Synthesis of In-DOTA labeled peptide

According to the fluorenyl-methoxy-carbonyl (FMOC) solid phase peptide synthesis strategy [1], the peptide was synthesized automatically on a batch peptide synthesizer (PSSM-8, Shimadzu, Kyoto, Japan) using FM-protected amino acid derivatives, catalyzed using 1,3-Diisopropylcarbodiimide (Fluka) /1-hydroxy-1,2,3-benzotriazole (Orpegen)/ diisopropylethylamine (Fluka) (DIC/HOBt/DIPEA) in 5-fold excess. The $B\beta_{15-42}$ analogue (BOC)D(tBu)R(Pbf)GHR(Pbf)PLD(tBu)K(BOC)K(BOC)R(Pbf)E(tBu)E(tBu)APS(tBu)LRPAPP PIS-(tBu)GGGY(tBu)R(Pbf)K(Dde)-NH2 was synthesized on a Tentagel Rink Amide resin (0,25 mmol g^{-1}, Rapp Polymere, Tübingen, Germany). Subsequently, the resin attached fully protected peptide was treated with 5 % hydrazine (Fluka) /dimethylformamide (Acros) to cleave the orthogonal protecting group 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (-Dde) and after removal of the cleavage solution $DOTA(tBu)_3$ was attached to the side chain amine of lysine in position 29 using 1H-Benzotriazol-1-yloxy)tripyrrolodino-phosphonium Hexafluorophosphate (PyBOP, VWR) and DIPEA. The peptide was deprotected with trifluoroacetic acid (TFA, Acros) containing water, with phenol (Fluka) and TIS (Acros) as scavengers, precipitated with di-ethylether (VWR), isolated in high yields and lyophilised. The purification was performed with preparative RP-HPLC column (20 x 250 mm) filled with a C-18 stationary phase (Kromasil, 15 µm particle size, Eka Chemicals AB**,** Bohus, Sweden) using an aqueous gradient of 10 to 60% acetonitrile in 0.1% TFA. Pure fractions were analysed and pooled before lyophilisation.

The purified peptide GHRPLDKKRE EAPSLRPAPP PISGGGYRK(DOTA)-NH2 was dissolved in 0,2 M sodium acetate buffer (pH=5) and, after addition of 3 equiv. $InCl_3$ (Sigma Aldrich, Vienna, Austria), refluxed for 25 min at 85 °C. Subsequent purification by prep. RP-HPLC gave GHRPLDKKRE EAPSLRPAPP PISGGGYRK(DOTA[In])-NH2 x TFA in good yields after lyophilisation. The homogeneity of the final products was estimated by analytical RP-HPLC. The identity was finally confirmed by MALDI-TOF-MS (Axima CFR, Kratos, Shimadzu) in positive reflectron mode.

[1] E. Atherton, R.C. Sheppard, Solid Phase Peptide Synthesis: A Practical Approach, 1989, IRL Press, Oxford, U.K.