktail by using trifluoroacetic acid (TFA, 92.5 %), triisopropylsilane (2.5 %), 1,4 dithiothritol (2.5 %) and water (2.5 %). 10 ml of the mixture was used for 1 g resin with 2 h agitation. The peptide was precipitated in dry diethyl ether and lyophilized. The purity of the peptide (overall, more than 93 %) was measured by Analytical Waters HPLC (Binary HPLC pump 1525 and UV-Waters 2489), (RPC18, Xbridge 4.6 mm × 250 mm), flow rate 0.5 ml/min, 220 nm.

3 Results and Discussion

Initially, the nitro-Michael reactions of propanal with *trans*-β-nitrostyrene in the presence of peptides, **1**, **2** and **3** (Fig. 1) were examined. The peptides were easily prepared from readily available amino acids using standard procedures of solid phase peptide synthesis [14, 15]. Catalytic activities of peptide **1** under various conditions were studied. A reaction with H_2O without any additives, failed, due to the insolubility of the substrate in water and the acidic moiety of peptide (Table 1, entry 1). For good catalytic activity, both catalyst and substrate should be soluble in the reaction media. To create a facile enamine intermediate, the pH needed to be adjusted. Therefore, iPrOH and *N*-methylmorpholine (NMM) were used to increase the solubility of the substrate and pH, respectively. The best result was obtained when the ratios of solvent and pH were (H_2O:iPrOH, 1:1) and pH 5.5–6.0 (Table 1, entries 4, 5).

These results demonstrated that high solubility of catalyst and substrates in solvents led to obtain high yield and enantioselectivity. Slightly acidic pH helps to increase enantioselectivity (Table 1, entries 4, 5). The influence of catalyst loading was also evaluated and it was found that

Table 1 Optimization screening of Michael adduct reaction



Entry	Cat. (mol%)	NMM (pH)	Time (h)	Solv.	Yield (%)[a]	ee (%)[b]
1	5	No NMM (pH 2)	72	H_2O	NR	NR
2	5	pH 4	24	H_2O/^{i}PrOH (8/2)	60	35
3	5	pH 5	24	H_2O/^{i}PrOH (6/4)	75	43
4	5	pH 5.5	24	H_2O/^{i}PrOH (5/5)	87	44
5	5	pH 6	24	H_2O/^{i}PrOH (5/5)	90	44
6	5	pH 7	24	H_2O/^{i}PrOH (5/5)	94	34
7	5	pH 5,5	24	H_2O/^{i}PrOH (3/7)	67	40
8	3	pH 5.5	24	H_2O/^{i}PrOH (5/5)	65	42
9	10	pH 5.5	20	H_2O/^{i}PrOH (5/5)	88	44
10	15	pH 5.5	15	H_2O/^{i}PrOH (5/5)	88	44

NR no reaction, *NMM N*-methylmorpholine

The Michael reaction between propanal (2 eq, 0.2 mmol, 11.6 mg) and *trans*-β-nitrostyrene (1 eq, 0.1 mml, 14.9 mg) in the presence of 5 mol% of peptide **1** at ambient temperature

[a] Yields of isolated products after column chromatography

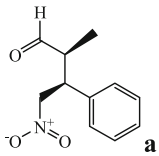
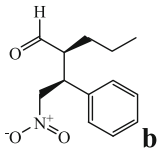
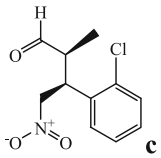
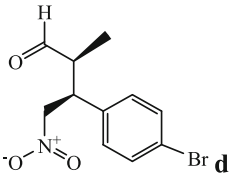
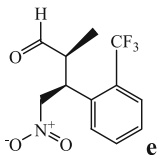
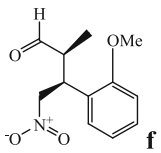
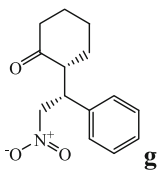
[b] Enantioselectivities were determined for syn-product by chiral-phase HPLC analysis (Daicel Chiralpak AD-H, OD-H)

5 mol% of catalyst furnished the best results (Table 1, entry 4). A reduction in the amount of catalyst **1** and H_2O to 3 mol% and (H_2O/iPrOH, 3/7) resulted in a relatively slow reaction and still produced 65 % yield, 42 % ee and 67 % yield, 40 % ee within 24 h (Table 1, entries 7, 8). Therefore, the reaction conditions were optimized with 5 mol% of peptides, NMM as pH adjustor (pH 5.5–6.0) and mixture of H_2O/iPrOH (1:1) as solvents for further studies. The scope of the reaction was probed for different aromatic nitroolefins and Michael donors with peptide **1** as catalyst and NMM as an additive on H_2O/iPrOH (Table 2). In all cases, reactions afforded syn products. Surprisingly, when a longer linear chain of aldehyde was applied, the enantioselectivity decreased sharply from 44 to 27 % ee (Table 2, entries 1 and 2). It can be explained that a longer chain caused a larger steric hindrance between the nitroolefins and catalyst to push it to both sides of the catalyst, resulting in a lower yield of enantioisomers. Both electron-rich and electron-deficient nitrostyrenes were shown to be good Michael acceptors in reacting with propanal and the reactions all occurred smoothly in aqueous media (Table 2, entries 3–7). Michael adducts (Table 2, entries 1–7) were obtained in good yields (81–93 %), moderate enantioselectivities (19–44 % ee), and high diastereoselectivities (syn/anti ratio up to 99:1). It was observed that reactions involving nitrostyrenes bearing electron-withdrawing substituents in the *ortho* position produced higher enantioselectivity, but slightly lower yields and diastereoselectivity when substituents in the *para* position were used (Table 2, entries 3, 5, 6 vs 4). A cyclic ketone donor—cyclohexanone—was tested under the previously optimized conditions. The reaction of cyclohexanone with *trans*-β-nitrostyrene resulted in a product with 85 % yield, 41 % ee and excellent diastereoselectivity syn/anti, 99/1 (Table 2, entry 7). The absolute configuration of **g** (depicted in Table 2, entry 7) was determined to be 2S, 3R by comparing the optical rotation with those reported elsewhere [16–18].

The influence of peptide length on stereoselectivity outcome was investigated. Heptapeptides, **2** and **3**, which were derived from peptide **1** were synthesized and tested on the model reaction under the optimum conditions (Fig. 1). The results exhibited higher yield and stereoselectivity (Table 3, entry 1). Although both shorter heptapeptides had better yield and stereoselectivity compared to peptide **1**, slightly higher stereoselectivity was observed when peptide **3** was applied as a catalyst. The difference between peptides **2** and **3** is that the second amino acid residue is glutamic acid in peptide **2** and is aspartic acid in peptide **3**. The difference between two amino acids is just one methylene (–CH_2–) group. It can be concluded that with peptide **3**, the carboxylic acid side chain is close enough to nitroolefin to make better hydrogen bonds and nucleophile attacks on the Michael acceptor at the opposite side easier than glutamic acid (Fig. 2).

Proline as the *N*-terminus secondary amino acid creates covalent interactions via enamine intermediates and hydrogen bonding, which facilitates keeping the substrate on one side of the catalyst, forcing the nucleophile to attack

Table 2 Examples of peptide 1 catalyzed nitro-Michael additions of different aldehyedes and ketone to nitroolefins

Entry	Product	Yield (%)[a]	dr (syn/anti)[b]	ee (%)[c]
1	 **a**	87	80/20	44
2	 **b**	88	81/19	27
3	 **c**	85	80/20	38
4	 **d**	93	95/5	19
5	 **e**	88	80/20	37
6	 **f**	81	66/34	32
7	 **g**	85	99/1	41

The Michael reaction between aldehydes/ketone (2 eq, 0.2 mmol) and *trans*-β-nitrostyrene (1 eq, 0.1 mmol) in the presence of 5 mol% of peptide **1** at ambient temperature

[a] Yields of isolated products after column chromatography

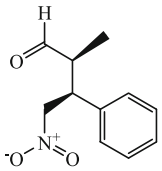
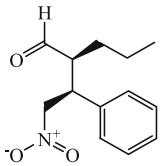
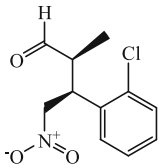
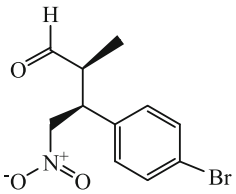
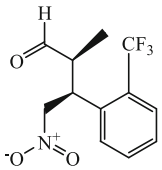
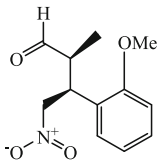
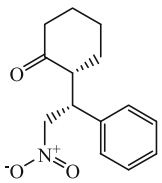
[b] Enantioselectivities were determined for syn-product by chiral-phase HPLC analysis (Daicel Chiralpak AD-H, OD-H)

[c] Determined by ^{1}H NMR analysis of the crude product of chiral-phase HPLC analysis (Daicel Chiralpak AD-H, OD-H)

the opposite side. The hydrogen bond between a water molecule and the amide oxygen atom of the peptide could increase the acidity of the amide bond and strengthen the related hydrogen bond with the nitro group of the *trans*-β-nitrostyrene. The additional positive effect of water could be ascribed to the hydrogen bonds formed between the nitro-group and the water molecule, which at the same time forms the hydrogen bond with the COO– group of the catalyst, thus, stabilizing the transition state.

The scope of the reaction included a variety of aldehydes as donors and ketones reacting with nitroolefins of variable electronic characteristics in the presence of catalysts, **2** and **3**, with good yield (80–90 %) and moderate to good enantioselectivities (39–71 %) (Table 3, entries 1–6). Catalyst **2** however, was found to exhibit moderate enantioselectivity and good reactivity for the α-α-disubstituted aldehydes and niroolefin acceptors. Catalyst **3** exhibits greater reactivity and selectivity, when compared to catalyst **2** for the Michael reaction of α-α-disubstituted aldehydes and niroolefins (Table 3). The reactions of substituted nitrostyrenes and linear aldehyde, *n*-pentanal, were studied. Slightly lower yields and enantioselectivities were obtained for both catalysts. The reactions between aldehydes and nitrostyrenes, bearing both electron-donating

Table 3 Scope and limitation studies of Michael addition between aldehydes/ketone and nitroolefins catalyzed by peptides 2 and 3

Entry	Product	Cat.	Yield (%)[a]	%dr (syn/anti)[b]	ee (%)[a]
1		3	90	80/20	60
		2	87	84/16	57
2		3	90	87/13	51
		2	87	90/10	46
3		3	88	73/27	45
		2	84	74/26	43
4		3	86	60/40	64
		2	84	60/40	57
4		3	89	85/15	44
		2	85	76/24	39
5		3	84	85/15	45
		2	80	82/18	44
6		3	85	85/15	71
		2	82	77/23	55

The Michael reaction between aldehydes/ketone (2 eq, 0.2 mmol) and *trans*-β-nitrostyrene (1 eq, 0.1 mmol) in the presence of 5 mol% of peptides **2** and **3** at ambient temperature

[a] Yields of isolated products after column chromatography

[b] Enantioselectivities were determined for syn-product by chiral-phase HPLC analysis (Daicel Chiralpak AD-H, OD-H)

[c] Determined by ^{1}H NMR analysis of the crude product of chiral-phase HPLC analysis (Daicel Chiralpak AD-H, OD-H)

and electron-withdrawing substituents produced high yields (80–89 %), moderate enantioselectivities (39–64 %), and high stereoselectivities (syn/anti ratio up to 85/15) for both catalysts (Table 3, entry 5). Obtained results demonstrated that peptide **3** produced relatively higher yield and enantioselectivity. The asymmetric addition of cyclohexanone to

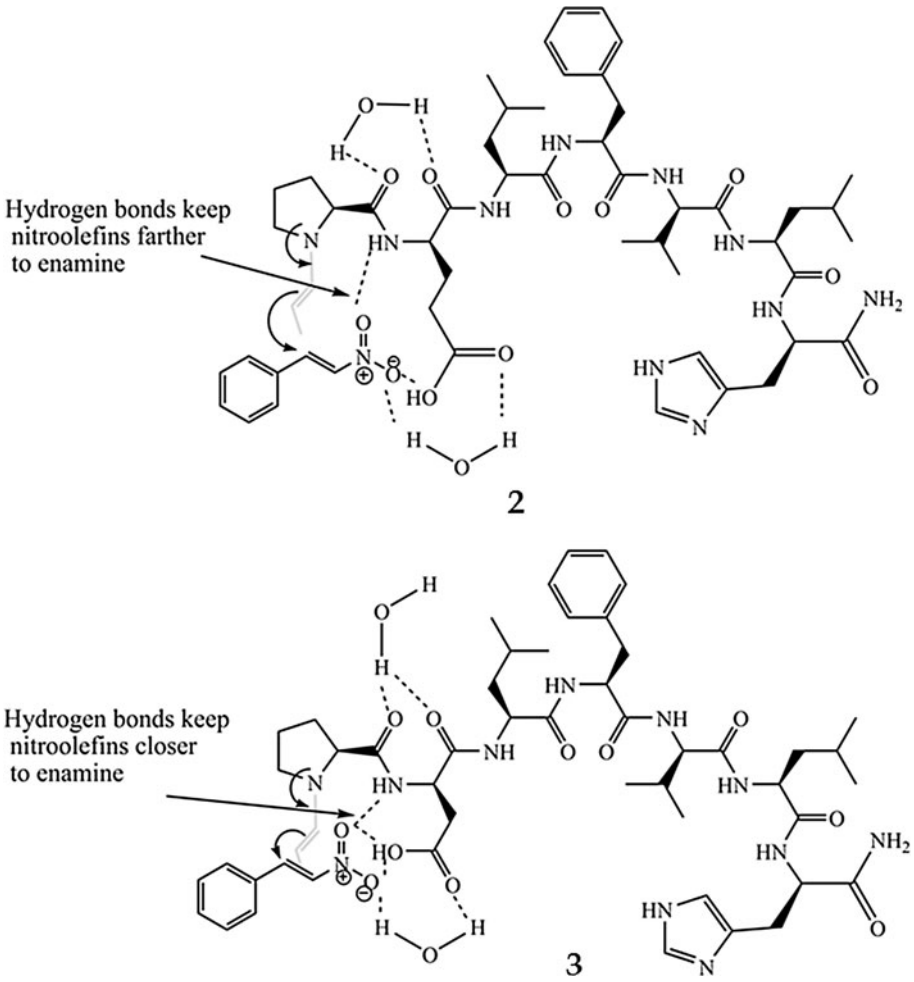


Fig. 2 Proposed transition state mechanism for Michael addition catalyzed by peptides **2** and **3**

trans-β-nitrostyrene using **2** and **3** as catalysts, respectively, was also investigated. Good enantioselectivity was obtained when **3** was applied (Table 3, entry 6, 71 % ee) and moderate enantioselectivity was attained for **2** (Table 3, entry 6, 55 % ee).

Peptide **2** exhibited moderate enantioselectivity and good reactivity for the α-α-disubstituted aldehydes and niroolefin acceptors. In comparison, peptide **3** exhibits greater reactivity and selectivity. The reactions of substituted nitrostyrenes and linear aldehyde n-pentanal were studied. Slightly, lower yields and enantioselectivity was obtained for both catalysts. The reactions between aldehydes and nitrostyrenes, bearing both electron-donating and electron-withdrawing substituents, produced high yields (80–89 %), moderate enantioselectivities (39–64 %), and high stereoselectivities (syn/anti ratio up to 85/15) for both catalysts (Table 3, entry 5). Results demonstrated that catalyst **3** produced relatively higher yield and enantioselectivity than catalyst **2**. The asymmetric addition of cyclohexanone to *trans*-β-nitro-styrene using **2** and **3** as catalysts, respectively, was also investigated. Good enantioselectivity was obtained when catalyst **3** was applied (Table 3, entry 6, 71 % ee) and moderate enantioselectivity was attained for **2** (Table 3, entry 6, 55 % ee).

4 Conclusion

In summary, three mimetic peptides were designed based on promiscuous AKR active sites. Their catalytic activities were evaluated in the Michael adduct reactions. Based on experimental results, heptapeptides **2** and **3** demonstrated higher reactivity. In order to investigate the role of very similar structures of amino acid in the catalysis **3** was synthesized. Aspartic acid as the second amino acid in peptide **3** exhibited better asymmetric catalytic activity in the Michael reaction than peptide **2**. Water, as an eco-friendly solvent also increased enantioselectivity.

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