Solid phase peptide synthesis of ‘difficult sequences’ using Pyoxim

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Introduction

Although SPPS has advanced significantly in terms of reagents, solid supports and instrumentation, the peptide chemist is still often faced by challenges, where optimization is required in order to obtain acceptable crude purities. In such optimization processes, the coupling reagent often play a crucial role. During the past five years a range of novel coupling reagents, based on the oxime auxiliary, has been introduced. One of these, namely Pyoxim, have shown highly effective in our laboratory, especially in the synthesis of peptaibols. Despite the reported efficacy of pyoxim in SPPS, not many publications, describing the utilization of this coupling reagent have appeared since its introduction back in 2010 [1]. In one of these studies [2] the performance of pyoxim did not match similar coupling reagents (PyLock, COMU), findings which are in stark contrast with our experience with Pyoxim. In the present study we sat out to elucidate the optimal coupling conditions using Pyoxim as coupling reagent in SPPS, along with the related coupling reagents HCTU and PyLock (fig. 1).

With HCTU we reported a crude purity (83%) similar to that previously reported (75%). However, a comparable crude purity was obtained when employing Pyoxim as coupling reagent (74%), an nearly four-fold increase in crude purity, compared to that previously reported (Table 1). When performing the synthesis under identical conditions, but omitting the pre-mixing of Pyoxim and NMM, a comparable crude purity was obtained.

Stability

The stability of coupling reagents has been examined in recent studies [3,4,5] and uranium based coupling reagents was shown to be more stable in solution compared to phosphonim based derivatives (with the exception of COMU). Hence, it seemed obvious to investigate the effect of pre-mixing the coupling reagent and base on the coupling efficacy. The coupling of Fmoc-Ile-OH onto H-DYING-resin was used as a representative example. The coupling reagent was mixed with the coupling base and used in the coupling at indicated time shown in Fig. 2.

Pyoxim was found to be far more susceptible to degradation compared to H-DYING and PyLock, when pre-mixed with the coupling base. In addition, the stronger base DIPEA (pKₐ: 10.4) accelerated the degradation of Pyoxim to a much higher degree compared to NMM (pKₐ: 7.4). Pylock performed best under the given conditions, and even after 24 hrs in solution with NMM, a reasonable level of coupling efficacy was observed (fig. 2).

Challenging Couplings

Next the performance of the three coupling reagents in the sterically demanding condensation of two consecutive Aib residues was investigated. The four residue C-terminal part of Alamethicin H-[H-UPUAUAQUVUGLUPVUUEQ-Phol] was used as a template. Fmoc-Aib-OH was double coupled for the indicated time using either DIPEA or NMM as base (fig.3). It is clear that Pyoxim is far superior to HCTU and PyLock with regards to the stability of sterically coupling reagents. In addition, the base strength is crucial, and the stronger base DIPEA outperformed NMM (fig.3).

‘Difficult sequences’

Finally Pyoxim was used in the synthesis of a range of sequences reported to be ‘difficult sequences’ (2-5). In all cases we were able to obtain similar results, and in the case of 3 and 5 the crude purity was improved 35% and 25%, respectively (table 2).

Table 2. Synthesis of "difficult sequences"

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Crude Purity (%)</th>
<th>Reported (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>85</td>
<td>83</td>
</tr>
<tr>
<td>3</td>
<td>88</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>59</td>
<td>62</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>43</td>
</tr>
</tbody>
</table>

*See methods for details.

Table 1. ‘Fast cycle’ synthesis of 65-74ACP

<table>
<thead>
<tr>
<th>Coupling reagent</th>
<th>Pre-mixing</th>
<th>Crude Purity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCTU</td>
<td>Yes</td>
<td>82 (75%)</td>
</tr>
<tr>
<td>Pyoxim</td>
<td>Yes</td>
<td>74 (20%)</td>
</tr>
<tr>
<td>PyLock</td>
<td>No</td>
<td>78</td>
</tr>
</tbody>
</table>

*See methods for details.

Methods

The AC(34-48)penta-tryptophan fragment, in DYING-Wg(9-15), used in fig. 2, was synthesized as described for (2) on a 100µmol scale. The synthesis of the individual residue is to be described in a future paper.

The Fast cycle (fig. 2) was synthesized as described for (2) on a 100µmol scale. The synthesis of the individual residue is to be described in a future paper.

Pyoxim was found to be far more susceptible to degradation compared to H-DYING and PyLock, when pre-mixed with the coupling base.

Conclusions

• It was shown that pyoxim is more susceptible towards degradation, resulting from pre-mixing coupling reagent and base, compared to Pylock and HCTU.

• In the coupling of the α-α-disubstituted amino acid Ab onto another Ab residue, Pyoxim was superior to HCTU and Pylock. In addition, the choice of base is crucial, and the stronger base DIPEA showed to be superior compared to NMM.

Pyoxim was shown to be as effective as HCTU and Pylock in the synthesis of 65-74ACP applying fast cycle times.

References


Fig 1. The coupling reagents used in this study.

Fig 2. Effect of pre-mixing coupling reagent and base*

*See methods for details.

Fig 3. ‘Fast cycle’ synthesis of 65-74ACP

*See methods for details.