Chemical synthesis of chemokines: accessing novel research reagents

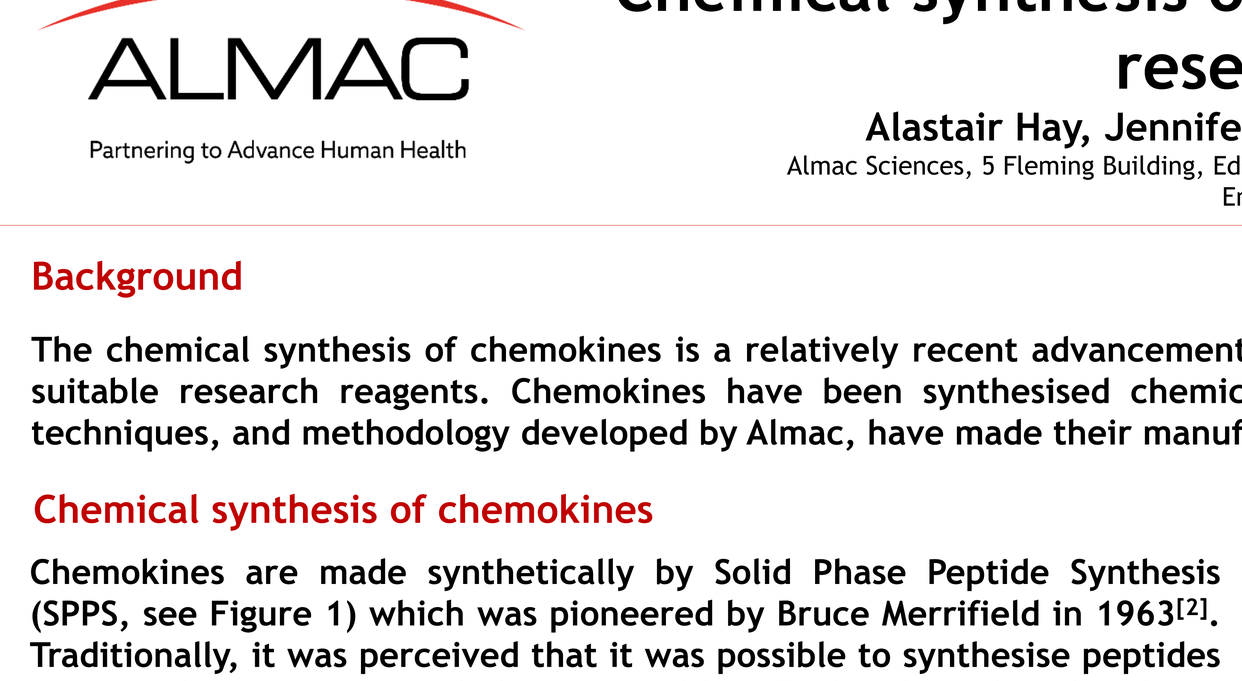
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Background

The chemical synthesis of chemokines is a relatively recent advancement, but one that should be strongly considered when identifying a source of suitable research reagents. Chemokines have been synthesised chemically since the early 1990s[1], but recent advances in peptide synthesis techniques, and methodology developed by Almac, have made their manufacture routine.

Chemical synthesis of chemokines

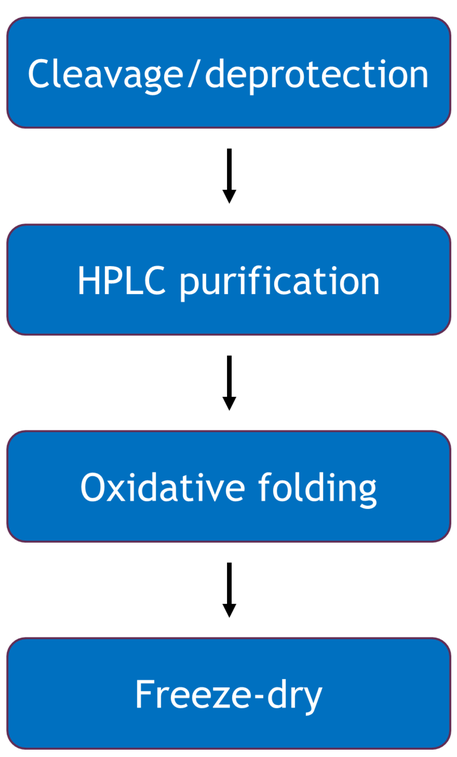
Chemokines are made synthetically by Solid Phase Peptide Synthesis (SPPS, see Figure 1) which was pioneered by Bruce Merrifield in 1963[2]. Traditionally, it was perceived that it was possible to synthesise peptides up to only 40 amino acids by SPPS, although that boundary has been pushed back considerably in the last 20 years. Since most chemokines are 70-80 amino acids in length, despite advances in methodology, chemokine synthesis remains challenging, not least because in addition to the number of amino acids, an oxidative folding step is required to ensure the correct disulfide bridge arrangement and protein tertiary structure.



SPPS is the process of building up a peptide on an insoluble solid support, and consists of a repeated cycle of amino acid coupling and deprotection steps. Each amino acid is orthogonally protected to enable chain elongation on the backbone only. Each amino acid in the sequence is added one at a time, and the cycle repeated until the full sequence is completed.

***Figure 1:** : Principles of solid phase peptide synthesis (SPPS)*

Downstream manufacturing steps



Cleavage of the crude peptide from the resin, and concomitant removal of side chain protecting groups is achieved by treatment of the resin-bound peptide with strong acid.

Impurities formed from incomplete amino acid coupling or by-products formed during the cleavage/deprotection step are removed by RP-HPLC purification.

The correct tertiary structure is vital for biological function. The chemokine is folded under oxidative conditions to ensure the correct disulfide bridge arrangement and tertiary structure. A second purification step is performed.

The final product is freeze-dried to produce free-flowing powder.

Advantages of chemical synthesis of chemokines

- **The process of SPPS lends itself to automation.**
- **The position of each amino acid is precisely defined, ensuring precise sequence integrity.**
- **Sequence modifications, such as incorporation of labels, is easily defined, and are incorporated in a site-specific manner.**
- **The process is quick, and rapidly produces 10s of mg in a few weeks.**

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References:

[1] Ian Clark-Lewis et al., Biochemistry 1991, 30(12), 3128-3135.

[2] R. B. Merrifield et al., Journal of American Chemical Society 1963, 85(14), 2149-2154

Incorporating labels onto chemokines

Chemical synthesis enables chemokines to be labelled with probes in a site-specific manner. Labels can be incorporated by two methods (i) on-resin or (ii) in solution.

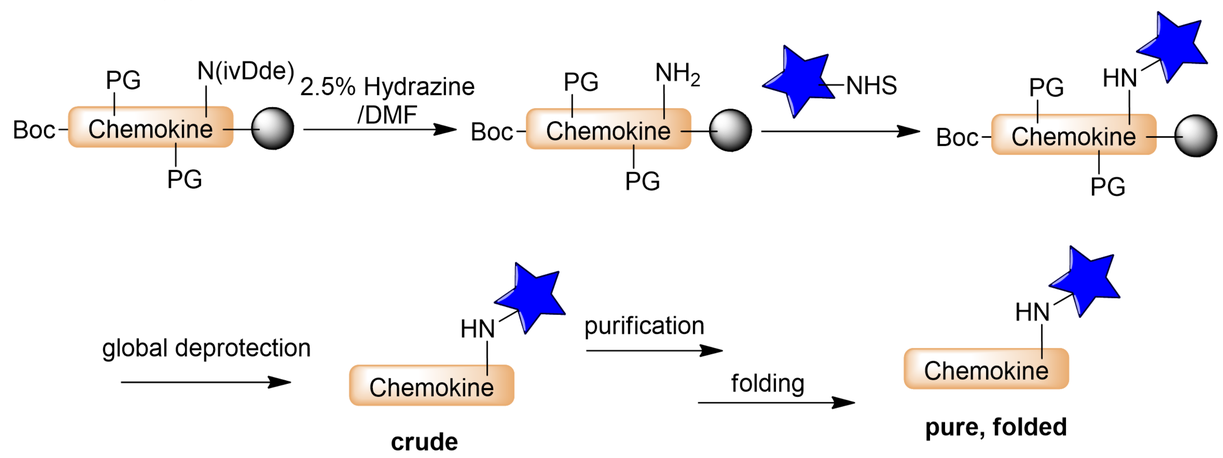
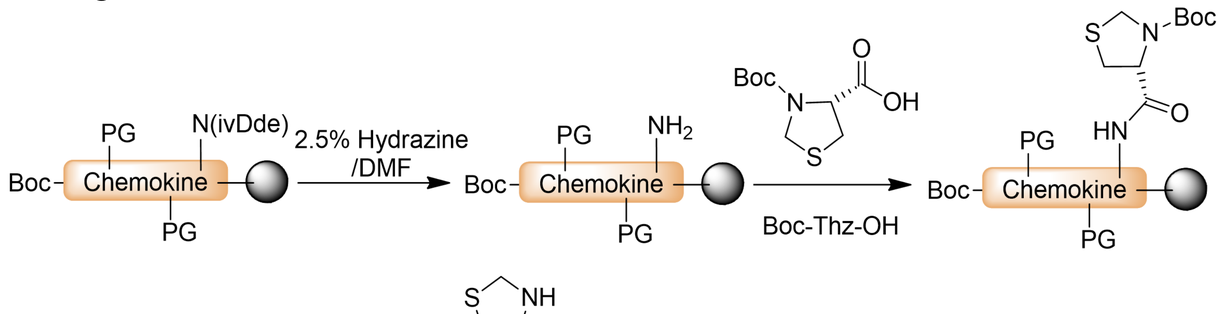


***Figure 2:** On-resin approach to chemokine labelling*

On-resin labelling is entirely site-specific, but results in synthetic impurities being labelled, and is relatively inefficient in terms of the label use.

An alternative approach is to label after purification and folding. Such an approach was developed by Almac, and requires the incorporation of a "masked" Cys (thiazolidine), which can be exposed under mild conditions for labelling, whilst keeping the required disulfide bridge arrangement intact.



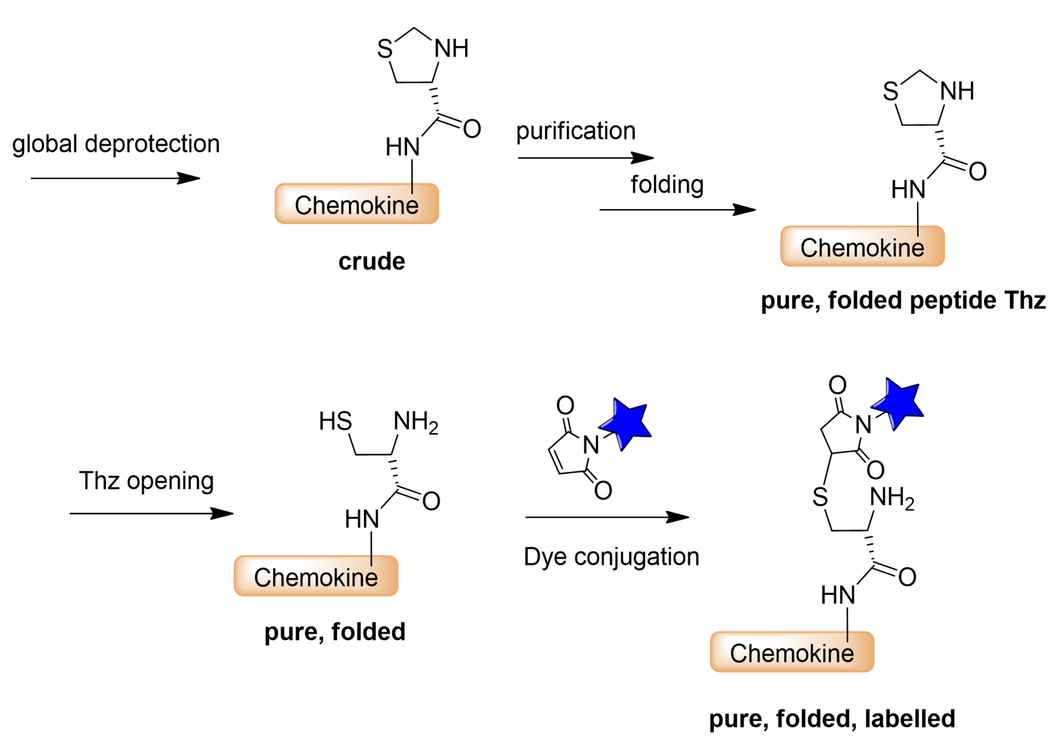


***Figure 3:** Chemokine labelling in solution*

The solution phase approach is more efficient than the on-resin approach as it means that peptide impurities are not labelled, there is no issue with scrambling the disulfide bridge connectivity, and is the more efficient route in terms of consumption of the label.

Biological assays confirm that chemokines produced by chemical synthesis are as active as the recombinantly produced counterparts, even after labelling.

Conclusion

Chemical synthesis opens the door to the rapid, low cost manufacture of chemokines. Specific advantage is afforded over classical recombinant methods by ensuring an animal and carrier-free source of reagents, enabling the incorporation of unnatural amino acids, and enabling the straightforward labelling with useful probes such as biotin and AF647®.