Design, synthesis of 4-hydroxyl-α-cyanocinnmaic acid derived compounds and their applications in chiral recognition of amino acids by mass spectrometry

LUO Xiaoyu[1], WANG Haixing[3], ZHANG Shu[3], CHEN Peng[1],
ZHENG Huanhuan[1], CHEN Heru[1,2], YAO Zhongping[3]
(1. aInstitute of Traditional Chinese Medicine and Natural Products, College of Pharmacy, Jinan University, Guangzhou 510632, China; 2. Guangdong Province Key Laboratory of Pharmacodynamic Constituents of TCM and New Drugs Research, Guangzhou 510632, China; 3. State Key Laboratory of Chirosciences and Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Hong Kong)

[Abstract] Four pairs of compounds designed based on scaffolds of proline and 4-hydroxy-α-cyanocynnmaic acid (CHCA) have been synthesized as the chiral selectors. Chiral recognition of these compounds towards 19 common amino acids was evaluated by investigating the ESI-MS/MS spectra of the protonated dimers and trimers formed between the amino acids and the chiral selectors. Effects of the structural change of the chiral selectors on chiral recognition towards the amino acids were discussed in this study.

[Key words] Chiral recognition; mass spectrometry; amino acid; proline; 4-hydroxy-α-cyano-cynnmaic acid
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4-羟基-α-氰基肉桂酸衍生物的设计合成及在质谱法手性识别氨基酸中的应用

罗小雨[1], 王海星[3], 张 舒[3], 陈 鹏[1], 郑欢欢[1], 陈河如[1,2], 姚钟平[3]
(1. 暨南大学 药学院中药及天然药物研究所, 广东 广州 510632;
2. 广东省中药药效物质基础及创新药物研究重点实验室, 广东 广州 310632;
3. 手性科学国家重点实验室, 香港科技大学应用生物与化学系, 香港)

[摘 要] 设计合成了 4 对以脯氨酸和 4-羟基-α-氰基肉桂酸(CHCA)为骨架、作为手性选择子的化合物。通过 ESI-MS/MS 质谱方法研究氨基酸与手性识别子所形成的质子化二聚体和三聚体,评估了这些化合物对 19 种氨基酸的手性识别作用。探讨了手性识别子结构变化对氨基酸手性识别作用的影响。

[关键词] 手性识别; 质谱; 氨基酸; 脯氨酸; 4-羟基-α-氰基肉桂酸
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[Authors] LUO Xiaoyu (1988-), male, Research interest: design and synthesis of new drug; these author contribute equally to this work.
[Corresponding authors] CHEN Heru (1967-), male, professor, E-mail: thrchen@jnu.edu.cn;
YAO Zhongping (1967-), male, professor, Email: bczpyao@polyu.edu.hk.

0 Introduction

Amino acids play a key role in living organisms as sources of energy and precursors of biosyntheses. In living organisms, the majority of amino acids exist in L-forms; however, several kinds of free D-forms have been discovered in species of lower animals, mammalian organs and biological fluids [1]. Detection of D-amino acids could also be used as an indication of aging and pathology [2-3]. Therefore, chiral analysis of amino acid enantiomers is of great interest and increasing importance in many fields such as chemistry, biology, physiology and pharmacy.

Polarimetry, circular dichroism, nuclear magnetic resonance and chromatography are conventional techniques for chiral analysis of organic compounds [1-5]. In recent years, mass spectrometry (MS) has become more and more popular in chiral analysis due to its many advantages such as short analysis time, high sensitivity and ability to analyze mixtures [6-7]. Various mass spectrometric techniques including chemical ionization (CI) [8-9], fast atom bombardment (FAB) [10-12] and electrospray ionization (ESI) [13-18] have been applied for chiral analysis, and ESI-MS/MS has been more commonly used since around 2000 [13-18]. Chiral enantiomers have identical mass, so they cannot be directly differentiated by MS. Chiral analysis by MS requires a chiral selector to form diastereomeric complexes with enantiomers, and the enantiomers are normally differentiated according to their differences in signal intensity of the formed complexes in MS or fragment ions of the complexes in MS/MS [6,17]. Selection of suitable chiral selectors is the key step for successful chiral analysis by MS. For chiral analysis of amino acids, modified chiral amino acids including *N*-*tert*-butoxycarbonylphenylalanine, *N*-*tert*-butoxycarbonylproline and *N*-*tert*- butoxycarbonyl-*O*-benzylserine as well as tertiary amine appended *trans*-4-hydroxyproline derivatives and *N*-(3,5-dinitrobenzoyl)-leucine have been successfully used as chiral selectors in chiral analysis by ESI-MS/MS [16-18] and ESI-MS [19-20] respectively; and transition metal ions together with chiral amino acids were reported as the chiral selectors for chiral analysis of amino acids by the kinetic method [6,13-15].

Based on the previous results that the rigid structure of proline could induce larger chiral discrimination [16-20], in the current study, we designed and synthesized a series of proline derivatives (Figure 1) as the chiral selectors for chiral recognition of amino acids. 4-Hydroxy-α-cyanocynnmaic acid (CHCA) was introduced as the building module since CHCA is a common matrix in matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) [21] and the chiral selector thus obtained may be directly used for chiral recognition by MALDI-MS. Chiral recognition of all the synthesized chiral selectors towards amino acids was evaluated using the ESI-MS/MS approach.

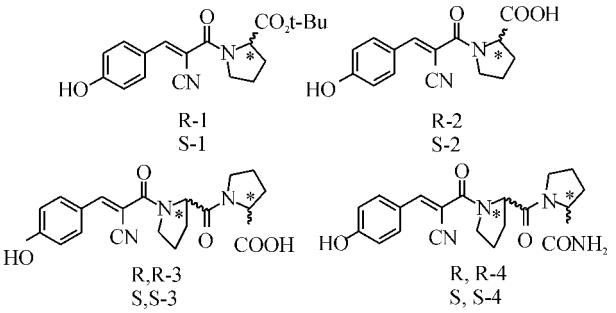


Figure 1　Chemical structures of all the designed compounds

1 Experimental

1.1 Materials

4-Hydroxyl-α-cyanocinnmaic acid was purchased from Sigma-Aldrich Co. in China. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) was purchased from Aladdin Reagent Database Inc., China. 1-Hydroxylbenzotriazol (HOBt), diisopropylethylamine (DIPEA), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetra- methyluronium hexafluorophosphate (TBTU), triethylsilane, D-proline *tert*-butyl ester, L-proline *tert*-butyl ester, and Rink amide resin were purchased from GL Biochem Shanghai Ltd., China. Silica gel for column chromatography was purchased from Qingdao Marine Chemicals Inc., China; All D- and L-α-amino acids were purchased from Sigma Chemical Co. (Milwaukee, WI) and used without further purification.

1.2 General Procedure

NMR spectra were recorded on a Bruker AV-300 (Bruker Biospin, Swiss) spectrometer with TMS as the internal standard. ESI-MS spectra for structural confirmation were recorded on a Finnigan LCQ Advantage MAX mass spectrometer. HPLC separation was performed on a Wufeng 100 liquid chromatography equipped with a UV detector. Pre-coated thin-layer chromatography (TLC) plates (Institute of Yantai Chemical Industry, China) were used for TLC separation. Spots on TLC plates were detected by either a ZF-7A portable UV detector or spraying $KMnO_4$ solution followed by heating. N, N-dimethylformamide (DMF) was refluxed over CaH_2 for 2 hrs and redistilled under reduced pressure before use. Tetrahydrofuran (THF) was dried over sodium thread and then redistilled before use. Dichloromethane (DCM) was dried over P_2O_5 for 2 hrs and redistilled before use.

1.3 Synthesis

(1) Syntheses of *S*-1 and *R*-1

To a solution of L-proline *tert*-butyl ester (124 mg, 0.6 mmol) in 5 mL DCM, Et_3N (0.1 mL, 0.97 mmol) was added. The mixture was then cooled down to −20 ℃, followed by addition of CHCA (100 mg, 0.53 mmol), HOBt (81 mg, 0.6 mmol), and EDCI (114.6 mg, 0.6 mmol) in *V*(DCM):*V*(DMF) = 5:1 (5 mL). The mixture was stirred for 1 hr and then warmed up to rt and kept at rt for 24 hrs. DCM was removed by evaporation under reduced pressure. The condensate was then submitted to lyophilization for removal of DMF. The resulted residue was then purified by column chromatography with petroleum ether/EtOAc (6:4, *v/v*) as the mobile phase. *S*-1 was then obtained in yellow oil (268 mg, 78.5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.79(s, 1H, ArH), 7.71 ~ 7.74 (d, J = 9.0 Hz, 2H, ArH), 6.85 ~ 6.88 (d, J = 9.0 Hz, 2H, ArH), 5.25 (s, 1H), 4.43 ~ 4.47 (m, 1H), 3.85 ~ 3.88 (m, 2H), 2.20 ~ 2.26(m, 2H), 1.92 ~ 2.01 (m, 2H), 1.45(s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.33, 162.67, 161.38, 154.16, 133.11, 123.70, 116.57, 116.22, 99.79, 82.18, 61.38, 49.13, 28.77, 27.76, 27.72, 25.18; ESI-MS (*m/z*) found: 365.1 ([M + Na]$^+$), 341.3 ([M − H]$^-$).

With the same procedure described above, replacement of L-proline *tert*-butyl ester with D-proline *tert*-butyl ester offered *R*-1 in yellow oil (261.2 mg, 76.5%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.80(s, 1H, ArH), 7.71 ~ 7.74 (d, J = 9.0 Hz, 2H, ArH), 6.85 ~ 6.88 (d, J = 9.0 Hz, 2H, ArH), 5.26 (s, 1H, ArOH), 4.44 ~ 4.48(m,1H), 3.88 ~ 3.90(m, 2H), 2.02 ~ 2.31(m, 2H), 1.96 ~ 1.99 (m, 2H), 1.46(s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.35, 162.66, 161.37, 154.18, 133.12, 123.72, 116.59, 116.24, 99.81, 82.19, 61.39, 49.14, 28.78, 27.73, 25.20; ESI-MS (*m/z*) found: 365.1 ([M + Na]$^+$), 341.3 ([M − H]$^-$).

(2) Syntheses of *S*-2 and *R*-2

To a solution of *S*-1 or *R*-1 (140 g, 0.41 mmol) in DCM (5 ml), trifluoroacetic acid (5 ml) and Et_3SiH (0.2 ml) were added at 0 ℃ under stirring. The reaction was allowed to last for 5 hrs, followed by removal of DCM under reduced pressure. The condensate was then submitted to lyophilization for removal of other impurities with high boiling points. The resulted residue was *S*-1 or *R*-1 in yellow solid (111.2 mg, 95.0%), with good purity confirmed by TLC. Both products were confirmed by mass spectrometry. ESI-MS (*m/z*) found: 309.1 ([M + Na]$^+$), 287.1 ([M + H]$^+$).

(3) Syntheses of *S*,*S*-3 and *R*,*R*-3

To a solution of L-proline *tert*-butyl ester (124 mg, 0.6 mmol) in 5 mL DCM, Et_3N (0.1 mL, 0.97 mmol) was added. The mixture was then cooled down to −20 ℃, followed by addition of *S*-2 (151.7 mg, 0.53 mmol), HOBt (81 mg, 0.6 mmol), and EDCI (114.6 mg, 0.6 mmol) in *V*(DCM):*V*(DMF) = 5:1 (5 mL). The mixture was stirred for 1 hr and then warmed up to rt and kept at rt for 24 hrs. DCM was removed by evaporation under reduced pressure. The condensate was then submitted to lyophilization for removal of DMF. The resulted residue was then treated with DCM (5 mL), trifluoroacetic acid (5 mL) and Et_3SiH (0.2 mL) for 5 hrs. Excess DCM and DMF solvents were removed in usual way and the resulted

residue was purified by RP-HPLC (column: Cosmosil C_{18}, 30 ~ 40 Å, 4.6 × 250 mm; Eluent: 40 vol% methanol in water) to offer *S*,*S*-3 in yellowish solid (136.5 mg, 67.2%). ^{1}H NMR (300 MHz, CD_3OD) δ: 7.89 (s, 1H, ArH), 7.82 ~ 7.86 (d, J = 12.0 Hz, 2H, ArH), 6.87 ~ 6.90 (d, J = 9.0 Hz, 2H, ArH), 4.77 ~ 4.81 (m, 1H), 4.46 ~ 4.478 (m, 1H), 3.81 ~ 3.88 (m, 2H), 3.66 ~ 3.69 (m, 2H), 2.13 ~ 2.35 (m, 2H), 2.00 ~ 2.09 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ: 172.28, 164.49, 163.45, 153.76, 134.09, 125.00, 122.93, 117.11, 102.02, 60.98, 51.03, 49.85, 48.46, 30.04, 29.28, 26.35, 25.86; ESI-MS (*m*/*z*) found: 406.3 ([M + Na]$^+$), 382.4 ([M - H]$^-$).

With the same procedure described above, replacement of L-proline *tert*-butyl ester and *S*-2 with D-proline *tert*-butyl ester and *R*-2 respectively offered *R*,*R*-3 in yellowish solid (129.7 mg, 63.8%). ^{1}H NMR (300 MHz, CD_3OD) δ: 7.89 (s, 1H, ArH), 7.82 ~ 7.86 (d, J = 12.0 Hz, 2H, ArH), 6.87 ~ 6.90 (d, J = 12.0 Hz, 2H, ArH), 4.76 ~ 4.79 (m, 1H), 4.46 ~ 4.49 (m, 1H), 3.83 ~ 3.88 (m, 2H), 3.66 ~ 3.69 (m, 2H), 2.22 ~ 2.39 (m, 2H), 2.00 ~ 2.14 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ: 175.28, 172.27, 164.49, 163.44, 153.77, 134.08, 125.00, 117.10, 102.01, 60.96, 60.39, 54.79, 51.02, 30.01, 29.27, 26.35, 25.85; ESI-MS (*m*/*z*) found: 406.3 ([M + Na]$^+$), 382.4 ([M - H]$^-$).

(4) Syntheses of *S*,*S*-4 and *R*,*R*-4

The synthesis was carried out on a 0.15 mmol scale, starting with 550 mg Rink amide resin (capacity: 0.2 mmol/g) in a 15 - mL peptide reactor. The resin was first treated with 25 vol% piperidine in DMF (*v*/*v*) twice to release the free amino group. Coupling reactions were carried out in 20 vol% DMF in DCM medium with a three-fold excess of TBTU/HOBt/DIPEA, in which they were TBTU (96 mg, 0.3 mmol), HOBt (40.5 mg, 0.3 mmol), and DIPEA (0.05 mL, 0.3 mmol) respectively, and a three-fold excess of Fmoc-Pro-OH and six fold excess of CHCA, in which they were Fmoc-Pro-OH (101 mg, 0.3 mmol) and CHCA (120 mg, 0.6 mmol) respectively. The mixture was agitated by an IKA-110 shaker at a speed of 240 times/min. The coupling reaction was allowed to run for 90 min. Solvent was removed by filtration under reduced pressure, and the resin was washed 6 times with fresh V(DMF):V(DCM) = 1:4 to remove excess starting materials and by-products. After coupling of CHCA, the resin was washed with DMF-DCM (1:4, *v*/*v*), methanol, and DCM respectively and blown with N_2 gas to dryness. The resin was then treated twice with a mixture of V(TFA):V(Et_3SiH):V(H_2O) = 14:0.75:0.45, at rt for 1.5 hrs under shaking at a speed of 240 times/sec. The solution was separated by filtration and the combined filtrate was concentrated by N_2-blowing. The condensate was submitted to lyophilization resulted in a residue, which was purified by RP-HPLC (column: Cosmosil C_{18}, 30 ~40 Å, 4.6 ×250 mm; Eluent: 40 vol% methanol in water) to offer *S*,*S*-4 or *R*,*R*-4.

S,*S*-4 in yellowish solid (86 mg, 35.6%). ^{1}H NMR (300 MHz, CD_3OD) δ: 7.88 (s, 1H, ArH), 7.81 ~ 7.85 (d, J = 12.0 Hz, 2H, ArH), 6.87 ~ 6.90 (d, J = 12.0 Hz, 2H, ArH), 4.75 ~ 4.79 (m, 1H), 4.43 ~ 4.47 (m, 1H), 3.85 ~ 3.88 (m, 2H), 3.64 ~ 3.68 (m, 2H), 2.31 ~ 2.35 (m, 2H), 1.93 ~ 2.23 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ: 177.01, 172.46, 164.44, 163.43, 153.70, 134.08, 124.96, 117.12, 101.98, 61.32, 61.01, 51.04, 30.75, 29.46, 26.43, 25.96; ESI-MS (*m*/*z*) found: 405.2 ([M + Na]$^+$), 381.3 ([M - H]$^-$).

R,*R*-4 in yellowish solid (70 mg, 28.9%). ^{1}H NMR (300 MHz, CD_3OD) δ: 7.87 (s, 1H, ArH), 7.81 ~ 7.85 (d, J = 12.0 Hz, 2H, ArH), 6.87 ~ 6.90 (d, J = 9.0 Hz, 2H, ArH), 4.75 ~ 4.79 (m, 1H), 4.43 ~ 4.47 (m, 1H), 3.85 ~ 3.88 (m, 2H), 3.66 ~ 3.68 (m, 2H), 2.31 ~ 2.39 (m, 2H), 1.84 ~ 2.22 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ: 172.47, 164.45, 163.41, 153.72, 134.08, 124.96, 117.11, 101.97, 61.33, 61.01, 54.80, 51.04, 49.85, 30.74, 29.46, 26.43, 25.96; ESI-MS (*m*/*z*) found: 405.4 ([M + Na]$^+$), 381.3 ([M - H]$^-$).

1.4 Mass spectrometry

Chiral recognition by mass spectrometry was performed in positive ion mode on a Q-TOF2 mass spectrometer (waters, manchester, UK) fitted with an ESI source. The instrument conditions were as following: capillary voltage 3.0 kV, cone voltage 30 V, desolvation gas temperature 150 ℃, ion source temperature 80 ℃. Sample solutions were introduced into the mass spectrometer by a syringe pump at a flow rate of 5.0 μL/min. Nitrogen was used as desolvation gas. Argon was used as the collision gas for CID at a pressure of 0.5 bar. Collision energy was set to a value to produce product ions and precursor ions of comparable intensities for the first time and kept constantly for all four chiral combinations (RS, RR, SS and SR) of chiral selectors and amino acids.

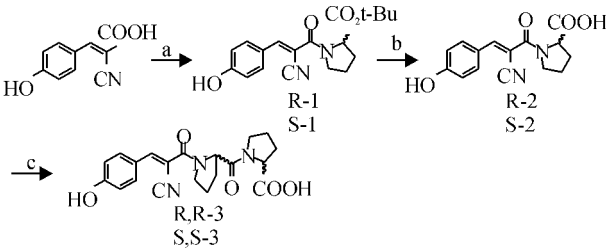
For measurement of chiral recognition, 2 mmol/L amino acids (*R* or *S*) were prepared in 50% methanol containing 0.2% formic acid and 2 mmol/L chiral selectors (*R*-1, *S*-1, *R*-2, *S*-2, *R*,*R*-3, *S*,*S*-3, *R*,*R*-4, and *S*,*S*-4) were prepared in methanol. The solutions of the amino acid and chiral selector were mixed in a ratio of 1∶1 prior to mass spectrometric analysis. Each chiral combination of the amino acid and chiral selector was measured six times for *R*-1 and *S*-1, and three times for *R*-2, *S*-2, *R*,*R*-3, *S*,*S*-3, *R*,*R*-4, and *S*,*S*-4.

2 Results and Discussion

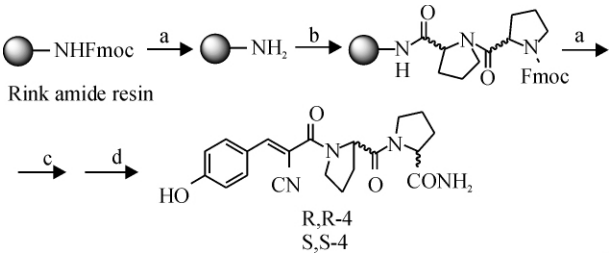
2.1 Syntheses of proline derivatives

The synthesis schemes of the designed compounds were outlined in Scheme 1 and Scheme 2. With the use of EDCI/HOBt, coupling of CHCA with chiral proline *tert*-butyl ester directly resulted in production of either *R*-1 or *S*-1. Protection of hydroxyl at 4-position of CHCA is normally required for such kind of reactions. However, our results showed that without protection of the 4-hydroxyl, a yield of 76.5% ~ 78.5% could be obtained for *R*-1 and *S*-1. Deprotection of *tert*-butyl ester on *R*-1 and *S*-1 led to production of *R*-2 and *S*-2 respectively. A high yield of 95% was obtained with *R*-2 and *S*-2 under mild reaction conditions. Coupling *R*-2 or *S*-2 with chiral proline *tert*-butyl ester produced the precursor of R,*R*-3 or S,*S*-3, which was treated with trifluoroacetic acid and Et_3SiH and R,*R*-3 or S,*S*-3 was then obtained.

Solid-phase synthesis based on t-Butyl/Fmoc strategy was employed here for the syntheses of *R*,*R*-4 and *S*,*S*-4 (Scheme 2). The advantage of this strategy was that tedious separation in each step was avoided and only a simple filtration was required for discarding excess starting materials and by-products. TBTU was chosen as the coupling reagent because it was tolerant to steric hindrance arisen from proline. The overall yields for *S*,*S*-4 and *R*,*R*-4 were 35.6% and 28.9% respectively, with *S*,*S*-4 a bit higher, indicating that the coupling with L-proline was easier than with D-proline.



Scheme 1 Synthesis of the designed compounds. Reagents and conditions: (a) D-Pro-Ot-Bu(HCl or L-Pro-Ot-Bu(HCl, Et_3N, EDCI/HOBt at −20 ℃ for 5 min, then DIPEA at rt for 1 hr, 76.5% ~ 78.5%; (b) DCM/TFA/Et_3SiH, 0 ℃(rt, 5 hrs, 95.0%; (c) repeat (a), and then (b).



Scheme 2 Synthesis of *R*,*R*-4 and *S*,*S*-4. Reagents and conditions: (a) 25 vol% piperidine, rt, 15 minutes, treated twice; (b) sequential coupling and removal of Fmoc; the sequence of amino acids was either Fmoc-D-Pro-OH, Fmoc-D-Pro-OH or Fmoc-L-Pro-OH, Fmoc-L-Pro-OH; coupling reagents used were TBTU/HOBt/DIPEA; reagent used for removal of

Fmoc group was 25 vol% piperidine in DMF; (c) CHCA, TBTU/HOBt/DIPEA, 1.5 hrs; (d) V(TFA):V(Et_3SiH):V(H_2O) = 14:0.45:0.75, 1.5 hrs, treated twice, then RP-HPLC purification.

2.2 Chiral recognition of the proline derivatives towards amino acids

All the synthesized chiral selectors could form oligomers including dimers and trimers with amino acids as observed in the ESI-MS spectra. For example, in the ESI-MS spectrum of *S*-1 and L-Trp, two peaks corresponding to the protonated dimer [L-Trp) + (*S*-1) + H]$^+$ and protonated trimer [L-Trp) + 2(*S*-1) + H]$^+$ were observed at *m/z* 547 and 889 respectively (Figure 2). Besides, ions of the amino acid and chiral selector were observed in the forms of [L-Trp) + H]$^+$ as well as [*S*-1) + H]$^+$, [*S*-1) + Na]$^+$ and [2(*S*-1) + Na]$^+$. No significant difference in peak intensities was observed between the spectra obtained with L- and D-amino acids or *R*- and *S*-chiral selectors, indicating that chiral recognition could not be studied with ESI-MS.

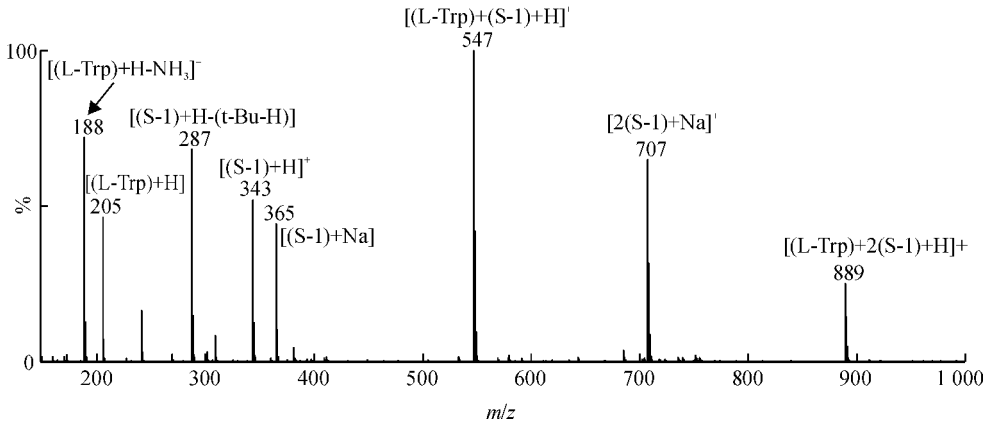


Figure 2　ESI-MS spectrum of a mixture of L-Trp and *S*-1

The collision-induced dissociations (CID) of the protonated dimer and trimer of amino acids and chiral selectors were then investigated, and the typical MS/MS spectra thus obtained were shown in Figure 3a and 3b. The protonated dimer [L-Trp) + (*S*-1) + H]$^+$ was dissociated to [L-Trp) + H]$^+$ and [*S*-1) + H]$^+$ in the CID spectrum (Figure 3a). [*S*-1) - (t-Bu - H)]H$^+$ at *m/z* 287 was also observed in the spectrum, indicating that the *tert*-butyl group was easily to lose. As for the protonated trimer [L-Trp) + 2(*S*-1) + H]$^+$, it lost one *S*-1 to form [L-Trp) + (*S*-1) + H]$^+$ in the CID spectrum (Figure 3b). No [2(*S*-1) + H]$^+$ was observed in the spectrum, suggesting that dimer of (L-Trp) and (*S*-1) has much higher proton affinity than dimer of (*S*-1).

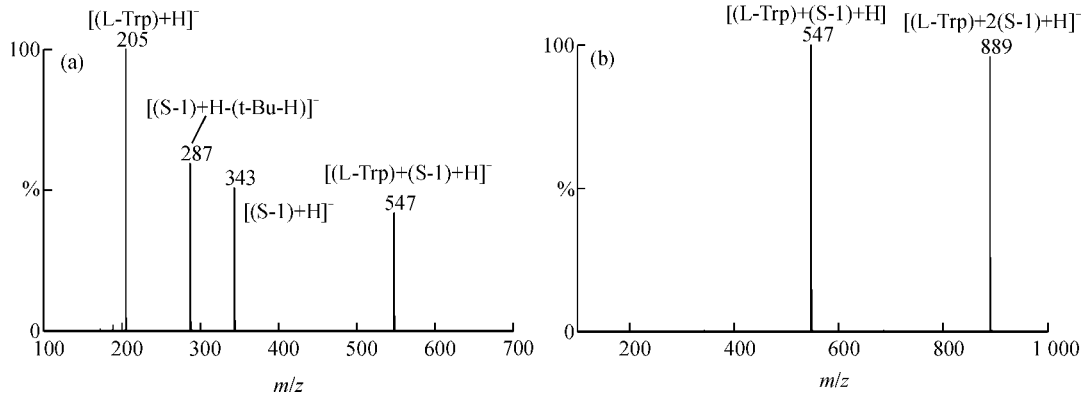


Figure 3　(a). The CID spectrum of the protonated dimer [L-Trp) + (*S*-1) + H]$^+$; (b) The CID spectrum of the protonated trimer [L-Trp) + 2(*S*-1) + H]$^+$

The dissociation of the protonated dimers and trimers could be described as follows:

$$AL_nH^+ \rightarrow AL_{n-1}H^+ + L \quad (1)$$

where A represents amino acid, L represents chiral selector and n = 1 and 2 represent dimer and trimer respectively. There are four chiral combinations of the chiral selectors and the amino acids: SS, RR, SR and RS. SS and RR are homochiral combinations, while SR and RS are heterochiral combinations. Chiral recognition (*CR*) factor could be calculated from the ion intensities in the MS/MS spectra using the following equation [17]:

$$CR = \frac{(AL_{n-1}H^+/AL_nH^+)_{SR} + (AL_{n-1}H^+/AL_nH^+)_{RS}}{(AL_{n-1}H^+/AL_nH^+)_{SS} + (AL_{n-1}H^+/AL_nH^+)_{RR}} \quad (2)$$

Similar to the previous study [17], a *CR* close or equal to 1 means little or no chiral recognition. A *CR* larger than 1 means that the dissociation of heterochiral combinations is more favorable, while a *CR* less than 1 means that the dissociation of homochiral combinations is more favorable.

Chiral recognition of chiral selector 1 (*S*-1 and *R*-1) towards 19 common amino acids as observed in the MS/MS spectra of their protonated dimers and trimers was summarized in Table 1. Chiral discrimination was observed for most amino acids. For MS/MS of the protonated dimers, a *CR* value less than 1 was obtained for amino acids except for His, Glu, Ala, Lys and Tyr, indicating that dissociation of the homochiral dimers was more favorable than that of the heterochiral dimers for most amino acids. Significant chiral discrimination was observed with Trp and Cys, which had a *CR* value of 0.881 ± 0.006 and 0.862 ± 0.013 respectively. For MS/MS of the protonated trimers, a *CR* value larger than 1 was obtained for amino acids except Pro, His, Glu, Cys, and Asp, indicating that dissociation of the heterochiral trimers was more favorable than that of the homochiral trimers for most amino acids. The most significant chiral discrimination was obtained with Lys, having a *CR* value of 1.357 ± 0.014. Comparison with the previous results obtained with *N*-*tert*-butoxycarbonylproline as the chiral selector [16-18] revealed that generally chiral discrimination was significantly reduced with the introduction of CHCA to the chiral selector. This result suggested that the achiral CHCA moiety of *S*-1 and *R*-1 might be involved in the interaction with the amino acids, and direct introduction of a chiral center to the CHCA moiety might be considered in order to enhance the chiral discrimination.

Table 1 ***CR*** **values of *R*-1 and *S*-1 towards 19 common amino acids**

Amino Acid	CR_{dimer}	CR_{trimer}
Pro	0.979 ± 0.010	0.943 ± 0.007
Trp	0.881 ± 0.006	1.020 ± 0.006
His	1.081 ± 0.006	0.986 ± 0.007
Met	0.956 ± 0.005	1.159 ± 0.004
Ala	1.019 ± 0.006*	1.001 ± 0.005
Asn	0.988 ± 0.006	1.068 ± 0.005
Phe	0.958 ± 0.010	1.019 ± 0.007
Arg	0.917 ± 0.005	1.117 ± 0.006
Ile	0.901 ± 0.009	1.082 ± 0.004
Cys	0.862 ± 0.013*	0.966 ± 0.003
Ser	0.958 ± 0.009*	1.006 ± 0.008
Gln	0.953 ± 0.007	1.038 ± 0.007
Val	0.910 ± 0.005*	1.016 ± 0.012
Thr	0.971 ± 0.003*	1.072 ± 0.005
Leu	0.953 ± 0.006*	1.089 ± 0.005
Glu	1.029 ± 0.005	0.786 ± 0.004
Lys	1.113 ± 0.005	1.357 ± 0.014
Asp	0.974 ± 0.005*	0.990 ± 0.006
Tyr	1.026 ± 0.006	1.034 ± 0.013

* Amino acid ion was not detected in the MS/MS spectrum and intensity of chiral selector ion was used for calculation of *CR* instead.

Seven representative amino acids Pro, Try, Phe, Arg, Leu, Glu, and Lys were selected to evaluate the chiral discrimination of the other designed chiral selectors and the results are summarized in Table 2. Compared to the results obtained with *S*-1 and *R*-1, absence of t-Bu in *S*-2 and *R*-2 did not induce significant differences in chiral recognition ability of the chiral selectors. However, reverse dissociation preference was observed with the protonated dimers of Pro and Trp as well as the protonated trimers of Trp and Lys. For example, the *CR* value for the protonated dimers of Trp was less than 1 (i.e., 0.881 ± 0.006) with *S*-1 and *R*-1 as the chiral selectors, indicating that dissociation of the homochiral dimers was more favorable than that of the heterochiral dimers; when *S*-2 and *R*-2 were used as the chiral selectors, a *CR* value larger than 1

(i.e., 1.062 ± 0.005) was obtained for the protonated dimers of Trp, indicating that dissociation of the heterochiral dimers was more favorable than that of the homochiral dimers. These results suggested that the presence and absence of the bulky t-Bu group might have pronounced effects on the structures of the protonated dimers of the chiral selectors with Pro and Trp as well as the protonated trimers of the chiral selectors with Trp and Lys. Incorporation of one more proline into *S*-2 and *R*-2 to increase structural rigidity (*S*-3 and *R*-3, and *S*-4 and *R*-4) and replacement of acidic carboxylic group with neutral amide group (*S*-4 and *R*-4) did not significantly enhance the chiral discrimination towards the amino acids. However, such structural changes of the chiral selectors induced reversed dissociation preference for the amino acids except Phe and Lys, indicating that the structural changes of the chiral selectors might cause changes of the chiral recognition sites of the corresponding dimers and trimers.

Table 2　*CR* values of four pairs of chiral selectors towards seven representative common amino acids

Amino	*R*-1 and *S*-1		*R*-2 and *S*-2		*R*,*R*-3 and *S*,*S*-4		*R*,*R*-4 and *S*,*S*-4	
Acid	CR_{dimer}	CR_{trimer}	CR_{dimer}	CR_{trimer}	CR_{dimer}	CR_{trimer}	CR_{dimer}	CR_{trimer}
Pro	0.979 ± 0.010	0.943 ± 0.007	1.019 ± 0.006	0.862 ± 0.006	1.025 ± 0.009	0.901 ± 0.005	0.990 ± 0.004	1.023 ± 0.008
Trp	0.881 ± 0.006	1.020 ± 0.006	1.062 ± 0.005	0.906 ± 0.006	0.896 ± 0.004	0.721 ± 0.004	0.887 ± 0.008	0.922 ± 0.006
Phe	0.958 ± 0.010	1.019 ± 0.007	0.968 ± 0.004	0.936 ± 0.011	0.990 ± 0.009	0.987 ± 0.008	0.954 ± 0.004*	0.982 ± 0.005
Arg	0.917 ± 0.005	1.117 ± 0.006	0.916 ± 0.004	1.122 ± 0.006	1.153 ± 0.005	1.001 ± 0.002	1.097 ± 0.005	0.812 ± 0.007
Leu	0.953 ± 0.006*	1.089 ± 0.005	0.983 ± 0.010	1.040 ± 0.005	0.988 ± 0.001*	0.984 ± 0.002	0.986 ± 0.005*	1.031 ± 0.006
Glu	1.029 ± 0.005	0.786 ± 0.004	1.057 ± 0.006	0.971 ± 0.004	0.945 ± 0.002	0.788 ± 0.002	0.943 ± 0.006*	1.057 ± 0.008
Lys	1.113 ± 0.005	1.357 ± 0.014	1.257 ± 0.009	0.848 ± 0.007	1.129 ± 0.007	0.945 ± 0.008	1.160 ± 0.008	0.755 ± 0.005

* Amino acid ion was not detected in the MS/MS spectrum and intensity of chiral selector ion was used for calculation of *CR* instead.

3　Conclusion

Four pairs of compounds designed based on scaffolds of proline and 4-hydroxy-α-cyanocynnmaic acid (CHCA) have been synthesized as the chiral selectors. Chiral recognition of these compounds towards 19 common amino acids was evaluated by investigating the ESI-MS/MS spectra of the protonated dimers and trimers formed between the amino acids and the designed compounds. Chiral discrimination was observed for most studied amino acids. Compared with the previous studies, the presence of CHCA in the chiral selectors reduced the chiral recognition ability. Modification to the proline moiety, including replacement of the carboxylic group with *tert*-butyl ester, incorporation of one more proline and replacement of the carboxylic group in the diproline derivatives with amide did not significantly increase the recognition ability of the selector, but caused reversed dissociation preference of some amino acids. These results provide useful information to optimizations of chiral selectors. Further design and synthesis of new chiral selectors and their applications particularly in chiral analysis by MALDI-MS are now in progress.

[References and Notes]

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