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## **Short Research Article**

# Tritium labelling of the GLP-1 analogue liraglutide<sup>†</sup>

UFFE S. LARSEN\*, HEIDI B. HANSEN, ANNE-METTE DAHL, LONE SØRENSEN and JESPER B. KRISTENSEN

Isotope Chemistry Group, Novo Nordisk A/S, Novo Nordisk Park, Maaloev DK-2760, Denmark Received 1 September 2006; Revised 19 January 2007; Accepted 21 January 2007

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# Introduction

Liraglutide, an analogue of glucagon-like peptide-1 (GLP-1), is currently in clinical trails for treatment of type 2 diabetes. Liraglutide is GLP-1 modified in the Lys<sub>26</sub> position with the addition of a N- $\varepsilon$ -( $\gamma$ -Lglutamyl(N-α-palmitoyl)) group and Lys<sub>34</sub> is replaced by Arg (Figure 1). This modification provides a prolonged duration of action and an overall enhanced stability of the protein, which is desirable for a 'once daily' dosing regime. Any modification in an indigenous protein will obviously be subject to comprehensive investigations in preclinical studies and consequently require isotope labelling of the compound.

# Results and discussion

For early metabolism and autoradiography studies Liraglutide was labelled with tritium or iodine-125 in Tyr<sub>19</sub> within the peptide backbone. Tritium was incorporated via iodination of Tyr<sub>19</sub> followed by tritio-dehalogenation (Scheme 1). The iodination was performed using two equivalents of sodium iodide with careful control of reaction time and pH ( $\leq$ 1 h, pH 7.4). This gave selective incorporation of iodine in  $Tyr_{19}$  with 30–50% conversion. Prolonged reaction time caused fibrillation of the peptide as well as incorporation of iodine in His7. Treatment of the crude precursor [Tyr<sub>19</sub>-<sup>127</sup>I] Liraglutide (2) with tritium gas provided the tracer [Tyr<sub>19</sub>-3H]Liraglutide (3) (>98% purity, SA 8 Ci/mmol).

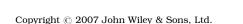
For the later metabolism studies isotope labelling of  $N-\varepsilon$ -( $\gamma$ -L-glutamyl( $N-\alpha$ -palmitoyl)) group requested. Consequently, an alternative strategy with tritium labelling of the palmitic moiety of the side chain was investigated (Scheme 2). As labelling close to the glutamine linker was required, palmitic acid (4) was  $\alpha$ -brominated by treatment with bromine in phosphorus trichloride. Successive treatment with potassium tert-butoxide provided 2-hexadecenoic acid (6).

The activated ester 7 was formed by treatment with DCC and HOSu, which, in turn, was treated with monobenzyl-protected glutamic acid to give the carboxylic acid 8. This shelf-stable intermediate was synthesised on a 1g scale in four steps from palmitic acid (4) in 45% overall yield. The carboxylic acid 8 then served as the precursor for a number of tracer productions for use in various preclinical studies. The activated ester 9 was formed from 8 by treatment with DCC and HOSu and was then catalytically reduced and deprotected in one pot by reaction with Pd/C and tritium for 2h. To avoid hydrolysis and transesterfication of 10 acidic conditions were necessary during reaction, HPLC purification and storage. Finally, coupling of the tritium-labelled activated ester 10 to [Arg<sub>34</sub>] GLP-1 (7-37) peptide provided [Pal-<sup>3</sup>H]Liraglutide (11) in 40% crude yield. RP-HPLC purification gave 11 in >99% purity with a specific activity of 40 Ci/mmol.

#### Conclusions

• The successful labelling of Liraglutide in both the peptide backbone and the side chain has been achieved.

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<sup>\*</sup>Correspondence to: Uffe S. Larsen, Isotope Chemistry Group, Novo Nordisk A/S, Novo Nordisk Park, Maaloev DK-2760, Denmark. E-mail: ula@novonordisk.com

Figure 1 Liraglutide.

$$\begin{array}{c} \text{Nal} \\ \text{Lactoperoxidase} \\ \text{Liraglutide} & \frac{H_2O_2}{H_2O} & \text{[Tyr}_{19}\text{-}^{127}\text{I]Liraglutide} & \frac{\text{Pd/C}}{\text{DMF}(1\%\text{TFA})} & \text{[Tyr}_{19}\text{-}^{3}\text{H]Liraglutide} \\ \mathbf{1} & \mathbf{2} & \mathbf{3} \\ \end{array}$$

### Scheme 1

# Scheme 2

- [Tyr<sub>19</sub>-<sup>3</sup>H]Liraglutide was synthesised in only two steps using Liraglutide as starting material.
- [Pal-<sup>3</sup>H]Liraglutide was synthesised in seven steps with tritium introduced in the final two steps.