Solid phase synthesis of monosaccharide-containing N-glycopeptides.

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Glycoproteins have attracted much attention in recent years owing to their role in many biological processes, especially as biological recognition markers on cell membranes [1-3]. The development of standard protocols for the chemical synthesis of glycopeptides is therefore of great interest. Most laboratories now synthesise peptides using a solid phase method [4]. This involves the covalent attachment of the C-terminal amino acid of the sequence of interest to a solid resin support followed by stepwise addition of amino acids in the C-N direction. This approach, incorporating glycosylated amino acids, has only recently been applied to glycopeptide synthesis [5-10].

The main problem in the synthesis of glycopeptides is the susceptibility of the glycosidic bond to acid and base. It is therefore preferable to adopt a peptide synthesis protocol that does not involve the use of strongly acidic or basic reagents. The Fmoc (9-fluorenylmethoxy-carbonyl) strategy is the obvious choice [11]. The Fmoc ω-amino protecting group is removed by a mild organic base and is used in conjunction with a solid phase that can be cleaved by moderate acid. There have been several reports of the use of strongly acidic or basic reagents. The Fmoc (9-fluorenylmethoxy-carbonyl) strategy is the obvious choice [11]. The Fmoc ω-amino protecting group is removed by a mild organic base and is used in conjunction with a solid phase that can be cleaved by moderate acid.

The first sugar residue in N-glycosylation is 2-acetamido-2-deoxy-b-D-glucopyranose (GlcNAc) attached to the amide group of an amino acid building block (I) and (II).

Peptide and glycopeptide synthesis was carried out using the continuous flow method starting with 1g of Fmoc-(amino acid)-derivatised resin (Pepsyn KA, Milligen Biosense). Fmoc groups were removed by a 10 min. treatment with 20% (v/v) piperidine in N,N-dimethylformamide (DMF) throughout. Fmoc-amino acids, except for Asn derivatives, were coupled as their prederivatised active esters (5 equiv.) with 1-hydrobenzotriazole (HOBt) added as an auxiliary nucleophile (5 equiv.). Fmoc-Asn derivatives, including the glycosylated amino acid building blocks (I) and (II), were coupled as their HOBT esters (5 equiv.) formed in situ with diisopropylcarbodiimide. The coupling time was 1 hour in all cases.

Cleavage of peptide or glycopeptide products from the resin and simultaneous amino acid side-chain deprotection was accomplished with 95% TFA for 2 hrs at room temperature. Precipitation of the cleaved product using diethyl ether yielded peptides (III & VI) and glycopeptides (IV, V & VII). De-O-acetylation of glycopeptides (IV) and (VII) was achieved with methanolic ammonia (2 days at room temperature). The glycosidic linkage is cleaved by acid.

<table>
<thead>
<tr>
<th>Peptide/Glycopeptide</th>
<th>Yield (%)</th>
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<tbody>
<tr>
<td>III Ala-Asn-Ala-Ser-Gly</td>
<td>95</td>
</tr>
<tr>
<td>(AcO)2GlcNAc</td>
<td></td>
</tr>
<tr>
<td>IV Ala-Asn-Ala-Ser-Gly</td>
<td>91</td>
</tr>
<tr>
<td>GlcNAc</td>
<td></td>
</tr>
<tr>
<td>V Ala-Asn-Ala-Ser-Gly</td>
<td>115 *</td>
</tr>
<tr>
<td>VI Asn-Ala-Asn-Ser-Ile-Glu</td>
<td>91 §</td>
</tr>
<tr>
<td>(AcO)2GlcNAc</td>
<td></td>
</tr>
<tr>
<td>VII Asn-Ala-Asn-Ser-Ile-Glu</td>
<td>84</td>
</tr>
</tbody>
</table>

* Solvent of crystallisation.
§ Sequence taken from an excretory-secretory protein of the nematode parasite Trichinella spiralis.

In conclusion, the solid phase synthesis of monosaccharide-containing N-glycopeptides is readily achievable using standard Fmoc peptide chemistry, giving good yields and high purity.

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