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Short Research Article

Synthesis and analysis of (^{3}H) salmon calcitonin (([^{3}H]SMC021)†

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Abstract: Tritium-labelled salmon calcitonin was synthesized successfully through a 5 step peptide synthesis. LC-MS performed on each step played an important role for in process control. For accuracy, Q-TOF MS was used to determine the specific activity of tritiated salmon calcitonin ([^{3}H]SMC021) using the triple charged ion. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: tritiation; tritium gas; salmon calcitonin

Introduction

Salmon calcitonin is a polypeptide hormone of 32 amino acids, which plays a crucial role in the treatment of osteoporosis and reduces the bone pain associated with osteoporosis and some bone tumors. Tritium labelled compound was used to help in the development of a new oral dosage form of salmon calcitonin. The synthesis and method of determination of the specific activity of tritum labelled salmon calcitonin are described.

Results and discussion

Tritium-labelled salmon calcitonin ([^{3}H]SMC021) was prepared by a five-step sequence. The synthesis began with the peptide TRICOSA, which was treated with iodine monochloride in methanol affording mainly the diiodide TRICOSA(I_2) with the iodo-substituents on the tyrosine aromatic ring. A small percentage of the material also contained a third iodo-substituent presumably on the histidine imidazole ring system. Electrospray MS(+) analysis of this iodo-compound showed mainly a mixture of diiodinated (*m*/*e*= 1511[M=2H$^+$/2) and triiodinated (*m*/*e*=1574 [M+2H$^+$/2) peptide products. Due to the acidic conditions of LCMS, the trityl group was lost on the probe. Treatment of this iodinated material with tritium gas in the presence of palladium on carbon and excess base in DMF cleanly yielded [^{3}H]TRICOSA, which was further used without purification. Electrospray MS (+) analysis showed the presence of mono-tritiated (*m*/*e*=1386[M+2H$^+$/2]), di-tritiated (*m*/*e*=1387) and tri-tritiated (*m*/*e*=1388) analogues in an approximate 30:50:20 ratio, respectively.

Removal of the trityl protecting group on [^{3}H]TRICOSA was effected by treatment with 2 N HCl in a mixture of water/trifluoroethanol affording [^{3}H]TRICOSA-H. The [^{3}H]TRICOSA-H was coupled with the disulfide-containing peptide β-1NONA01 using DCC as the coupling agent in the presence of HOBT and Hunig's base in DMF to give [^{3}H]DOTRIA-ROH, which was purified by semi-preparative HPLC. MS(+) analysis showed good formation of the labelled coupled peptide (*m*/*e*=1896, 1897, 1898 [M+2H$^+$/2]) and no [^{3}H]TRICOSA remaining. Treatment of [^{3}H]DOTRIA-ROH with TFA in methylene chloride removed all protecting groups affording pure [^{3}H]SMC021 with radiochemical purity >95%. Q-TOF-MS analysis showed triple charged peaks at [M+3H$^+$]/3=1144.5, 1145.16, 1145.83 and 1146.5 which correspond to non-labelled, mono-, di- and triply-charged SMC021. To calculate the specific activity of [^{3}H]SMC021 the enrichment of

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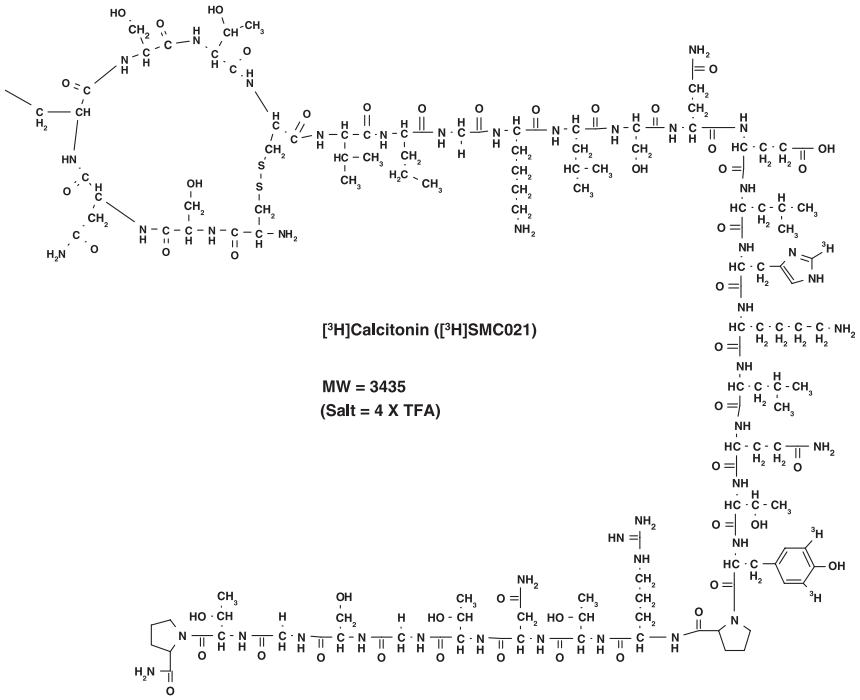

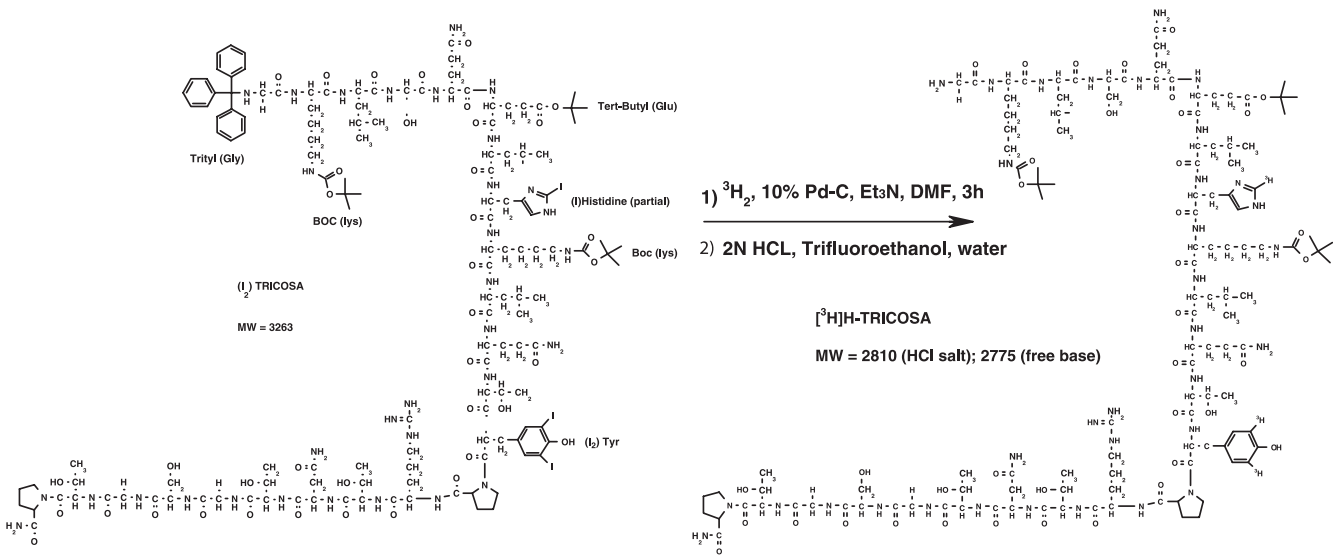

triple charged peaks (1145.16, 1145.83, 1146.5) was used to compare with the non-labelled peak 1144.5. Based on the above data the specific activity was calculated as 43 Ci/mmol.

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