Synthesis of N-Urethane Protected α-Aminoalkyl-α’-cyanomethyl Ketones; Application to the Synthesis of 3-Substituted 5-Amino-1H-pyrazole Tethered Peptidomimetics

M. K. Sharnabai, G. Nagendra, Vommina V. Sureshbabu*

#109, Peptide Research Laboratory, Department of Studies in Chemistry, Central College Campus, Bangalore University, Dr. B. R. Ambedkar Veedhi, Bangalore 560 001, India

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Abstract: The preparation of N-protected amino/peptide α-cyanomethyl ketones through cyanation of the corresponding α-bromo-methyl ketones is described. The utility of the resulting α-cyanomethyl ketones in the synthesis of 3-substituted-5-amino-1H-pyrazoles has also been demonstrated. In both steps a wide range of N-protected amino/peptide acids has been employed and the products are obtained in good yield. The enantiomeric purity of both the α-cyanomethyl ketones and pyrazoles were confirmed by chiral HPLC analysis of the corresponding Z-protected α- and 1-Ala-OH as model substrates. The synthesis of peptide pyrazolecarboxamides is also delineated.

Key words: peptidomimetics, amino acid mimics, ketones, pyrazole

An important aspect of peptidomimetic design involves the use of suitable building blocks. To this end, either the -NH₂ or -COOH group of enantiopure α-amino acids are converted into the desired functionality. Among them, azides,1 isonitriles,2 nitriles,3 and acetylenes4 have been converted into the desired functionality. Among them, amides is also delineated.

As model substrates. The synthesis of peptide pyrazolecarboxamides is also delineated.

Boc/Ac-amino thioamide with methyl triflate and the resulting intermediate was treated with a nucleophile. Boc/Ac-amino thioamide with methyl triflate and the resulting intermediate was treated with a nucleophile. N-Protected α-aminoalkyl-α’-halomethyl ketones9 have emerged as attractive targets for the design of peptidomimetics.10–12 Our group has developed a simple route for the preparation of N-protected α-aminoalkyl-α’-halomethyl ketones and employed them for the construction of thiazole,13 selenazole,14 and triazole15 tethered peptidomimetics (Scheme 1).

In the present letter we describe the synthesis of N-urethane protected α-aminoalkyl-α’-cyanomethyl ketones and their utility in the synthesis of amino acid derived 3-substituted 5-amino-1H-pyrazoles. The α-cyanomethyl ketone is an important scaffold that is found in many pharmaceutical compounds16 with a broad spectrum of biological activity.17,18 Sauve et al. reported amino acid derived α-cyanomethyl ketones and carboxy group modified dipeptides.19 Boc/Ac-protected Phe/Leu-Phe derived cyanomethyl ketones were synthesized through alkylation of Boc/Ac-amino thioamide with methyl triflate and the resulting intermediate was treated with a nucleophile. N-Acetyl protected cyanomethyl ketones have also been prepared by the reaction of activated carboxylic acids and the carbanion of tert-butyl cyanacetate, and the resulting enols were then subjected to hydrolysis followed by decarboxylation.20 α-Cyanomethyl ketones derived from N,N′-bisbenzyl protected benzyl phenyl alaninate was prepared by the reaction of MeCN and NaNH₂.21 Some of the above approaches either require a cumbersome protocol or are incompatible with the use of urethane-type protecting groups. We describe herein a convenient method for the synthesis of urethane-protected α-cyanomethyl ketones and their conversion into N-Boc/Z-protected α-aminoalkyl-5-amino pyrazoles. Pyrazole22 derivatives of α-amino acids have received considerable attention because of their diverse range of biological properties such as potent angiotensin II antagonist activity both in vitro and in vivo,23 anti-hypertensive, anti-bacterial, and anti-inflammatory activity,24 muscle relaxant properties, and inhibition of cyclin dependent kinases.25 They have also been used as building blocks for the synthesis of peptidomimetics.26,27

The required urethane-protected α-aminoalkyl-α’-bromomethyl ketone precursors were prepared by using a two-step procedure reported by our group.13 A similar approach was employed for the preparation of bromomethyl ketones containing the Boc-protected compounds with suitable modifications.28 In all cases, bromomethyl ke-
tones were obtained as stable solids without the need for column purification. The resulting α-bromomethyl ketones were then converted into α-cyanomethyl ketones. We initially investigated the use of several cyanating reagents by using different solvents; the results are summarized in Table 1. Boc-Ala-[CH₂Br]₂a (Scheme 2) was used as a model substrate to optimize the reaction conditions, with the progress of the reaction being monitored by TLC. Treatment of 2a with TMSCN in MeOH failed to give 3a at room temperature (Table 1, entry 1), but under reflux conditions the product was obtained in 20% yield (Table 1, entry 2). We then explored the use of NaCN, HgCN, K₃Fe(CN)₆ and KCN at room temperature in MeOH (Table 1, entries 3–6). KCN turned out to be the most useful to obtain 3a. Furthermore, we examined a range of solvents with the aim of stabilizing the reaction conditions and found that use of THF and CH₃CN led to the formation of 3a in moderate yield (Table 1, entries 7 and 8), DMF and DMSO gave acceptable yields of 3a (68 and 72% respectively; Table 1, entries 9 and 10), but the use of MeOH provided an excellent yield of 3a at room temperature (Table 1, entry 6).

Under the optimized conditions, reactant 2a was completely consumed, as evidenced by IR and RP-HPLC analysis. The disappearance of the strong IR absorption band at 1730 cm⁻¹ for the α-bromomethyl ketone 2a and the appearance of strong bands at 1650 and 2243 cm⁻¹ for the carbonyl and adjacent nitrile groups, respectively, confirmed the formation of 3a. The protocol was further applied to various N-protected amino/peptide α-bromomethyl ketones to obtain the corresponding cyanomethyl ketones (Table 2). The procedure worked well even for amino acids Ser and Thr, which contain free hydroxyl groups (Table 2, entries 9, 13, and 15).

Next, we turned our attention to the synthesis of a hitherto unreported class of Nα-Boc/Z-aminoalkyl-5-amino pyrazoles. The 3-substituted 5-amino-1H-pyrazole derivatives 4 were prepared by reaction of α-cyanomethyl ketones 3 with hydrazine hydrate under reflux in MeOH (Scheme 3). In a typical experiment, Boc-Ala-[CH₂CN]₃a was added to a solution of 99–100% hydrazine hydrate in MeOH. The reaction mixture was heated to reflux at 40 °C for approximately two hours. After completion of the reaction (TLC analysis), the solvent was evaporated under reduced pressure and the residue was purified by column chromatography to afford 4a. Several Boc and Z-protected α-aminoalkyl-α'-cyanomethyl ketones were...
converted into their respective pyrazole derivatives 4a–i in good yield and purity (Table 3).

**Table 2** List of Nα-Z/Boc-Protected α-Cyanomethyl Ketones 3a–r

<table>
<thead>
<tr>
<th>Entry</th>
<th>Product 3</th>
<th>[α]D 25 (c 1, CHCl3)</th>
<th>Yield (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3a</td>
<td>–14.2</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>3b</td>
<td>–12.1</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>3c</td>
<td>–21.2</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>3d</td>
<td>–15.3</td>
<td>87</td>
</tr>
<tr>
<td>5</td>
<td>3e</td>
<td>–13.4</td>
<td>89</td>
</tr>
<tr>
<td>6</td>
<td>3f</td>
<td>–16.7</td>
<td>90</td>
</tr>
<tr>
<td>7</td>
<td>3g</td>
<td>–18.3</td>
<td>91</td>
</tr>
<tr>
<td>8</td>
<td>3h</td>
<td>+21.6</td>
<td>90</td>
</tr>
<tr>
<td>9</td>
<td>3i</td>
<td>–22.1</td>
<td>88</td>
</tr>
<tr>
<td>10</td>
<td>3j</td>
<td>–12.5</td>
<td>80</td>
</tr>
<tr>
<td>11</td>
<td>3k</td>
<td>–13.9</td>
<td>78</td>
</tr>
<tr>
<td>12</td>
<td>3l</td>
<td>–17.2</td>
<td>89</td>
</tr>
<tr>
<td>13</td>
<td>3m</td>
<td>–14.8</td>
<td>79</td>
</tr>
</tbody>
</table>

a Isolated yield.

**Scheme 3** Synthesis of N-protected 3-substituted 5-amino-1H-pyrazoles 4

Similarly, two examples of Nα-Boc/Z-protected peptidyl 3-substituted 5-amino-1H pyrazoles 4j–k were also prepared (Figure 2). Both cyanomethyl ketones and pyrazole derivatives were characterized by mass, IR and NMR analyses.

**Figure 2** Dipeptidyl pyrazoles synthesized

In order to gain insight into the possibility of racemization, enantiomeric Z-protected D- and L-Ala-OH were
converted into cyanomethyl ketones and pyrazoles under the developed reaction conditions, and their enantiomeric excess was determined by chiral HPLC analysis. As shown in Figure 3, compounds 3g and 3h showed single peaks with retention times of 28.1 and 22.5 min, respectively.

In contrast, the constituents of the racemic mixture of 3g and 3h were separated with retention times of 27.8 and 21.9 min. Similarly, HPLC profiles for pyrazoles 4g and 4h showed retention times of 16.9 and 10.1 min, respectively. Thus, these results confirm that both α-cyanomethyl ketones and pyrazoles were prepared in optically pure form.

Finally, the synthesis of pyrazole-linked dipeptidomimetics was undertaken. Pyrazolecarboxamide derivatives are known for their use in the treatment of pain and inflammation and also play a vital role in the β-sheet stabilization of peptides. Thus, pyrazoles 4 were employed to synthesize peptide-derived pyrazolecarboxamides 6.

As a test case, a solution of Fmoc-Val-Cl in anhydrous THF, Boc-Ala-pyrazole amine 4a and NMM were reacted at 0 °C. The coupling was found to be complete in three hours, as observed by TLC analysis (Scheme 4).

![Scheme 4](This image is not provided in the text. It is implied to be a diagram showing the synthesis of pyrazole-tethered peptidomimetics.)

The desired N,N'-orthogonally protected dipeptidomimetic 6a was isolated in 88% after column purification. As a test case, a solution of Fmoc-Val-Cl in anhydrous THF, Boc-Ala-pyrazole amine 4a and NMM were reacted at 0 °C. The coupling was found to be complete in three hours, as observed by TLC analysis (Scheme 4).

### Table 3 Data for 3-Substituted 5-Amino-1H-pyrazole Derivatives 4a-i

<table>
<thead>
<tr>
<th>4</th>
<th>PG</th>
<th>R</th>
<th>HRMS [M + Na]⁺</th>
<th>Yield (%)</th>
<th>[α]D 25 (c 1, CHCl₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>Boc</td>
<td>Me</td>
<td>249.1327</td>
<td>85</td>
<td>−12.1</td>
</tr>
<tr>
<td>4b</td>
<td>Boc</td>
<td>i-Pr</td>
<td>277.1640</td>
<td>88</td>
<td>−15.3</td>
</tr>
<tr>
<td>4c</td>
<td>Boc</td>
<td>Bn</td>
<td>325.1638</td>
<td>92</td>
<td>−17.2</td>
</tr>
<tr>
<td>4d</td>
<td>Boc</td>
<td>i-Bu</td>
<td>291.1793</td>
<td>94</td>
<td>−14.5</td>
</tr>
<tr>
<td>4e</td>
<td>Boc</td>
<td>CH₂OH</td>
<td>355.1748</td>
<td>92</td>
<td>−10.9</td>
</tr>
<tr>
<td>4f</td>
<td>Z</td>
<td>CH₃COOR-Bu</td>
<td>505.1849</td>
<td>91</td>
<td>−29.5</td>
</tr>
<tr>
<td>4g</td>
<td>Z</td>
<td>Me</td>
<td>359.1481</td>
<td>90</td>
<td>−13.5</td>
</tr>
<tr>
<td>4h</td>
<td>Z</td>
<td>Me b</td>
<td>248.1241</td>
<td>88</td>
<td>−14.9</td>
</tr>
<tr>
<td>4i</td>
<td>Z</td>
<td>CH(CH₃)OH</td>
<td>445.1856</td>
<td>87</td>
<td>−16.2</td>
</tr>
</tbody>
</table>

*a Isolated yield.

b Z-D-Ala-OH was used as starting compound.

![Figure 3](This image is not provided in the text. It is implied to be a series of chromatograms showing the separation of pyrazole derivatives.)

**Figure 3** Chiral HPLC analysis. Chromatograms shown are: (a) Z-L-Ala-CH₂CN 3g; (b) Z-D-Ala-CH₂CN 3h; (c) Prepared 1:1 mixture of 3g and 3h; (d) Z-L-Ala-pyrazole 4g; (e) Z-D-Ala-pyrazole 4h; (f) Prepared 1:1 mixture of 4g and 4h.

shown in Figure 4, the same procedure was extended to the synthesis of compounds 6b-e.

In summary, a simple and easily accessible route has been established for the synthesis of enantiopure N-urethane-protected amino acid/peptidyl cyanomethyl ketones. The resulting cyanomethyl ketones were utilized for the construction of amino acid derived 3-substituted-5-amino-1H-pyrazoles. The protocol has also been extended to prepare five N,N'-orthogonally protected pyrazolecarboxamide tethered peptidomimetics in good yield.

Acknowledgment

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References and Notes


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(28) Preparation of Boc-Protected Bromomethyl Ketones; Typical Procedure for 2a:

To a solution of diazomethyl ketone (1.8 mmol, 0.4 g) in THF, aq 47% HBr (2–3 mL) at 0 °C, was added Cbz-protected-Ala-5-amino-pyrazole. The reaction mixture was stirred for another 2–3 min until the starting material was completely consumed. The reaction mixture was diluted with excess H2O and the precipitated solid was filtered. A simple recrystallization (THF–H2O) led to the analytically pure product.

(29) Preparation of Boc-Ala-[CH2CN] 3a; Typical Procedure:

To a solution of Boc-Ala-CH2Br (1.8 mmol, 0.5 g) in MeOH (5 mL), KCN (3.7 mmol, 0.24 g) was added at r.t. The reaction mixture was stirred for 3 h (reaction followed by TLC analysis). After completion of the reaction, the solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc (2 × 10 mL) and, to dispose of any excess KCN, the reaction mixture was quenched with sat. K MnO4 solution and washed with excess H2O. The organic layer was washed with brine (10 mL) and the solution was dried over anhydrous Na2SO4. The solvent was filtered and evaporated under reduced pressure and the product 3a was isolated by column chromatography.

Compounds 3a

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
<th>HPLC (% area)</th>
<th>λmax (nm)</th>
<th>Chemical purity (ESI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>89%</td>
<td>23.1</td>
<td>255, 285</td>
<td>99.9</td>
</tr>
</tbody>
</table>

(30) Goodman, M.; Felix, A.; Moroder, L.; Toniolo, C.


(31) Preparation of Boc-Ala-5-amino-pyrazole 4a; Typical Procedure:

To a solution of Nα-protected Boc-Ala-[CH2CN] 3a (1.8 mmol, 0.4 g) in MeOH (5 mL), hydrazine hydrate (14 mmol, 0.7 mL) was added. The reaction mixture was heated at reflux at 40 °C for 2 h (progress monitored by TLC). After cooling, the solvent was removed under reduced pressure to obtain the crude product, which was purified by column chromatography (silica gel 100–200 mesh; CHCl3–MeOH, 9:1).