Iodine-accelerated synthesis of $N^\alpha$-urethane protected amino/peptide hydroxamic acids from amino/peptide thioacids

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A facile and mild synthesis of $N^\alpha$-protected amino/peptide hydroxamic acids is accomplished from the corresponding $N^\alpha$-protected amino/peptide thioacids using I$_2$, 1-hydroxybenzotriazole (HOBt) and NH$_2$OH at RT. The protocol can be extended to bifunctional as well as sterically hindered amino acids, to afford amino/peptide hydroxamic acids in good yields.

Keywords: Iodine, $N^\alpha$-protected amino/peptide thioacids, hydroxamic acids

The $N$-hydroxamide or [CO-N(OH)] link is an oxidized form of the amide group present in a number of metabolites from microorganisms with a wide spectrum of biological activities

The versatile biological activity of hydroxamates is due to the strong chelating ability with the metal ions such as Zn(II)$^3$, Fe(II), Fe(III)$^4$, their NO-releasing properties and provide sites for acylations.

Hydroxamic acids have generally been synthesized by the acylation of carboxylic acids with hydroxylamine using various coupling reagents like cyanuric chloride$^5$ and propylphosphonic anhydride (T3P)$^6,7$, reaction of O/N-protected hydroxylamines such as O-benzylhydroxylamine, N,N,O-tris(trimethylsilyl)-hydroxylamine, N-Boc-O-THP hydroxylamine, N-Boc-O-TBDMS-hydroxylamine and O-trimethylsilyl-hydroxylamine$^8$ with activated carboxylic acids, reaction of esters with hydroxylamine$^9$, Lewis acid mediated detritylation in the synthesis of $N$-hydroxylamine derivatives$^{10}$, treatment of N-acloyoxazolidines with hydroxylamine in the presence of samarium triflate$^{11}$. McAllister group synthesized cyclic hydroxamic acids through nitro group reductive cyclisation$^{12}$. Efficient methods for the synthesis of O-alkyl hydroxamic acids using phosphoric acid diethyl ester 2-phenyl-benzenimidazol-1-yl ester$^{13}$ and 2-acryl-pyridazin-3(2H)-ones$^{14}$ were reported. While some of these methods have attractive features, their general utility is often limited by longer duration$^6,7$, utilization of highly expensive reagents$^8$, difficulty in purification and convenient methods have to be employed for deprotection after acylation of hydroxamic acids, which limit their synthetic utility in multistep synthesis of hydroxamic acids$^{8,13,14}$.

Inspite of the continuous progress in hydroxylamidation, still there is a need to develop an efficient, simple and an alternative protocol for the preparation of hydroxamic acids. Herein, we report a novel and facile method for the preparation of hydroxamic acids from corresponding thioacids using readily available iodine and hydroxylamine. Due to the wide utility and limited commercial availability, the preparation of thioacids and their applications became significant in organic synthesis. When compared to carboxylic acids, their thioacid counterparts have numerous advantages with respect to acidity, nucleophilicity and solubility in organic solvents, afford less epimerized products and mild reaction conditions needed for activation during coupling.

Results and Discussion

Thioacids are an interesting class of compounds which play significant role in peptide bond formation and native chemical ligation (NCL) reactions due to their specific reactivity with aziridines$^{15}$, sulfonamides$^{16}$, isocyanates/isothiocyanates$^{17}$, azides$^{18}$, imidotriazoles$^{19}$, alkyl halides$^{20}$. Danishefsky et al.$^{21}$, have developed an efficient protocol for ligating peptides and glycopeptides by exploiting the reactivity of thioacids
and demonstrated that thioacids themselves have some intermolecular acyl donor capacity which can be significantly increased in the presence of I$_2$. Recently Gopi et al. described Cu(II) mediated synthesis of peptides in methanol using thioacids and amines in less than five minutes. Highly selective N-acylation of amines with thioacids in methanol at room temperature was demonstrated by the same group using copper sulfate. An efficient protocol was reported by our group for the synthesis N$\alpha$-protected amino/peptide thioacids from the corresponding carboxylic acids using propylphosphonic anhydride in combination with Na$_2$S$_2$O$_5$.

In the initial studies, the Fmoc-Phe-SH was treated with DIPEA, HOBt, I$_2$ and NH$_2$OH at ambient temperature in CH$_3$CN. After 3 hr as the reaction mixture was monitored by TLC, the corresponding hydroxamic acid 2b was obtained in lower yield (60%) (Table I, entry 1). To improve the yield, the reaction conditions were investigated with respect to the equivalence of I$_2$ and different organic solvents. Lower yields were observed with DMSO and DMF (entries 2-5). However performing the reaction in THF using I$_2$ (0.8 equiv) drastically reduced the reaction time and afforded the desired product 2b in 85% yield (entry 7). Similar results were noticed even with the rise in equivalence of I$_2$ in THF (entry 8). When similar reaction conditions were carried without HOBt, lower yield and longer duration was observed (entry 9). The progress of the reaction was monitored by TLC and after completion of the reaction, 2b was isolated using column chromatography.

Compared to carboxylic acid, its thioacid counterpart serves as better acyl donor and additionally the usage of I$_2$ acts as an oxidizing agent where it enhances the intermolecular acyl donor capacity of thioacid. HOBt employed suppresses the possible epimerization which may occur at C-terminal of an acid. The formed HOBt ester can be easily N-acylated by the addition of NH$_2$OH. The present methodology is advantageous since it proceeds readily at RT, in short duration and is compatible for the amino acids such as Ser 2d, Tyr 2f, Thr 2j without protection of the hydroxy group. Having the optimized reaction conditions in hand, we then turned our attention towards the preparation of a series of N$\alpha$-urethane protected amino hydroxamic acids 2 from thioacids 1. The reaction is shown in Scheme I and the results are summarized in Table II.

Using chiral HPLC, racemization studies of the diastereomeric products obtained from the Fmoc protected L-Ala-SH and D-Ala-SH were analyzed. The prepared hydroxamic acids 2a and 2a* showed peaks at R$_f$ = 12.67 min (2a, l-isomer) and R$_f$ = 16.44 min (2a*, d-isomer). However the equimolar mixture of 2a and 2a* (1D-isomer) showed distinct peaks at R$_f$ = 12.98 min and R$_f$ = 16.70 min. Thus, it was proved that the present protocol was free from racemization.

In the next part of the study, the protocol was further extended to the synthesis of peptide hydroxamic acids from corresponding peptide thioacids. For this, the required Fmoc, Boc and Z protected dipeptide thioacids were prepared using known procedure. Then, as described previously, to a solution of a dipeptide thioacid in THF, DIPEA, HOBt, I$_2$ were added at RT. Then the reaction mixture was treated with neutral NH$_2$OH. In this study, the reaction was found to complete within half an hour and isolation of product was facilitated through column chromatography. All the compounds were characterized by IR, mass and NMR analyses.

### Table I — Optimization of reaction conditions for the preparation of N$\alpha$-protected amino hydroxamic acid 2b

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>I$_2$ (equiv)</th>
<th>Reaction time</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH$_3$CN</td>
<td>0.8</td>
<td>3.0 h</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>DMSO</td>
<td>0.4</td>
<td>4.0 h</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>DMSO</td>
<td>0.8</td>
<td>3.5 h</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>DMF</td>
<td>0.4</td>
<td>4.0 h</td>
<td>55</td>
</tr>
<tr>
<td>5</td>
<td>DMF</td>
<td>0.8</td>
<td>3.0 h</td>
<td>73</td>
</tr>
<tr>
<td>6</td>
<td>THF</td>
<td>0.4</td>
<td>60 min</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>THF</td>
<td>0.8</td>
<td>30 min</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>THF</td>
<td>1.2</td>
<td>30min</td>
<td>86</td>
</tr>
<tr>
<td>9</td>
<td>THF</td>
<td>0.8</td>
<td>3.0h$^b$</td>
<td>40</td>
</tr>
</tbody>
</table>

$^a$ reactions were conducted at rt with DIPEA, HOBt, I$_2$.

$^b$ reaction was conducted at rt with DIPEA, I$_2$.

### Experimental Section

All solvents were freshly distilled before use. Amino acids were used as received from Sigma-Aldrich Company having L-configuration. $^1$H and $^{13}$C NMR

![Scheme I — Synthesis of N$\alpha$-protected amino hydroxamic acids 2](image-url)

Pg=Fmoc, Boc, Chz, ; R = amino acid side chain
spectra were recorded at 300 and 75 MHz respectively, with DMSO-$d_6$ as an internal standard. Splitting patterns described apparent multiplicities and were designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiple), or br s (broad singlet). Mass spectra were recorded using high resolution mass spectrometer (HRMS). Melting points were measured with Veego (Model: VMP-DS) melting point apparatus and the samples were dried under vacuum before analysis.

**General procedure for the synthesis of $N^\alpha$-urethane protected amino/peptide hydroxamic acids 2, 4**

To a solution of $N^\alpha$-urethane protected amino/peptide thioacid (1.0 equiv) in THF, DIPEA (1.5 equiv), HOBt (2.0 equiv), I$_2$ (0.8 equiv) were added at RT and to the reaction mixture molecular sieves (MS 4 Å) were added. The reaction mixture was treated with neutral NH$_2$OH (prepared by treating the NH$_2$OH.HCl with methanolic NMM) which was allowed to stir at RT. After completion of the reaction as monitored by TLC, the solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc. The organic layer was washed with water (2 × 10 mL), brine (2 × 10 mL) and dried over anhydrous Na$_2$SO$_4$. The solvent was removed under reduced pressure to afford the product, which was purified by column chromatography using CHCl$_3$/MeOH.

**Spectral data**

$N^\alpha$-Fmoc-Ala-NHOH 2a: $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.36 (d, $J = 5.9$ Hz, 3H), 4.38 (m, 1H), 4.27 (t, $J = 6.2$ Hz, 1H), 4.52 (d, $J = 6.5$ Hz, 2H), 5.30 (s, 1H), 7.22-7.84 (m, 8H), 8.48 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.9, 45.6, 46.8, 64.5, 126.6, 128.2, 128.4, 128.8, 139.6, 42.8, 158.0, 166.8; HRMS: Calcd m/z C$_{18}$H$_{18}$N$_2$O$_4$: 326.1267. Found: m/z 349.1169 [M+Na]$^+$.  

$N^\alpha$-Fmoc-Phe-NHOH 2b: $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.36 (d, $J = 5.9$ Hz, 3H), 4.38 (m, 1H), 4.27 (t, $J = 6.2$ Hz, 1H), 4.52 (d, $J = 6.5$ Hz, 2H), 5.30 (s, 1H), 7.22-7.84 (m, 8H), 8.48 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 16.9, 45.6, 46.8, 64.5, 126.6, 128.2, 128.4, 128.8, 139.6, 42.8, 158.0, 166.8; HRMS: Calcd m/z C$_{18}$H$_{18}$N$_2$O$_4$: 326.1267. Found: m/z 349.1169 [M+Na]$^+$.  

### Table II — List of $N^\alpha$-protected amino hydroxamic acids 2

<table>
<thead>
<tr>
<th>Entry</th>
<th>Hydroxamic acid (2)</th>
<th>m.p.({\degree}C)</th>
<th>Yield (%)</th>
<th>Entry</th>
<th>Hydroxamic acid (2)</th>
<th>m.p.({\degree}C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>FmocHN$\text{O}$$\text{ONHOH}$</td>
<td>126-28</td>
<td>80</td>
<td>g</td>
<td>BocHN$\text{O}$$\text{ONHOH}$</td>
<td>Oil</td>
<td>79</td>
</tr>
<tr>
<td>b</td>
<td>FmocHN$\text{ONHOH}$</td>
<td>153</td>
<td>82</td>
<td>h</td>
<td>BocHN$\text{O}$$\text{ONHOH}$</td>
<td>Oil</td>
<td>77</td>
</tr>
<tr>
<td>c</td>
<td>FmocHN$\text{ONHOH}$</td>
<td>130-32</td>
<td>68</td>
<td>i</td>
<td>O$\text{Bn}$</td>
<td>132</td>
<td>68</td>
</tr>
<tr>
<td>d</td>
<td>FmocHN$\text{ONHOH}$</td>
<td>163-65</td>
<td>70</td>
<td>j</td>
<td>CbzHN$\text{ONHOH}$</td>
<td>Oil</td>
<td>78</td>
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<tr>
<td>e</td>
<td>FmocHN$\text{ONHOH}$</td>
<td>195</td>
<td>95</td>
<td>k</td>
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<td>75</td>
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<td>f</td>
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<td>90</td>
<td>l</td>
<td>N$\text{Cbz}$</td>
<td>gum</td>
<td>69</td>
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Table III — List of N-terminal peptide hydroxamic acids 4

<table>
<thead>
<tr>
<th>Entry</th>
<th>Pg</th>
<th>R¹</th>
<th>R²</th>
<th>Yield (%)</th>
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<tbody>
<tr>
<td>4a</td>
<td>Fmoc</td>
<td>CH₃</td>
<td>CH₃</td>
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<tr>
<td>4b</td>
<td>Fmoc</td>
<td>CH(CH₃)₂</td>
<td>CH₃CH₃</td>
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<tr>
<td>4c</td>
<td>Boc</td>
<td>CH(CH₃)₂</td>
<td>CH₂CH₂CH₃</td>
<td>68</td>
</tr>
<tr>
<td>4d</td>
<td>Boc</td>
<td>CH(CH₃)₂</td>
<td>CH(CH₃)CH₂CH₃</td>
<td>70</td>
</tr>
<tr>
<td>4e</td>
<td>Cbz</td>
<td>CH(CH₂CH₃)₂</td>
<td>CH₃CH₃CH₃</td>
<td>69</td>
</tr>
</tbody>
</table>

Scheme II — Synthesis of N-terminal peptide hydroxamic acids 4

Pgc,Fmoc, Boc, Cbz; R¹, R²=amino acid side chain
$N^\alpha$-Cbz-Pro-NHOH 2f: $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.58-1.88 (m, 4H), 3.32-3.49 (m, 2H), 4.15 (m, 1H), 5.16 (s, 2H), 7.12-7.28 (m, 5H), 8.54 (brs, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 22.6, 28.5, 44.3, 56.4, 65.4, 126.2, 126.8, 128.8, 142.4, 156.5, 169.5; HRMS: Calcd m/z C$_{23}$H$_{28}$N$_2$O$_6$: 427.2107. Found: m/z 427.1400 [M+Na]$^+$. $N^\alpha$-Cbz-Gly-Val-NHOH 4f: $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.16 (d, $J = 5.9$ Hz, 6H), 2.44 (m, 1H), 3.64 (s, 2H), 4.28 (d, $J = 6.1$ Hz, 1H), 5.14 (s, 2H), 6.16 (brs, 1H), 6.76 (s, 1H), 7.08-7.28 (m, 5H), 7.86 (s, 1H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 15.6, 32.4, 46.4, 57.6, 68.6, 125.4, 125.7, 127.2, 144.4, 159.4, 165.4, 173.8; HRMS: Calcd m/z C$_{19}$H$_{22}$N$_2$O$_6$: 323.1481. Found: m/z 324.1374 [M+Na]$^+$. Conclusion

The work presented herein describes a simple, facile and efficient synthesis of $N$-protected $\alpha$-amino/peptide hydroxamic acids from corresponding $\alpha$-amino/peptide thioacids. The protocol employed can serve as an alternative to the existing methods available for the synthesis of simple, bifunctional and sterically hindered amino/peptide hydroxamic acids.

Acknowledgement

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References

25 Chiral HPLC analysis of isomers was carried out by Lux 5 μ Amylose-2, 250 × 4.60 mm; solvent system: hexane-2-propanol (7:3); flow rate: 0.5 mL/min.