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A generally applicable synthesis of amino acid p-nitroanilides as synthons

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Introduction

Amino acid p-nitroanilides are versatile chromogenic substrates for proteolytic enzymes [1]. Their synthesis is problematic because of the low nucleophilicity of p-nitroaniline, since the current coupling methods of peptide synthesis appear inadequate. In an earlier paper we mentioned the applicability of phosphorus oxychloride as condensing agent in the synthesis of protected arginine p-nitroanilides [2]. We now describe here the use of this reagent in the synthesis of orthogonally protected p-nitroanilides and their transformation into synthons for chromogenic substrates.

Results and Discussion

We found that phosphorus oxychloride [3] is an excellent condensing agent for amines and alcohols (J. Broos, personal communication) of low nucleophilicity (Scheme 1). Virtually all Boc- and Z-protected amino acid p-nitroanilides were obtained in high yield (70–90%) in an optically pure form, some being given in Table I. We also found Boc-Ala-pNA to be excellently stable in 50% piperidine in DMF, which prompted us to synthesize Fmoc-amino acid p-nitroanilides, which have not been mentioned in the literature until now (Table I). The use of Fmoc as α-amino protection allows side chain protections which can be cleaved off under mildly acidic conditions at the end of the synthesis. As an example, Scheme 2 depicts the synthesis of the chromogenic substrate (S2238) used in the determination of thrombin.

Bz-Ile-Glu-Gly-Arg-pNA.HCl (S2222), Bz-Ile-Glu(N-piperidyl)-Gly-Arg-pNA.HCl (S2337), from Boc-Arg-pNA.HCl and H-D-Val-Leu-Lys-pNA.2HCl

Scheme 1. Equimolar amounts of phosphorus oxychloride and carboxylic acid are required in this procedure. X symbolizes an α-amino protective group, X' a side-chain protection and AA stands for an amino acid.
Table 1  
Some protected amino acid p-nitroanilides synthesized with phosphorus oxychloride as condensing agent

<table>
<thead>
<tr>
<th>Y (%)</th>
<th>m.p. (°C)</th>
<th>αD</th>
</tr>
</thead>
<tbody>
<tr>
<td>84</td>
<td>foam</td>
<td>-7.9°</td>
</tr>
<tr>
<td>84</td>
<td>foam</td>
<td>-8.1°</td>
</tr>
<tr>
<td>91</td>
<td>187</td>
<td>-12.8°</td>
</tr>
<tr>
<td>96</td>
<td>174</td>
<td>-10.7°</td>
</tr>
<tr>
<td>65</td>
<td>amorph</td>
<td>-12.4°</td>
</tr>
<tr>
<td>89</td>
<td>212-213</td>
<td>+13.3°</td>
</tr>
<tr>
<td>70</td>
<td>189-191</td>
<td>-27.2°</td>
</tr>
<tr>
<td>79</td>
<td>183</td>
<td>-39.1°</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Y (%)</th>
<th>m.p. (°C)</th>
<th>αD</th>
</tr>
</thead>
<tbody>
<tr>
<td>83</td>
<td>98</td>
<td>-26.8°</td>
</tr>
<tr>
<td>79</td>
<td>94 (dec.)</td>
<td>+24.5°</td>
</tr>
<tr>
<td>88</td>
<td>96 (dec.)</td>
<td>-45.5°</td>
</tr>
<tr>
<td>81</td>
<td>116-117</td>
<td>-23.3°</td>
</tr>
<tr>
<td>90</td>
<td>foam</td>
<td>-18.8°</td>
</tr>
<tr>
<td>95</td>
<td>foam</td>
<td>-6.1°</td>
</tr>
<tr>
<td>71</td>
<td>214-215</td>
<td>-25.3°</td>
</tr>
</tbody>
</table>

* c = 1, MeOH.
* b = 1, DMF.

(S2251), from Boc-Lys(Z)-pNA or Fmoc-Lys(Boc)-pNA, were synthesized in high yield as substrates for factor Xa and plasmin, respectively, and exhibited the known kinetic data. Our conclusion, that phosphorus oxychloride is a powerful condensing agent in the synthesis of p-nitroanilides, is confirmed by these results.

Reference:


Results:

C-terminal recently purpose followed by These an applied t

Introduce

C-terminus is o of reduc

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Acet Glu/OtO

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amount