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Synthesis of Cross-linkable 2,5-Diketopiperazine Derivatives.

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Abstract: Synthesis of cross-linkable diketopiperazine derivatives is described. Cross-linkable α-amino acid methyl esters were subjected to peptide synthesis with Boc-protected glycine or L-tyrosine. No protections of cross-linkable functional groups (catechol and phenylazide) are necessary for constructions of diketopiperazine skeleton.

Keywords: cross-link, cyclic dipeptide, diketopiperazine, DOPA, phenylazide, photoaffinity label

1. INTRODUCTION

2,5-Diketopiperazine (2,5-DKP) scaffolds are core templates for numerous bioactive compounds [1]. Stereo-controlled intramolecular cyclization of dipeptide is one of the effective ways to construct 2,5-DKP skeletons [2-5]. Cross-link between bioactive compounds and their target biomolecules are attractive methods to elucidate molecular mechanism of bioactivity. DOPA, which has catechol (o-hydroxyphenol) moiety, can be utilized as a cross-linkable α-amino acid in the presence of periodate [6-8]. Two examples for DOPA containing 2,5-DKP derivatives have been reported previously. First is enzymatic hydroxylation of cyclo(Tyr-Tyr) in cultured cell to afford cyclo(DOPA-DOPA) [9]. The another one is condensation of all hydroxyl groups protected Tyr and DOPA followed by deprotection to afford cyclo(Tyr-DOPA) [10]. The protections of phenolic and catechol moieties are conducted to prevent the side reactions. To date, diketopiperazine formations without unprotected catechol moiety have not been reported yet. Photoaffinity labeling is the most attractive method for creating a crosslinking method between ligand and biomolecule interactions [11, 12]. Photoreactive aromatic α-amino acid derivatives have been used to investigate bioactive peptides interactions [13-17]. Although aryldiazides are one of the common photophores for the photoaffinity labeling but no description of 2,5-DKP derivatives has been reported. In this report, we aim to describe the novel synthesis of cross-linkable 2,5-DKP derivatives, based on glycine or tyrosine with catechol and phenylazide, which can be utilized to elucidate their biological activity.

2. RESULTS AND DISCUSSIONS

L-DOPA 1 is converted to corresponding methyl ester treated with thionyl chloride in methanol at room temperature [18]. L-DOPA-OMe hydrochloride 2 was condensed with Boc-Gly 3 in the presence of DCC in acetonitrile and DMF at 0 °C. The dipeptide was purified with silica column chromatography. Boc-Gly-L-DOPA-OMe 4 was subjected to deprotection of Boc group followed by intramolecular cyclization in the presence of AcOH and N-methylmorpholine in 2-BuOH under reflux for several hours [2]. The reaction mixture became suspension and the precipitates was washed with methanol for several times to purify cyclo(Gly-L-DOPA) 5 with moderate yield. During the course of the synthesis, protection of catechol hydroxyl groups were not necessary to form 2,5-DKP skeleton. Boc-L-Tyr 6 was treated with L-DOPA-OMe hydrochloride 2, followed by treatment of dipeptide 7 with identical manner described above, afforded cyclo(L-Tyr-L-DOPA) 8 with moderate yield.

Figure 1. Synthesis of 2,5-DKP containing L-DOPA

Different protecting groups for L-Tyr and L-DOPA have been utilized in the previous synthesis of compound 8 [10] to inhibit byproduct formations via hydroxyl groups. But our results indicated that no protection of phenolic and catechol
hydroxyl group was essential to construct 2,5-DKP derivatives (Figure 1).

Photophore containing derivatives, L-Phe(4-N₃) 9 [19], was converted corresponding methyl ester hydrochloride 10 with thionyl chloride in methanol at room temperature. Compound 10 was subjected to peptide coupling with Boc-Gly 3 or Boc-L-Tyr 6 in the presence DCC in acetonitrile and DMF. The dipeptides 11 and 13 were deprotected under acidic condition and intramolecular cyclization in aforementioned conditions to 2,5-DKP derivatives 12 and 14, respectively. IR measurements of compound 12 and 14 indicated that azide moiety was not influenced by synthetic conditions.

![Diagram of synthesis of 2,5-DKP containing L-Phe(4-N₃)](image)

Many diketopiperazines are dissolved in acidic media. The cyclo(Gly-Phe(4-N₃)) was easily soluble in trifluoroacetic acid-d to measure NMR spectrum [3]. But the compound 12 was decomposed during NMR measurement and this decomposition was still continued even though the sample was decomposed at -20 °C for several days. The 1H-NMR analysis indicated that the compound 12 was decomposed completely after 70 hours at -20 °C (Table 1). The acidic solution of 12 was concentrated and subjected to IR spectrometry. The azide moiety was decomposed under the treatment. In spite of this result, DOPA containing diketopiperazine 8 was stable in trifluoroacetic acid at room temperature over 24 hours. The result consistent with our previous result, that the arylazide moiety was unstable under strong acid conditions [20]. Finally, compound 12 in 1% DCl-D₂O is subjectable to ¹H- and ¹³C-NMR measurement without decomposition of azide moiety.

### 4. EXPERIMENTAL

**General** FTIR spectra were recorded on a JASCO FT-IR 4100 spectrometer. ¹H (270 MHz) and ¹³C NMR (67.5 MHz) spectra were recorded on a JEOL EX-270 spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter.

**5-(5)-Methyl 2-amino-3-(3,4-dihydroxyphenyl)propanoate hydrochloride (L-DOPA-OMe, 2)** Thionyl chloride (13.5 mL, 184 mmol) was added dropwise to a solution of L-DOPA 1 (4.02 g, 20.4 mmol) in methanol (100 mL) at 0 °C. After 21 hours at room temperature, the solvent was evaporated, and the residue was concentrated to afford pale yellow mass (5.01 g, 99%). ¹H NMR (CD₃OD): δ 3.00 (dd, J = 14.5, 7.3 Hz, 1H), 3.11 (dd, J = 14.5, 5.9 Hz, 1H), 3.82 (s, 3H), 4.21 (dd, J = 7.3, 5.9 Hz, 1H), 6.55 (dd, J = 8.2, 2.0 Hz, 1H), 6.66 (d, J = 2.0 Hz, 1H), 6.75 (d, J = 8.2 Hz, 1H); ¹³C NMR (CD₃OD): 36.7, 53.6, 55.4, 116.9, 117.3, 121.9, 126.3, 146.1, 146.7, 170.4; HRMS (ESI): calcd. for C₁₀H₁₄NO₄ \((M⁺H)²⁺\): 212.0928, found 212.0923; [α]₀̣ +40.6 (c 5.0, CH₃OH).

**5-(5)-Methyl 2-(2-((tert-butoxycarbonyl)amino)acetamido)-3-(3,4-dihydroxyphenyl)propanoate (Boc-Gly-L-DOPA-OMe, 4)** A solution of L-DOPA methyl ester hydrochloride 2 (1.02 g, 4.11 mmol) and N-(tert-butoxycarbonyl)-glycine 3 (0.72 g, 4.11 mmol) in DMF (15 mL) and acetonitrile (60 mL) was cooled in ice. With stirring, triethylamine (0.5 mL, 3.6 mmol) was added followed by dicyclohexylcarbodiimide (0.89 g, 4.33 mmol). After 5 h of continued stirring at 0 °C, the reaction mixture was placed in the freezer for overnight. The insoluble material was filtered off and washed with ethyl acetate. The combined filtrate was evaporated in vacuo leaving a gummy residue, which was taken up in ethyl acetate (100 mL) and water (40 mL). The organic layer was washed successively with 40 mL portions of 0.5 N HCl, H₂O, 0.5 N NaHCO₃ and brine. The organic layer was washed with ethyl acetate. The combined filtrate was evaporated in vacuo leaving a gummy residue, which was taken up in ethyl acetate (100 mL) and water (40 mL). The organic layer was washed successively with 40 mL portions of 0.5 N HCl, H₂O, 0.5 N NaHCO₃ and brine. The organic layer was dried over MgSO₄ and the filtrate was evaporated. The residue was subjected to silica column chromatography (ethyl acetate/hexane = 1:1, 3:2 then 2:1) to afford a colorless amorphous mass (0.91 g, 60%). ¹H NMR (CDCl₃): δ 1.44 (s, 9H), 2.93 (dd, J = 14.0, 5.4 Hz, 1H), 3.05 (dd, J = 14.0, 4.1 Hz, 1H), 3.72 (s, 3H), 3.77 (d, J = 5.6 Hz, 2H), 4.78 (m, 1H), 5.53 (m, 1H), 6.45 (dd, J = 7.9 Hz, 1.6 Hz, 1H), 6.59 (d, J = 1.6 Hz, 1H), 6.73 (d, J = 7.9 Hz, 1H), 6.86 (br d, J = 8.2 Hz, 1H); ¹³C NMR (CDCl₃): 62.283, 36.7, 44.0, 52.5, 53.4, 81.0, 115.3, 116.7, 121.2, 127.3, 143.7, 143.8, 156.5, 169.8, 172.0; HRMS (ESI): calcd. for C₁₇H₂₅N₂O₇ (M⁺H): 369.1662, found 369.1667; [α]₀̣ +9.8 (c 2.0, CH₃OH).

### Table 1. Stability of cyclo(Gly-L-Phe(4-N₃)) 12 in trifluoroacetic acid-d at -20 °C

<table>
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<th>Time (h)</th>
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<tr>
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<tr>
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<td>24</td>
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<tr>
<td>50</td>
<td>14</td>
</tr>
<tr>
<td>70</td>
<td>0</td>
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*Compound 12 (5 mg) was dissolved in trifluoroacetic acid-d (0.5 mL). The proportion of residual 12 was calculated from ¹H-NMR.*
The reaction mixture was cooled at -20 °C overnight. The precipitates were centrifuged and washed with methanol to afford colorless amorphous mass (0.34 g, 58%). 1H NMR (CDCl3): δ 1.34 (s, 3H), 2.67 (m, 1H), 2.90 (m, 3H), 3.35 (s, 3H), 3.66 (s, 3H), 4.05 (t, J = 7.9 Hz, 1H), 4.68 (d, J = 7.9 Hz, 1H), 6.62 (s, 1H), 6.69 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 8.6 Hz, 2H). 13C NMR (CDCl3): δ 28.6, 37.9, 38.4, 52.6, 55.2, 57.5, 80.7, 116.1, 116.3, 117.2, 121.7, 129.1, 131.1, 123.1, 143.5, 146.2, 157.1, 173.2, 174.2; HRMS (ESI): calcd. for C17H24N5O5 (M++H) 378.1777, found 378.1771; ν(C=O) 2136 cm⁻¹.

(S)-Methyl 2-amino-3-(4-azidophenyl)propanoate hydrochloride (L-Phe(4-N3)-OMe, 10) A solution of Phe(4-N3) methyl ester hydrochloride (10 (0.45 g, 1.77 mmol) and N-(tert-butauxcarbonyl)glycine (3) (0.32 g, 1.84 mmol) in DMF (3.5 mL) and acetonitrile (14 mL) was cooled in ice. With stirring, triethylamine (0.25 mL, 1.79 mmol) was added followed by dicyclohexylcarbodiimide (0.25 g, 1.11 mmol). After 5 h of continued stirring at 0 °C, the reaction mixture was warmed to room temperature, then concentrated to afford colorless amorphous mass (0.45 g, 71%). 1H NMR (CD3OD): δ 1.54 (s, 3H), 3.50 (d, J = 8.6 Hz, 2H), 7.40 (d, J = 8.6 Hz, 2H); 13C NMR (CD3OD): δ 53.6, 55.1, 120.6, 130.2, 132.1, 141.2, 170.3; HRMS (ESI): calcd. for C17H24N5O5 (M++H) 378.1777, found 378.1771; [α]D +55.8 (c 1.0, CHCl3); IR (solid): ν(C=O) 2123 cm⁻¹.
119.8, 131.4, 133.8, 139.4, 167.4, 175.0; HRMS (ESI): calcd. for C_{11}H_{12}N_{5}O_{2} (M+H) 246.0991, found 246.0988; [α]_{D}^{20} +58.4 (c 1.0, 1% HCl); IR (solid): ν 2125 cm\(^{-1}\).

(S)-Methyl 3-(4-azidophenyl)-2-((S)-2-((tert-butoxycarbonyl)amino)-3-(4-hydroxybenzyl)carbonyl)amino)-3-(4-hydroxybenzyl)propanamido)-propanoate (Boc-L-Tyr-L-Phe(4-N\(_{3}\))-OMe, 13) A solution of Phe(4-N\(_{3}\)) methyl ester hydrochloride 10 (0.45 g, 1.75 mmol) and N-(tert-butoxycarbonyl)-L-tyrosine 6 (0.51 g, 1.80 mmol) in DMF (3.5 mL) and acetonitrile (14 mL) was cooled in ice. With stirring, triethylamine (0.25 mL, 1.79 mmol) was added followed by dicyclohexylcarbodiimide (0.37 g, 1.81 mmol). After 2 h stirring at 0 °C, the reaction mixture was refluxed for three hours. After cooling, the precipitates were collected with centrifuge and washed with methanol to afford colorless amorphous mass (0.64 g, 30%). 1H NMR (DMSO-d\(_{6}\)): δ 1.42 (s, 9H), 2.93 (d, J = 6.3 Hz, 2H), 3.02 (t, J = 6.6 Hz, 1H), 3.07 (m, 1H), 3.87 (m, 1H), 3.97 (m, 1H), 6.72 (d, J = 8.6 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 7.00 (d, J = 7.9 Hz, 2H), 7.09 (d, J = 7.9 Hz, 2H); 13C NMR (DMSO-d\(_{6}\)): δ 28.2, 37.3, 37.4, 52.4, 53.3, 56.1, 80.6, 115.6, 119.1, 127.7, 130.4, 130.6, 132.3, 138.9, 155.3, 155.5, 171.1, 171.2; HRMS (ESI): calcd. for C\(_{25}\)H\(_{30}\)N\(_{5}\)O\(_{6}\) (M+H) 484.2196, found 484.2195; [α]_{D}^{20} +28.9 (c 3.0, CHCl\(_{3}\)); IR (solid): ν 2130 cm\(^{-1}\).

CONCLUSION
It is well known that 2,5-DKP skeltons were pivotal roles for many bioactivities, but the derivatives for tools to elucidate their bioactivities have been reported yet. The cross-linkable 2,5-DKP derivatives described in this report will be utilized for this purpose.

CONFLICT OF INTEREST
The authors confirm that this article content has no conflict of interest.

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REFERENCES