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Enantioselective synthesis of an unnatural bipyridyl amino acid and its incorporation into a peptide

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Abstract

The synthesis of a bipyridyl amino acid, 2-amino-3-(4′-methyl-2,2′-bipyridin-4-yl) propanoic acid, is described. A short three step synthesis from commercially available 4,4′-dimethyl-2,2′-bipyridine provides the amino acid in 65% enantiomeric excess (ee). An enzyme-mediated chiral resolution increases the ee to 95% in two additional steps. The amino acid was incorporated into a 22 amino acid peptide composed predominantly of alanine. The peptide was found to be 88% α-helical in aqueous solution at 1°C by circular dichroism (CD) spectropolarimetry, indicating a high helical propensity for this amino acid. This amino acid can provide a means to incorporate a metal into structure-forming peptides.

1. Introduction

There is an enormous interest in the synthesis of unnatural amino acids, especially those that can bind metals. These amino acids are used in the *de novo* design of metalloproteins[1,2] and metallopeptides[3–5] and for the study of electron transfer in peptides.[6,7] They can also be used to modulate the structure of the protein or peptide. Syntheses for both bipyridine[8–10] and phenanthroline[11,12] amino acids have been reported. For bipyridine amino acids, the point of attachment to the bipyridine ring can have profound effects on metal affinity. In particular, attachment through the 6 position of the bipyridine ring leads to steric hindrance and poor metal binding.[12] However, attachment through the 4 or 5 positions allows metals to bind well.[12]

The existing synthesis[9] of a bipyridine amino acid with the attachment through the 4 position of the bipyridine ring requires the relatively arduous formation of the bipyridine ring system from acyclic precursors. Use of the hazardous oxidizing agent, selenium dioxide, is also required. To avoid these difficulties, we have designed a synthesis that utilizes a commercially available bipyridine, 4,4′-dimethyl-2,2′-bipyridine, thus significantly reducing the number of synthetic steps. To enhance the yield of the

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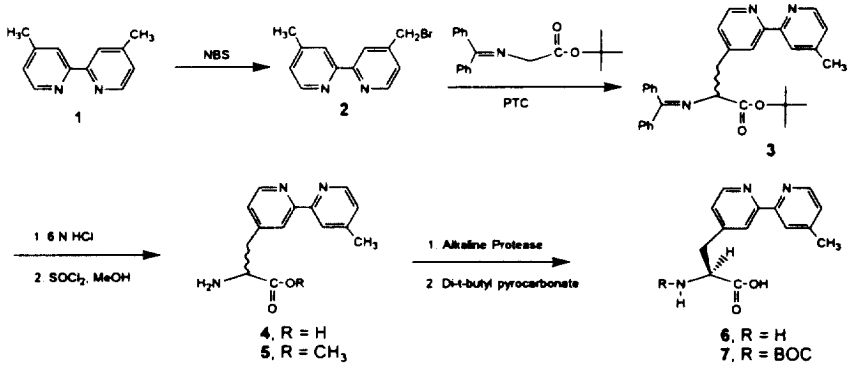
desired L-isomer, a commercially available phase transfer catalyst was used. To achieve % ee adequate for peptide synthesis, an enzyme-based chiral resolution was employed. Once synthesized, the amino acid was incorporated into a peptide.

2. Results and discussion

The synthesis (Scheme 1) originates from commercially available 4,4′-dimethyl-2,2′-bipyridine **1**, which is converted into 4-bromomethyl-4′-methyl-2,2′-bipyridine, as previously reported.[13] The brominated bipyridine **2** was then coupled to *N*-(diphenylmethylene) *tert*-butyl glycinate.[9,14] A phase transfer catalyst (PTC), (8*S*,9*R*)-(−)-*N*-benzylcinchonidinium chloride, was used to stereoselectively enhance the formation of the desired L-isomer, thereby increasing its overall yield.[9] Compound **3** was hydrolyzed with 6N HCl to obtain amino acid **4**. The enantiomeric excess (ee) was determined to be 65%, by using a CrownPak(+) chiral column.



Scheme 1.

The separation of the desired L-isomer from the D-isomer was accomplished by enzyme-mediated resolution.[14] First, the amino acid was converted to the methyl ester **5**.[15] Then, the enzyme, alkaline protease, was used to selectively hydrolyze the L-methyl ester to the L-amino acid **6**.[9,12] The D-methyl ester was separated from the amino acid by extraction with chloroform. The % ee was found to be 95% after resolution.

For peptide synthesis, the amino acid **4** was protected with a *tert*-butyloxycarbonyl (Boc) group. The amino acid was treated with di-*t*-butyl pyrocarbonate, in dioxane and 1N NaOH to obtain the protected amino acid **7**.[16] The NMR spectrum reveals a splitting of the β-proton peaks at 3.31 and 3.45 ppm, indicating restricted rotation about one of the bonds to the β-carbon.

The amino acid was then incorporated into a peptide by solid phase peptide synthesis (SPPS), using Boc/benzyl chemistry.[17] The peptide has a similar design to a set of peptides synthesized by Baldwin and coworkers.[18–20] These peptides form an α-helix in water, due to the high content of alanine, which has a high helical propensity. Our peptide, with the sequence Ac–AKAAAAKAAAABAAAAHAAAHA–NH_2 (A=alanine, K=lysine, H=histidine, B=bipyridine amino acid, Ac=acetyl), was also found to form an α-helix in water. Secondary structure was characterized by circular dichroism (CD) spectropolarimetry. The distinctive double minima at 209 nm and 222 nm (Fig. 1), are indicative of an α-helix. Identical molar ellipticities at 222 nm were obtained for 15 μM and 133 μM solutions, demonstrating that the peptide forms a monomeric helix. Temperature also strongly affects the secondary structure of the peptide. At 25°C, the percent helicity is 48%, as determined by trifluoroethanol titration. The same peptide at 1°C exhibits 88% helicity. Thus, helix formation is enthalpy driven, meaning the peptide is more helical at

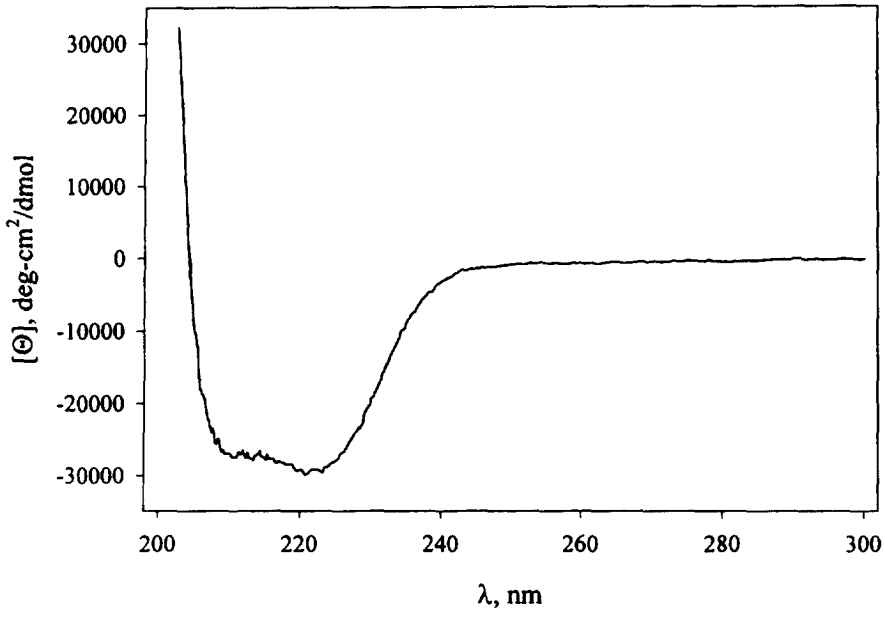


Fig. 1. CD spectrum in the far UV at 25°C of the peptide incorporating amino acid **6**. Peptide concentration is 15 μM. Buffer is 1 mM sodium borate, 1 mM sodium citrate, 1 mM sodium phosphate, 1 M NaCl, pH 7.0 and contains 25% (v/v) TFE

lower temperatures. The degree of helicity found at 1°C is comparable to the 78% helicity found for a similar alanine–lysine peptide studied by Baldwin,[20] suggesting that the helix propensity of this amino acid is similar to that of lysine.

In conclusion, a short, efficient synthesis of 2-amino-3-(4′-methyl-2,2′-bipyridin-4-yl) propanoic acid has been accomplished. It has been incorporated into a peptide, which forms an α-helix in aqueous solution as determined by CD spectropolarimetry. Thus, the amino acid is compatible with the formation of an α-helix. The side chain of this amino acid can be used as a template for introduction of a metal into a peptide. We have recently prepared a metallated form of this amino acid and have incorporated it into a peptide, using SPPS.[21]

3. Experimental

1,4-Dioxane was run down an alumina column (dried in a 110°C oven overnight) to remove any peroxides. Methanol was dried over 3 Å molecular sieves overnight. The rest of the chemicals were used as purchased. ^{1}H NMR spectra were obtained on either a Chemagnetics 200 MHz spectrometer or a Varian Mercury 400 MHz spectrometer, using tetramethylsilane as an internal standard. Peptide purification was performed on a Pharmacia LKB 2248 dual pump HPLC system, with a VWM 2141 detector, using an acetonitrile:water gradient. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was performed on a Kratos MALDI II mass spectrometer, using α-cyanocinnamic acid as the matrix. Flash chromatography was performed with 200–400 mesh silica gel from Aldrich. Optical rotation was measured on a JASCO DIP-4 digital polarimeter.

3.1. N-(Diphenylmethylene)-2-amino-3-(4′-methyl-2,2′-bipyridin-4-yl) tert-butylpropionate 3

N-(Diphenylmethylene) *tert*-butyl glycinate (262 mg, 0.89 mmol) and the chiral phase transfer catalyst, (8*S*,9*R*)-(−)-*N*-benzylcinchonidinium chloride (37 mg, 8.88×10^{-5} M, 10% catalyst) were dissolved in dichloromethane (16 mL). 4-(Bromomethyl)-4′-methyl-2,2′-bipyridine (300 mg, 1.14 mmol) was dissolved in 1 mL of dichloromethane and added to the reaction. Then, a 50% solution of NaOH (1.4 mL) was added. The reaction was stirred overnight at room temperature. After the reaction, the desired product was extracted with dichloromethane (3×15 mL). The organic layers were pooled and dried over $MgSO_4$ and then the solvent was removed. The desired product was purified by flash chromatography (silica, hexanes:ethyl acetate=5:1 with 0.5% TEA, silica TLC: R_f=0.1 for the product). Yield: 83%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.51 ppm (d, J=2.2 Hz, 1H), 8.49 (d, J=2.2 Hz, 1H), 8.18 (s, 1H), 8.10 (s, 1H), 7.60 (bd, 1H), 7.57 (d, J=2.2 Hz, 1H), 7.26 (m, 8H), 6.73 (m, 2H), 4.25 (dd, J=8.8 Hz, J=4.4 Hz, 1H), 3.31 (m, 2H), 2.43 (s, 3H), 1.45 (s, 9H).

3.2. DL-2-Amino-3-(4′-methyl-2,2′-bipyridin-4-yl) propanoic acid 4

Compound **3** (710 mg, 1.48 mmol) was dissolved in 6N HCl (17 mL). The reaction was stirred under reflux for 4 h. After the reaction, the mixture was cooled to room temperature. Then, it was lyophilized overnight to yield a reddish solid. Yield, 100%. ^{1}H NMR (400 MHz, CD_3OD): δ 8.86 ppm (d, J=5.0 Hz, 1H), 8.73 (d, J=6.2 Hz, 1H), 8.72 (s, 1H), 8.60 (s, 1H), 7.91 (d, J=6.2 Hz, 1H), 7.73 (d, J=5.0 Hz, 1H), 4.60 (t, J=6.4 Hz, 1H), 3.56 (dd, ^{3}J=6.4 Hz, ^{2}J=14.4 Hz, 1H), 3.47 (dd, ^{3}J=6.4 Hz, ^{2}J=14.4 Hz, 1H), 2.77 (s, 3H).

After deprotection, the percent enantiomeric excess (% ee) was determined. The mobile phase was a solution of 10% *t*-butanol in 0.1N $HClO_4$. The amino acid was run down a CrownPak(+) chiral column (Daicel) at 0.7 mL/min. The retention times were 9.78 min for the D-isomer and 11.69 min for the L-isomer. By integrating the peaks, it was found that the % ee was 65%.

3.3. DL-2-Amino-3-(4′-methyl-2,2′-bipyridin-4-yl) methyl propionate 5

The amino acid **4** (500 mg, 1.70 mmol) was dissolved in dry MeOH (13 mL). Thionyl chloride (0.73 mL, 10.2 mmol) was carefully added dropwise to the reaction mixture. The flask was capped with a calcium chloride drying tube and was stirred overnight at room temperature. The solvent was removed, methanol was readded and removed.

The crude product was dissolved in dichloromethane (20 mL). The unreacted amino acid was extracted with saturated $NaHCO_3$ (3×15 mL). The solvent was removed, leaving a brown oil. Yield: 60%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.60 ppm (d, J=5.1 Hz, 1H), 8.53 (d, J=5.1 Hz, 1H), 8.28 (s, 1H), 8.22 (s, 1H), 7.16 (m, 2H), 3.85 (dd, J=5.1 Hz, J=7.2 Hz, 1H), 3.73 (s, 3H), 3.18 (dd, ^{3}J=5.1 Hz, ^{2}J=11 Hz, 1H), 2.72 (dd, ^{3}J=7.2 Hz, ^{2}J=11 Hz, 1H), 2.44 (s, 3H).

3.4. L-2-Amino-3-(4′-methyl-2,2′-bipyridin-4-yl) propanoic acid 6

The methyl ester **5** (550 mg, 2.03 mmol) was suspended in 10% *t*-butanol in 0.1M $NaHCO_3$ (62 mL). Then, the enzyme, alkaline protease, was added (30 mg). The reaction mixture was shaken for 1.5 h. The progress of the reaction was checked by HPLC, using the same conditions for determining % ee, until the ratio of D-methyl ester to L-methyl ester was near 50:1 (approximately 90 minutes). The enzyme was deactivated with the addition of chloroform. The D-methyl ester was extracted from the desired L-amino

acid with chloroform (3×25 mL). The aqueous layer was then lyophilized to isolate the L-isomer. The % ee was determined to be 95% using the method described in Section 3.2. $[\alpha]_D^{26}$=+2.9 (c=0.24, in MeOH).

3.5. N-*Boc-l-2-amino-3-(4′-methyl-2,2′-bipyridin-4-yl) propanoic acid* 7

The resolved amino acid **6** (740 mg, 2.88 mmol) and di-*t*-butyl pyrocarbonate (691 mg, 3.16 mmol) were dissolved in dioxane (3.2 mL) and water (6.4 mL). Then, 3.2 mL of 1N NaOH was added. The reaction was stirred at room temperature for 4 h.

After the reaction, the pH was adjusted to between 2 and 3 with 0.2M $KHSO_4$. The desired product was extracted with ethyl acetate (3×15 mL). The organic layers were combined, dried over $MgSO_4$, and the solvent removed. A white, powdery solid remained. Yield: 70%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 ppm (d, J=5.2 Hz, 1H), 8.62 (d, J=5.2 Hz, 1H), 8.23 (s, 1H), 8.14 (s, 1H), 7.22 (m, 2H), 5.29 (d, J=6.4 Hz, 1H), 4.70 (m, 1H), 3.45 (dd, ^{3}J=5.2 Hz, ^{2}J=13.6 Hz, 1H), 3.31 (dd, ^{3}J=4.8 Hz, ^{2}J=13.6 Hz, 1H), 2.47 (s, 3H), 1.45 (s, 9H). Melting point: 192–193.5°C. Anal. calcd for $C_{19}H_{23}N_3O_4$: C, 63.85; H, 6.49; N, 11.76. Found: C, 63.99; H, 6.66; N, 11.52. MALDI-TOF MS: calcd for M+1: 358.4. Found: 358.8. $[\alpha]_D^{24}$=−0.55 (c=0.78, MeOH).

3.6. *Solid-phase peptide synthesis (SPPS)*

The peptide was synthesized using Boc/benzyl chemistry and manual SPPS methods.[17] The completion of each coupling was verified by using the Kaiser test. If there was a positive result to the test, then the residue was recoupled. The peptide was cleaved from the resin with HF and purified by HPLC on a preparatory C18 reversed-phase column, using a water/acetonitrile gradient. The purity of the sample was verified by running the sample through an analytical C18 reversed-phase column. Peptide analytical data: MALDI-TOF MS: calcd (M+1): 2037.1. Found: 2036.7. Amino acid analysis: expected: A, 17; K, 2; H, 2; B, 1. Found: A, 17.004; K, 1.959; H, 2.037; B, 0.151. The bipyridine amino acid decomposes under amino acid analysis conditions, similar to tryptophan.

3.7. *Circular dichroism (CD) and 2,2,2-trifluoroethanol (TFE) titrations*

A Jasco J500C spectropolarimeter was used to determine the ellipticity of the peptide. TFE is an organic solvent that has been found to induce helical structure in peptides.[20] The titrations were performed by increasing the concentration of TFE, at either 1°C or 25°C, in 5% increments until the ellipticity had clearly leveled off. Peptide concentrations were maintained at either 15 μM or 133 μM during the titrations. The spectropolarimeter was calibrated with D-10-camphorsulfonic acid (222 μM).[22]

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