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Rational design of mimetic peptides based on aldoketoreductase enzyme as asymmetric organocatalysts in aldol reactions†

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Peptides as a kind of important chiral scaffold are broadly identified for their obvious advantages, diverse structures and accessibility. Based on promiscuous aldoketoreductase 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 (>98%) under mild reaction selectivity and enantioselectivity. The secondary structures of peptide catalysts provide an understanding of their mechanism.

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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 have always suffered from the problem of limitation in a 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 Despite the emerging promiscuous enzymes for catalyzing different chemical transformations of natural or non-natural substrates, their low activity in terms of both yield and stereoselectivity impelled the chemists to design small molecules inspired from enzymes.⁵

List *et al.* demonstrated that L-proline itself could catalyze a 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 secondary amine like proline.^{20–22} In addition, Wennemers *et al.* employed tripeptides 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 evidence showed that in the active center of natural aldolases, a primary amino group of a lysine residue that lies in a hydrophobic pocket plays the main role in enamine intermediate construction. In this study, aldoketoreductase'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 the catalytic activity, stereoselectivity of the peptide, the design of a peptide to attain high yield of enantiomer excess was particularly considered. Thus, several mimetic peptides were designed based on the 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 a reaction medium is another attractive research subject, mainly due to the low cost, safety and the environmentally benign nature of water.³⁹ In the present study, the designing of the best structure based on an enzyme which can be employed in

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different kinds of asymmetric organic reactions, particularly in C–C bond-forming reactions, was considered. To fulfil this purpose, an 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, peptides may be an ideal compromise between small rigid organocatalysts and enzymes. Designing and synthesizing peptides capable of catalyzing a wide range of substrates asymmetrically, has still remained an immense challenge. The strategy of this study lies in the inspiration of nature's enzymes. According to similarities of the active site of AKRs to aldolase, a series of peptides were designed and synthesized based on AKRs (PDB = 1VBJ) active site, using a Fmoc-solid phase protocol.

The active site of AKRs contains several hydrophilic and hydrophobic amino acid residues which scaffold surround the ligand. As exhibited in Fig. 1, these residues have been distributed in different places and the locations are far from each other. It is very difficult to synthesise this polypeptide by using a 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 (Fig. 2).

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 peptide catalysts in aqueous medium (H₂O : iPrOH, 1 : 1, pH = 5.5). The 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

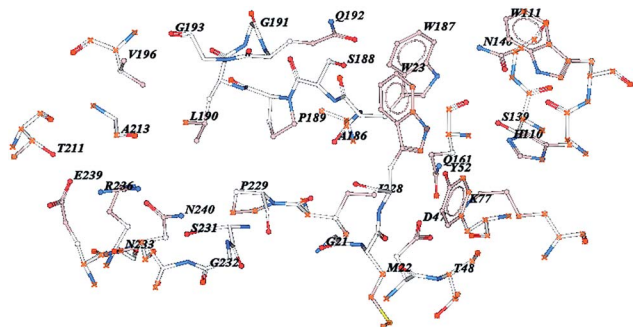


Fig. 1 All amino acid residues in AKR's active site.

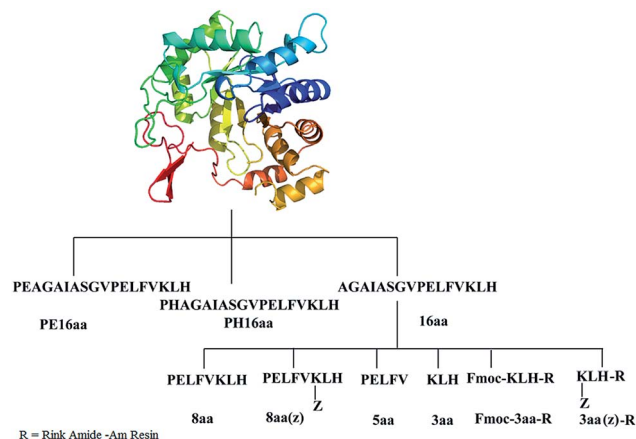
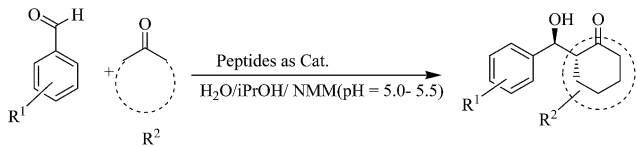


Fig. 2 A series of synthesized peptides mimicked from AKRs.

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 high yield (up to 89%) of the corresponding products, 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 enantioselectivity due to hydrogen bonding and the imidazole group is more effective than the carboxylic group.⁴⁰ Interestingly, when **PH16aa** was applied as a catalyst in the reaction between aromatic aldehydes and cyclohexanone, the 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 as 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 for creating strong hydrogen bonds with substrates. The aldol reaction between *p*-nitrobenzaldehyde and cyclohexanone produced moderate yield and poor enantioselectivity (Table 1, entry 1).

For further investigation of the peptide length efficacy on an asymmetry aldol reaction, **16aa** was cleaved to a shorter peptide to obtain a peptide with a secondary amine at the N-terminus

Table 1 Results of the reaction of various aldehydes with cyclohexanone catalyzed with **16aa**, **PE16aa** and **PH16aa**^a


Entry	R ¹	R ²	Cat.	Yield ^b (%)	dr ^c (%)	ee ^d (%)
1	4-NO ₂	Cyclohexanone	16aa	67	97/3	39
2	4-NO ₂	Cyclohexanone	PE16aa	88	99/1	63
3	2-NO ₂	Cyclohexanone	PE16aa	87	98/2	61
4	4-Cl	Cyclohexanone	PE16aa	89	96/4	62
5	4-CN	Cyclohexanone	PE16aa	87	97/3	67
6	4-CF ₃	Cyclohexanone	PE16aa	89	99/1	65
7	4-Br	Cyclohexanone	PE16aa	86	97/3	57
8	2-Cl	Cyclohexanone	PE16aa	84	99/1	68
9	4-NO ₂	Cyclohexanone	PH16aa	95	95/5	86
10	2-NO ₂	Cyclohexanone	PH16aa	94	99/1	81
11	4-Cl	Cyclohexanone	PH16aa	92	99/1	78
12	2-Cl	Cyclohexanone	PH16aa	95	99/1	71
13	4-CN	Cyclohexanone	PH16aa	88	99/1	81
14	4-CF ₃	Cyclohexanone	PH16aa	93	99/1	80
15	4-Br	Cyclohexanone	PH16aa	94	99/1	84
16	H	Cyclohexanone	PH16aa	92	99/1	76
17	4-NO ₂ (Solv. = iPrOH/1% SDS)	Cyclohexanone	PH16aa	94	99/1	81
18	4-NO ₂	Acetone	PH16aa	93	—	56
19	2-NO ₂	Acetone	PH16aa	92	—	45
20	4-Cl	Acetone	PH16aa	92	—	43
21	4-CF ₃	Acetone	PH16aa	94	—	39
22	4-Br	Acetone	PH16aa	94	—	35
23	4-NO ₂	Cyclohexanone	No cat.	—	—	—

^a Reaction conditions: aldehydes (30 mg, 0.198 mmol, 1 eq.) with cyclohexanone or acetone (23.3 mg, 0.238 mmol, 1.2 eq. or 1 mL), catalyst (3 mol%), solvent (H₂O : iPrOH, 1 : 1 or H₂O : acetone, 1 : 1), pH = 5.5, RT, 24 h. ^b Isolated yield. ^c dr values were determined by the crude product ¹H NMR. ^d ee values were determined by HPLC using a Chiral OD-H and AD-H columns.

(see the sequences in Fig. 2). The sixteen amino acid polypeptide (**16aa**) was shortened to eight amino acids (**PELFVKLH**, **8aa**) and was used as a 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 result achieved prompted the researchers to investigate the influence of shorter peptides derived from **8aa** on the catalytic activity of catalysts in an aldol reaction. Later, five new peptides were designed and synthesized, named **8aa-z**, **5aa**, **3aa**, **Fmoc-3aa-R**, and **3aa(z)-R** (Fig. 2). The theory behind these designs was as following:

8aa-z was designed with the purpose of knowing if the side chain of the primary amine of lysine does have any effect on the enhancement of stereoselectivity. In order to accomplish this aim, the primary amine was protected by benzylchloroformate (Fig. 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 the catalytic activity, and on the lack of hydrophilic residues such as lysine and histidine. Furthermore, in order to find to what extent the primary amino acid attached to the hydrophobic amino acid followed by a polar amino acid can influence stereoselectivity, the

tripeptide **3aa** was designed. In **Fmoc-3aa-R**, the Fmoc group protected the N-terminus of lysine while the primary amine side chain of lysine was free. This tripeptide was attached to a resin. The purpose was to identify the role of free amines on 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 the peptides **8aa**, **8aa-z**, **5aa**, **3aa**, **Fmoc-3aa-R** and **3aa(z)-R** as asymmetric catalysts in the defined conditions. Compared to the catalyst **8aa**, the yield and enantioselectivity remarkably diminished by almost 10% while the catalyst **8aa-z** was utilized as an asymmetry catalyst (Table 2, entry 3). From a mechanistic point of view, this examination showed that primary amine of lysine has an impressive synergy with other amino acid residues to accelerate both the yield and stereoselectivity (Table 2, entry 3). Likewise, the pentapeptide **5aa** was evaluated for a 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

Table 2 Aldol reaction catalyzed by fragmented peptides^a

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

^a 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 (H₂O : iPrOH, 1 : 1), pH = 5.5, RT.

was that it was envisaged the hydrophobic amino acid residues also play a key role in the enhancement of the yield and stereoselectivity due to steric hindrance. Based on a study by Kofoed *et al.*, Pro-Glu-NH₂ and Pro-Asp-NH₂ were ineffective both in terms of catalysis and enantioselectivity.¹⁶ Surprisingly, when the tripeptide **3aa** was employed as a catalyst for a direct aldol reaction between *p*-nitrobenzaldehyde and cyclohexanone, good yield and stereoselectivity were obtained (Table 2, entry 5) but still lower than **8aa**.

On the other hand, although **Fmoc-3aa-R** gave high yield of the corresponding aldol product, the enantioselectivity dropped to 3% (Table 2, entry 6). This demonstrates that in **3aa**, the N-terminus of the lysine formed enamine intermediate properly and the substrate made a hydrogen bonding with histidine. In **Fmoc-3aa-R**, the distance between the enamine intermediate and histidine is far enough to form a hydrogen-bonding interaction with the imidazole group. Therefore, the corresponding almost racemic aldol product was obtained. The Fmoc protecting group was then removed and the side chain amine group was protected to achieve **3aa(z)-R**. High yield and good stereoselectivity were observed (Table 2, entry 7). The flexibility of the free peptide induced higher enantioselectivity, in contrast to the peptide attached to the resin (Table 2, entry 7 vs. 5).

For subsequent reactions, the 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 the 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%).

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,

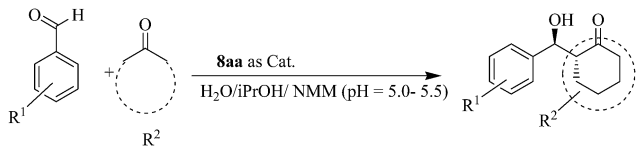
such as water, dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide (DMF) and *N*-methyl-2-pyrrolidone (NMP). No reaction occurred when toluene, chloroform, and THF were used (Table 3, entries 1–3). Additionally, high yield and enantioselectivity were obtained when DMF, DMSO, and NMP were utilized (Table 3, entries 4–6). 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 H₂O/DMF (3 : 1) produced the highest stereoselectivity (Table 3, entry 7). This result indicates that water (as an eco-friendly 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 shown 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 **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). The 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, >98% ee, and excellent yield (Table 4, entry 4), while benzaldehyde itself reacted with good yield and enantioselectivity (Table 4, entry 9). Pyridinecarboxaldehyde 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 both the *para*- and *ortho*-substitutions resulted in high yields with good to excellent enantio- and diastereoselectivities.

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

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 ₂ O/DMF (3 : 1)	24	96	97
8	Brine/DMF (3 : 1)	24	91	95
9	1% SDS/iPrOH(3 : 2)	24	93	82

^a 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.

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


Entry	R ¹	R ²	Time (h)	Yield ^b (%)	dr ^c (%)	ee ^d (%)
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-Pyridinecarboxyaldehyde	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	—	—	—

^a 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 1 mL), catalyst (3 mol%), solvent (H₂O : iPrOH, 1 : 1 or H₂O : acetone, 1 : 1), pH = 5.5, RT. ^b Isolated yield. ^c dr values were determined by crude product ¹H NMR. ^d ee values were determined by HPLC using a Chiral OD-H and AD-H columns.

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 the reaction was faster and completed in only 15 h. The lower ee% is due to the flexibility and free rotation of acetone. As illustrated by the transition state (Fig. 2), the specific rigid conformation of cyclohexanone results in a remarkably higher enantio- and diastereoselectivity ratio as compared to acyclic ketones. However, the difference in enantioselectivity ratio between *p*-nitrobenzaldehyde and benzaldehyde in the 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 a nitro group, and a 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 and 4).

The next stage of the investigation was the exploration of the reusability of **8aa** as asymmetric catalyst in an 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**^a

Entry	Cat.	Time (h)	Yield ^b (%)	ee ^c (%)
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	Run 10	24	83	76

^a 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 (H₂O : iPrOH, 1 : 1), pH = 5.5, RT. ^b Isolated yield. ^c ee values were determined by HPLC using a Chiral OD-H and AD-H columns.

3. Mechanism study

These findings suggest a plausible mechanism, similar to that proposed by the groups of List and Houk for proline catalysis, involving enamine formation, a 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 obtaining very high yield and stereoselectivity. The proposed mechanism is presented in Fig. 3.

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

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 can be seen in the CD spectra (Fig. 4), a random peptide structure was revealed. The peptide was dissolved in water and the pH was acidic due to the TFA residue. When SDS (1%) was used as a solvent, two negative peaks at approximately 205 and 220 nm were observed, representing a α -helix structure for the peptide, which indicated that

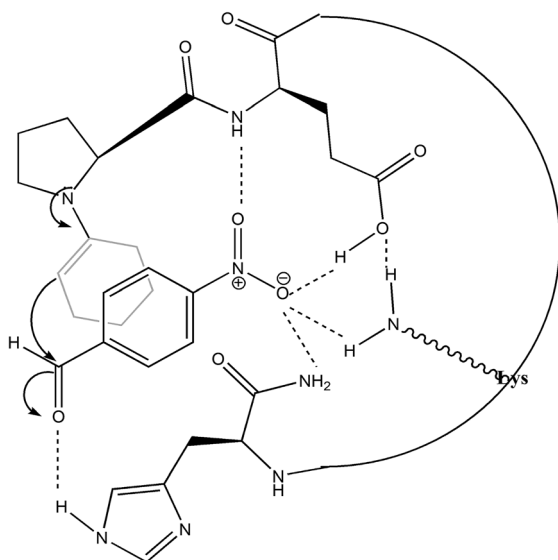


Fig. 3 Proposed aldol reaction mechanism catalyzed by **8aa**.

the structure of the peptide could change. According to these results, both structures can be catalytically active. The CD data were analyzed by <http://perry.freeshell.org/raussens.html>, the free online program. The obtained results in water showed that the highest percentage of the peptide's structure was random (40%), followed by β 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 β sheets (14%) and β -turns (12%) (Fig. 4).

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. Amides 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 1655 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 1668 cm^{-1} was assigned to β -turns. Antiparallel β -sheets (1698 cm^{-1}) were not observed. **8aa** had an intense

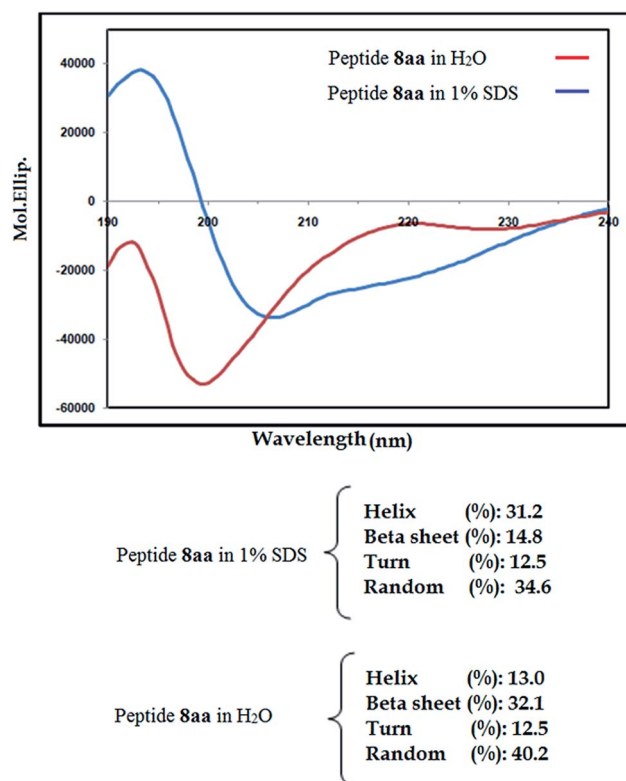


Fig. 4 CD spectrum of peptide **8aa**, in water and 1% SDS.

absorption peak at $1624\text{--}1642\text{ cm}^{-1}$, corresponding to its high content of β -sheet conformers, which is consistent with amide II band absorptions around 1534 cm^{-1} .⁵⁰ The 1662 cm^{-1} signal was assigned to β -turn conformers, which overlapped with a β -sheet. The FT-IR spectra of the octapeptide displayed a large band in the range of $1590\text{ to }1700\text{ cm}^{-1}$, with maximum absorption at $1648 \pm 2\text{ cm}^{-1}$ (random coil overlap with the β -sheet), and $1668 - 62\text{ cm}^{-1}$ (β -sheet, β -turn, overlap with α -helix). The 1656 and 1668 cm^{-1} bands correlated with important amide II bands at 1541 , 1545 and 1528 cm^{-1} (Fig. 5). 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 the beta sheet (28%) and the amounts of α -helix and β -turn structures, 15 and 12%, respectively (Fig. 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 Fig. 6 and those for **PE16aa** and **16aa** are in the ESI.[†]

As can be seen from the FT-IR spectrum related to **PH16aa**, the band observed at 1654 cm^{-1} was assigned to an α -helix. Antiparallel β -sheets (1698 cm^{-1}) were not observed. **PH16aa** had an intense absorption at 1624 cm^{-1} , corresponding to its high content of β -sheet conformers which is consistent with

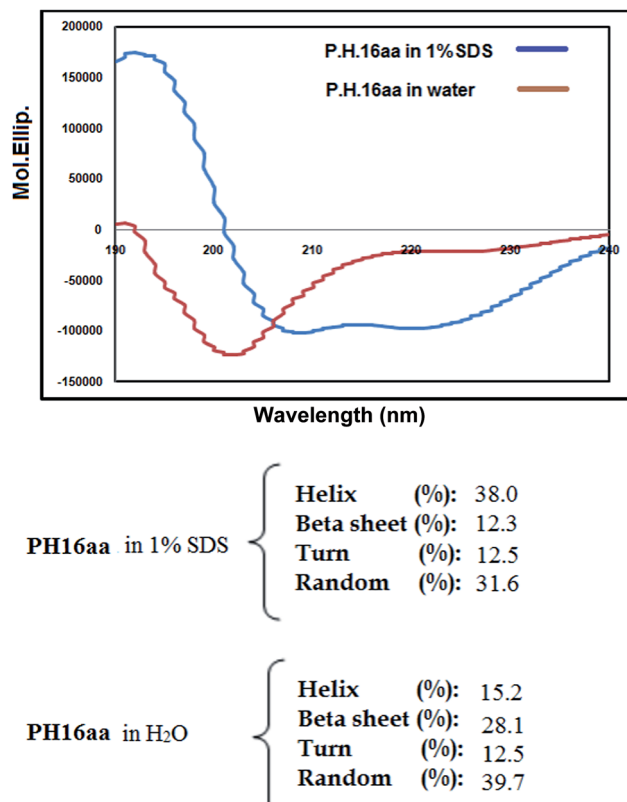


Fig. 6 CD spectrum of peptide PH16aa, in water and 1% SDS.

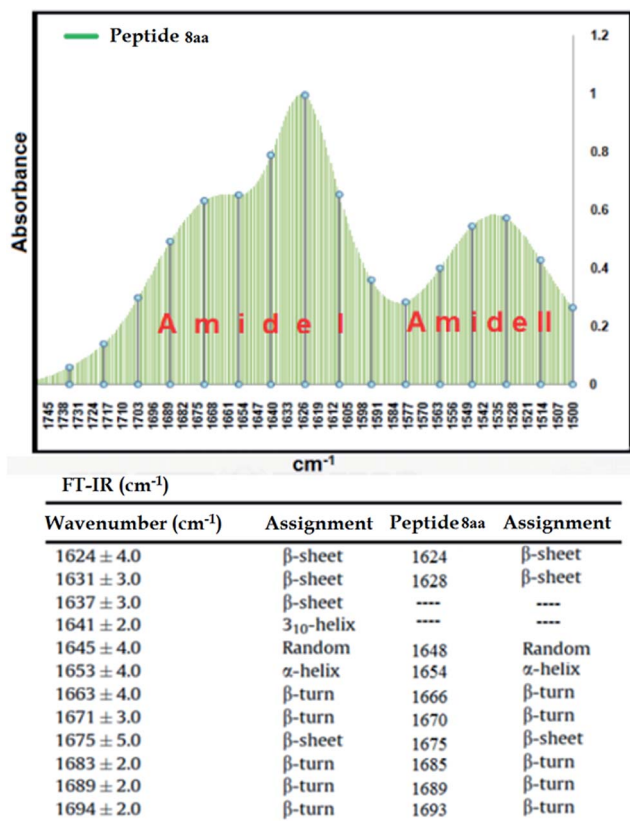


Fig. 5 Assignment of the FT-IR spectrum of peptide 8aa.

amide II band absorptions around 1536 cm^{-1} . The 1662 cm^{-1} signal was assigned to β -turn conformers which overlapped with the α -helix (Fig. 7). The FT-IR spectra of **PH16aa** displayed a large band in the range of $1590\text{ to }1700\text{ cm}^{-1}$, with some maxima of absorption at $1648 \pm 2\text{ cm}^{-1}$ (a random coil overlap with the β -sheet and α -helix), and $1668 - 62\text{ cm}^{-1}$ (β -sheet, β -turn, overlap with the α -helix).⁵¹ The 1654 and 1668 cm^{-1} bands correlate with important amide II bands at $1541\text{--}1546\text{ cm}^{-1}$ (Fig. 7). The FT-IR spectra of **16aa** and **PE16aa** confirmed that the structures of these oligopeptides are mostly α -helix and random. The results were almost identical to **PH16aa** (their spectra can be seen in the ESI[†]). 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.

4. Experimental

4.1 General information

All chemicals were purchased and used without further purification. Analytical thin layer chromatography (TLC) was performed using a Merck 60 F254 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 the functional groups. NMR data were recorded at 500 MHz for ^1H NMR and at 100 MHz for ^{13}C NMR (JEOL JNM ECA) spectrometer. The relative and absolute configurations

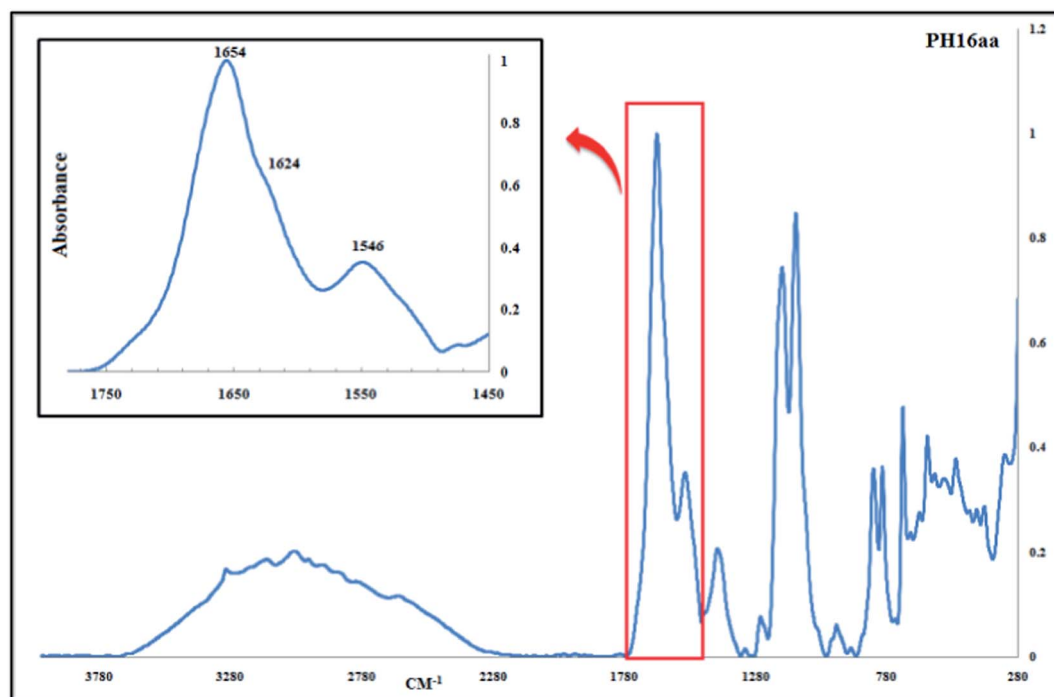


Fig. 7 Secondary structure study of PH16aa using the FT-IR technique.

(dr) of the Aldol reactions were determined through a comparison using ^1H NMR spectroscopic analysis. The mass spectra (MS) were measured with a spectrometer (DIMS QP5050A SHIMADZU). The enantioselectivity was determined using HPLC (Waters 1525 Binary Pump and UV-Water 2489) and Chiral OD-H or AD-H columns. The 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 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 h. 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 4.6 mm \times 250 mm).

4.3 CD spectra

The CD spectra were measured on a JASCO J-810 automatic recording spectropolarimeter calibrated with camphorsulfonic acid. The spectra were recorded over 190–240 nm using a 0.1 cm path length quartz cuvette. The scan speed was 100 nm min^{-1} . The 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^{-1} and the CD spectra were run at 20 $^\circ\text{C}$. The program which was retrieved from <http://perry.freeshell.org/raussens.html> was used for secondary structure analysis.

4.4 General procedure for aldol reactions

The corresponding catalysts (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 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 treated with saturated aqueous ammonium chloride. The mixture was extracted with ethyl acetate (3 \times 2 mL). 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. Conclusions

Several new peptide catalysts which mimic based on promiscuous enzyme AKRs were designed and synthesized, and proved to be excellent multifunctional organocatalysts 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 a proof-of-principle and indicates that proline/glutamic acid and histidine 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 the advantage of functioning as a powerful asymmetric organocatalyst in aqueous medium and of being reusable.

References

- 1 V. Bisai, A. Bisai and V. K. Singh, Enantioselective organocatalytic aldol reaction using small organic molecules, *Tetrahedron*, 2012, **68**(24), 4541–4580.
- 2 M. Nielsen, D. Worgull, T. Zweifel, B. Gschwend, S. Bertelsen and K. A. Jørgensen, Mechanisms in aminocatalysis, *Chem. Commun.*, 2011, **47**(2), 632–649.
- 3 M. M. Heravi and S. Asadi, Recent applications of organocatalysts in asymmetric aldol reactions, *Tetrahedron: Asymmetry*, 2012, **23**(20), 1431–1465.
- 4 R. Mahrwald and D. Evans, *Modern aldol reactions*, Wiley-VCH, Weinheim, vol. 2, 2004.
- 5 P. I. Dalko and L. Moisan, In the golden age of organocatalysis, *Angew. Chem.*, 2004, **43**(39), 5138–5175.
- 6 B. List, R. A. Lerner and C. F. Barbas, Proline-catalyzed direct asymmetric aldol reactions, *J. Am. Chem. Soc.*, 2000, **122**(10), 2395–2396.
- 7 Z. G. Hajos and D. R. Parrish, Asymmetric synthesis of bicyclic intermediates of natural product chemistry, *J. Org. Chem.*, 1974, **39**(12), 1615–1621.
- 8 H. Wennemers, Asymmetric catalysis with peptides, *Chem. Commun.*, 2011, **47**(44), 12036–12041.
- 9 H. Wennemers, Peptides as asymmetric catalysts and templates for the controlled formation of Ag nanoparticles, *J. Pept. Sci.*, 2012, **18**(7), 437–441.
- 10 S. B. Ötvös, I. M. Mándity and F. Fülöp, Asymmetric aldol reaction in a continuous-flow reactor catalyzed by a highly reusable heterogeneous peptide, *J. Catal.*, 2012, **295**, 179–185.
- 11 K. Akagawa and K. Kudo, Construction of an All-Carbon Quaternary Stereocenter by the Peptide-Catalyzed Asymmetric Michael Addition of Nitromethane to β -Disubstituted α , β -Unsaturated Aldehydes, *Angew. Chem., Int. Ed.*, 2012, **51**(51), 12786–12789.
- 12 K. Kudo, K. Akagawa and R. Suzuki, Effect of the Helical Tether of a Resin-Supported Peptide Catalyst for Friedel–Crafts-Type Alkylation in Water, *Adv. Synth. Catal.*, 2012, **354**(7), 1280–1286.
- 13 G. Szöllösi, A. Csámpai, C. Somlai, M. Fekete and M. Bartók, Unusual enantioselectivities in heterogeneous organocatalyzed reactions: Reversal of direction using proline di- versus tri-peptides in the aldol addition, *J. Mol. Catal. A: Chem.*, 2014, **382**(0), 86–92.
- 14 S. Bahmanyar, K. Houk, H. J. Martin and B. List, Quantum mechanical predictions of the stereoselectivities of proline-catalyzed asymmetric intermolecular aldol reactions, *J. Am. Chem. Soc.*, 2003, **125**(9), 2475–2479.
- 15 B. List, Proline-catalyzed asymmetric reactions, *Tetrahedron*, 2002, **58**(28), 5573–5590.
- 16 J. Kofoed, J. Nielsen and J.-L. Reymond, Discovery of new peptide-based catalysts for the direct asymmetric aldol reaction, *Bioorg. Med. Chem. Lett.*, 2003, **13**(15), 2445–2447.
- 17 W. Zou, I. Ibrahim, P. Dziedzic, H. Sundén and A. Córdova, Small peptides as modular catalysts for the direct asymmetric aldol reaction: ancient peptides with aldolase enzyme activity, *Chem. Commun.*, 2005, (39), 4946–4948.
- 18 W. Huang, H. Tian, H. Xu, L. Zheng, Q. Liu and S. Zhang, L-Valine dipeptide organocatalysts with two amide units for the direct asymmetric aldol reaction in brine, *Catal. Lett.*, 2011, **141**(6), 872–876.
- 19 P. Dziedzic, W. Zou, J. Háfren and A. Córdova, The small peptide-catalyzed direct asymmetric aldol reaction in water, *Org. Biomol. Chem.*, 2006, **4**(1), 38–40.
- 20 A. Córdova, W. Zou, P. Dziedzic, I. Ibrahim, E. Reyes and Y. Xu, Direct asymmetric intermolecular aldol reactions catalyzed by amino acids and small peptides, *Chem.-Eur. J.*, 2006, **12**(20), 5383–5397.
- 21 E. Bellis and G. Kokotos, Proline-modified poly(propyleneimine) dendrimers as catalysts for asymmetric aldol reactions, *J. Mol. Catal. A: Chem.*, 2005, **241**(1–2), 166–174.
- 22 J. Gao, J. Liu, D. Jiang, B. Xiao and Q. Yang, L-Prolinamide functionalized mesoporous silicas: Synthesis and catalytic performance in direct aldol reaction, *J. Mol. Catal. A: Chem.*, 2009, **313**(1–2), 79–87.
- 23 P. Krattiger, R. Kovasy, J. D. Revell, S. Ivan and H. Wennemers, Increased structural complexity leads to higher activity: peptides as efficient and versatile catalysts for asymmetric aldol reactions, *Org. Lett.*, 2005, **7**(6), 1101–1103.
- 24 J. D. Revell, D. Gantenbein, P. Krattiger and H. Wennemers, Solid-supported and pegylated H-Pro-Pro-Asp-NHR as catalysts for asymmetric aldol reactions, *Pept. Sci.*, 2006, **84**(1), 105–113.
- 25 J. D. Revell and H. Wennemers, Functional group requirements within the peptide H-Pro-Pro-Asp-NH₂ as a catalyst for aldol reactions, *Tetrahedron*, 2007, **63**(35), 8420–8424.
- 26 J. D. Revell and H. Wennemers, Peptidic catalysts developed by combinatorial screening methods, *Curr. Opin. Chem. Biol.*, 2007, **11**(3), 269–278.
- 27 E. A. Davie, S. M. Mennen, Y. Xu and S. J. Miller, Asymmetric catalysis mediated by synthetic peptides, *Chem. Rev.*, 2007, **107**(12), 5759–5812.
- 28 B. R. Linton, M. H. Reutershan, C. M. Aderman, E. A. Richardson, K. R. Brownell, C. W. Ashley, C. A. Evans and S. J. Miller, Asymmetric Michael addition of α -nitroketones using catalytic peptides, *Tetrahedron Lett.*, 2007, **48**(11), 1993–1997.
- 29 S. J. Miller, In search of peptide-based catalysts for asymmetric organic synthesis, *Acc. Chem. Res.*, 2004, **37**(8), 601–610.

- 30 F. Chen, S. Huang, H. Zhang, F. Liu and Y. Peng, Proline-based dipeptides with two amide units as organocatalyst for the asymmetric aldol reaction of cyclohexanone with aldehydes, *Tetrahedron*, 2008, **64**(40), 9585–9591.
- 31 C. Andreu and G. Asensio, Effect of addition of Lewis/Brönsted acids in the asymmetric aldol condensation catalyzed by trifluoroacetate salts of proline-based dipeptides, *Tetrahedron*, 2012, **68**, 7966–7972.
- 32 D. Zhao and K. Ding, Recent Advances in Asymmetric Catalysis in Flow, *ACS Catal.*, 2013, **3**(5), 928–944.
- 33 M. Wiesner, J. D. Revell and H. Wennemers, Tripeptides as Efficient Asymmetric Catalysts for 1,4-Addition Reactions of Aldehydes to Nitroolefins—A Rational Approach, *Angew. Chem., Int. Ed.*, 2008, **47**(10), 1871–1874.
- 34 X. Y. Gong, D. Dobrunz, M. Kümin, M. Wiesner, J. D. Revell, H. Wennemers and P. C. Hauser, Separating stereoisomers of di-, tri-, and tetrapeptides using capillary electrophoresis with contactless conductivity detection, *J. Sep. Sci.*, 2008, **31**(3), 565–573.
- 35 S. Bayat, B. A. Tejo, A. B. Salleh, E. Abdmalek, Y. M. Normi and M. B. A. Rahman, Various Polar Tripeptides as Asymmetric Organocatalyst in Direct Aldol Reactions in Aqueous Media, *Chirality*, 2013, **25**(11), 726–734.
- 36 S. S. Hoog, J. E. Pawlowski, P. M. Alzari, T. M. Penning and M. Lewis, Three-dimensional structure of rat liver 3 α -hydroxysteroid/dihydrodiol dehydrogenase: a member of the aldo-keto reductase superfamily, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**(7), 2517–2521.
- 37 J. Jez, M. Bennett, B. Schlegel, M. Lewis and T. Penning, Comparative anatomy of the aldo-keto reductase superfamily, *Biochem. J.*, 1997, **326**, 625–636.
- 38 S. D. Copley, Enzymes with extra talents: moonlighting functions and catalytic promiscuity, *Curr. Opin. Chem. Biol.*, 2003, **7**(2), 265–272.
- 39 C.-J. Li and L. Chen, Organic chemistry in water, *Chem. Soc. Rev.*, 2006, **35**(1), 68–82.
- 40 C. Allemann, J. M. Um and K. N. Houk, Computational investigations of the stereoselectivities of proline-related catalysts for aldol reactions, *J. Mol. Catal. A: Chem.*, 2010, **324**(1–2), 31–38.
- 41 X. Q. Yu, Z. B. Xie, N. Wang and G. F. Jiang, Biocatalytic asymmetric aldol reaction in buffer solution, *Tetrahedron Lett.*, 2013, **54**(8), 945–948.
- 42 N. K. Rana, R. Unhale and V. K. Singh, Enantioselective sulfa-Michael addition of thioacids to α,β -unsaturated ketones with bifunctional organocatalyst, *Tetrahedron Lett.*, 2012, **53**(16), 2121–2124.
- 43 J. G. Hernández and E. Juaristi, Efficient ball-mill procedure in the ‘green’ asymmetric aldol reaction organocatalyzed by-proline-containing dipeptides in the presence of water, *Tetrahedron*, 2011, **67**(36), 6953–6959.
- 44 K. N. Houk and B. List, Asymmetric organocatalysis, *Acc. Chem. Res.*, 2004, **37**(8), 487.
- 45 E. Saguer, P. Alvarez and A. A. Ismail, Heat-induced denaturation/aggregation of porcine plasma and its fractions studied by FTIR spectroscopy, *Food Hydrocolloids*, 2012, **27**(1), 208–219.
- 46 E. Goormaghtigh, J.-M. Ruyschaert and V. Raussens, Evaluation of the information content in infrared spectra for protein secondary structure determination, *Biophys. J.*, 2006, **90**(8), 2946–2957.
- 47 J. Alsina, C. Chiva, M. Ortiz, F. Rabanal, E. Giralt and F. Albericio, Active carbonate resins for solid-phase synthesis through the anchoring of a hydroxyl function. Synthesis of cyclic and alcohol peptides, *Tetrahedron Lett.*, 1997, **38**(5), 883–886.
- 48 F. Albericio, Developments in peptide and amide synthesis, *Curr. Opin. Chem. Biol.*, 2004, **8**(3), 211–221.
- 49 P. I. Haris and D. Chapman, The conformational analysis of peptides using Fourier transform IR spectroscopy, *Biopolymers*, 1995, **37**(4), 251–263.
- 50 W. K. Surewicz, H. H. Mantsch and D. Chapman, Determination of protein secondary structure by Fourier transform infrared spectroscopy: a critical assessment, *Biochemistry*, 1993, **32**(2), 389–394.
- 51 J. Kong and S. Yu, Fourier transform infrared spectroscopic analysis of protein secondary structures, *Acta Biochim. Biophys. Sin.*, 2007, **39**(8), 549–559.
- 52 R. B. Merrifield, Solid phase peptide synthesis. I. The synthesis of a tetrapeptide, *J. Am. Chem. Soc.*, 1963, **85**(14), 2149–2154.