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<td>Author(s)</td>
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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 a-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 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.

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 chroide 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.

\[
\text{N}_3
\]

\[
\text{COOH}
\]

\[
\text{rt}
\]

\[
\text{MeOH}
\]

\[
\text{BocHN}
\]

\[
\text{H}_2\text{N}
\]

\[
\text{SOCl}_2
\]

\[
\text{N}_3
\]

\[
\text{H}_2\text{N}
\]

\[
\text{COOMe}
\]

\[
\text{Boc-Gly 3}
\]

\[
\text{or}
\]

\[
\text{Boc-L-Tyr 6}
\]

\[
\text{TEA, DCC}
\]

\[
\text{DMF, CH}_3\text{CN}
\]

\[
\text{1H- and 13C- NMR measurement without decomposition of azide moiety.}
\]

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

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Residual 12 (%)</th>
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<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>58</td>
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<tr>
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<td>26</td>
<td>24</td>
</tr>
<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.

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.

\((S)-\text{Methyl 2-aminoo-3-(3,4-dihydroxyphenyl)propanoate hydrochloride (L-DOPA-OMe, 2)}\)

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, \(J = 14.5, 7.3 \text{ Hz, } 1\text{H})\), 3.31 (dd, \(J = 14.5, 5.9 \text{ Hz, } 1\text{H}\)), 3.82 (dd, \(J = 8.2, 2.0 \text{ Hz, } 1\text{H}\)), 6.55 (dd, \(J = 8.2, 2.0 \text{ Hz, } 1\text{H}\)). \(^13C\) NMR (CD₃OD): 36.7, 53.6, 55.4, 116.9, 117.3, 121.9, 126.3, 143.7, 143.8, 156.5, 161.0 ppm. Optical rotations were measured on a JASCO DIP-370 polarimeter.

\((S)-\text{Methyl 2-(2-((tert-butoxycarbonyl)amino)acetamido)-3-(3,4-dihydroxyphenyl)propanoate (Boc-Gly-L-DOPA-OMe, 4)}\)

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 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, \(J = 14.0, 5.4 \text{ Hz, } 1\text{H})\), 3.05 (dd, \(J = 14.0, 4.1 \text{ Hz, } 1\text{H})\), 3.72 (s, 3H), 4.21 (dd, \(J = 7.3, 5.9 \text{ Hz, } 1\text{H})\), 6.55 (dd, \(J = 8.2, 2.0 \text{ Hz, } 1\text{H}\)), 6.66 (d, \(J = 2.0 \text{ Hz, } 1\text{H}\)), 6.75 (d, \(J = 8.2 \text{ Hz, } 1\text{H}\)). \(^13C\) NMR (CDCl₃): 36.7, 53.6, 55.4, 116.9, 117.3, 121.9, 126.3, 143.7, 146.1, 146.7, 170.4 ppm. HRMS (ESI): calcd. for C₁₀H₁₄NO₄ (M⁺+H): 212.0923, found 212.0928; [\(α\)]D +9.8 (c 2.0, CH₃OH).

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 stored at -20 °C for several days. The \(^1H\)-NMR analysis
(S)-3-(3,4-Dihydroxybenzyl)piperazine-2,5-dione  
(cyclo(Gly-L-DOPA), 5) Boc-Gly-L-DOPA-OMe 4 (0.36 g, 0.97 mmol) was suspended in 4M HCl-dioxane (5 mL) and the reaction mixture was stirred at room temperature for 2 hours, then concentrated. The residue was co-evaporated with dioxane three times and was dissolved in 0.1 M AcOH-2-butanol (12.5 mL), then N-methylmorpholine (0.09 mL) was added. The reaction mixture was refluxed for three hours. After cooling to rt, the mixture was dissolved in ethyl acetate (40 mL) and water (25 mL). The combined filtrate was evaporated and the residue was filtered off and washed with ethyl acetate. The residue was dissolved in 0.1 M AcOH - 2-butanol (5 mL). After 7 h of continued stirring at 0 °C, the reaction mixture was stirred at room temperature for 2 hours, then concentrated. The residue was co-evaporated with dioxane three times. The residue was precipitated with dioxane (15 mL), then MgSO4. After filtration and concentration, the crude yellow precipitates were centrifuged and washed with methanol to afford colorless amorphous mass (0.12 g, 39%). 1H NMR (DMSO-d6): δ 7.07 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.6 Hz, 2H); 13C NMR (CF3COOD): δ 41.2, 41.3, 58.8, 58.9, 118.7, 119.5, 120.2, 125.9, 129.7, 130.4, 133.8, 145.0, 145.8, 156.1, 172.8, 172.9; HRMS (ESI): calcd. for C18H19N2O5 (M++H) 343.1294, found 343.128; [α]D +11 (c 1.0, CF3COOH).

(5)-Methyl 2-amino-3-(4-azidophenyl)propanoate hydrochloride (L-Phe-(4-N3)-OMe, 10) L-Phe-(4-N3) 9 (0.51 g, 2.47 mmol) was dissolved in mehanol (25 mL). Thionyl chloride (1.5 mL, 20.8 mmol) was added to the solution dropwise at 0 °C and the reaction mixture was warmed to room temperature, then concentrated to afford colorless amorphous mass (0.45 g, 71%). 1H NMR (CD3OD): δ 3.9 (dd, J = 13.5, 5.9 Hz, 1H), 3.27 (dd, J = 13.5, 5.8 Hz, 1H), 3.80 (s, 3H), 4.33 (t, J = 6.8 Hz, 1H); 13C NMR (CD3OD): δ 36.6, 53.6, 55.1, 120.6, 132.0, 132.1, 141.2, 170.3; HRMS (ESI): calcd. for C18H19N2O5 (M++H) 343.128, found 343.128; [α]D +15 (c 1.0, CH3OH); IR (neat): ν 2136 cm⁻¹.

(5)-Methyl 3-(4-azidophenyl)-2-(tert-butyxycarbonylamino)propanoate (Boc-L-Phe-(4-N3)-OMe, 11) A solution of Phe(4-N3) methyl ester hydrochloride 10 (0.45 g, 1.77 mmol) and N-(tert-butyxycarbonyl)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, 1.51 mmol) was added followed by dicyclohexylcarbodiimide (0.25 g, 1.11 mmol). After 7 h of continued stirring at 0 °C, the reaction mixture was cooled in the freezer overnight. The insoluble material was filtered off and washed with ethyl acetate. The combined filtrate was evaporated and the residue was dissolved in ethyl acetate (40 mL) and water (25 mL). The organic layer was washed successively with 25 mL portions of 0.5 N HCl, H2O, 0.5 N NaHCO3 and brine and dried over MgSO4. After filtration and concentration, the crude yellow solid was subjected to silica chromatography (ethyl acetate/hexane = 1:1, then 3:1) to afford a colorless amorphous mass (0.34 g, 64%). 1H NMR (CD3OD): δ 1.34 (s, 3H), 2.67 (m, 1H), 2.90 (m, 3H), 3.35 (s, 3H), 3.66 (s, 3H), 4.23 (t, J = 6.9 Hz, 1H), 4.59 (t, J = 6.8 Hz, 1H), 6.48 (d, J = 7.9 Hz, 1H), 6.62 (s, 1H), 6.68 (d, J = 7.9 Hz, 1H), 6.69 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 8.6 Hz, 2H); 13C NMR (CD3OD): δ 28.6, 37.9, 38.4, 52.6, 55.2, 57.5, 80.7, 116.1, 116.3, 117.2, 121.7, 129.0, 131.1, 143.5, 146.2, 157.1, 157.2, 173.2, 174.2; HRMS (ESI): calcd. for C18H19N2O5 (M++H) 475.2087, found 475.2085; [α]D -21 (c 2.0, CH3OH).

(3S,6S)-3-(3,4-Dihydroxybenzyl)-6-(4-hydroxybenzyl)piperazine-2,5-dione (cyclo(L-Tyr-L-DOPA), 8) Boc-L-Tyr-L-DOPA-OMe 7 (0.47 g, 1.02 mmol) was treated with 4M HCl-dioxane (2.5 mL) and the suspension turned to solution with stirring at room temperature, then concentrated. The residue was dissolved in 0.1 M AcOH-2-butanol (15 mL). N-Methylmorpholine (0.11 mL) was added. The reaction mixture was refluxed for three hours. After cooling to rt, the reaction mixture was cooled at -20 °C overnight. The precipitates were centrifuged and washed with methanol to afford colorless amorphous mass. (0.20 g, 58%). 1H NMR (CF3COOD): δ 2.19 (dd, J = 12.9, 7.6 Hz, 1H), 2.47 (dd, J = 12.9, 6.8 Hz, 1H), 2.97 (d, J = 13.8 Hz, 1H), 3.06 (d, J = 13.8 Hz, 1H), 4.48 (m, 1H), 4.55 (m, 1H), 6.66 (d, J = 7.9 Hz, 1H), 6.80 (s, 1H), 6.98 (d, J = 7.9 Hz, 1H), 6.99 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.6 Hz, 2H); 13C NMR (CF3COOD): δ 41.2, 41.3, 58.8, 58.9, 118.7, 119.5, 120.2, 125.9, 129.7, 130.4, 133.8, 145.0, 145.8, 156.1, 172.8, 172.9; HRMS (ESI): calcd. for C18H19N2O5 (M++H) 343.1294, found 343.1281; [α]D -11 (c 1.0, CF3COOH).

(5)-Methyl 2-amino-3-(3-azidophenyl)propanoate hydrochloride (L-Phe-(4-N3)-OMe, 10) L-Phe-(4-N3) 9 (0.51 g, 2.47 mmol) was dissolved in mehanol (25 mL). Thionyl chloride (1.5 mL, 20.8 mmol) was added to the solution dropwise at 0 °C and the reaction mixture was warmed to room temperature, then concentrated to afford colorless amorphous mass (0.45 g, 71%). 1H NMR (CD3OD): δ 3.9 (dd, J = 13.5, 5.9 Hz, 1H), 3.27 (dd, J = 13.5, 5.8 Hz, 1H), 3.80 (s, 3H), 4.33 (t, J = 6.8 Hz, 1H); 13C NMR (CD3OD): δ 36.6, 53.6, 55.1, 120.6, 132.0, 132.1, 141.2, 170.3; HRMS (ESI): calcd. for C18H19N2O5 (M++H) 343.128, found 343.128; [α]D +15 (c 1.0, CH3OH); IR (neat): ν 2136 cm⁻¹.
The authors confirm that this article content has no conflict of interest.

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REFERENCES


