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Short Research Article

Labelling of a potent glucagon receptor antagonist with tritium, carbon-14 and stable isotopes†

STEEN K. JOHANSEN*, SUSSI H. WAGNER, CLAUS U. JESSEN, JACOB S. VALSBORG and LARS MARTINY

Isotope Chemistry Group, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Maaloev, Denmark

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Introduction

3-{4-[1-(4-Cyclohex-1-enylphenyl)-3-(3,5-dichlorophenyl) ureidomethyl]-benzoylamino}propionic acid (**1**) is a potent glucagon receptor antagonist, which has been investigated in pre-clinical trials for the treatment of type 2 diabetes (Scheme 1). Here, we present the syntheses of tritium, carbon-14 and stable isotope labelled **1** for use in a range of preclinical studies.

Results and discussion

Tritium labelling was achieved using Crabtree's catalyst in good yield (36%) and high specific activity (95 Ci/mmol) (Scheme 1). Tritium NMR determined the labelling positions to be in the *ortho*-positions of the benzoic acid moiety as well as in the β-positions of the β-alanine moiety.

Carbon-14 labelling was achieved in two steps starting from [1-^{14}C]β-alanine ethyl ester (Scheme 1). First, [1-^{14}C]β-alanine ethyl ester was reacted with the benzoic acid **2** using standard peptide coupling chemistry followed by hydrolysis of the ester group to give [^{14}C]L in 41% overall yield and with a specific activity of 52 mCi/mmol.

Stable isotope labelling was achieved using a convergent route employing a total of ten steps with eight steps in the longest linear sequence. At least seven additional masses were required for the internal mass spectroscopy standard with six of the additional masses introduced from [U-^{13}C]benzene and another two from [$^{2}H_5$]aniline.

For the first intermediate, [U-^{13}C]benzene was converted to 1,4-bis(chloromethyl)benzene (**3**) by reaction with paraformaldehyde, $ZnCl_2$ and thionyl chloride (Scheme 2). Oxidation with HNO_3 then gave 4-formylbenzoic acid (**4**) along with several oxidative by-products, which were removed after protection of the acid moiety to provide 4-formylbenzoic acid methyl ester (**5**) in 5.1% overall yield (3 steps). For the second intermediate, [$^{2}H_5$]aniline was reacted with cyclohexanone to give the adduct **6**. This was then treated with concentrated HCl and cracked under distillation to provide 4-cyclohex-1-enylphenylamine (**7**) in 30% overall yield (2 steps).

The convergent step in the synthesis was the reductive amination of aldehyde **5** with amine **7** to give the benzoic acid derivative **8** (85%). This was then reacted with 3,5-dichlorophenyl isocyanate followed by hydrolysis to give the benzoic acid **10** (68%). Finally, reaction of **10** with β-alanine methyl ester using standard peptide coupling chemistry followed by hydrolysis provided [$^{13}C_6$,$^{2}H_2$]**1** (79%). The overall yield was 2.4% based on the longest linear sequence (eight steps) with the isotopic purity found to be >99% by LCMS analysis.

*Correspondence to: Steen K. Johansen, Isotope Chemistry Group, Novo Nordisk A/S, Novo Nordisk Park, DK-2760 Maaloev, Denmark. E-mail: skjo@novonordisk.com

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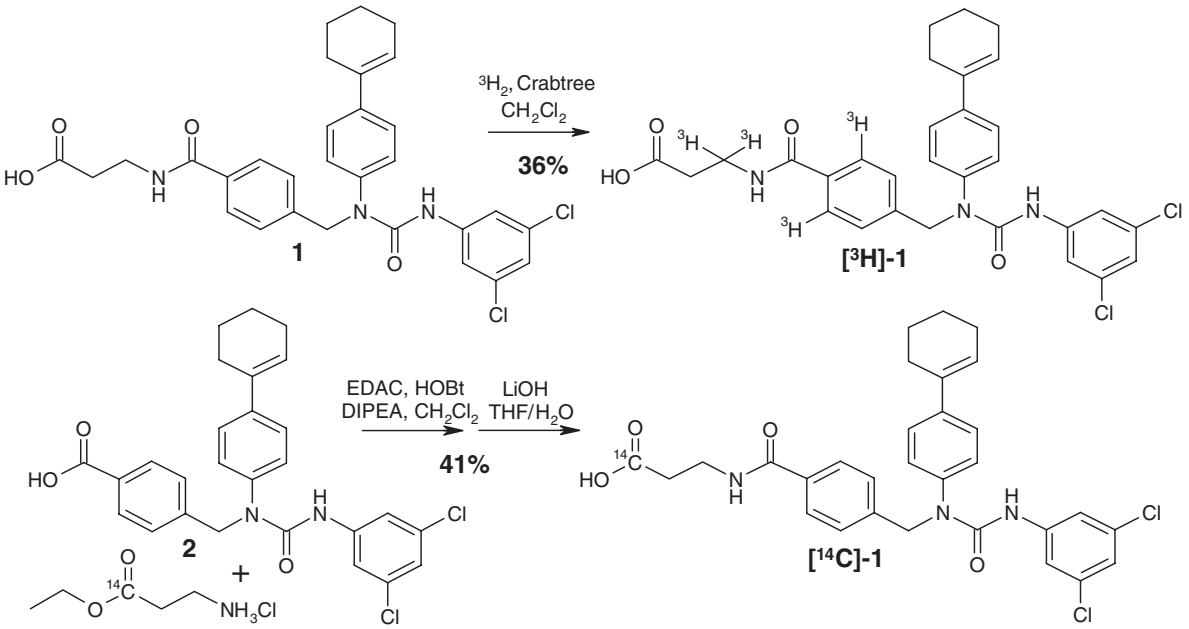
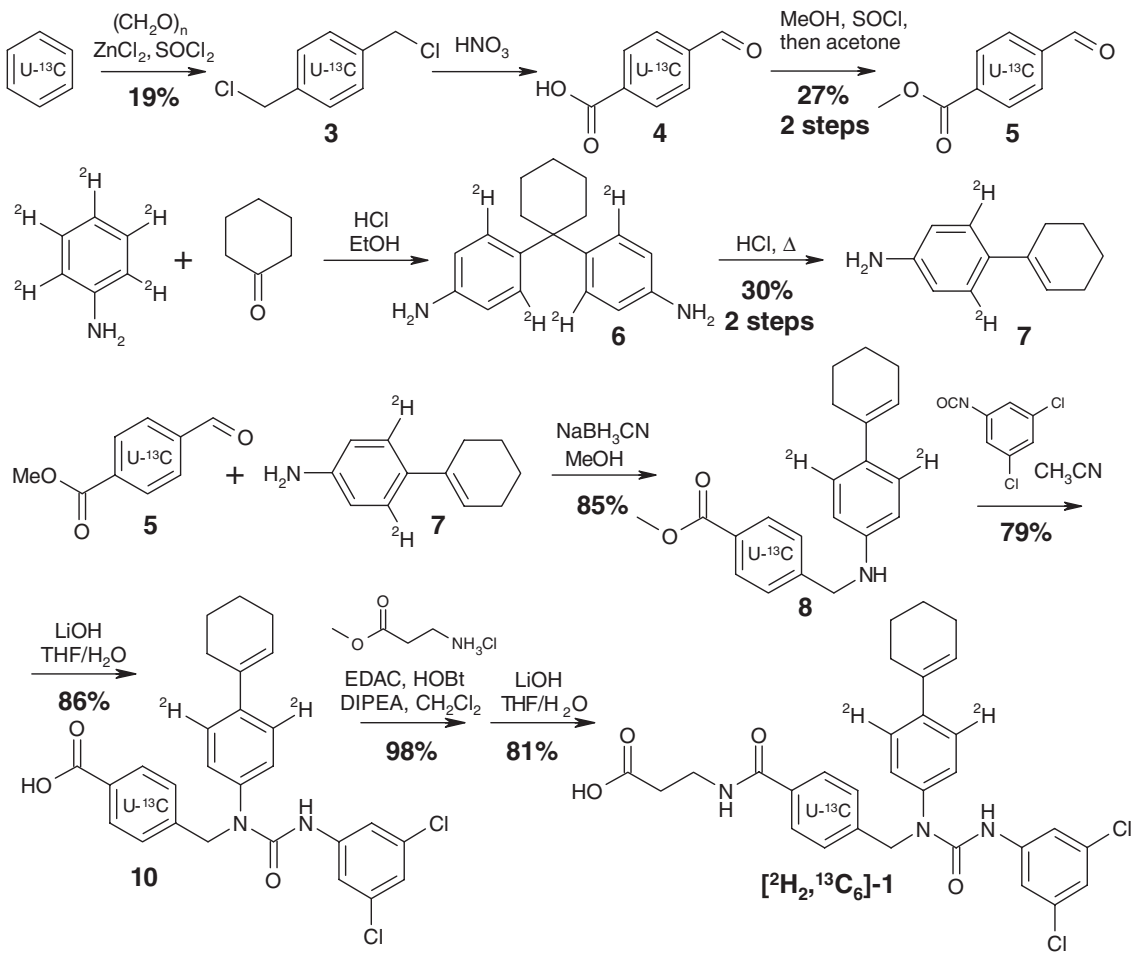


Scheme 1



Scheme 2

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