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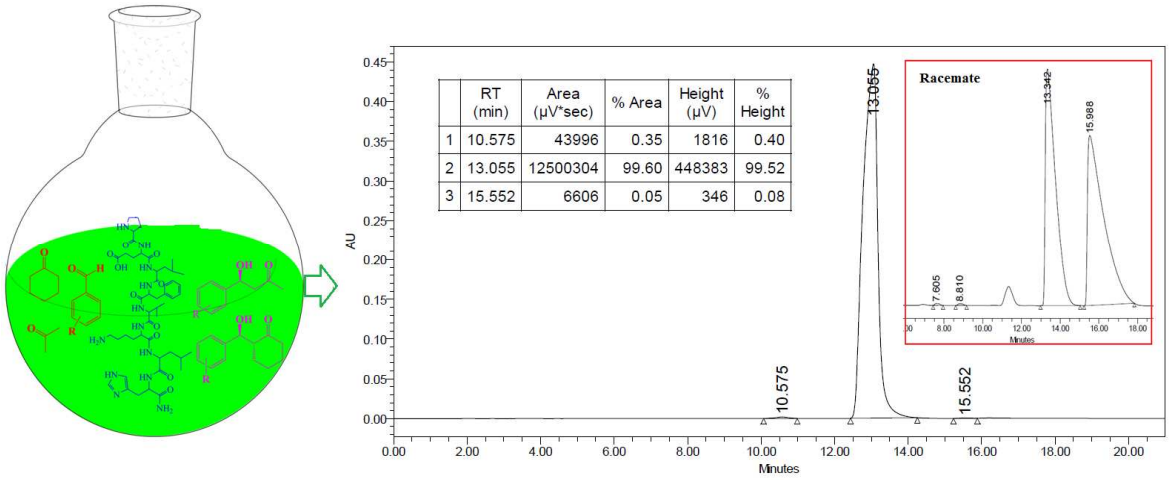
Graphic Abstract



Excellent yield (up to 97%), enantioselectivity (up to 99%) and diastereoselectivity (up to 99/1) were obtained by using mimetic peptides.

Rational Design of Mimetic Peptides Based on Aldo-Ketoreductase Enzyme as Asymmetric Organocatalyst in Aldol Reactions

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Abstract: Peptides as a kind of important chiral scaffold are broadly identified for their obvious advantages, diverse structures and readily accessibility. Based on promiscuous aldo-keto-reductase enzymes, several mimetic peptides were designed which were synthesized and tested as multifunctional organocatalysts in direct asymmetric aldol reactions. The corresponding aldol products were produced with high yields (up to 97%) and excellent diastereoselectivities (up to 99/1) and enantioselectivities (up to 99.9) under mild reaction conditions. These peptides exhibit excellent catalytic activity in terms of yield, diastereoselectivity and enantioselectivity. The secondary structures of peptide catalysts provide an understanding of their mechanism.

Keywords: Mimetic peptide • aldo-ketoreductase enzyme, asymmetric aldol reactions • diastereoselectivity • enantioselectivity

1. Introduction

The aldol reaction is a fundamental organic reaction in the construction of the C-C bond.[1-3] Although, for a couple of decades, enzymes as practical catalysts have been increasingly utilized for organic synthesis due to their simple processing requirements, high selectivity and mild reaction conditions, they always suffer from the problem of the limmitaion in broad substrate scope. Type I aldolases, which are natural enzymes, employ the primary amine of a lysine residue under aqueous conditions for enamine formation and aldol reactions.[4] Dispite the promiscuous enzymes emerged for catalyzing different chemical transformation of natural or non-natural substrates, their low activity in terms of both yield and stereoselectivity impeled chemists to design small molecules inspired from enzymes.[5]

List et al. demonstrated that L-proline itself could catalyze the direct intermolecular asymmetric aldol reaction with an enamine intermediate.[6-7] The use of peptides as organocatalysts significantly increased for various reasons:[8-15] they emulate the action of natural enzymes;[16-17] their building blocks with inherent chirality and functional diversity are easily available; they are very easy to synthesize via solid phase methodology; finally, they provide a high degree of stability. Some of the derivatives, used as catalysts in aldol reactions, are dipeptides,[18-19] which have terminal end primary amino acids or the secondary amine like proline.[20-22] In addition, Wennemers et al. employed tripeptides by using the combinatorial method in asymmetry C-C bond formation with excellent stereoselectivity.[23-26] Thus, peptides can be ideal asymmetric organocatalysts

due to their diverse structures and functionality and because they are great alternatives to small, rigid organocatalysts and enzymes.[27-31] Mimetic peptides and oligopeptides have gained a great deal of attention due to their asymmetric catalytic properties, availability and similarity to natural enzymes.[25, 32-35] The main challenge in developing asymmetric catalysis lies in how to design the peptides in the mimicking of natural enzymes. Mimetic peptides as asymmetric catalyst should be both efficient and capable of accepting a broad range of substrates. The evidences showed that in the active center of natural aldolases, primary amino group of a lysine residue that lies in a hydrophobic pocket plays the main role in enamine intermediate construction. In this study, Aldo-keto reductase's (AKRs) active site is very similar to aldolase enzyme.[36-37] Therefore, all amino acids, which have an important role in the catalytic mechanism of AKRs, were determined.

In order to probe catalytic activity, stereoselectivity of peptide, the design of peptide to attain high yield of enantiomer excess, was particularly considered. Thus, several mimetic peptides were designed based on active site of AKRs. To realize such a reaction in a single flask, since water is generally a suitable solvent for enzymatic reactions, the organocatalyst should be activated in water.[38] Using water as reaction medium is another attractive research subject, mainly due to the low cost, safety and environmentally benign nature of water.[39] In the present study, the designing of the best structure based on enzyme which can be employed in different kind of asymmetric organic reactions, particularly in C-C bond-forming reaction, was considered. To fulfil this purpose, aldol reaction, which is one of the most important carbon–carbon bond-forming reactions in organic chemistry, was chosen as a model.

2. Results and Discussion

2.1. Design of mimetic peptides based on AKRs

In the field of asymmetric organocatalysis, short peptides and peptide-based molecules have emerged as promising catalysts for a rapidly growing body of reactions. Peptides offer many sites for functional and structural diversities that can be used to generate optimized catalysts. Therefore, peptide may be an ideal compromise between small rigid organocatalysts and enzymes. Designing and synthesizing peptides, which are capable of catalyzing a wide range of substrates asymmetrically, have still remained as an immense challenge. The strategy of this study lies on the inspiration of nature's enzymes. According to similarities of active site of AKRs to aldolase, a series of the peptides were designed and synthesized based on AKRs (PDB = 1VBJ) active site, using Fmoc-solid phase protocol.

The active site of AKRs contains several hydrophilic and hydrophobic amino acids residues which scaffold surround the ligand. As exhibited in Figure 1, these residues have been distributed in different places and the location are far from eachother. It is very difficult to synthesise this polypeptide by using manual or automated peptide synthesis method. Therefore, the number of amino acids were reduced to eighteen just by removing some amino acids. In the initial study, three mimetic peptides derived from AKRs, which have subsequent sequences PEAGAIASGVPELFVKLH, PHAGAIASGVPELFVKLH and AGAIASGVPELFVKLH, and called peptides **PE16aa**, **PH16aa** and **16aa**, respectively, were designed and synthesized. The secondary structure of these peptides was predicted by LOMETS as random and α-helix (Figure 2).

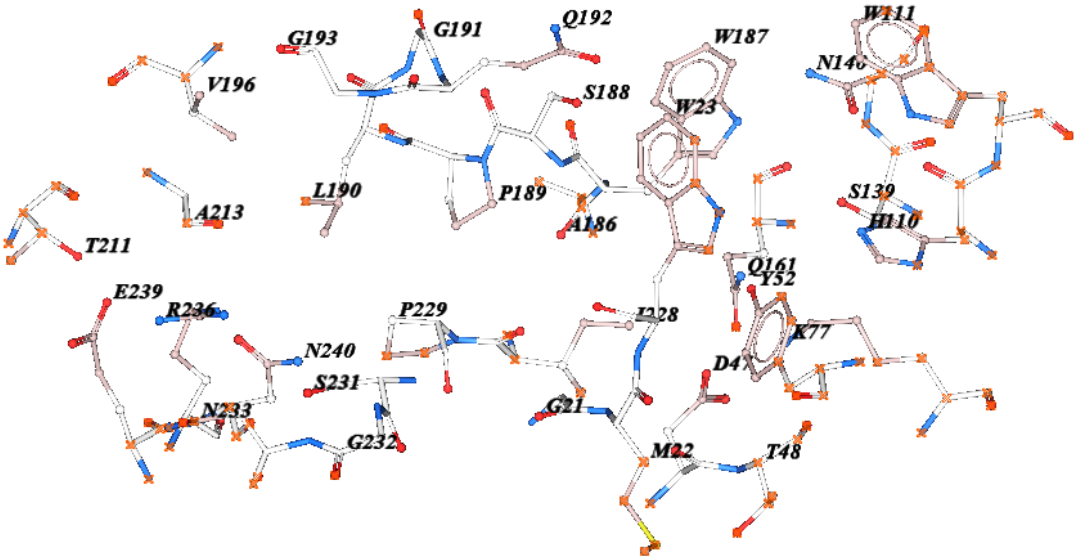


Figure 1. All amino acid residues in AKR's active site

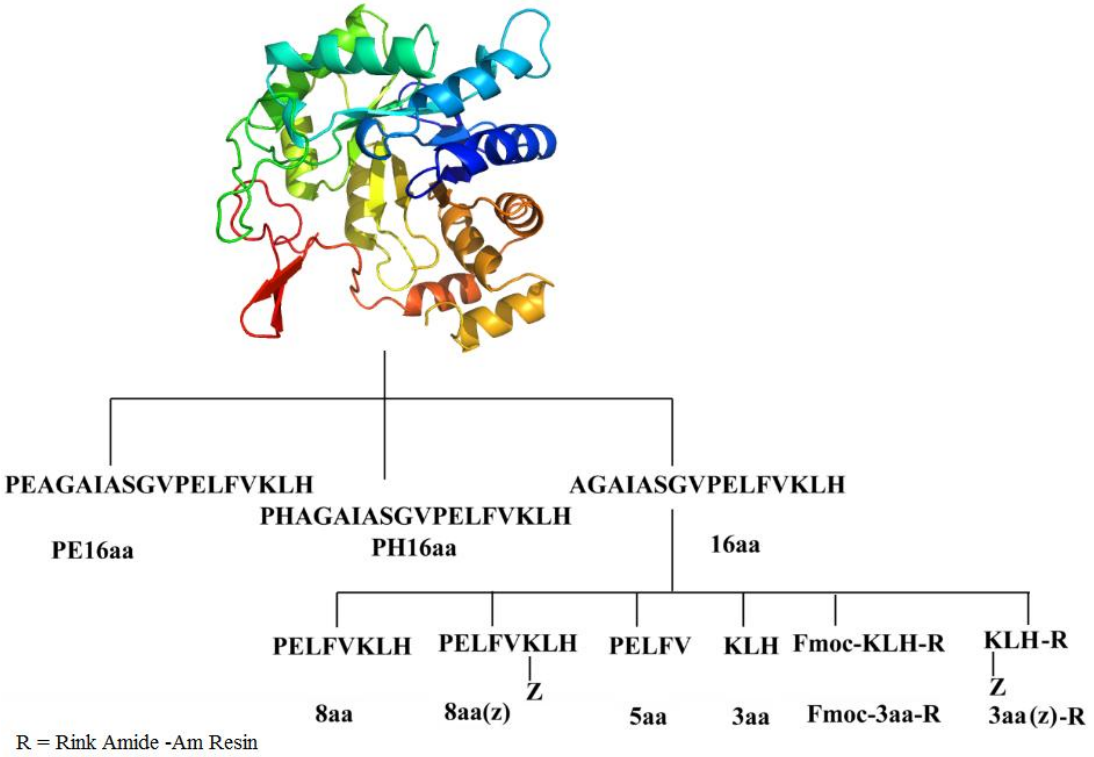
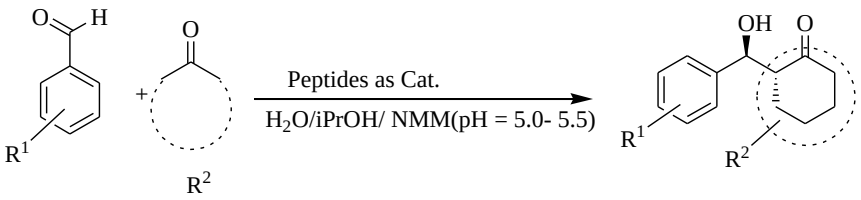


Figure 2. A series of synthesized peptides mimicked from AKRs

The reaction of *p*-nitrobenzaldehyde (30 mg, 0.198 mmol, 1 eq) with cyclohexanone (23.3 mg, 0.238 mmol, 1.2 eq) was performed as an initial test in the presence of 3 mol% of the peptide catalysts in aqueous medium (H_2O:iPrOH, 1:1, pH = 5.5). Catalytic activity of **PH16aa**, **PE16aa** and **16aa** was evaluated (Table 1). In order to broaden the range of substrates, the best catalytic efficiency for the defined conditions of the reaction with **PH16aa** was found (Table 1, entries 9-22). Using **PE16aa** as an organocatalyst under defined conditions, it was observed that the aldol reaction gave the corresponding products high yield (up to 89%), and with moderate enantioselectivity (up to 68%) and diastereoselectivity *(*anti/syn, up to 99:1) (entries 2-8). These results suggest that the carboxylic side chain of glutamic acid and the imidazole group of histidine

can influence on enantioselectivity due to hydrogen bonding and imidazole group is more effective than carboxylic group.[40] Interestingly, when **PH16aa** was applied as a catalyst in the reaction between aromatic aldehydes and cyclohexanone, yield and stereoselectivity increased (Table 1, entries 9-17, up to 95% yield, up to 99/1 dr, up to 86% ee). The reaction between *p*-nitrobenzaldehyde and cyclohexanone was also performed in the presence of 1% sodium dodecyl sulfate (SDS) as an additive, and the results exhibited almost equal yield and %ee compared to those of the reactions in which SDS was not used. Notably, in most cases, the anti-aldol products were obtained with excellent diastereoselectivity and good enantioselectivity, regardless of the electronic nature of the aromatic aldehydes and ketones. The substitution of aromatic aldehydes and acetone as acyclic ketone catalyzed by **PH16aa** produced excellent yields and moderate enantioselectivity up to 56% (Table 1, entries 18-22). To investigate the effect of peptide length on diastereo- and enantioselectivity in aldol reactions, a peptide with sixteen amino acids was synthesized, which consisted of a sequence derived from the active site of the AKR enzyme (**16aa**). This fragment was initiated by a primary amino acid. This polypeptide was designed in order to investigate the roles of the primary amine and side chain in the enhancement of enantioselectivity. The second amino acid was glycine which did not contain carboxylic, imidazol or hydrophilic side function groups to create strong hydrogen bonds with substrates. The aldol reaction between *p*-nitrobenzaldehyde and cyclohexanone produced a moderate yield and poor enantioselectivity (Table 1, entry 1).

Table 1. Results of reaction of various aldehydes with cyclohexanone catalyzed by 16aa, PE16aa and PH16aa



Entry	R^1	R^2	Cat.	Yield (%)[a]	dr (%)[b]	ee (%)[c]
1	4-NO_2	cyclohexanone	16aa	67	97/3	39
2	4-NO_2	cyclohexanone	PE-16aa	88	99/1	63
3	2-NO_2	cyclohexanone	PE-16aa	87	98/2	61
4	4-Cl	cyclohexanone	PE-16aa	89	96/4	62
5	4-CN	cyclohexanone	PE-16aa	87	97/3	67
6	4-CF_3	cyclohexanone	PE-16aa	89	99/1	65
7	4-Br	cyclohexanone	PE-16aa	86	97/3	57
8	2-Cl	cyclohexanone	PE-16aa	84	99/1	68
9	4-NO_2	cyclohexanone	PH-16aa	95	95/5	86
10	2-NO_2	cyclohexanone	PH-16aa	94	99/1	81
11	4-Cl	cyclohexanone	PH-16aa	92	99/1	78
12	2-Cl	cyclohexanone	PH-16aa	95	99/1	71
13	4-CN	cyclohexanone	PH-16aa	88	99/1	81
14	4-CF_3	cyclohexanone	PH-16aa	93	99/1	80
15	4-Br	cyclohexanone	PH-16aa	94	99/1	84
16	H	cyclohexanone	PH-16aa	92	99/1	76
17	4-NO_2 (Solv.= ^{i}PrOH/1% SDS)	cyclohexanone	PH-16aa	94	99/1	81
18	4-NO_2	acetone	PH-16aa	93	-	56
19	2-NO_2	acetone	PH-16aa	92	-	45
20	4-Cl	acetone	PH-16aa	92	-	43
21	4-CF_3	acetone	PH-16aa	94	-	39
22	4-Br	acetone	PH-16aa	94	-	35
23	4-NO_2	cyclohexanone	no cat.	-	-	-

Reaction conditions: Aldehydes (30 mg, 0.198 mmol, 1 eq) with cyclohexanone or acetone (23.3 mg, 0.238 mmol, 1.2 eq or 1mL), catalyst (3 mol%), solvent (H_2O:iPrOH, 1:1 or H_2O:acetone, 1:1), pH = 5.5, RT, 24 h. [a]Isolated yield. [b] dr values were determined by crude product ^{1}H NMR. [c]ee values were determined by HPLC using a Chiral OD-H and AD-H columns

For further investigation of peptide length efficacy on asymmetry aldol reaction, 16aa was cleaved to shorter peptide to obtain a peptide with a secondary amine at the *N*-terminus (see the sequences in Figure 2). The sixteen amino acids polypeptide (**16aa**) was shortened to eight amino acids (PELFVKLH, **8aa**) and was used as catalyst in the model reaction. The corresponding product catalyzed by this peptide was attained with excellent yield and enantioselectivity (yield = 97%, ee = 97%). This significant achieved result fascinated the researchers to investigate the influence of shorter peptides derived from **8aa** on catalytic activity of catalysts in aldol reaction. Later, five new peptides were designed and synthesized which are named **8aa-z**, **5aa**, **3aa**, **Fmoc-3aa-R**, and **3aa(z)-R** (Figure 2). The theory behind these designs was as following:

The **8aa-z** was designed with the purpose of knowing if the side chain of the primary amine of lysine does have any effect in the enhancement of stereoselectivity. In order to accomplish this aim, the primary amine was protected by benzylchloroformate (Figure 2, **8aa-z**).

Pentapeptide **5aa** was designed to investigate the effect of hydrophobic residues such as leucine, phenylalanine, valine, attached to a polar amino acid like glutamic acid on catalytic activity, and the lack of hydrophilic residues such as lysine and histidine. Furthermore, in order to find to what extend the primary amino acid attached to the hydrophobic amino acid followed by polar amino acid can influence the stereoselectivity, the tripeptide **3aa** was designed. In **Fmoc-3aa-R**, Fmoc group protected the N-terminus of lysine while the primary amine side chain of lysine was free. This tripeptide was attached to resin. The purpose was to identify the role of free amine in the side chain of lysine in the stereoselectivity of the carbon-carbon forming reaction. Finally, **3aa(z)-R** was designed to contain an N-terminus which is free, a carboxybenzyl group which protected the primary amine in the side chain of lysine, and a C-terminus attached to the rink amide-am-resin. The reaction of *p*-nitrobenzaldehyde with cyclohexanone was conducted using peptides **8aa**, **8aa-z**, **5aa**, **3aa**, **Fmoc-3aa-R** and **3aa(z)-R** as asymmetric catalysts in defined conditions. Compared to catalyst **8aa**, the yield and enantioselectivity remarkably diminished by almost 10% while catalyst **8aa-z** was utilized as asymmetry catalyst (Table 2, entry 3). From the mechanistic point of view, this examine showed that primary amine of lysine has an impressive synergy with other amino acids residues to accelerate both yield and stereoselectivity (Table 2, entry 3). Likewise, pentapeptide **5aa** was evaluated for direct aldol reaction between *p*-nitrobenzaldehyde and cyclohexanone in the defined conditions. Although, high to excellent yields and stereoselectivities were achieved, its catalytic activity was lower than 8aa (Table 2,entry 4 vs entry 2). The reason behind this design was that it was envisaged the hydrophobic amino acids residues also play key role in the enhancement of yield and stereoselectivity due to steric hindrance. Based on a study by Kofoed et al., Pro-Glu-NH_2 and Pro-Asp-NH_2 were ineffective both in terms of catalysis and enantioselectivity.[16] Surprisingly, when the tripeptide **3aa** was employed as a catalyst for direct aldol reaction between *p*-nitrobenzaldehyde and cyclohexanone, good yield and stereoselectivity were obtained (Table 2, entry 5) but still lower than **8aa**.

Table 2. Aldol reaction catalyzed by fragmented peptides

Entry	Cat.	Yield (%)	ee (%)
1	16aa	67	39
2	**8aa**	**97**	**97**
3	8aa-z	89	86
4	5aa	86	90
5	3aa	90	84
6	Fmoc-3aa-R	90	3
7	3aa(z)-R	85	75

Reaction conditions: *p*-nitrobenzaldehydes (30 mg, 0.198 mmol, 1 eq) with cyclohexanone (23.3 mg, 0.238 mmol, 1.2 eq), catalyst (3 mol%), solvent (H_2O:iPrOH, 1:1), pH = 5.5, RT.

On the other hand, although **Fmoc-3aa-R** gave a high yield of corresponding aldol product, the enantioselectivity dropped to 3% (Table 2, entry 6). This demonstrates that in 3aa, the *N*-terminus of lysine formed enamine intermediate properly and the substrate made the hydrogen bonding with histidine. In the **Fmoc-3aa-R**, the distance between enamine intermediate and histidine is far enough to form hydrogen-bonding interaction with imidazole group. Therefore, the almost racemic

corresponding aldol product was obtained. The Fmoc protecting group was then removed and the side chain amine group was protected to achieve **3aa(z)-R**. The high yield and good stereoselectivity were observed (Table 2, entry 7). The flexibility of free peptide induced higher enantioselectivity, in contrast with peptide attached to the resin (Table 2, entry 7 vs 5).

For subsequent reactions, catalyst **8aa** was used to optimize the experimental parameters of the condition, scope, limitations, and solvents. The optimized reaction conditions were screened for enantioselective aldol reactions between cyclohexanone (23.3 mg, 0.238 mmol, 1.2 eq) and *p*-nitrobenzaldehyde (30 mg, 0.198 mmol, 1 eq) in the presence of the octapeptide. TFA salt was chosen as an asymmetric catalyst (3.0 mol%). The iPrOH was applied as a solvent and NMM (*N*-methylmorpholine) was used to adjust pH = 5.0 - 5.5. In the presence of just iPrOH, no reaction occurred due to the insolubility of the catalyst. Various additives, such as water, amines, and carboxylic acids, increased the yield and ee of proline catalyzed aldol reactions. [41] The best mole ratio was the mixture of 0.6 ml water with 0.4 ml iPrOH. The reaction proceeded with high yield and enantioselectivity (97%).

Table 3. Aldol reaction of cyclohexanone with *p*-nitrobenzaldehyde catalyzed by **8aa** in different solvents

Entry	solvent	Time (h)	Yield (%)	ee (%)
1	$CHCl_3$	72	NR	-
2	Toluene	72	NR	-
3	THF	72	NR	-
4	DMF	24	94	86
5	DMSO	24	95	87
6	NMP	24	92	89
7	H_2O/DMF (3:1)	24	96	97
8	Brine/DMF (3:1)	24	91	95
9	1%SDS/iPrOH(3:2)	24	93	82

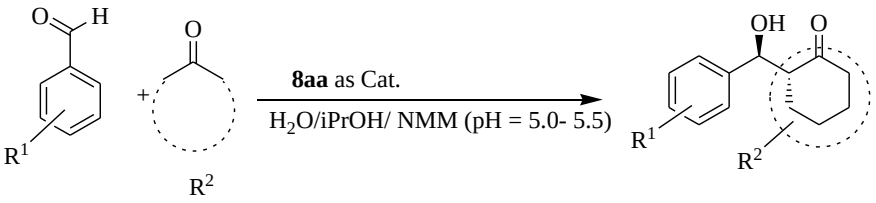
Reaction conditions: *p*-nitrobenzaldehydes (30 mg, 0.198 mmol, 1 eq) with cyclohexanone (23.3 mg, 0.238 mmol, 1.2 eq), catalyst (3 mol%), solvent, pH = 5.5, RT.

The effects of several solvents on the reaction with cyclohexanone and *p*-nitrobenzaldehyde were also investigated (Table 3). Due to the poor solubility of **8aa** in most solvents, reactions mediated by **8aa** are limited to polar organic solvents, such as water, dimethyl sulfoxide (DMSO), *N*,*N*-dimethylformamide (DMF) and *N*-methyl-2-pyrrolidone (NMP). No reaction occurred when toluene, chloroform, and THF were used (Table 3, entries 1-3). Additionally, high yield and enantioselectivity were obtained when DMF, DMSO, and NMP were utilized (Table 3, entries 4, 5, 6). While DMSO and DMF are commonly used for aldol reactions catalyzed by peptides, these solvents are generally considered problematic for large-scale reactions due to the inconvenient work-up and solvent removal and recovery. The mixture of water/DMF (3:1) produced the highest stereoselectivity (Table 3, entry 7). This result indicates that water (as an ecofriendly solvent) is requisite for achieving excellent diastereoselectivity and enantioselectivity. Similar results were achieved with brine (Table 3, entry 8).

The model reaction was also performed in SDS (1% w/v)/ iPrOH (3:2). As it is showed in Table 3, entry 9, this mixture is also a reasonable choice for a reaction which will have high yield and good enantioselectivity. The scope and limitations of a direct aldol reaction of cyclohexanone with various aromatic aldehydes, catalyzed by the **8aa**, were explored (Table 4). Benzaldehydes were substituted with *p*-nitro, *p*-trifluoromethyl, *p*-cyano, *p*-chloro, *o*-chloro and *o*-nitro as electron-withdrawing groups and *p*-methoxy as an electron-donating group. Electron-withdrawing groups on aromatic ring substrates tend to accelerate the reaction. This is illustrated by the high turnover (3 mol% of catalyst loading) and short reaction time (24 h; Table 4, entries 1-8 and 10). Electron-donating substituent resulted in high enantioselectivity with moderate yield (Table 4, entry 11). The aldol reaction of o-chlorobenzaldehyde and cyclohexanone produced 96:4 dr, 99.9% ee, and excellent yield (Table 4, entry 4). While benzaldehyde itself reacted with good yield and enantioselectivity (Table 4, entry 9). Pyridinecarboxyaldehyde is a good substrate, as well, affording high ee's (Table 4, entry 8). The aldol reactions of electron-deficient benzaldehydes with cyclohexanone proceeded smoothly to produce aldol adducts with excellent diastereoselectivities (99/1) and enantioselectivities (> 98%). It is also interesting to note that the both *para*- and *ortho*-substitutions resulted in high yields with good to excellent enantio- and diastereoselectivities.

To examine the generality of the current process, the aldol reactions between aromatic aldehydes and different ketones, including cyclic and acyclic ketones were tested (Table 4, entries 10, 12-16). The diastereo- and enantioselectivities are significantly influenced by the ketone structure. Acyclic ketones produced excellent yields and moderate to good stereoselectivities (Table 4, entries 12-16, up to 77% ee). When acetone was utilized, the enantioselectivity diminished notably, but reaction was faster and completed in only 15h. The lower % ee is due to the flexibility and free rotation of acetone. As illustrated by the transition state (Figure 2), the specific rigid conformation of cyclohexanone results in a remarkably higher enantio- and diastereoselectivity ratio compared to acyclic ketones. However, the difference in enantioselectivity ratio between p-nitrobenzaldehyde and benzaldehyde in reaction with cyclohexanone could be due to an electron-withdrawing group; however, it is important to note the key role of strong hydrogen bonding between carboxylic acid and nitro group, and carbonyl group with an imidazol group to produce higher ee.[42] It seems that the side chain amine group of lysine has a significant role in the transition state to enhance enantioselectivity. When the peptides 8aa(z) and 5aa were employed in the reaction between p-nitrobenzaldehyde and cyclohexanone as catalysts under defined conditions, the enantioselectivity reduced by 10% (Table 4, entries 3, 4).

Table 4. Direct aldol reaction catalyzed by **8aa** in aqueous medium



Entry	R^1	R^2	Time (h)	Yield (%)[a]	dr (%)[b]	ee (%)[c]
1	4-NO_2	cyclohexanone	24	97	90/10	97
2	2-NO_2	cyclohexanone	24	94	90/10	89
3	4-Cl	cyclohexanone	24	95	93/7	96
4	2-Cl	cyclohexanone	24	96	96/4	>98
5	4-CN	cyclohexanone	24	92	99/1	86
6	4-CF_3	cyclohexanone	24	94	98/2	86
7	4-Br	cyclohexanone	24	93	99/1	80
8	4-pyridinecaboxyaldehyde	cyclohexanone	24	95	99/1	98
9	H	cyclohexanone	24	94	98/2	87
10	4-NO_2	cycloheptanone	30	71	92/8	88
11	4-OMe	cyhexanone	48	65	99/1	95
12	4-NO_2	acetone	15	97	-	77
13	2-NO_2	acetone	15	96	-	59
14	4-Cl	acetone	15	96	-	52
15	4-CF_3	acetone	15	96	-	74
16	4-Br	acetone	15	96	-	73
17	4-NO_2 (no cat.)	cyclohexanone	24	-	-	-

Reaction conditions: *p*-nitrobenzaldehydes (30 mg, 0.198 mmol, 1 eq) with cyclohexanone or acetone (23.3 mg, 0.238 mmol, 1.2 eq or 1mL), catalyst (3 mol%), solvent (H_2O:iPrOH, 1:1 or H_2O:acetone, 1:1), pH = 5.5, RT. [a]Isolated yield. [b] dr values were determined by crude product ^{1}H NMR. [c]ee values were determined by HPLC using a Chiral OD-H and AD-H columns

The next stage of the investigation was the exploration of the reusability of **8aa** as asymmetric catalyst in aldol reaction. The catalyst can be easily separated through precipitation by adding diethyl ether, ethyl acetate or other low polar solvents. The reusability of the catalyst was evaluated through using cyclohexanone with *p*-nitrobenzaldehyde. The recovered **8aa**, could be reused ten times without an obvious loss of enantioselectivity and decreased activity (Table 5, entries 1–10). After the 10th cycle, the activity began to decrease, which could be attributed to the loss of catalyst during recycling.

Table 5. Investigated reusability of 8aa

Entry	Cat.	Time (h)	Yield (%)	ee (%)
1	Run 1	24	94	97
2	Run 2	24	94	96
3	Run 3	24	93	96
4	Run 4	24	92	94
5	Run 5	24	92	93
6	Run 6	24	93	93
7	Run 7	24	92	91
8	Run 8	24	90	87
9	Run 9	24	87	81
10	Run10	24	83	76

Reaction conditions: *p*-nitrobenzaldehydes (30 mg, 0.198 mmol, 1 eq) with cyclohexanone or Acetone (23.3 mg, 0.238 mmol), catalyst (3 mol%), solvent (H_2O:iPrOH, 1:1), pH = 5.5, RT. [a]Isolated yield. [b]ee values were determined by HPLC using a Chiral OD-H and AD-H columns

3. *Mechanism Study*

These finding suggest a plausible mechanism, similar to that proposed by the groups of List and Houk for proline catalysis, involving enamine formation, subsequent reaction with the ketone and proton transfer from the carboxylic acid.[43-44]

In conclusion, the multifunctionality properties of **8aa**, which is comprised of hydrophilic and hydrophobic amino acid residues, may cause to obtain very high yield and stereoselectivity. The proposed mechanism is presented in Figure 3.

The secondary structural study of **8aa**, showed the random structure. One of the configurations of 8aa is β-turn. Therefore, aldehyde can take place in the pocket of peptides. Based on experimental data, primary amine and carboxylic acid of the side chain of lysine and glutamic acid residues had impressive hydrogen bond interaction with nitro group of aldehyde on one side, and on the other side, hydrogen bond interaction between imidazole group of histidine and carbonyl group of aldehyde helped to keep substrate in a favoured position. Finally, the nucleophile will attack to the carbonyl group on the opposite side to produce a corresponding anti-Aldol product.

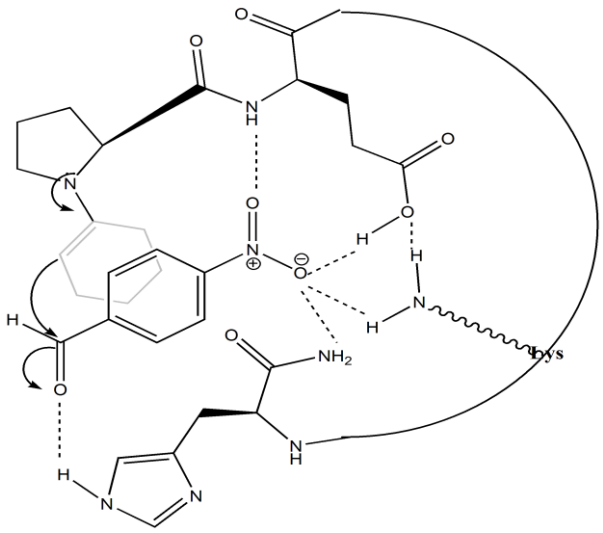
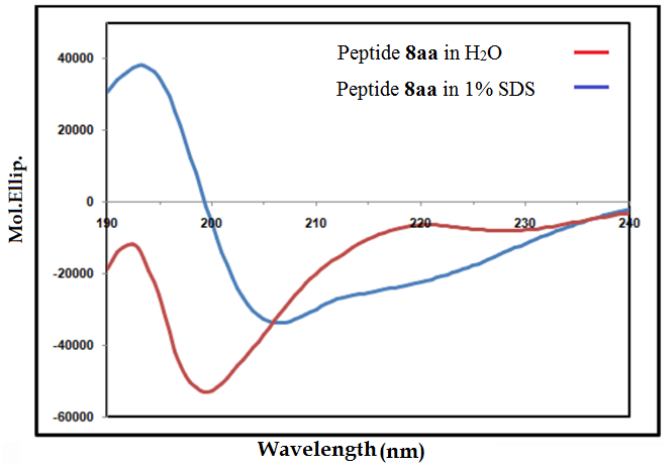


Figure 3. Proposed Aldol Reaction Mechanism Catalyzed by 8aa

3.1 Peptide structure studies

Circular dichroism (CD) spectropolarimetry and FT-IR spectroscopy were applied to find the secondary structure of 8aa. The results from computational modelling predicted by LOMETS (LOcal MEta-Threading-Server), indicated a random structure for the catalyst. The CD spectra were performed in two solvents, namely water and 1%SDS. As it can be seen in the CD spectra (Figure 4), a random peptide structure was revealed. Peptide was dissolved in water and the pH was acidic due to TFA residue. When SDS (1%) was used as solvent, two negative peaks at approximately 205 and 220 nm were observed, representing a α-helix structure for the peptide which indicated that the structure of the peptide could change. According to these results, both structures can be catalytically active. The CD data were analyzed by *www.perry.freeshell.org*, the free online program. The obtained results in water showed that the highest percentage of the peptide's structure was random (40%), followed by beta sheets (32%) and almost equal amounts of α-helix and β-turn structures (12%). In 1% SDS, the highest percentages of the structures were random followed by α-helix (34% and 31% respectively) with almost equal amounts of beta sheets (14%) and β-turns (12%) (Figure 4)



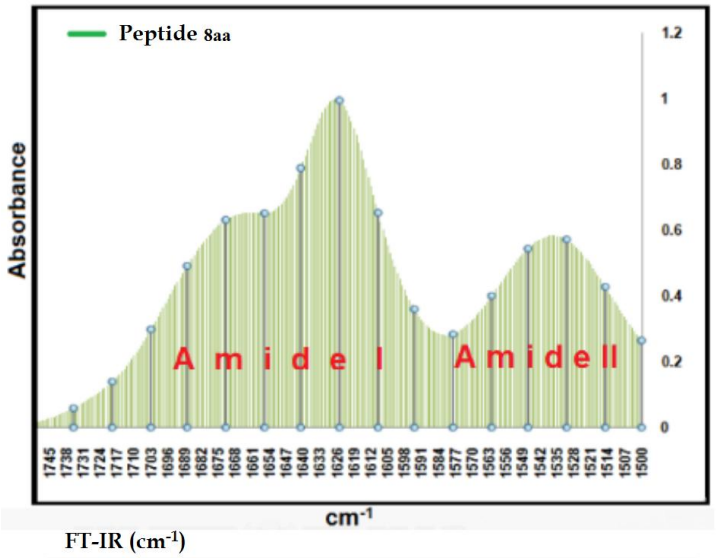
Peptide **8aa** in 1% SDS
Helix (%): 31.2
Beta sheet (%): 14.8
Turn (%): 12.5
Random (%): 34.6

Peptide **8aa** in H_2O
Helix (%): 13.0
Beta sheet (%): 32.1
Turn (%): 12.5
Random (%): 40.2

Figure 4. CD spectrum of peptide 8aa, in water and 1%SDS

In addition to CD, the secondary structure of 8aa was investigated via Infrared (IR) spectroscopy, as it is also one of the oldest and well-established techniques for the analysis of the secondary structures of polypeptides and proteins.[45-46] The amide I band between 1600 and 1700 cm^{-1} was the most intense absorbance band for all the investigated proteins and peptides, being mainly associated with the C=O stretching vibration and directly related to the backbone conformation. This band had a characteristic shape for each peptide investigated. Amide I and II (1500-1600 cm^{-1}) are the two major bands of the protein infrared spectrum and are conformationally sensitive. Amide II results mostly from the N—H bending vibration and from the C—N stretching vibration (18-40%).[47-48] Generally, the 1,655 cm^{-1} peak, which is assigned to the α-helix conformation, was positively correlated with bands at 1175, 1305, 2950 and 3330 cm^{-1}, as previously reported.[49] The band

observed at 1,668 cm^{-1} was assigned to β-turns. Antiparallel β-sheets (1,698 cm^{-1}) were not observed. 8aa had an intense absorption peak at 1624-1642 cm^{-1}, corresponding to its high content of β-sheet conformers, which is consistent with amide II band absorptions around 1,534 cm^{-1}.[50] The 1,662 cm^{-1} signal was assigned to β-turn conformers, which overlapped with β-sheet. FT-IR spectra of octapeptide displayed a large band in the range of 1,590 cm^{-1} to 1,700 cm^{-1}, with maximum absorption at 1,648±2 cm^{-1} (random coil overlap with β-sheet), and 1,668-62 cm^{-1} (β-sheet, β-turn, overlap with α -helix). The 1,656 cm^{-1} and 1,668 cm^{-1} bands correlated with important amide II bands at 1,541 cm^{-1},545 cm^{-1} and 1,528 cm^{-1} (Figure 5).

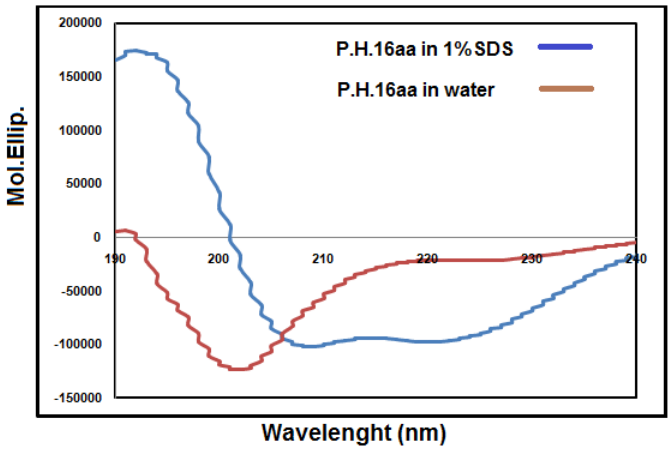


FT-IR (cm^{-1})

Wavenumber (cm^{-1})	Assignment	Peptide 8aa	Assignment
1624 ± 4.0	β-sheet	1624	β-sheet
1631 ± 3.0	β-sheet	1628	β-sheet
1637 ± 3.0	β-sheet	----	----
1641 ± 2.0	3_{10}-helix	----	----
1645 ± 4.0	Random	1648	Random
1653 ± 4.0	α-helix	1654	α-helix
1663 ± 4.0	β-turn	1666	β-turn
1671 ± 3.0	β-turn	1670	β-turn
1675 ± 5.0	β-sheet	1675	β-sheet
1683 ± 2.0	β-turn	1685	β-turn
1689 ± 2.0	β-turn	1689	β-turn
1694 ± 2.0	β-turn	1693	β-turn

Figure 5. Assignment of FT-IR spectrum of peptide 8aa

The FT-IR spectroscopy complements CD-spectroscopy according to the conformational studies of peptides and proteins, and its results strongly confirm the CD spectroscopy's results. The secondary structure of **PH16aa** in water indicated that the majority was structurally random (39%), followed by beta sheet (28%) and amounts of α-helix and β-turn structures, 15% and 12 %, respectively (Figure. 6). In 1% SDS, most of the structures were random and α-helix (31% and 38% respectively), with almost equal amounts of beta sheets (12%) and β-turns (12%). The CD spectra for **PE16aa** and **16aa** in both environments (water and SDS) are roughly similar to **PH16aa**. A CD spectrum for **PH16aa** is shown in Figure 6 and for **PE16aa** and **16aa** are in supplementary content.



PH16aa in 1% SDS

Helix	**(%):**	38.0
Beta sheet	**(%):**	12.3
Turn	**(%):**	12.5
Random	**(%):**	31.6

PH16aa in H_2O

Helix	**(%):**	15.2
Beta sheet	**(%):**	28.1
Turn	**(%):**	12.5
Random	**(%):**	39.7

Figure 6. CD spectrum of peptide PH.16aa, in water and 1%SDS

As can be seen from FT-IR spectrum related to **PH16aa**, the band observed at 1,654 cm^{-1} was assigned to α-helix. Antiparallel β-sheets (1,698 cm^{-1}) were not observed. **PH16aa** had an intense absorption at 1,624 cm^{-1}, corresponding to its high content of β-sheet conformers which is consistent with amide II band absorptions around 1,536 cm^{-1}. The 1,662 cm^{-1} signal was assigned to β-turn conformers which overlapped with α-helix (Figure 7).

FT-IR spectra of **PH16aa** displayed a large band in the range of 1,590 cm^{-1} to 1,700 cm^{-1}, with some maxima of absorption at 1,648±2 cm^{-1} (random coil overlap with β-sheet and α–helix), and 1,668-62 cm^{-1} (β-sheet, β-turn, overlap with α -helix).[51] The 1,654 cm^{-1} and 1,668 cm^{-1} bands correlate with important amide II bands at 1,541-1546 cm^{-1} (Figure 7). The FT-IR spectra of **16aa** and **PE16aa** confirmed that the structure of these oligopeptides are mostly α–helix and random. The results were almost identical to **PH16aa** (their spectra can be seen in the supplementary content). FT-IR spectroscopy complements CD-spectroscopy according to the conformational studies of peptides and proteins, and its results strongly confirm the CD spectroscopy's results.

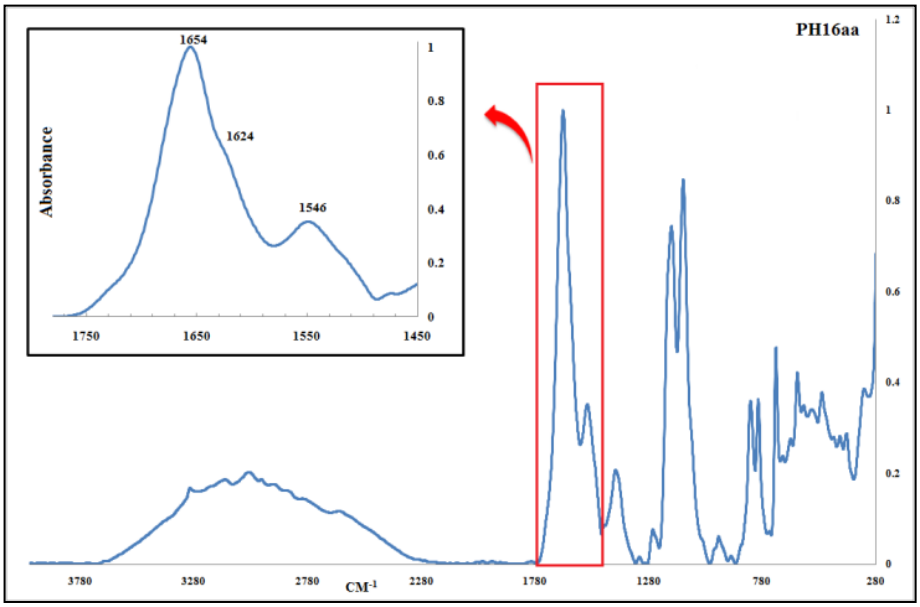


Figure 7. Secondary structure study of PH16aa using FT-IR technique

4. Experimental section

4.1 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 (FTIR), Perkin Elmer Spectrum 100, was used for identification of functional groups. NMR data were recorded on 500 MHz for 1HNMR and on 100 MHz for 13C NMR (JEOL JNM ECA) spectrometer. The relative and absolute configurations (dr) of the Aldol reactions were determined through doing the comparison using 1H NMR spectroscopic analysis. Mass spectra (MS) were measured with a spectrometer (DIMS QP5050A SHIMADZU). Enantioselectivity was determined using HPLC (Waters 1525 Binary Pump and UV-Water 2489) and Chiral OD-H or AD-H columns. CD spectra were measured by a JASCO J-810 automatic recording spectropolarimeter.

4.2 Catalyst Preparation

All peptides which were used in this study, were synthesized according to the Fmoc solid-phase strategy.[52] 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 double coupling cycles. At the end of the synthesis, the peptide was separated from the resin through using a mixture of trifluoroacetic acid (TFA, 92.5%), triisopropylsilane (2.5%), 1,4 dithiothreitol (2.5%) and water (2.5%). 10 ml of this solution was utilized with 1 g of resin and agitated for 2 hrs. The peptide was precipitated in dry diethylether and lyophilized. The purity of the peptides was measured by analytical Waters HPLC (Binary HPLC pump 1525 and UV-Waters 2489), (RPC18, Xbridge 4.6mm ×250mm).

4.3 CD Spectra

CD spectra were measured on a JASCO J-810 automatic recording spectropolarimeter calibrated with camphorsulfonic acid. Spectra were recorded over 190-240 nm using a 0.1cm path length quartz cuvette. The scan speed was 100 nm/min. Curves were digitally recorded and fed through a data processor for signal averaging (3 times) and baseline subtraction. The concentration of the samples was 0.0006 mol/L and the CD spectra was run at 20°C. The program which was retrived from *http://perry.freeshell.org/raussens* was used for secondary structure analysis.

4.4 General Procedure for Aldol Reactions

The corresponding catalyst (3 mol%), NMM (N-methylmorpholine) (1 drop, pH = 5.0 -5.5) and ^{i}PrOH (0.4 mL) were added to 0.6 mL of water. The reaction mixture was stirred for 20 min followed by the addition of the corresponding ketone (0.168 mmol, 1.2 eq). The requisite aldehyde (0.14 mmol, 1 eq) was added to the reaction mixture. The resulting mixture was stirred at room temperature for 24 h and then was treated with saturated aqueous ammonium chloride. The mixture was extracted with ethyl acetate (3×2mL). The combined organic extract was washed with brine, dried over Na_2SO_4, and concentrated in-vacuo. NMR analysis determined the diastereomeric ratio, and the residue was purified by flash column chromatography with hexane/ethyl acetate (3:1) to produce the aldol products that were subjected to chiral HPLC analysis to determine enantiomeric excesses.

5. Conclusion

Several new peptide catalysts which mimic based on promiscuous enzyme AKRs were designed and synthesized, and proved to be an excellent multifunctional organocatalyst for direct asymmetric aldol reactions. High enantioselectivities were observed for most of the aldol reactions. Direct aldol reactions between selected ketones and aromatic aldehydes indicated a very impressive selectivity over a range of direct aldol reactions. The present study offers the proof-of-principle and indicates that proline/glutamic acid and hystidine lysine as a hydrophilic residue and leucine, phenylalanine, valine as hydrophobic residues in **8aa** have significant potentials in catalysis. The carboxyl group of glutamic acid, lysine and histidine provided an obvious site for facile attachment of structural units, which can provide alternate hydrogen bonding arrays. The secondary structures of the peptides were studied using CD spectra and FT-IR. **8aa** has advantages to work as a powerful asymmetric organocatalyst in aqueous media and to be reusable.

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