Synthesis and Conformational Study of Ser and Cys Derivatives of N-Hydroxy Diketopiperazine

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Received September 6, 2007

N-Hydroxy diketopiperazine derivatives from L- and D-forms of Ser and Cys were synthesized for the first time, and the ring conformation of both analogues in water was determined to be close to flat via NMR and \textit{ab initio} calculations. However, the side chain of the Ser analogue was oriented toward a pseudo-equatorial position while that of the Cys analogue was slightly oriented toward a pseudo-axial direction, with a slightly distorted boat-shaped ring conformation. Among them, the D-Cys analogue was found to be a weak inhibitor of \(\alpha\)-glucosidase.

**Key Words:** N-Hydroxy diketopiperazine, Serine, Cysteine, Conformation, Glucosidase

**Introduction**

2,5-Diketopiperazines, known as cyclic dipeptides, are frequently formed from both the enzymatic and nonenzymatic processes of peptides/proteins. They are also well-known as by-products that are formed during removal of the temporal protecting group of the second amino acid. Many cyclic dipeptides are found in nature and their derivatives show interesting biological activities, including antiviral, antifungal, antitumour, antimicrobial, and antibacterial properties. N-Hydroxylation of diketopiperazines is a known biological process in the protist and plant kingdoms during the syntheses of iron chelators and dehydro amino acids. Natural products like aspergillic acid, pulcherriminic acid, and sarcodonin contain several structural features of N-hydroxy diketopiperazine and it is likely that they are the oxidized metabolites or intermediates of diketopiperazine derivatives.  

Although various syntheses of racemic N-hydroxy diketopiperazine derivatives have been studied, only a few syntheses of asymmetric N-hydroxy analogues have been reported. More recently, M. Akiyama et al. described a convenient synthesis and the spectrocopropic properties of N-hydroxy diketopiperazines derived from L-forms of Ala, Val, Pro, Phe, and Asp. Early studies, however, revealed that handling these types of compounds is a nontrivial process with low cyclization yields. In addition, the purification of a highly polar hydroxamate with a hydrophilic side chain is a problematic step for the synthesis.

Here we report the first synthesis of N-hydroxy diketopiperazine analogues from L- and D-forms of Ser and Cys, respectively. Nothing has been reported, so far, regarding the synthesis of Ser and Cys analogues or their biological activities. We also determined the preferred conformation via NMR and \textit{ab initio} calculations and evaluated inhibitory activities against \(\alpha\)-glucosidase because there are some structural similarities between hexose and N-hydroxy diketopiperazine, especially from Ser or Cys (Fig. 1). The conformational study of the new N-hydroxy diketopiperazines may provide us some background of understanding biological activities. In a study with implications for our compounds, Nishimura’s group reported the synthesis and conformational analysis of eight isomers of D-glycono-\(\delta\)-lactam (Fig. 1). They found that five isomers, including three with boatlike conformations, exhibited inhibitory activities against \(\beta\)-glucosidase.

**Results and Discussion**

**Synthesis.** In our synthetic efforts, we first tried the

[Scheme 1: Synthesis of protected N-hydroxy diketopiperazine derivatives from 1-Ala and 1-Ser.]
following strategy (Scheme 1). Starting from Boc-protected L-Ala and L-Ser, we prepared the benzylamide 1a and 1b, respectively. After removal of the Boc group, bromoacetic acid was attached to give 2a and 2b with yields of about 50%. Intramolecular cyclization of the compounds 2a and 2b under various basic conditions gave the desired products, 3a and 3b, in below 50% yields with decomposed byproducts. Since the yields of the two reactions were unsatisfactory, we modified the synthetic scheme to utilize the cyclization precursors, 4b and 4c (Scheme 2).

Selective mono-N-alkylation of the benzylamide compounds 1b and 1c was successful, giving yields of 64 to 66%. After removal of the Boc-protecting group, the crude product was cyclized under basic conditions to give the diketopiperazine backbones 3b and 3c. Deprotection of the benzyl group from the Ser analogue 3b was accomplished using Pd/C, H2, and MeOH in the presence of 2 N HCl. Under the same conditions but without HCl, the monodebenzylation product on the N-OH group was obtained. For the Cys derivative 3c, no product was obtained. By applying Li in liquid NH3 to the Cys derivatives, debenzylation was complete based on NMR analysis of the crude reaction mixture, but purification step was problematic. Temporary protection of the crude product with Boc group followed by silica gel purification gave the pure bis-protected 5c. However, deprotection of the Boc group under acidic condition (10% TFA in CH2Cl2) gave a mixture of desired product and unidentified product having same molecular mass. The pure product 5c could be obtained only with RP-HPLC purification under an elution of 0.1% TFA in H2O.

Since the configuration of the stereocarbon of the products 5b and 5c was opposite to the corresponding natural carbohydrates, D-forms of Ser and Cys were subjected to the same reactions shown in Scheme 2. The yields of the reactions were more or less the same as in the reactions of the L-forms of Ser and Cys derivatives. The proton NMR data of the enantiomers obtained from L- and D-amino acids were exactly same.

Conformation. Three conformations are known to exist for the diketopiperazine ring and the N-hydroxy diketopiperazine ring, depending on the folding angle $\beta$, a flat ring conformation ($\beta = 0$), a boat-shaped conformation with a pseudo-axial side chain ($\beta < 0$), and a boat-shaped conformation with a pseudo-equatorial side chain ($\beta > 0$). The preferred conformations of the two compounds, 5b and 5c, were determined from ab initio calculations with the Gaussian03 package. After generating the conformations of both equatorial and axial side chains with a molecular mechanics-based force field, the ab initio structure optimization was carried out with the MP2/6-31G(d) level in a vacuum. The resulting structures were further optimized in the DMSO solvation environment (dielectric constant $\varepsilon = 47.6$) with the polarized continuum model (IEFPCM) using the MP2/6-31+(d) level. We also repeated the same calculation with the B3LYP/6-31G+(d) level using the CPCM solvation model. This time, the molecule was in the water (dielectric constant $\varepsilon = 80$) environment and the hydrogen atom at N-OH was removed in consideration of the likely protonation state. As far as the structure was concerned, the lowest energy conformations from two different calculations were essentially the same, with a slightly distorted flat ring as shown in Figure 2. The folding angle $\beta$ was 5.3 for the Ser analogue 5b and -5.8 for the Cys analogue 5c. These results implied that the side chain of 5b was close to the pseudo-equatorial position, while the side chain of 5c was closer to the pseudo-axial orientation. Regarding the rotation of side chains, the energy differences among the three rotamers were calculated to be less than 1 kcal/mol.

A NMR study was performed to obtain information on solution conformation. Magnetic nonequivalence for the Gly-CH2 protons of the two analogues was an indication that
the ring had a rigid conformation, but distinct differences between the two compounds could not be found (Table 1). In the 2D-NMR study, ROE cross peaks between the two residues (H or H' and Hb or Hb') were not obtained. Temperature coefficients of the Ser analog in DMSO-d6 was determined to give similar values (4.6 × 10⁻⁵ ppm/K for N-OH, 4.8 × 10⁻⁵ ppm/K for N-H, 4.4 × 10⁻³ ppm/K for O-H), indicating that no intramolecular H-bonded or shielded polar protons are present.

We evaluated the inhibitory activities of synthesized N-hydroxy diketopiperazines against rice α-glucosidase. The activities of L-Cys analogue 5c and its D-Cys analogue enant (5d) were very weak with IC₅₀ values of 220 and 100 μM respectively, while the analogues of L- and D-Ser have no activity. The reason is probably due to the conformational difference from half-chair geometry and the lack of a positive charge at the ring oxygen position, mimicking the transition state of an enzyme-substrate complex.

**Conclusion**

We prepared new N-hydroxy diketopiperazine derivatives from L- and D-forms of Ser and Cys in satisfactory yields.

The ring conformation of both analogues in water was determined to be close to flat via NMR and ab initio calculations. However, the side chain of the Ser analogue was oriented toward a pseudo-equatorial position while that of the Cys analogue was slightly oriented toward a pseudo-axial direction, with a slightly distorted boat-shaped ring conformation. Among the compounds we prepared, the D-Cys analogue exhibited very weak activity against rice α-glucosidase.

**Experimental Section**

**General procedures and methods.** All reagents were obtained from commercial suppliers and used without purifications unless specified. ¹H and ¹³C-NMR spectra were collected on a Bruker AVANCE II 500 spectrometer at resonance frequencies of 500.1 MHz and 125.7 MHz, respectively. Solvents used were CDCl₃ and D₂O. The chemical shifts were reported in ppm from tetramethylsilane and the resonance frequencies of 500.1 MHz and 125.7 MHz, respectively. Solvents used were CDCl₃ and D₂O.

**Table 1.** Proton chemical shifts and coupling constants for 5b and 5c in D₂O

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Gly residue*</th>
<th>Other residue*</th>
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<tbody>
<tr>
<td>5b</td>
<td>4.34, 4.20 (17.2)</td>
<td>4.11, 3.89, 3.68</td>
</tr>
<tr>
<td>5c</td>
<td>4.46, 4.27 (17.2)</td>
<td>4.43, 3.06, 2.85</td>
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*H, H' denote two α-protons of Gly; Hb, Hb' denotes α-proton, and Hb and Hb' denote two β-protons of Ser or Cys.

(2-Benzoyloxy-1-benzoylcarbamoyl-ethyl)-carbamic acid tert-butyl ester (1b)

**L-Form:** A mixture of Boc-L-Ser(Bzl)-OH (2.4 g, 8.1 mmol), DCC (1.5 g, 7.5 mmol), and HOBt (0.19 mg, 1.3 mmol) was stirred in CH₂Cl₂ (100 mL) at 0 °C for 15 min. Subsequently, BnONH₂·HCl (1.0 g, 6.2 mmol) and DIPEA (2.3 mL, 13.2 mmol) were added. After 1 h at 0 °C, the mixture was stirred at room temperature for 17 h. The precipitated urea was filtered off and the solution was concentrated in vacuo. The residue was dissolved in EtOAc and the precipitated urea was filtered off again. The organic layer was washed with saturated aqueous NaCl solution and dried with MgSO₄. The solvent was removed in vacuo and the crude product was purified by chromatography on silica gel column (3:1 hexane/EtOAc) to give a white solid. Yield: 2.14 g (88%). ¹H NMR (500 MHz, CDCl₃): δ 8.93 (bs, 1H), 7.33-7.21 (m, 10H), 5.30 (bs, 1H), 4.87 (s, 2H), 4.49, 4.44 (ABq, J = 11.5 Hz, 2H), 4.22 (bs, 1H), 3.79-3.78 (m, 1H), 3.50-3.47 (m, 1H), 1.40 (s, 9H); ¹³C NMR (125.76 MHz, CDCl₃): δ 168.15, 155.34, 137.13, 135.02, 129.14, 128.66, 128.49, 128.44, 127.89, 127.70, 80.34, 78.21, 73.38, 69.38, 52.11, 28.21; HRMS (FAB) m/z calculated for C₂₉H₂₅N₂O₇ [M+H]⁺, found 401.2080.

**D-Form:** By applying the same procedure described above, starting with Boc-D-Ser(Bzl)-OH (2.4 g, 8.1 mmol), the enantiomer of 1b was obtained. Yield: 2.18 g (88%). ¹H NMR spectrum was the same as for compound 1b.
acid tert-butyl ester (1c)

1-Form: By using a similar procedure as described above, starting with 2.6 g of Boc-L-Cys(Bzl)-OH, the desired compound was obtained as a white solid. Yield: 2.15 g (82%). 1H NMR (500 MHz, CDCl3): δ 9.14 (bs, 1H), 7.38-7.22 (m, 10H), 5.29 (bs, 1H), 4.89 (s, 2H), 4.10 (bs, 1H), 4.49, 4.44 (ABq, J = 11.5 Hz, 2H), 2.77-2.67 (m, 2H), 1.42 (s, 9H); 13C NMR (125.76 MHz, CDCl3): δ 168.15, 155.34, 137.13, 137.02, 129.14, 128.86, 128.49, 128.44, 127.89, 127.70, 80.34, 78.21, 73.38, 69.38, 52.11, 82.57; HRMS (FAB) m/z calculated 487.2444 for C25H22N2O3[M+H]+, found 487.2455.

D-Form: By applying the same procedure described above, starting with Boc-D-Cys(Bzl)-OH (2.6 g, 8.1 mmol), the enantiomer of 1c was obtained. Yield: 2.23 g (85%). 1H NMR spectrum was same as for compound 1c.

By applying the same procedure described above, starting with 1 g of 4c, the desired compound was obtained as a white solid. Yield: 0.53 g (75%). 1H NMR (500 MHz, CDCl3): δ 8.11 (s, 1H), 7.38-7.22 (m, 10H), 5.29 (bs, 1H), 4.89 (s, 2H), 4.10 (bs, 1H), 4.49, 4.44 (ABq, J = 11.5 Hz, 2H), 2.77-2.67 (m, 2H), 1.42 (s, 9H); 13C NMR (125.76 MHz, CDCl3): δ 168.15, 155.34, 137.13, 137.02, 129.14, 128.86, 128.49, 128.44, 127.89, 127.70, 80.34, 78.21, 73.38, 69.38, 52.11, 82.57; HRMS (FAB) m/z calculated 487.2444 for C25H22N2O3[M+H]+, found 487.2455.

D-Form: By applying the same procedure described above, starting with the enantiomer of 4b, the enantiomer of 3b was obtained. Yield: 0.57 g (80%); [α]D25 = -1.9 (c = 0.1 in MeOH). 1H NMR spectrum was same as for compound 3b.

By applying the same procedure described above, starting with the enantiomer of 4c, the enantiomer of 3e was obtained. Yield: 0.55 g (77%); [α]D25 = +8.4 (c = 0.1 in MeOH). 1H NMR spectrum was same as for compound 3e.

1-Hydroxy-3-benzylsulfonylmethyl-piperazine-2,5-dione (5b)

L-Form: Compound 3b (200 mg, 0.58 mmol) was stirred in 2 N HCl (0.2 mL) and methanol (10 mL) at room temperature for 30 min with 10% Pd/C (20 mg) under an atmosphere of H2 balloon. After filtration over celite and concentration under reduced pressure, the product was recrystallized in ether/MeOH to give a white solid. Yield: 88 mg (93%). 1H NMR (500 MHz, D2O): δ 4.34, 4.20 (ABq, J = 13.1 Hz, 2H), 2.77-2.67 (m, 2H), 1.42 (s, 9H); 13C NMR (125.76 MHz, D2O): δ 167.37, 163.08, 62.85, 56.69, 52.57; HRMS (FAB) m/z calculated 357.1273 for C14H13N2O3S[M+H]+, found 357.1293; [α]D25 = +16.4 (c = 0.14 in MeOH).

D-Form: By applying the same procedure described above, starting with the enantiomer of 3b, the enantiomer of 5b was obtained. Yield: 83 mg (90%); [α]D25 = -22.8 (c =
0.14 in MeOH). \(^1H\) NMR spectrum was same as for compound 5b.

1-Hydroxy-3-mercaptopethyl-pipeazine-2,5-dione (5c)

\(1\text{-Form: NH}_3\) (30 mL) was collected into a solution of 3c (200 mg, 0.53 mmol) in THF (10 mL) at \(-78^\circ\text{C}\). Li (18.4 mg, 2.7 mmol) was added and stirred until a deep blue color was obtained. The reaction mixture was stirred for about 20 min until the deep blue color disappeared and then allowed to warm to RT. \(\text{N}_2\) was blown over the solution until it reached dryness. The yellowish-white solid was dissolved in methanolic \(\text{HCl}, \text{evaporated in vacuo}\) and then purified by using semi-preparative RP-HPLC with an isocratic elution of 0.1% TFA in \(\text{H}_2\text{O}\) over 15 min to give a white solid. (Flow rate = 3 mL/min, RI-detector, \(t_{R} = 6.2\) min). Yield: 56 mg (60%). \(^1\text{H}\)-NMR (500 MHz, D\(2\text{O}\)): \(\delta\) 4.46, 4.27 (ABq, \(J = 17.2\) Hz, 2H), 4.43 (bs, 1H), 3.06 (dd, 1H, \(J = 2.6\) Hz, 14.7 Hz); \(^13\text{C}\)-NMR (125.76 MHz, D\(2\text{O}\)): \(\delta\) 167.27, 162.87, 55.60, 52.66, 28.88; HRMS \([\text{M}+\text{H}]^+\), found 241.14 in MeOH).

\(2\text{-Form:}\) By applying the same procedure described above, starting with the enantiomer of 3c, the enantiomer of \(5c\) was obtained. Yield: >90% by NMR; \(\alpha_{D}^{25} = -2.4\) (c = 0.1 in MeOH). \(^1\text{H}\) NMR spectrum was same as for compound 5c.

**Enzyme assay.** Inhibition activity was assayed at \(37^\circ\text{C}\) in a total volume of 150 mL containing 0.33 mM maltose and 0.03 unit/mL of \(\alpha\text{-glucosidase.}\) After incubation for 15 min, the reaction was stopped by heating at 100 °C for 3 min. At this point, 100 mL of glucosidase oxidase/peroxidase (\(\alpha\text{-dianisidin}\) reagent (Sigma) was added to each tube and the reaction was carried out exactly 30 min at \(37^\circ\text{C}\). After quenching the enzyme reaction by adding 100 mL of \(\text{H}_2\text{SO}_4\) solution, absorbance at 505 nm was measured to determine the amount of \(\alpha\text{-glucose}\) released. Data interpretation by Grafit 4.0 gave us IC\(_{50}\) values to evaluate the concentration needed to inhibit 50% of \(\alpha\text{-glucosidase}\) activity.

**Acknowledgments.** This work was supported by grants No. R01-2000-000-00049-0 from the Basic Research Program of the Korea Science & Engineering Foundation and MarineBio21, Ministry of Maritime Affairs and Fisheries, Korea.

**References**