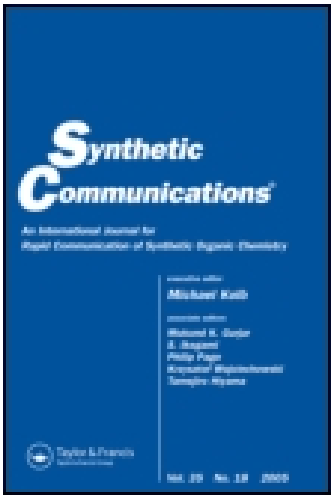

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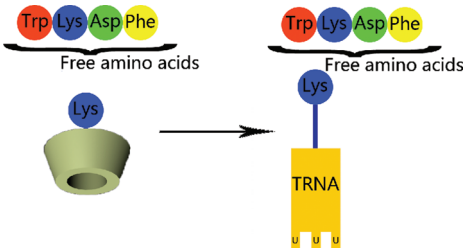
GRAPHICAL ABSTRACT



Abstract *A new method was developed to construct a special amido bond in the presence of β-cyclodextrin. This process is similar to peptide synthesis in organisms. NMR experiments were performed to investigate the possible mechanism. This work has potential application in biomimetic peptide synthesis.*

[Supplementary materials are available for this article. Go to the publisher's online edition of Synthetic Communications® *for the following free supplemental resource(s): Full experimental and spectral details.]*

Keywords Amino acids; cyclodextrin; tRNA

INTRODUCTION

Peptides, which are short polymers of amino acid monomers linked by peptide bonds, have recently gained prominence in molecular biology and drug synthesis for several reasons. First, peptides are involved in many diseases such as human immunodeficiency virus-1 (HIV-1) and Alzheimer's.[1] Second, polypeptide drugs play important roles in various treatments of disease.[2] Third, peptide antibodies allow molecular cell biologists to design antibodies for a specific assay of altered forms of proteins. Because they are amenable to specific immunolocalization of highly similar species, peptide

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antibodies can be considered as subcellular probes of cell expression and posttranslational modification.[3] As the building blocks of various biomolecules ranging from small peptides to large proteins, amino acids, including different kinds and sequences, are the key factor of peptide properties. To get sequence-specific peptides, solid-phase peptide synthesis (SPPS) and fermentation from recombinant organisms are the current primary methods.[4] Despite many improvements in the area of SPPS the general concept is still valid: The synthesis proceeds in contrast to peptide biosynthesis from the C-terminus to the N-terminus and the specificity is ensured by the reaction of a selected activated amino acid building block with an N-terminally unprotected solid supported peptide of choice.[5] For the amino acids such as L-lysine or L-ornithine, the two amino groups almost have the same chemical activity under normal conditions. Complicated steps, extra reagents, and long times are necessary in the synthesis of mono-Cbz-Cl-protected L-lysine or L-ornithine.[6] The key point of the mono-Cbz-protected L-lysine or L-ornithine is the selected activated amino group, which is similar to peptide synthesis in human.

The key element in peptide formation is the amino acid activation domain, which selects substrate amino acids and forms adenylates.[7] In organisms, sequence-specific peptides can be synthesized in the presence of free amino acids. Transfer RNA (tRNA), which carries special amino acids to the protein synthetic machinery of a cell (ribosome) as directed by a three-nucleotide sequence in messenger RNA (mRNA), is a necessary component in the synthesis of peptides (Fig. 1).[8] tRNA can selectively transfer amino acids from free amino acids in organisms. Mimicing the ability of tRNA is not only important in peptide synthesis but also significant in the origin of life.

Benzyl chloroformate (Cbz-Cl), which can react with an amino group to form an amido bond and be easily removed by catalytic hydrogenation, is widely used in the protection of amino acids and synthesis of peptides.[9] Without additional agents, the selectivity of Cbz-Cl is poor.[10] In our previous work, we found that β-cyclodextrin (β-CD) (Fig. 2) can catalyze the reaction between Cbz-Cl and special amino groups of L-lysine.[11] The different orientations of L-lysine in the cavity of β-CD make the amino groups have different chemical activities under special conditions. High chemoselectivity

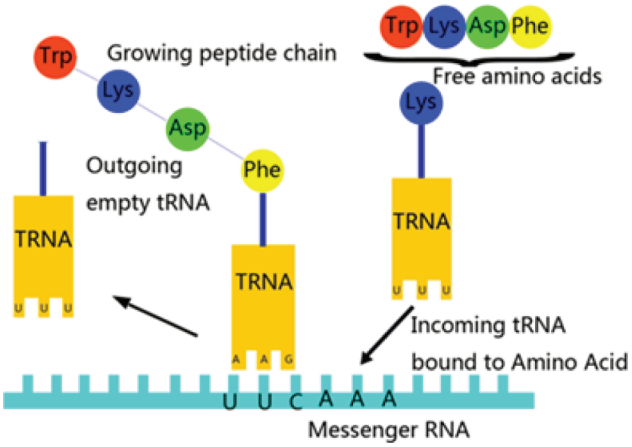
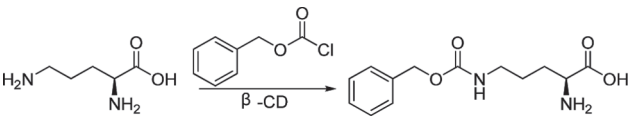


Figure 1. Position of tRNA in peptide synthesis. (Figure is provided in color online.)



Scheme 1. L-ornithine reacts with Cbz-Cl in the presence of β-CD.

(>99%) and yield (90%) are acquired in short times (10 min). L-Ornithine has a structure similar to L-lysine and is necessary in the ornithine cycle which converts toxic ammonia to less toxic water-soluble urea. Based on previous study, we envisioned that β-CD can form inclusions with L-ornithine that may affect the chemical activity of amino groups. The results confirmed our proposal, as only ε-amino groups reacted with Cbz-Clin the presence of β-CD (Scheme 1, selectivity >99% yield 90%). These observations inspired us to investigate the selectivity of β-CD to form inclusions with amino acids in the construction of amido bonds, which is similar to the ability of tRNA in peptide synthesis in organisms. Chain and aromatic amino acids were applied to investigate the selectivity separately.

Cyclodextrins (CDs) are cyclic oligomers of a-1, 4-linked D-glucopyranose.[12] The hydrophobic and hydrophilic features are what make the CDs extremely attractive components of mimic enzyme systems. In this way CDs provide a chemically and sterically specific reaction field, to provide by multiple molecular recognition means of different binding forces as required for an effective enzymelmodel.[13] The CDs and their derivative are wieldy used in the recognition of peptides and targeted delivery of DNA.[14] Among the CDs, β-CD is widely used for its water solubility, low cost, commercial availability, biocompatibility, and easy functionalization.[15] β-CD can form inclusion complexes with numerous guest molecules. The different inclusion stability constants allow β-CD to have potential applications in molecular recognition.

We studied a wide range of easily available and human essential amino acids for testing selectivity. The result was interesting. In the presence of chain amino acids, both the L-lysine and L-ornithine showed good selectivity. The selectivity was still good even when two chain amino acids existed (Table 1). This is similar to tRNA, which transfers special amino acids in the course of peptide synthesis. In the presence of an aromatic amino acid, the selectivity decreased. In theory, the cavity size of

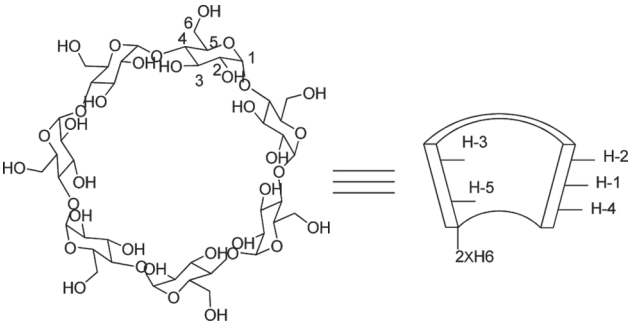
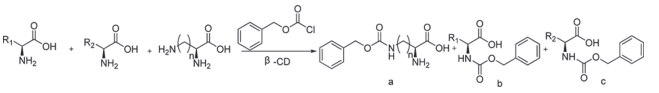


Figure 2. Sketch of the proton positions in a CD molecule.

Table 1. Chemoselectivity between L-lysine/L-ornithine and other amino acids in the presence of β-CD



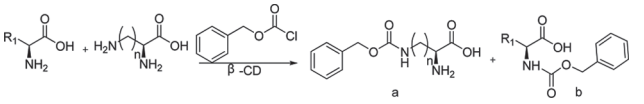
Entry	n	R_1	R_2	Yield (a)[a] %	Yield (b)	Yield (c)
1	4	$HOCH_2$	$(CH_3)_2CHCH_2$	80	ND	ND
2	3	$HOCH_2$	$(CH_3)_2CHCH_2$	85	ND	ND

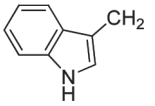
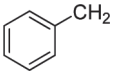
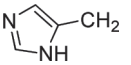
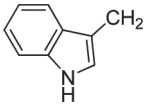
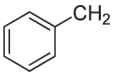
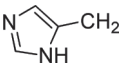
[a]Isolated yield. ND, not determined.

β-CD is fit for a benzene ring,[16] but L-ornithine still showed good selectivity when L-tryptophane was applied in the system. In the same condition the selectivity of L-lysine was poor (Table 2, entries 3, 8).

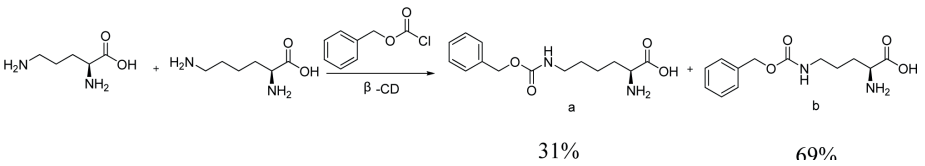
We propose that the β-CD plays two distinct roles in the reactions. The first is selectivity. The amino groups of L-lysine and L-ornithine almost have the same

Table 2. Chemoselectivity between L-lysine/L-ornithine and amino acid in the presence of β-CD



Entry	n	R_1	Yield (a)[a] %	Yield (b)[a] %
1	4	$HOCH_2$	90	ND
2	4	$(CH_3)_2CHCH_2$	90	ND
3	4		10	90
4	4		ND	ND
5	4		40	60
6	3	$HOCH_2$	90	ND
7	3	$(CH_3)_2CHCH_2$	90	ND
8	3		80	ND
9	3		ND	ND
10	3		50	50

[a]Isolated yield. ND, not determined.



Scheme 2. Chemoselectivity between L-lysine and L-ornithine in the presence of β-CD. Conversion determined from ^{1}H NMR spectrum of crude reaction mixture.

chemical activities under normal conditions, but mono-Cbz-protected L-lysine and L-ornithine can be observed in the presence of β-CD. The second is catalysis. The reaction between Cbz-Cl and amino groups are slow in the absence of β-CD.[17] The catalysis could play a major factor in Table 2, entry 3; the selectivity could play a major factor in Table 2, entry 8. This could be the reason that the selectivities in entries 3 and 8 are quite different.From the experimental results, it was proposed that the selectivity of L-ornithine is stronger than L-lysine. To confirm this, the reaction with L-ornithine and L-lysine were performed simultaneously and the main product of L-ornithine was observed (Scheme 2).

NMR spectroscopy is one of the most important tools to obtain inclusion modes and geometries. It is very useful in providing evidence because the proton environment in both host and guest will be reflected by chemical shifts. From the chemical shifts, we can conclude the orientations in the inclusions.^{1}H NMR experiments were used to investigate the inclusion between β-CD and L-lysine with different molar ratios.

The inclusion of a guest molecule in a CD generally provokes a change in the environment of only the protons inside the cavity.[18] From the spectra (Fig. 3), the chemical shifts of protons d and e significantly changed in the β-CD lysine solution (1:1 molar ratio). Compared to protons d and e, little chemical shift of protons a, b, and c were observed in the spectra (Tables 3 and 4). These observations unambiguously

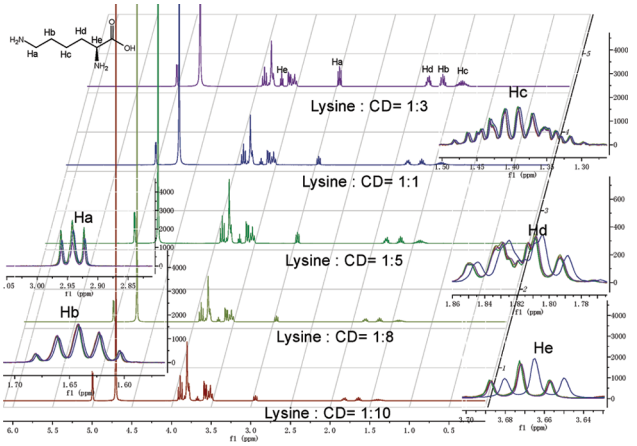


Figure 3. ^{1}H NMR (400-MHz) spectra in D_2O at 298K of β-CD (10^{-3}mol·L^{-1}) upon addition of L-lysine. Chemical shifts (δ) are reported in ppm and referenced to the residual solvent peak as an internal standard (δ=4.7000 ppm for 1H NMR in D_2O). (Figure is provided in color online.)

Table 3. δ of L-lysine protons in L-lysine/β-CD complex (1:1) and alone

δ (ppm) of L-lysine protons	Alone	In complex (1:1)	Δδ
a	2.9452	2.9451	–0.0001
b	1.6451	1.6450	–0.0001
c	1.3929	1.3928	–0.0001
d	1.8258	1.8200	–0.0058
e	3.6753	3.6651	–0.0102

Table 4. δ of β-CD protons in L-lysine/β-CD complex (1:1) and alone

δ (ppm) of β-CD	Alone	In complex (1:1)	Δδ
H1	4.9892	4.9893	–0.0001
H2	3.5684	3.5685	–0.0001
H3	3.8857	3.8911	–0.0054
H4	3.5032	3.5033	–0.0001
H5	3.7426	3.7497	–0.0071
H6	3.7975	3.7976	–0.0001

proved that the protons d and e of L-lysine are inside the cavity of β-CD, which allows only one amino group to react with Cbz-Cl. This is also in accorded with the observed experimental results. The similar results were also observed in the β-CDL-ornithine ^{1}H NMR spectrums (Fig. 4). The chemical shifts of L-ornithine protons next to α-amino groups significantly changed. Little chemical shifts of other L-ornithine protons were observed (Table 5 and 6).

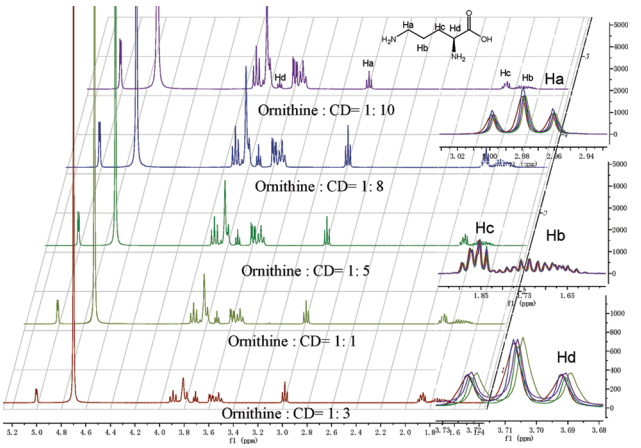


Figure 4. ^{1}H NMR (400 MHz) spectra in D_2O at 298K of β-CD (10^{-3} mol·L^{-1}) upon addition of L-ornithine. Chemical shifts (δ) are reported in ppm and referenced to the residual solvent peak as an internal standard (δ=4.7000 ppm for ^{1}H NMR in D_2O). (Figure is provided in color online.)

Table 5. δ of L-ornithine protons in L-ornithine/β-CD complex (1:1) and alone

δ (ppm) of L-ornithine protons	Alone	In complex (1:1)	Δδ
a	2.9800	2.9799	–0.0001
b	1.7395	1.7394	–0.0001
c	1.8557	1.8556	–0.0001
d	3.7067	3.7037	–0.0030

Table 6. δ of β-CD protons in L-ornithine/β-CD complex (1:1) and alone

δ (ppm) of β-CD	Alone	In complex (1:1)	Δδ
H1	4.9892	4.9893	–0.0001
H2	3.5684	3.5685	–0.0001
H3	3.8857	3.8921	–0.0064
H4	3.5032	3.5033	–0.0001
H5	3.7426	3.7506	–0.0080
H6	3.7975	3.7976	–0.0001

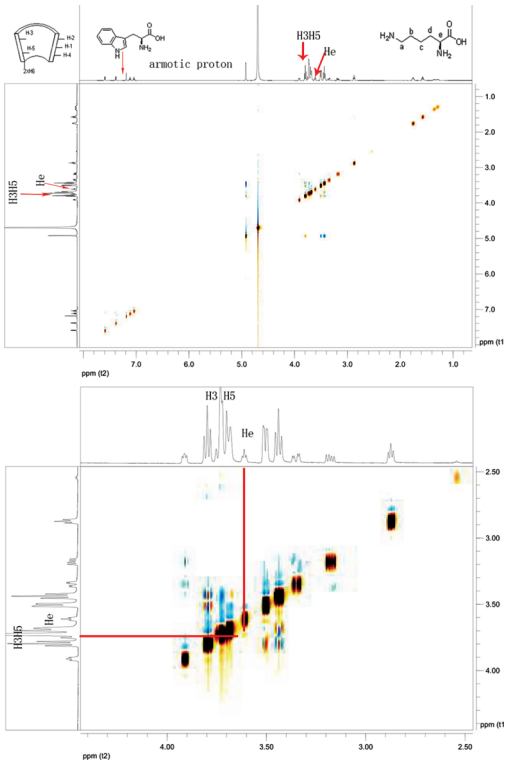


Figure 5. NOESY 2D spectra of β-CD/L-lysine/L-tryptophane (1:1:1 mol/mol/mol) in D_2O at room temperature. (Figure is provided in color online.)

A cross peak in nuclear Overhause effect spectroscopy (NOESY) or rotating frame NOESY (RNOESY) spectra occurs if two hydrogen atoms are within ~5 Å.[19] In L-tryptophane β-CD water solution, a cross peak between aromatic protons and inner proton of β-CD (H3, H5) occurred, which proved that the inclusion was observed in the two-dimensional (2D) NMR spectrum.[20] To further investigate inclusion selectivity, a 2D NMR experiment of β-CD, L-tryptophane, and L-lysine water solution was performed. No cross peaks between the inner proton of β-CD (H3, H5) and the aromatic proton of L-tryptophane were observed. Cross peaks between the inner proton of β-CD and proton e were observed in the spectrum, which proved the inclusion between β-CD and L-lysine (Fig. 5). It seems that L-lysine expelled the benzene ring of L-tryptophane from the cavity of β-CD. Similar results can be observed from the 2D NMR spectrum of β-CD, L-tryptophane, and L-ornithine water solution (Fig. 6).No cross peaks between the inner proton of β-CD (H3, H5) and the aromatic proton of L-tryptophane were observed. Cross peaks between the

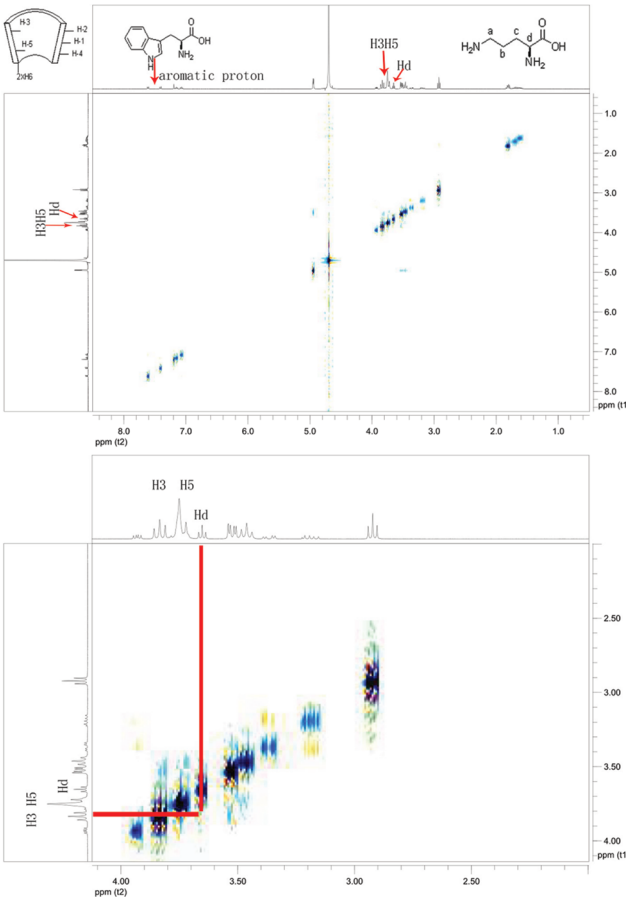


Figure 6. NOESY 2D spectra of β-CD/L-ornithine/L-tryptophane (1:1:1 mol/mol/mol) in D_2O at room temperature. (Figure is provided in color online.)

inner proton of β-CD and proton e were observed in the spectrum, which proved the inclusion between β-CD and L-ornithine. These observations were in accord with the experiment results. A computational study of host–guest inclusion complexes among β-CD, 20 natural L-amino acids, and some selected pentapeptides also proved that there were different stability constants in different inclusions.[21] Liu and coworkers also proved the molecular recognition abilities and enantioselectivity for different amino acid guests.[22]

EXPERIMENTAL

^{1}H NMR spectra were measured on a Bruker AM-400 spectrometer at room temperature with D_2O as the solvent. ^{1}H-^{1}H NOESY 2D experiments were recorded using an Inova-600 (600-MHz) spectrometer at ambient temperature. A mixing time of 0.200 s, a relaxation delay time of 1.000 s, and an acquisition time of 0.228 s were used. All plus sequences were set according to the manufacturer's standards. Tetramethylsilane (TMS) was used as internal standard. β-CD and derivatives were purchased from ShangdongZiyuan Chemical Reagent Co., China. Other reagents were all commercially available from Country Medicine Reagent Co. Ltd, Shanghai, China. Liquid chromatography was recorded on an Agilent 1200 series. The separation was performed using mobile phase consisting of MeOH, H_2O (50: 50 v/v), with 0.1% triethylamine (TEA). The column used was a Waters C_{18} column (250×4.6 mm internal diameter with 5-μm particle size). The flow rate was 1.0 ml/min. Mass spectrawere recorded on an Agilent 6510 Q-TOF, equipped with an electrospray ionization source (ESI). The capillary temperature was 350 °C, dryinggas is 12 L/min, and the spray voltage was 4.0 kV for all applications.

β-Cyclodextrin (0.1 mmol) was dissolved in 0.1 M carbonate buffer (pH = 8) (10 mL) at room temperature, the L-lysine (1 mmol) and other amino acids (1 mmol) were added, and the reaction mixture was stirred for 5 min. Cbz-Cl (1 mmol) was added and stirring was continued at room temperature until the reaction was complete. The reaction mixture was extracted with ethyl acetate (2×5 ml). The solvent was removed under vacuum and the products were obtained.

CONCLUSIONS

The inclusion complexes between β-CD and amino acids were studied. Special amino acids and activation domain were achieved in the construction of an amido bond, which is similar to the ability of tRNA in peptide synthesis. The ^{1}H NMR experiments in D_2O confirmed the inclusion and gave information on the position of guests in the CD cavity. The NOESY experiments gave information of inclusion complexes in mixtures. These studies showed that special amino acids can selectively form inclusion with β-CD. These properties have potential applications in mimic peptide synthesis. By accurate modification of CD, special selectivity can be accessed.

ACKNOWLEDGMENT

Thanks go to Edward C. Mignot, Shandong University, for linguistic advice. The authors declare no competing financial interests.

SUPPORTING INFORMATION

Full experimental detail, ^{1}H NMR spectra, and HPLC traces can be found via the "Supplementary Content" section of this article's Web page.

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