Carbon-14 Labelled Peptides: Solid Phase Peptide Synthesis, Biotinylation & PEGylation

Sean Kitson, William Watters
Almac Sciences, 20 Seaoge Industrial Estate, Craigavon, UK, BT63 5QD
Email: william.watters@almacgroup.com

Background

The radiolabelling group at Almac have synthesised a number of peptide APIs containing carbon-14 amino acid residues using the solid phase peptide synthesis (SPPS) approach. A number of these carbon-14 labelled peptides were modified by the addition of polyethylene glycols (PEGs) to produce a new chemical entity with a different pharmacological profile. In some cases carbon-14 labelled peptides can undergo biotinylation to provide targeted drug substances. This poster gives a general overview of Solid Phase Peptide Synthesis (SPPS), PEGylation & Biotinylation towards the synthesis of carbon-14 labelled peptides.

Almac has prepared a number of carbon-14 labelled peptides using solid phase peptide synthesis (SPPS) techniques (Figure 1)[1]. Key to the design of a labelled peptide synthesis is selection of a suitable amino acid to label. Almac's radiochemists are assisted by our highly experienced Protein and Peptide Technology (PPT) group to design a viable synthesis strategy and to run the initial trials[2].

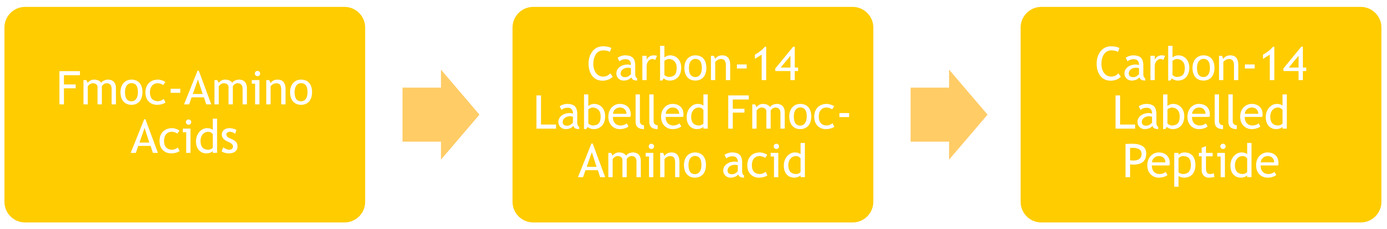


***Figure 1**: Solid Phase Approach*

Further expertise is contributed by Almac's peptide R & D operation near Edinburgh, Scotland who use solid-phase technologies developed in-house to manufacture pharmaceutical-grade peptides of up to 200 amino acid residues.

Choosing the right carbon-14 amino acid

The simplest way to introduce carbon-14 into a peptide is via a glycine residue. The chemical synthesis of the achiral carbon-14 labelled glycine is straightforward and high yielding since no resolution of unwanted enantiomers is required. Glycine can be labelled on one or both carbon atoms to yields specific activities up to 120 mCi/mmol.

If other carbon-14 labelled amino acids are required for the synthesis of a peptide, selection of amino acids that do not require side chain protection helps avoid an increase in the number of synthetic steps.

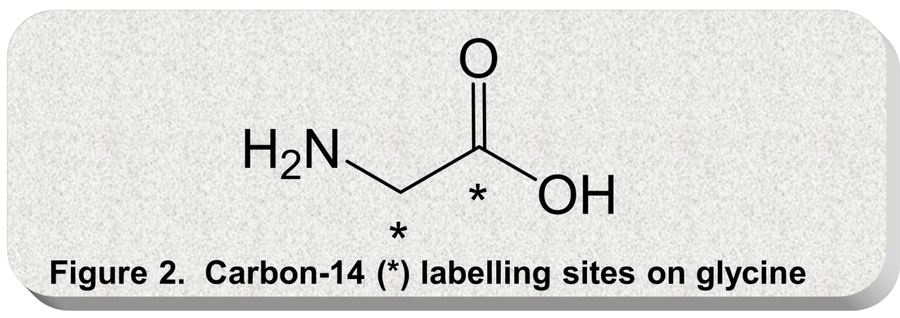
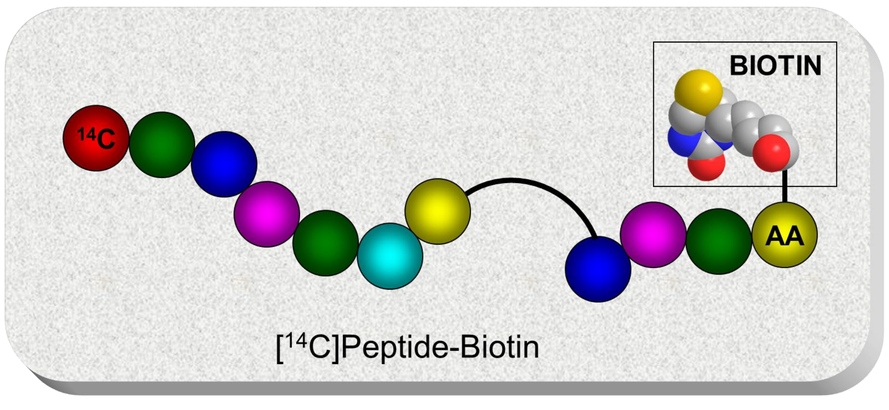


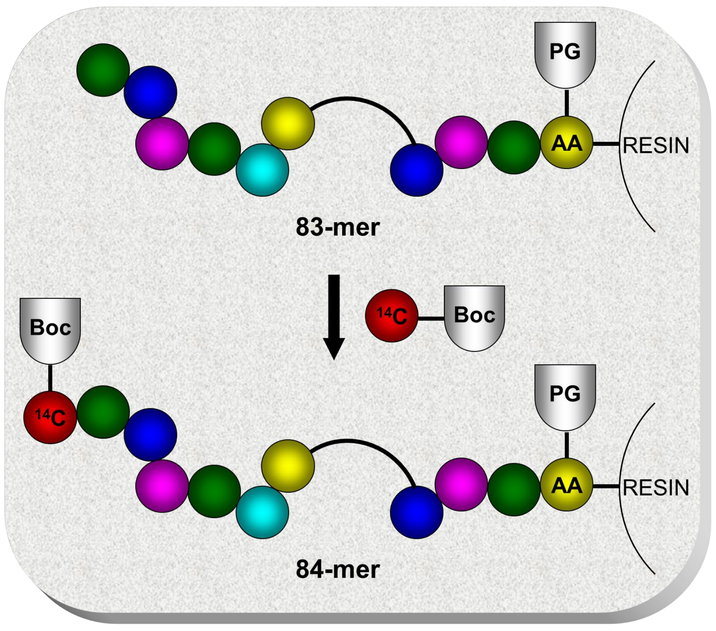
Figure 2. Carbon-14 (*) labelling sites on glycine

Case Study 1 - Target: [^{14}C]Peptide-Biotin



Requirements:

- **Solid phase synthesis of 83-mer peptide**
- **Conversion of *L*-[U-^{14}C]isoleucine to *N*-Boc-*L*-[U-^{14}C]isoleucine**
- **Terminal coupling of *N*-Boc-*L*-[U-^{14}C]isoleucine to unlabelled peptide on resin**



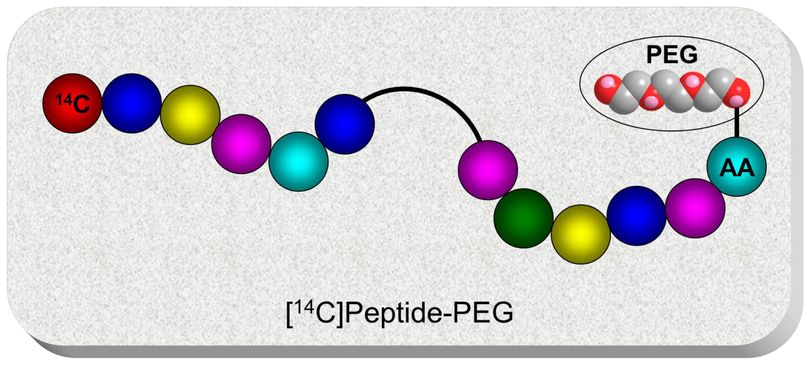
Synthesis Steps

1. **Unlabelled 83-mer resin bound peptide synthesis**
2. **Fmoc cleavage**
3. **Coupling of the *N*-Boc-*L*-[U-^{14}C]isoleucine**
4. **Cleavage of protecting group (PG)**
5. **Biotinylation**
6. ***N*-Boc cleavage**
7. **Resin cleavage to release [^{14}C]Peptide-Biotin**
8. **Purification & lyophilisation**

Deliverables:

- **~ 10 mg of [^{14}C]Peptide-Biotin was produced for ADME studies**
- **Radiochemical purity (HPLC) > 98 area%**
- **Chemical purity (HPLC) > 98 area%**
- **Specific Activity >300 mCi/mmol**

Case Study 2 - Target: [^{14}C]Peptide-PEG



Requirements:

- **Solid phase synthesis approach**
- **Conversion of [^{14}C]glycine to *N*-Boc-[^{14}C]glycine**
- **Terminal coupling of *N*-Boc-[^{14}C]glycine**

Synthesis Steps

1. **Coupling of amino acid residues to the resin**
2. **Fmoc cleavage**
3. **Coupling of *N*-Boc-[^{14}C]glycine**
4. **Resin cleavage & Purification**
5. **PEGylation**
6. **Deprotection & purification**

Deliverables:

- **[^{14}C]Peptide-PEG was produced for ADME studies**
- **Radiochemical purity (HPLC) > 98 area**
- **Chemical purity (HPLC) > 98.0 area%**
- **Specific Activity >20 mCi/mmol**

References
1. Kitson, S. L; Accelerated Radiochemistry, *PMPS Manufacturing*, May 2010, 68-70.
2. www.almacgroup.com