A facile synthesis of cysteine-based diketopiperazine from thiol-protected precursor

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1. Introduction

The use of biomass-based feedstock to replace fossil fuel-based chemicals is a current main tendency of research and commercial activities [1]. L-Cysteine with abundant resource, is one of the renewable and desirable alternatives. It is readily available on a commercial scale and conventionally derived from hydrolysis of poultry feathers or human hair. It is also available now by large-scale microbial fermentation [2]. As a unique amino acid, L-cysteine possessing a highly reactive thiol moiety, not only has the property and reactivity of the basic amino acid, but also can undergo the famous thiol-ene click reaction under a mild condition with high efficiency and orthogonality [3]. Moreover, thiol and amino acid groups readily form the chelating complexes with silver, lead, copper and other metal ions [4]. L-Cysteine is therefore a versatile naturally-occurring material with a great potential for use as a building block in the synthesis of multifunctional compounds.

L-Cysteine is one of the most promising biomass-based building blocks with great potential applications. Herein, we report a versatile synthetic route to produce cysteine-based 2,5-diketopiperazine (DKP) with good yield from the thiol-ene click reaction of L-cysteine and commercially available acrylates, followed by dimerization of the amino acid intermediates. The achieved DKP diastereomers were successfully separated and fully characterized by spectroscopic methods. Moreover, the chiroptical property of DKP in the presence of various metal ions was investigated by circular dichroism spectroscopy. The potential application of the optically active cysteine-based DKP as a chiral probe for detection of silver ion in water has been demonstrated.
2,5-Diketopiperazines (DKPs) are derived directly from amino acids and are also a class of naturally occurring compounds existing in a variety of foods and organisms [5]. They are structurally more rigid than linear amino acids, which can lead to the DKP-containing materials with much improved properties [6]. Moreover, owing to their biodegradability and potential biocompatibility, DKPs have been successfully employed in the preparation of green polymers and in the pharmacological industry for drug delivery and other medical purposes [5–8]. Accordingly, it is highly desirable to develop multifunctional compounds derived from cysteine-based DKP. Most DKPs are formed directly through self-condensation of amino acids or esters by heating [9]. However, the over-reactivity of the thiol group in cysteine makes its DKP synthesis time-consuming and low yield, and limited the research on cysteine-based DKP [10]. Thus, the development of cysteine-based DKP is urgent and of great significance.

Herein, we report the synthesis and chiroptical property of cysteine-based DKPs. Since survival of the thiol group is difficult during the DKP formation, we envision a reverse sequence, in which the thiol group in cysteine is protected first. Thus, the synthetic route involves a simple two-step process; a thiol-ene click reaction with commercially available acrylates and subsequent dimerization of the thiol-protected precursor. The cysteine-based DKPs are optically active and its chiroptical properties are affected specifically by silver ions.

2. Results and discussion

2.1. Synthesis and characterization of cysteine-based 2,5-diketopiperazines

To avoid the interference of the thiol group during the dimerization process, a thiol-ene click reaction, which is famous for its high efficiency and mild conditions, was first applied to modify the property. A thiol-acrylate Michael addition reaction was reported to proceed in water with almost 100% yield [11]. Thus, the reaction of L-cysteine with acrylates was selected to be carried out in water at ambient temperature for 3h until a large amount of white solids precipitated out. The S-alkylated cysteine derivatives 1a–1e were then obtained with nearly quantitative yield (scheme 1).

The structures of compounds 1a–1e were fully characterized by spectroscopic means (electronic supplementary material) [12–13]. Take compound 1a as an example, in the 1H nuclear magnetic resonance (NMR) spectra, the α-proton of amino acid was shifted to the lower field at 3.85 ppm in comparison with L-cysteine. The peaks for the two carbonyl’s carbons appear at 175.06 ppm and 172.71 ppm in the 13C NMR spectra, further confirming the successful synthesis of S-alkylated cysteine derivative 1a. In addition, molecular ion peaks in mass spectra (MS) is consistent with its structure. Besides, compounds 1a–1e are chiral and optically active, as characterized by circular dichroism (CD) spectroscopy. The CD spectra show a peak at 225 nm with a positive Cotton effect (electronic supplementary material).

The synthesis of DKP 2a–2e involves the dimerization of compound 1a–1e through intermolecular amide formation and intramolecular lactamization (scheme 2). Countless experiments were carried out to identify the feasible reaction condition including reaction catalysts, solvents and temperatures. First, a series of known coupling reagents for amide bond formation were tested in our work, such as dicyclohexylcarbodiimide, N,N'-diisopropylcarbodiimide, and O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate [14]. However, these peptide catalysts lack activity for intermolecular cyclization and only afforded linear dipeptide. By contrast, P2O5 has been reported as an effective coupling reagent in several DKP formations and could not only speed up the reaction rate, but also improve the yields significantly [15,16]. Therefore, P2O5 is an ideal catalyst with features including cost-effective, environmental-friendly and can easily be removed by water washing was used in our work. In terms of the reaction solvent, several factors were taken into account including the solvent boiling points, polarity and inherent natures [17]. Most of the common solvents available in the laboratory were carefully examined. Finally, 1,2-dichlorobenzene which is a polar, protophilic solvent with high-boiling point, was proven to be the only applicable solvent for the cyclization reaction. Accordingly, the dimerization reaction was finally carried out in 1,2-dichlorobenzene in the presence of P2O5 as a catalyst and dehydrating reagent. The reaction was first test run at different temperatures from 40 to 120°C. It was found that the reaction did not go to completion when the temperature was lower than 80°C and at high temperatures the starting materials and final products tend to decompose. Thus, the reaction was best carried out at 80–110°C.
2.2. Stereochemistry of cysteine-based 2,5-diketopiperazine

The course of the dimerization reaction could be monitored by the changes of the α-proton from amino acid in compound 1 at 3.8 ppm in $^1$H NMR spectra. Take compound 1a as an example, after 1 h of dimerization, a new peak appeared at 4.15 ppm along with the peak at 3.8 ppm (spectrum (b) in figure 1), indicating a low degree of conversion at the early stage of reaction (around 1 h). The peak at 4.25 ppm gradually increases as the reaction proceeds (spectrum (c) in figure 1). After 6 h, the $^1$H NMR spectrum shows the presence of two intense peaks at 4.25 and 4.15 ppm and absence of the α-proton resonance at 3.8 ppm (spectrum (d) in figure 1).

It is known that the dimerization of amino acid leads to the formation and isomerization of the cis/trans-DKP isomers, where the cis-DKP is $C_2$-symmetric and the trans-isomer is centre-symmetric (scheme 2) [9]. It is assumed that the formation of diastereomers results from the epimerization of the chiral α-proton at high temperature. Thus, at the beginning of the reaction, the formation of LL-isomer predominated because of the presence of a large amount of l-stereo isomeric precursor. As the dimerization reaction proceeds, isomerization between the cis-isomer and the trans-isomer also takes place, leading to the formation of two diastereomers or LL-/DD- and LD-DKP. Therefore, the peak at 4.25 ppm can be assigned to trans-DKP 2a and the one at 4.15 ppm is from the cis-isomer (figure 1). To confirm our hypothesis, research on synthetic DKP from various amino acids were investigated and it is found that little racemization took place in the beginning of the dimerization where only cis-isomer presented, and as time went by, the ratio of cis/trans gradually decreased [9,18]. Moreover, the α-proton of trans-DKP is normally located at the lower field region than that of cis-DKP in the $^1$H NMR spectra in different literature, which is same as the results of cysteine DKPs and the distinction was attributed to the conformational difference of the cyclic and intramolecular hydrogen bonding [19–21]. Therefore, the assignments of peak at 4.25 ppm to trans-DKP 2a and the one at 4.15 ppm to cis-isomer are reasonable.

It was reported that at the higher temperatures the dimerization is faster and more racemization takes place [18]. The same observations were taken in the preparations of cysteine-based DKPs. It was found that there were three possible factors that can influence the diastereomeric ratios, namely temperature, time and catalyst. Therefore, for a given reaction time of 6 h, the effect of $P_2O_5$ catalyst and reaction temperature on the compositions of cis/trans isomers were investigated for the dimerization of compound 1a. The ratios of cis and trans isomers were determined by the $^1$H NMR method and listed in table 1. It was clear that the temperature and catalyst concentration had a significant impact on the reaction outcomes. When there was an increase in the temperature or catalyst concentration, the trans-isomer was formed more than the cis-isomer or the LD-diastereomeric DKP was a major product.
Figure 1. $^1$H NMR spectra (300 MHz, DMSO-d$_6$) showing the progress of dimerization reaction of compound 1a to form DKP 2a at different reaction times.

Table 1. Influence of temperature and P$_2$O$_5$ catalyst on the formation of cis/trans isomers of DKP 2a.

| run | time (h) | catalyst (%) | temperature (°C) | cis : trans ratio |
|-----|----------|--------------|------------------|------------------|
| 1   | 6        | 10           | 80               | 1 : 0.8          |
| 2   | 6        | 20           | 80               | 1 : 1.2          |
| 3   | 6        | 10           | 120              | 1 : 1.5          |

It is known that diastereomers have different physical and chemical properties, and thus are worthy of separation. Chromatography is the most popular method of separating DKP diastereomers [22,23]. Because the DKPs from 2b to 2e are more soluble in organic solvents with the increase of the chain lengths, these compounds could be separated by column chromatography. But interestingly, the diastereomeric isomer of DKP 2a was able to be separated by selective precipitation in methanol. Owing to the different conformations of the rigid DKP rings and hydrogen bond interactions, the dominant isomer was prone to aggregate in methanol and precipitate afterwards. Thus, the desired pure isomer of DKP 2 could be easily obtained through controlling the ratio of the diastereomers formed during the dimerization process, followed by a simple filtration work-up. For example, when the reaction stopped after 1 h, the cis-isomer of DKP 2a was the main product. Since it could self-aggregate and precipitate from methanol, the pure cis-isomer was successfully isolated by simple filtration. Likewise, when the trans-isomer became the major component, the product collected from methanol precipitation would be pure trans-isomer. Moreover, it was found that the isomers of compound 2a had obvious differences in physical properties. For example, the cis-isomer has a melting point at 220°C, which is slightly higher than its trans-counterpart. In addition, the solubility of trans 2a in dichloromethane is better than its cis-counterpart, which was attributed to the fact that the cis-isomer has greater tendency in self-assembling through intermolecular hydrogen bonds (N–H–O) between adjacent molecules.

The diastereomer structures of DKP 2 were fully characterized by spectroscopic means (electronic supplementary material). Take the $^1$H of DKP 2a as an example (figure 2). A clear difference was observed...
Figure 2. $^1$H NMR (300 MHz, dimethylsulfoxide-$d_6$) spectra of (a) cis-2a and (b) trans-2a.

from the signals of CH$_2$ in amino acid, where the peaks of the cis-isomer was more narrow, which was attributed to the conformational differences of the cyclic rings and intramolecular hydrogen bonds. Therefore, to distinguish the diastereomers of cysteine-based DKP, one could simply compare the specific data in $^1$H NMR spectra.

Studies on DKPs having aliphatic side chains revealed that the information on isomer structures could be obtained from the $^3$J coupling constants of H-α-C-β-C-H. In NMR analysis, cis-DKP normally has a larger coupling constant compared to the trans-isomer [24]. Thus, the $^3$J coupling constants of H-α-C-β-C-H are 4.8 or 4.2 Hz for the cis-isomer and 4.2 or 3.3 Hz for the trans-isomer of DKP 2a, is also consistent with previous analysis. Moreover, the results from heteronuclear single quantum coherence, correlation spectroscopy NMR experiments draw the same conclusion on the assignment of cis- and trans-isomers.

It has been reported that the DKP ring could exist in a flat or a slightly puckered boat conformation. Furthermore, the cis-isomer normally exists in twist-boat conformation, while the trans-isomer prefers a planar ring [25–27]. Since DKP conformations can aid the structure assignments, nuclear overhauser effect spectroscopy and nuclear overhauser effect (NOE) measurements were applied to identify the conformations. It can be seen that a 16.5% NOE on NH was observed for the cis-isomer and 9.1% NOE...
was registered for the trans-isomer (electronic supplementary material). The observations are accordance with the speculation of DKP conformations and further confirms the stereochemistry distribution.

2.3. Chiroptical property of cysteine-based 2,5-diketopiperazine

L-Cysteine contains a chiral carbon and is capable of producing chiral derivatives. Many L-cysteine derivatives are known to have chiroptical activity for metal sensing application [28,29]. Therefore, the chiroptical property of the DKP-containing compound 2a was measured by CD spectroscopy. First, it was found that compound 1a is chiral and optically active, as characterized by CD spectroscopy (figure 3a). The CD spectra of compound 1a shows a peak at 225 nm with a positive Cotton effect. This result is consistent with the point that there is no racemization detected during the synthesis of S-alkylated compound [30]. Then, CD measurements on DKP diastereomers were performed. It is known that the C2-symmetric cis-DKP is optically active and centre-symmetric trans isomer is not optically active (scheme 2). When the dimerization reaction of compound 1a was terminated at the early stage where the cis-isomer was mainly produced, the isolated cis-isomer of DKP 2a was optically active with a specific optical rotation of $-88^\circ$ (C = 0.05, CH$_3$OH). Its CD spectrum displays a negative Cotton effect at 220 nm (figure 3b), which can be assigned to the n-$\pi^*$ transition of the amide group with reference to other known cyclic amino acids [22]. As expected, the trans isomer of DKP 2a shows no optical activity (figure 3b).

However, when the dimerization reaction went to nearly completion, the final products became totally optical inactive, indicating the complete racemization of each chiral isomer.

Cysteine-based DKP contain the amine, carbonyl and sulfide groups that are able to bind some metal ions [31]. If the metal binding is selective or specific and leads to a significant change in chiroptical property, the optically active cysteine-based DKP is potentially useful for chiroptical sensor of certain metal ions. The binding behaviour of the isolated optically active cis isomer of 2a with different metal ions was studied by CD measurement. Solutions of various metal ions (Zn$^{2+}$, Cu$^{+}$, Pd$^{2+}$, Ni$^{2+}$, Mg$^{2+}$, Ca$^{2+}$, Ba$^{2+}$, Co$^{2+}$, Li$^+$, Mn$^{2+}$, Pb$^{2+}$, Na$^+$ and Ag$^+$) were added and CD spectra were taken immediately afterwards. It was found that for the un-chelated ions, for example Pt$^{2+}$, there was no signal changing observed. For most of the chelated ions, chelation induced only slightly shift or decrease in CD signals, for example, Cu$^{2+}$. When Cu$^{2+}$ was added, the CD band shifted from 217 nm to 233.7 nm accompanying a significant decrease (electronic supplementary material). But the chelation with silver ions induced a significant change in CD spectra (figure 4a). As shown, upon addition of silver ions ($1 \times 10^{-5}$ M), a new CD band with an opposite sign appeared at 230 nm. In addition, by changing the molar ratios of Ag$^+$ to the optically active cis-isomer of 2a, the maximal binding ratio of 1 : 2 was found (figure 4b).

Sensing and monitoring silver ions becomes important as silver pollution in the environment increasingly threatens a broad range of microorganisms and marine invertebrates. Various chemosensors have been developed for Ag$^+$ detection in aqua systems which are normally based on organic fluorophores and semiconductor quantum dots. However, these chemosensors have some drawbacks such as hard preparative, low water solubility, and/or poor selectivity toward Ag$^+$ [32]. Thus, water-soluble, optically active cysteine-based DKP could be used as a sensitive CD probe for detection of silver ions in water with a concentration as low as $1 \times 10^{-5}$ M. In addition, since the cysteine-based
We have submitted an electronic supplementary document that includes all the data supporting the Data accessibility.

isolated enantiomers of DKP compounds has the potential to be used for sensing silver ions in aqua achieved successfully. Moreover, the chiroptical activity of the diastereoisomers combined with the over-reactivity of the thiol group. A series of DKP containing small molecules were successfully derivatives, were demonstrated, where a thiol-acrylae Michael addition reaction was used to suppress the over-reactivity of the thiol group. A series of DKP containing small molecules were successfully synthesized with the existence of diastereoisomers. The separations of DKP diastereoisomers were achieved successfully. Moreover, the chiroptical activity of the diastereoisomers combined with the isolated enantiomers of DKP compounds has the potential to be used for sensing silver ions in aqua systems.

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Authors’ contributions. D.Z. carried out the laboratory work, data analysis and drafted the manuscript.

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3. Conclusion

DKP compounds are potentially biodegradable, the DKP could also function as an environmental-friendly sensor.

Figure 4. CD spectra of (a) cis-2a with Ag$^{+}$; (b) cis-2a with various molar ratios of Ag$^{+}$.

3. Conclusion

Facile synthesis of cysteine-based DKP from thiol-protected precursors, mainly s-alkylated cysteine-derivatives, were demonstrated, where a thiol-acrylae Michael addition reaction was used to suppress the over-reactivity of the thiol group. A series of DKP containing small molecules were successfully synthesized with the existence of diastereoisomers. The separations of DKP diastereoisomers were achieved successfully. Moreover, the chiroptical activity of the diastereoisomers combined with the isolated enantiomers of DKP compounds has the potential to be used for sensing silver ions in aqua systems.

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