Radiolabelling in the New Age of Peptide Therapeutics

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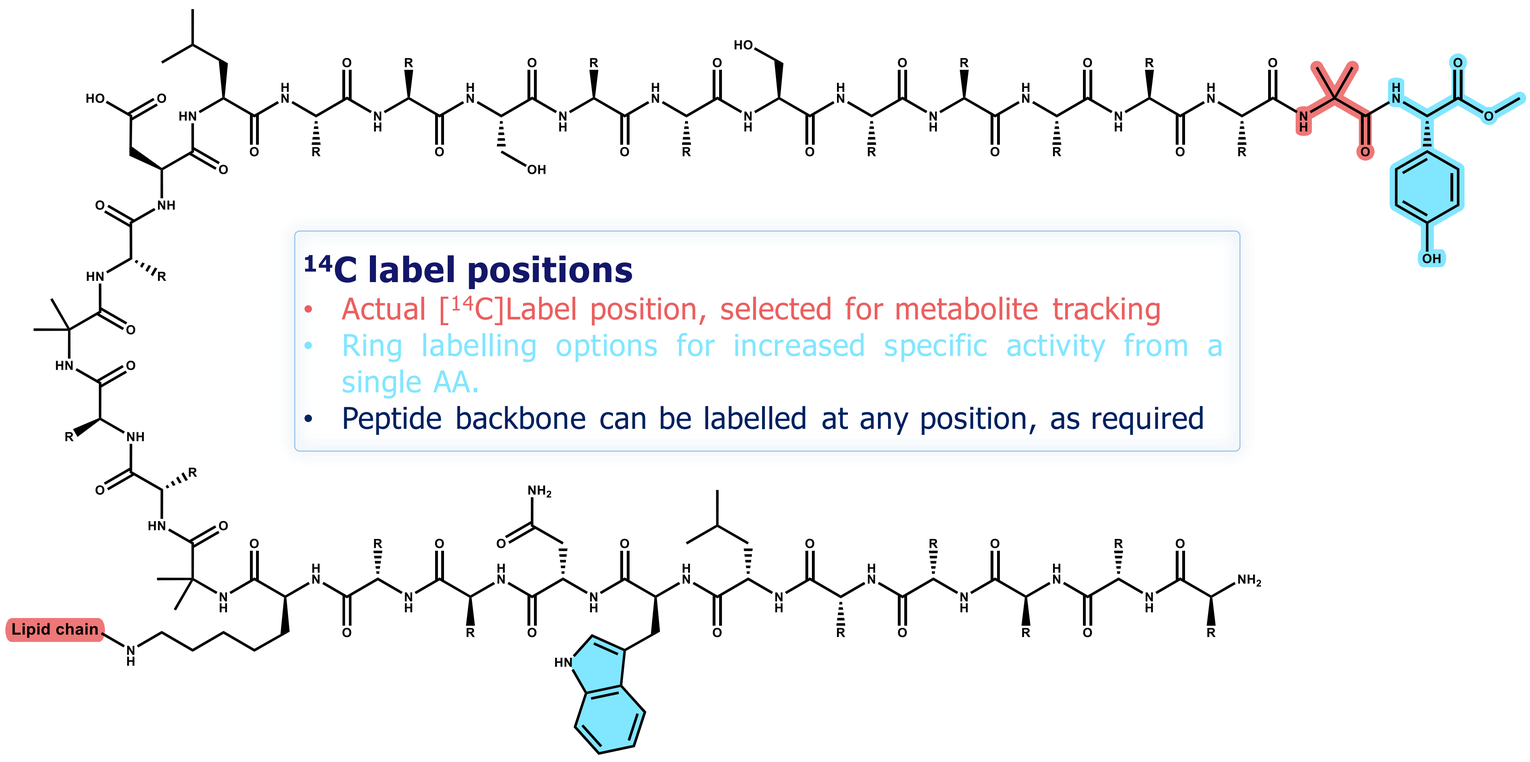
https://doi.org/10.17952/38EPS.2026.P449

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[^{14}C]TAK-510

[^{14}C]TAK-510 is a dual-radiolabelled, lipidated 31-amino acid peptide developed as a glucose-dependent insulinotropic polypeptide receptor (GIPR) agonist for the treatment of cyclic vomiting syndrome (CVS). The molecule is presented here as a case study demonstrating contemporary radiolabelling approaches for peptide therapeutics used in preclinical and early clinical development, including quantitative whole-body autoradiography (QWBA), mass balance, ADME, and human ADME (hADME) studies. Developed in partnership with Takeda to support a mass balance study, [^{14}C]TAK-510 incorporates two strategically positioned radiolabels: one within the peptide backbone at aminoisobutyric acid (AIB^{2}) and a second within the lipid moiety attached to Lys^{21}. This dual-labelling strategy enabled comprehensive profiling of metabolites and confirmed the presence of parent TAK-510 as the sole circulating drug-related component in plasma.



^{14}C label positions

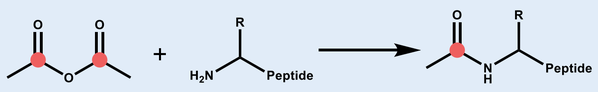
- Actual [^{14}C]Label position, selected for metabolite tracking
- Ring labelling options for increased specific activity from a single AA.
- Peptide backbone can be labelled at any position, as required

Peptide backbone

The traditional [^{14}C] labelling strategy for linear unmodified peptides is to position the label near the N-terminus or in an n-terminus acetylation, for convenience of synthesis. However, metabolic data may require labels to be incorporated towards the middle of the chain or in multiple locations in the sequence. If a high specific activity is required, more than one label could be required. Ring labelled amino acids such as phenylalanine and tyrosine can provide high specific activity by incorporating universally labelled aromatic rings which can be tuned to >120 mCi/mmol if required.

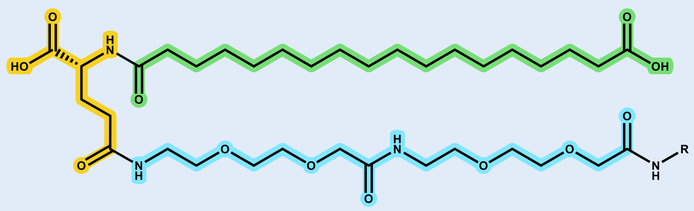
The high specific activity achieved with ring labelled compounds can prove suitable for tests that usually require high radioactivity/weight ratios, such as QWBA studies, which usually rely on the relatively high specific activity associated with tritiated compounds instead.

End capping the *N*-terminus with [^{14}C]acetic anhydride is one of the simplest ways to introduce a label into a peptide backbone



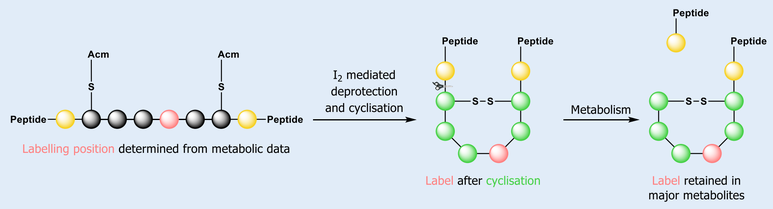
Lipidation

Lipidation is an increasingly common technique for extending peptide half-life and circulation time in the body. It has also provided additional convenient radiolabelling options. The spacers, γ-glutamic acid and lipids themselves can all be labelled. It is common for the lipid to be built on the resin after the completion of the main peptide chain. This is convenient for radiolabelling as it minimises the number of radioactive steps required. Below we've illustrated the lipid side chain of Semaglutide with the potential label sites highlighted. Most lipidation strategies use the similar functionalities which has prompted the development of synthetic strategies for [^{14}C] label incorporation into any length lipid, suitably protected γ-glutamic acid and 2-aminoethoxy-2-ethoxyacetic acid (AEEA).



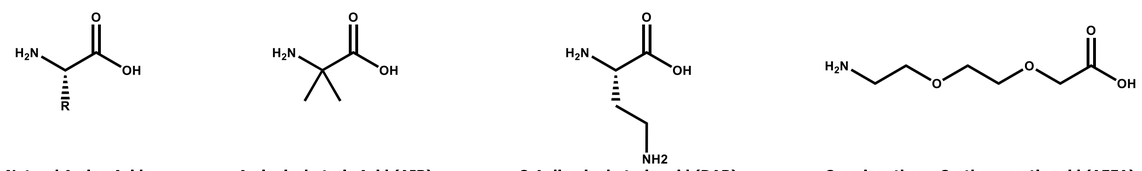
Cyclisation

Although not a consideration for TAK-510, the increase in cyclic therapeutic peptides with improved circulating half-life, target specificity, and conformational stability has required a shift in labelling strategy. From a preclinical study perspective, these metabolically robust cyclic structures can persist in vivo for extended periods and are often present in most identifiable metabolites. Therefore, they require appropriate radiolabelling to enable comprehensive metabolite identification and profiling during preclinical development. This frequently necessitates incorporation of the radiolabel at positions located deep within the loop/s to ensure retention of the label.



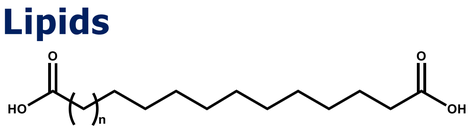
Library of available labelling locations

Natural and non-natural amino acids



We have the capability to label at multiple sites in all natural amino acids as well as a host of non-natural amino acids. Our library of non-natural amino acids is continuously being expanded.

Lipids



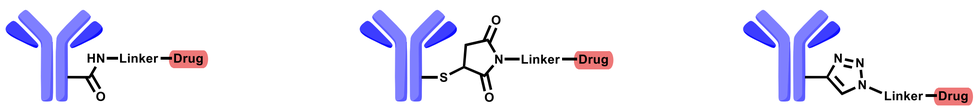
As lipid side chains have become an important part of increasing the bioavailability and reducing degradation of peptide-based drugs radiolabelling at various sites on the lipid has become more important. With the capacity to label throughout the chain and at either of the carbonyl groups, the entire lipid can be labelled based on customer needs or convenience.

PEGs



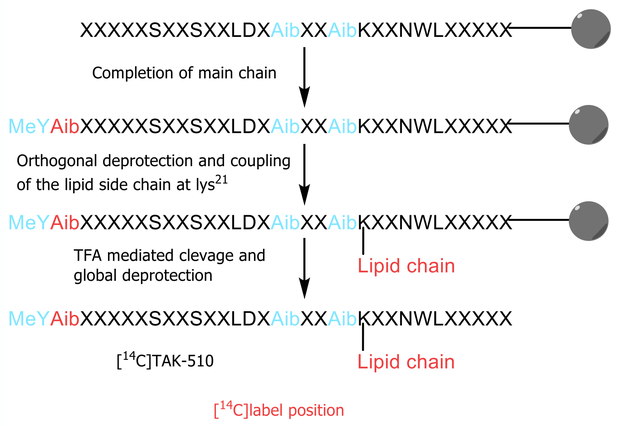
PEGylation is another common technique of adjusting the target specificity or circulating half-life of peptides in vivo. We've developed highly flexible labelling strategies that can accommodate linear or branched PEGs of any size. Labelling sites include the main chain, terminal residues and branched linkers.

Antibody Drug Conjugates (ADCs)



We can label ADC drug payloads using lysine-peptide ligation, thiomaleimide chemistry, or (2+3) cycloaddition. ADCs are purified using diafiltration, tangential flow filtration (TFF), and size exclusion chromatography (SEC). We determine ^{14}C-labelled drug-to-antibody ratios (DAR) and provide SEC-HPLC, RP-HPLC, and mass spectrometry analysis. We also operate with OEB 5 containment capability.

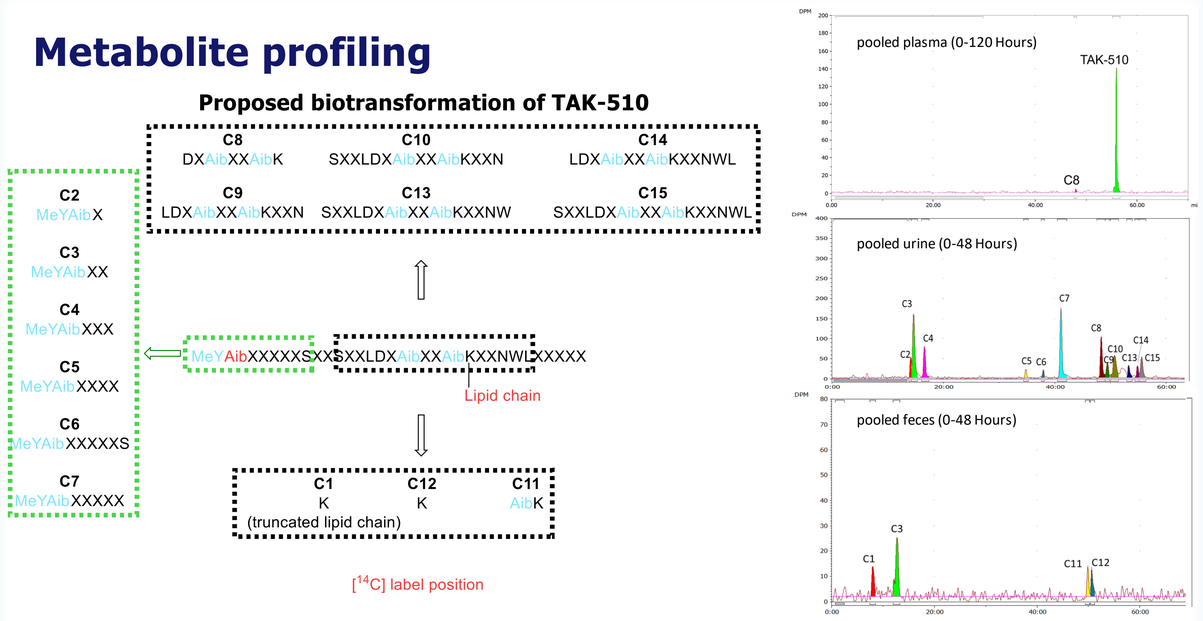
Synthesis



[^{14}C]TAK-510 was synthesised on resin using orthogonal protection on lys^{21} to build the peptide backbone, coupling the [^{14}C]aib^{2} and MeY^{1} to complete the main chain. Once complete, lys^{21} was deprotected and the lipid side chain was built up using standard Fmoc chemistry. Global cleavage and deprotection under TFA conditions yielded crude TAK-510 which was purified by preparative HPLC.

While this was a convenient strategy for obtaining the desired label positions it was noted that there was significant wastage of labelled material when coupling onto the side chain due to its proximity to the C-terminus. This required more lipid-labelled material than anticipated to achieve complete coupling of the exposed lysine side chain.

Metabolite profiling



The above data was first published at ISSX 2024 by Yukuang Guo, Eric Ballard, Sean Zhu: **Takeda Development Center Americas, Inc., Cambridge, MA**

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