Synthesis of a technetium-99m labeled L-tyrosine derivative with the *fac*-^{99m}Tc(I)(CO)$_3$-core

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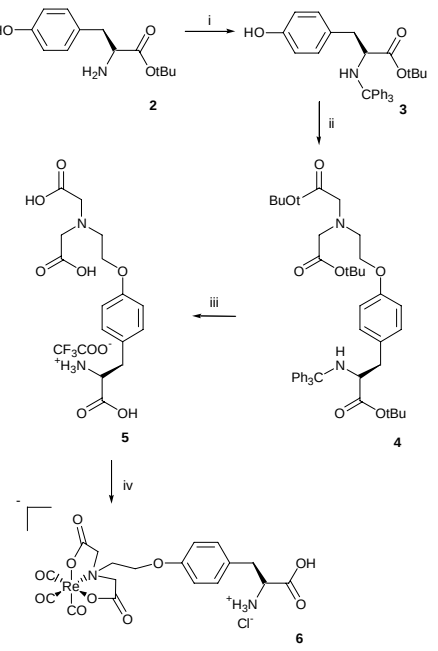
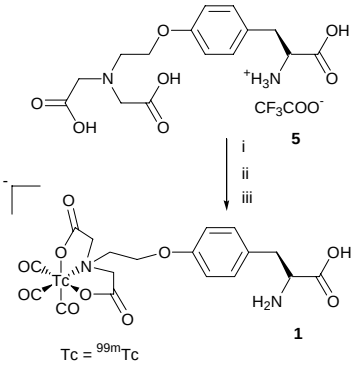
Introduction: The *fac*-[^{99m}Tc(I)(CO)$_3$]$^+$ carbonyl moiety is extremely interesting due to its high *in vitro* and *in vivo* stability when connected to various biomolecules. It has been reviewed in detail for its use in the second generation of single photon emission computed tomography (SPECT) radiopharmaceuticals.[1] Introduction of the Tc(I) can be achieved by convenient use of the *fac*-[^{99m}Tc(I)(OH$_2$)$_3$(CO)$_3$]$^+$ complex which can be synthesized easily from a commercially available kit formulation (Isolink®, Mallinckrodt) following Alberto's method of synthesizing *fac*-[^{99m}Tc(I)(OH$_2$)$_3$(CO)$_3$]$^+$ from [^{99m}TcO$_4$]$^-$ in aqueous solution.[2] The three water ligands can be replaced by mono-, bis- and tridentate ligands connected to the radiopharmaceutical, forming complexes of high stability.[3] This new labeling concept has been proven valuable in the synthesis of a large number of novel radiolabeled compounds.[4]

Aim: We intended to label the amino acid tyrosine with *fac*-[^{99m}Tc(I)(CO)$_3$]$^+$ by means of connecting a suitable tridentate ligand such as 2-[N,N-bis(tert.-butyloxycarbonylmethyl)amino)-1-bromoethane to the para-OH moiety of tyrosine (Scheme 1). Tyrosine seems to be a particulary suitable candidate because it has been demonstrated that derivatisation at the para-OH funtionality by ^{18}F-fluoroethylation does not affect its binding to amino acid transporters. This would suggest that a small technetium-containing structure like *fac*-[^{99m}Tc(I)(CO)$_3$]$^+$ might be tolerated as well. This application would be important because the use of ^{99m}Tc-radiopharmaceuticals are predominant in nuclear medicine due to their availability via a commercially available ^{99m}Tc-generator system.

Synthesis: The synthesis of the final labeled compound O-(^{99m}Tc(I)-tricarbonyl-N,N-bis(carboxymethyl)aminoethyl)-L-tyrosine potassium salt (**1**) started from L-tyrosine t.-butyl ester (**2**), which was reacted with triphenylmethyl chloride and triethylamine in DMF at 25°C to yield the tritylated compound **3** following a similar published procedure. The structural element 2-[N,N-bis(tert.-butyloxycarbonyl-methyl)amino)-1-bromoethane for complexation of the Tc(I)-tricarbonyl core was connected to the HO-moiety of the tyrosin (**3**) in acetone/TEA at RT to give **4** in 87% yield. Subsequent deprotection with trifluoroacetic acid yielded the derivatised tyrosine (**5**)[§] in quantitative yields for subsequent labeling with radioactive *fac*-[^{99m}Tc(I)(OH$_2$)$_3$(CO)$_3$]$^+$. The analoguous reaction with [NEt$_4$]$_2$[ReCl$_3$(CO)$_3$][9] was also performed for analytical purposes such as HPLC conditions for final isolation of **1**, since Tc and Re are of similar chemical behaviour (Scheme 1). For the synthesis of the analoguous Re-compound (**6**), **5** was reacted with [NEt$_4$]$_2$[ReCl$_3$(CO)$_3$] in dry methanol at 25°C for 30 min and purified by column chromatography. Radioactive labeling was conducted using the labeling precursor **5**, (1 mg, 2.2 µmol) and *fac*-[^{99m}Tc(I)(OH$_2$)$_3$(CO)$_3$]$^+$ at 100°C for 30 min which had been synthesized using the Isolink® -kit formulation and freshly eluted ^{99m}TcO$_4^-$ (200-560 MBq) from a commercially available ^{99}Mo/^{99m}Tc-generator (Scheme 2). The radiochemical yield was >98% which was proven both with radio-HPLC and radio-TLC[J]. To obtain an injectable sterile solution of the radiopharmaceutical, the reaction mixture was diluted with water and compound **1** was extracted using a 18C-SepPack cartridge® (Merck, Darmstadt, germany), rinsed with water and eluted with a hot aqueous sodium glycinate (0.5 N) solution. To this solution, isotonic NaCl was finally added and passed through a sterile filter. This injectable solution did not contain any precursor material (**5**) as proven by HPLC which could be attributed to the higher lipophilicity of the ^{99m}Tc-complex (**1**). Thus **1** was retained on the solid phase in contrast to the more hydrophilic labeling precursor **5** which was eluted during the washing step. Due to the basic labeling conditions the NH$_3^+$-moiety is deprotonated and the toxic CF$_3$COO$^-$ anion was removed by washing. Using 250-600 MBq ^{99m}TcO$_4^-$, between 180 and 500 MBq of **1** as a sterile aqueous solution could be obtained in a overall radiochemical yield of 70-80% with a radiochemical purity >98%. Enantiomeric purity of **1** was proven by chiral HPLC (CHIREX^{TM}, phenomenex, Aschaffenburg, Germany).



Scheme 1
*i, triphenylmethylchloride, TEA, DMF; ii, 2-[N,N-bis(t.-butyloxy-carbonylmethyl)amino)-1-bromoethane, TEA, acetone; iii, TFA; iv, [NEt$_4$]$_2$[ReCl$_3$(CO)$_3$], MeOH, ([NEt$_4$]$^+$ as a counter ion of **6**).*



Scheme 2.
i, ^{99m}TcO$_4^-$, Isolink®-kit, 30 min, 100°C; ii, dilution with H$_2$O, 18C-SepPack cartridge®, washing with H$_2$O; iii, elution with sodium glycinate (0.5 N), isotonic saline, sterile filtration.

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