# LABELLING OF $(DOTA)_n$-OCTROETIDE DERIVATIVES WITH $^{68}Ga$

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**Introduction:** Radiometal(III)labelled peptidic derivatives of somatostatin provide substantial impact in nuclear medicine diagnosis and therapy. Recently, $^{68}Ga$ has contributed to the molecular imaging of endocrine tumors mainly using DOTA-octreotide derivatives. An important characteristic of positron emitting $^{68}Ga$ is its availability via the $^{68}Ge/^{68}Ga$ radionuclide generator system. However, the specific activities achieved both by conventional or microwave-supported heating are limited. Further increase in specific activities might be achieved by introducing $(DOTA)_n$-octreotide derivatives with n=2 or 4. In this study, the labelling yields, kinetics and specific activities of DOTA-octreotide, $(DOTA)_2$-Lys-$Sar_5$-octreotate (DS94), $(DOTA)_4$-$apg_3$-octreotate (DS72) and $(DOTA)_4$-$apg_3$-$sar_5$-octreotate (DS112) with $^{68}Ga$ have been investigated.

**Experimental:** The peptides were synthetised on solid phase using the Fmoc strategy. pentasarcosine was used as primary spacer and aminopropylglycine(apg) to introduce divalency and tetravalency. DOTA was coupled as the tris(tBu) ester. After full assembly the peptide was cyclised on the resin, cleaved and deprotected followed by HPLC-purification.

Pre-concentration and purification of the initial $^{68}Ge/^{68}Ge$ generator eluate was performed utilizing a miniaturized column with organic cation exchanger resin (50 W-X8 200-400 mesh) and HCl / acetone media. The purified fraction was used for labelling of DOTA-octreotate derivatives.

First, various amounts of 3.5, 7.0, 10.5, 14.0 nmol of DOTA-$Tyr^3$-octreotide (DOTATOC) were used. Next, 3.5 nmol of $(DOTA)_n$ (n=2, 4) peptides, 3.5 nmol each were compared. Labelling was performed at 100 ºC for up to 15 min using pure water / aqueous HCl solution and pH 2.3. Reaction yields were determined using TLC.

Results and Discussion: SPPS gave < 10% overall yield of n=2 and n=4 DOTA derivatives. Reproducible and high yields for $^{68}Ga$-labelling of DOTATOC are obtained using 15 µg and 20 µg after 3 to 10 min reaction time, for example. At amounts of 5 µg (3.5 nmol), yields are significantly lower even at 15 min reaction time. For $(DOTA)_n$ derivatives at 3.5 nmol, both yields and kinetics of $^{68}Ga$ labelling improve according to the number of chelators. Specific activities increase by factors of about 2 (n=2) and 3 (n=4) at 3, 10 and 15 min reaction time, and of about 2-3 (n=2) and 5-6 (n=4) at 1 min, respectively.

**Conclusions:** Labelling of DOTATOC is defined by its amount and its kinetics. At low peptide amounts, $^{68}Ga$ labelling yields are low due to the amounts of metallic impurities present [Fig. 1].

This is compensated by an increasing number of DOTA chelators per peptide. Labelling yields, kinetics and specific activities are higher if compared to n=1 analogs. Provided that binding affinities and pharmacokinetics of the $(DOTA)_n$-octreotide derivatives (n=2 or 4) are adequate, these derivatives are superior [Fig. 2].

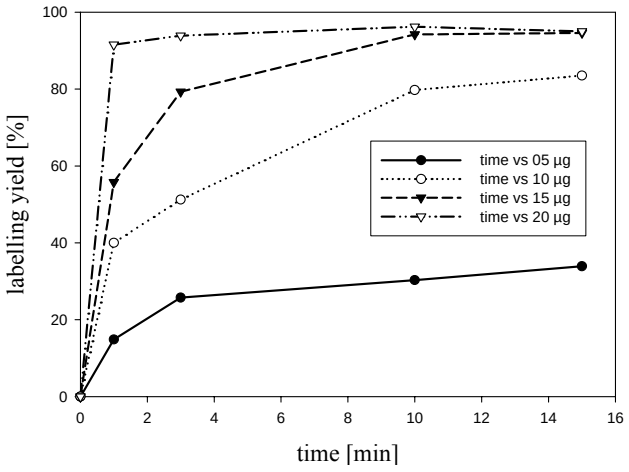


Fig 1: $^{68}Ga$-labelling yields depending on the mass of DOTA-TOC (1 µg / µl volume concentrations)

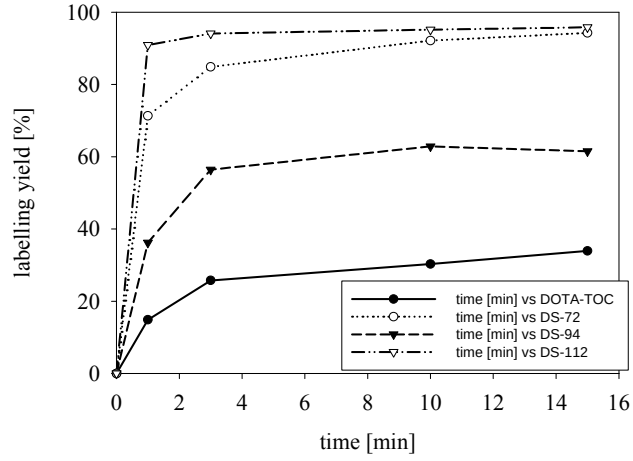


Fig 2: $^{68}Ga$-labelling yields for $(DOTA)_n$ derivatives at constant 3.5 nmol, depending on the number n of DOTA chelators per peptide