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Short Research Article

Tritium labelling of the GLP-1 analogue liraglutide†

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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_{26} position with the addition of a *N*-ε-(γ-L-glutamyl(*N*-α-palmitoyl)) group and Lys_{34} 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_{19} within the peptide backbone. Tritium was incorporated via iodination of Tyr_{19} followed by tritio-dehalogenation (Scheme 1). The iodination was performed using two equivalents of sodium iodide with careful control of reaction time and pH ($\leqslant$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 His_7. Treatment of the crude precursor [Tyr_{19}-^{127}I] Liraglutide (**2**) with tritium gas provided the tracer [Tyr_{19}-^{3}H]Liraglutide (**3**) (>98% purity, SA 8 Ci/mmol).

For the later metabolism studies isotope labelling of the *N*-ε-(γ-L-glutamyl(*N*-α-palmitoyl)) group was 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 α-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 1 g 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 2 h. 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_{34}] GLP-1 (7-37) peptide provided [Pal-^{3}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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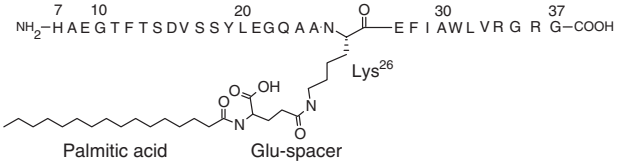
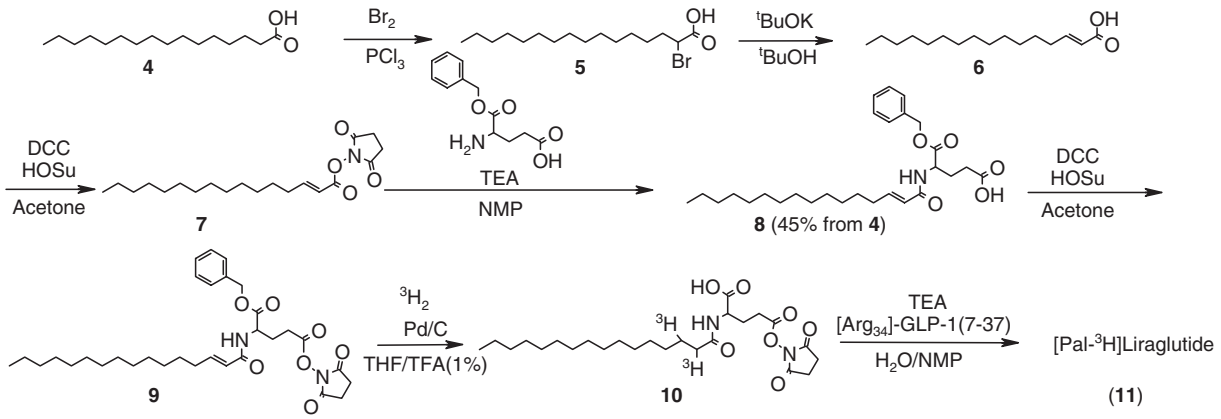


Liraglutide (1)

Figure 1 Liraglutide.

Scheme 1



Scheme 2

- [Tyr_{19}-^{3}H]Liraglutide was synthesised in only two steps using Liraglutide as starting material.
- [Pal-^{3}H]Liraglutide was synthesised in seven steps with tritium introduced in the final two steps.

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