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Research Article

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

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Summary

The synthesis of a novel technetium-99m labelled derivative of L-tyrosine as a potential tumour imaging agent for nuclear medicine diagnosis is reported. The synthesis involved the labelling precursor *fac*-[^{99m}Tc(OH$_2$)(CO)$_3$]$^+$ which was synthesized using the commercially available Isolink®-labelling kit and the tyrosine derivative *O*-(*N*,*N*-*bis*(carboxymethyl)aminoethyl)-L-tyrosine trifluoroacetate. The labelled compound *O*-(^{99m}Tc(I)-tricarbonyl-*N*,*N*-*bis*(carboxymethyl)aminoethyl)-L-tyrosine was obtained in a radiochemical yield of 70–80% within 60 min with a radiochemical purity greater than 98% without any HPLC purification step. Purification was achieved merely by solid phase extraction. Chemical as well as chiral purity was determined using gradient- and chiral HPLC. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: tyrosine; Tc-99m; Isolink®-labelling kit

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.[1–3] 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.[4] The three water ligands can be replaced by mono-,

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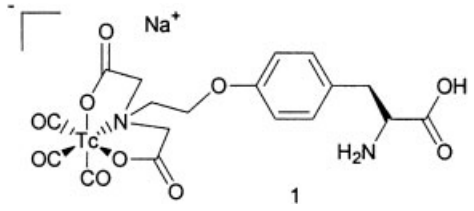


Figure 1. ^{99m}Tc-labelled tyrosine derivative (Tc = ^{99m}Tc, Na^+ as a counter ion)

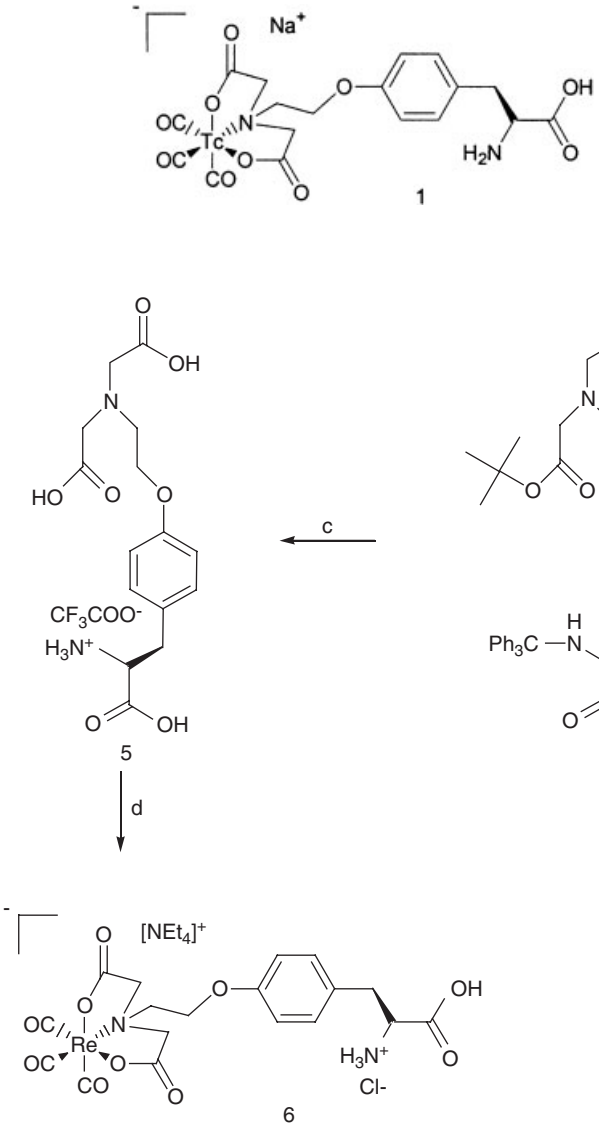
bis- and tridentate ligands connected to the radiopharmaceutical, forming complexes of high stability.[5] This new labelling concept has proved to be valuable in the synthesis of a large number of novel radiolabelled compounds.[6] The labelling of amino acids with this novel Tc(I)-core for imaging cancer has to our best knowledge not been described so far. Furthermore, compound 1 may be suitable for the labelling of peptides due to its simple and high yield preparation.

We intended to label the amino acid tyrosine with *fac*-[^{99m}Tc(I)$(CO)_3]^+$ by connecting a suitable tridentate ligand such as 2-[*N*,*N*-*bis*(*tert*.-butyloxycarbonylmethyl)amino)-1-bromoethane to the *para*-OH moiety of tyrosine (Figure 1). Tyrosine seems to be a particularly suitable candidate because it has been demonstrated that derivatization at the *para*-OH functionality by ^{18}F-fluoroethylation does not affect its binding to amino acid transporters.[7,8] 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.

Results and discussion

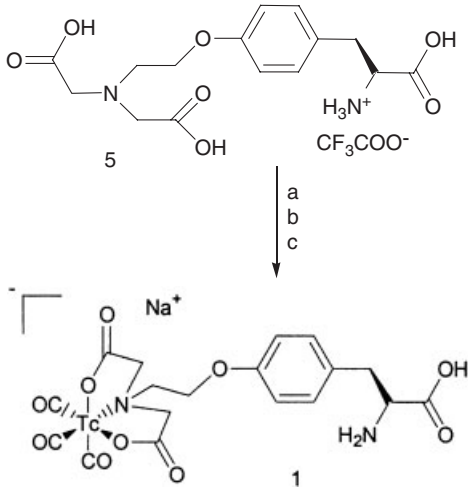
The synthesis of the final labelled compound *O*-(^{99m}Tc(I)-tricarbonyl-*N*,*N*-*bis*(carboxymethyl)aminoethyl)-L-tyrosine potassium salt (1) started from L-tyrosine *t*.-butyl ester (2) which was reacted with triphenylmethyl chloride and triethylamine in DMF at 25°C to yield the tritylated compound 3 following a similar published procedure.[9] The structural element 2-[*N*,*N*-*bis*(*tert*.-butyloxycarbonyl-methyl)amino)-1-bromoethane[10] for complexation of the Tc(I)-tricarbonyl core was connected to the HO-moiety of the tyrosine (3) in acetone/TEA at room temperature to give 4 which was not isolated but subsequently deprotected with trifluoroacetic acid to yield the derivatized tyrosine (5) in high yields for subsequent labelling with radioactive *fac*-[^{99m}Tc(I)$(OH_2)_3(CO)_3]^+$. The analogous reaction with $[NEt_4]_2[ReCl_3(CO)_3]$[11] was also performed for analytical purposes such as HPLC conditions for final isolation of 1, since Tc and Re have similar chemical properties (Scheme 1).

For the synthesis of the analogous 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



Scheme 1. (a) triphenylmethylchloride, TEA, DMF; (b) 2-[*N*,*N*-*bis*(*t*.-butyloxy-carbonylmethyl)amino)-1-bromoethane, TEA, acetone; (c) TFA; (d) $[NEt_4]_2$ $[ReCl_3(CO)_3]$, MeOH

column chromatography. Radioactive labelling was conducted using the labelling precursor 5, (1 mg, 2.2 μmol) and 1 ml of the *fac*-$[^{99m}Tc(I)(OH_2)_3(CO)_3]^+$-solution 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 precursor can be reduced to 0.3 mg (0.66 μmol) to guarantee a radiochemical yield of >98% which was determined using radio-HPLC. To obtain an injectable



Scheme 2. (a) $^{99m}TcO_4^-$, Isolink®-kit, 30 min, 100°C; (b) dilution with H_2O, 18C-SepPack cartridge®, washing with H_2O; (c) elution with sodium glycinate (0.5 N), isotonic saline, sterile filtration (Tc = ^{99m}Tc)

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 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 established by HPLC which can 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 labelling precursor 5 which was eluted during the washing step. Due to the basic labelling conditions the NH_3^+-moiety is deprotonated and the toxic CF_3COO^- anion was removed by washing the cartridge with water. Using 250–600 MBq $^{99m}TcO_4^-$, between 180 and 500 MBq of 1 as a sterile aqueous solution could be obtained in an overall radiochemical yield of 70–80% with a radiochemical purity >98% within 60 min. Enantiomeric purity of 1 was determined using chiral HPLC (CHIREX™, phenomenex, Aschaffenburg, Germany).

Experimental

*N-triphenylmethyl-*L*-tyrosine t.-butyl ester (3)*

L-Tyrosine *t.*-butyl ester (5 g, 21 mmol), triphenylmethylchloride (5 g, 18 mmol) and triethylamine (4.2 g, 41.5 mmol) were dissolved in DMF (60 ml) and stirred at room temperature for 6 h. The crude reaction mixture was evaporated to half its original volume and poured into 1000 ml of ice water. The precipitating

white solid was filtered off, washed with water and dried under vacuum. Yield: (8.4 g, 17.6 mmol, 98%). ^{1}H NMR (d_6-DMSO) δ 1.0 (s, 9H), 2.35–2.42 (dd, 1H), 2.54–2.60 (dd, 1H), 2.64 (d, 1H), 3.15 (m, 1H), 6.62 (d, 2H), 6.85 (d, 2H), 7.1–7.42 (m, 15H), 9.15 (s, 1H); ^{13}C NMR (d_6-DMSO) 26.9, 36.0, 58.4, 71.0, 79.8, 114.9, 123.7, 126.8, 127.7, 130.4, 146.3, 156.1, 173.2; MS ESI: *m/z* 502.72 $[M + Na]^+$; Anal. Calcd for $C_{32}H_{33}NO_3$: C, 80.14; H, 6.94; N, 2.92. Found: C, 79.87; H, 6.75; N, 3.12. A CHIREX™ chiral HPLC-column 250 × 4.6 mm (Phenomenex, Aschaffenburg, Germany) was used to determine the enantiomeric purity. Eluent: 1 M Cu (II) SO_4 in water; flow: 1 ml/min.

*O-(N,N-bis(carboxymethyl)amino ethyl)-*L*-tyrosine trifluoroacetate (5)*

N-triphenylmethyl-L-tyrosine *t*.-butyl ester (3) (1.03 g, 2.15 mmol), *N,N-bis* [(*t*.-butoxycarbonyl)methyl]-2-bromoethylamine (1.05 g, 3 mmol) and triethylamine (0.4 g, 4 mmol) were dissolved in acetone (50 ml) and stirred at room temperature for 18 h. The mixture was filtered, the solvent was evaporated off under vacuum and the remainder dissolved in TFA (20 ml) and allowed to stand for 24 h. After partition of the crude reaction mixture between $CHCl_3$ and water, the water phase was evaporated to half its original volume, ethanol added and the product was obtained as the CF_3COO^--salt after standing overnight at 4°C. Yield: (0.63 g, 1.42 mmol, 85%) ^{1}H NMR (d_6-DMSO, 400 MHz) δ 2.95 (d, 2H), 3.25 (t, 2H), 3.74 (s, 4H), 3.9 (s, 1H), 4.0–4.2 (m, 3H), 6.85 (d, 2H), 7.1 (d, 2H), 8.2 (bs, 3H); ^{13}C NMR (d_6-DMSO, 400 MHz) δ 35.4, 53.6, 53.7, 65.7, 115.0, 127.3, 130.9, 157.7, 170.8, 171.0; ^{19}F NMR (d_6-DMSO, 400 MHz) δ −74.6; FD MS: *m/z* 341.5 $[M^+]$; Anal. Calcd for $C_{15}H_{20}N_2O \cdot CF_3COOH$: C, 44.94; H, 4.66; N, 6.17. Found: C, 44.75; H, 4.72; N, 6.3. The enantiomeric purity was determined using a CHIREX™ chiral HPLC-column 250 × 4.6 mm (Phenomenex, Aschaffenburg, Germany). Eluent: 1 M Cu (II) SO_4 in water; flow: 1 ml/min.

*Tetraethylammonium O-(Re(I)-tricarbonyl-N,N-bis(carboxymethyl)aminoethyl)-*L*-tyrosine hydrochloride (6)*

$[NEt_4]_2[ReCl_3(CO)_3]$ (50 mg, 78.4 μmol) and 5 (35.7 mg, 78.4 μmol) were dissolved in methanol (1.5 ml) and stirred for 30 min. The solvent was evaporated off under reduced pressure and column chromatography on Si-60 was used for final purification: (acetonitrile/water 75:25; R_f: 0.55). The product was obtained as a double salt. $[NEt_4]^+$ as a counterion to the $[Re(I)(CO)]^-$ core and Cl^- as a counter ion to the NH_3^+ group. The presence of the CF_3COO^- anion could be excluded by means of ^{19}F NMR. ^{1}H NMR (d_6-DMSO, 400 MHz) δ 1.1 (tt, 12H), 2.86 (m, 1H), 3.06 (d, 2H), 3.15 (q, 8H), 3.42 (d, 2H), 3.56 (t, 2H), 3.64 (d, 2H), 4.19 (t, 2H), 6.84 (d, 2H), 7.19 (d, 2H), 7.5–8.0 (bs, 3H); ^{13}C NMR (d_6-DMSO, 400 MHz) δ 7.36, 39.7, 51.64, 53.9,

63.77, 64.48, 103.07, 114.65, 129.90, 130.87, 156.89, 178.97, 186.8 (C≡O), 190.2 (C≡O), 193.6 (C≡O); IR (KBr) *v* 2025 (s), 1903 (vs), 1890 (sh); Anal. Calcd for $[C_{18}H_{18}N_2O_{10}Re]$-$[NEt_4]^+ \cdot HCl$: C, 40.28; H, 5.07; N, 5.42. Found: C, 38.96; H, 5.17; N, 5.12. A CHIREX™ chiral HPLC-column 250 × 4.6 mm (Phenomenex, Aschaffenburg, Germany) was used to determine the enantiomeric purity. Eluent: 1 M Cu (II) SO_4 in water; flow: 1 ml/min.

O-(^{99m}*Tc(I)-tricarbonyl-N,N-bis(carboxymethyl)aminoethyl)*-L-*tyrosine potassium salt (1)*

To the Isolink®-kit formulation freshly eluted $^{99m}TcO_4^-$ (200–560 MBq) from a commercially available $^{99}Mo/^{99m}Tc$-generator was added and the solution heated for 20 min at 100°C. To this solution precursor 5 (1 mg, 2.2 μmol) was added and heated for 30 min. The solution was diluted with water (20 ml) and passed through a 18C-Sep-Pack cartridge® (Merck, Darmstadt, Germany), washed with water and eluted with hot aqueous sodium glycinate (0.5 N) solution (2 ml). Additionally, isotonic NaCl (1 ml) was added and the solution was passed through a sterile filter. This final solution contained between 180 and 500 MBq of 1 as a sterile aqueous solution with a radiochemical purity > 98%.

Column: LiChrosorb® RPselectB 250 × 10 (Merck, Darmstadt, Germany), gradient eluent: 100% H_2O + 0.1% TFA after 30 min 100% acetonitrile + 0.1% TFA; retention time: R_t = 21.0 min. A CHIREX™ chiral HPLC-column 250 × 4.6 mm (Phenomenex, Aschaffenburg, Germany) was used to determine the enantiomeric purity. Eluent: 1 M Cu (II) SO_4 in water; flow: 1 ml/min.

Summary

In conclusion we have synthesized a ^{99m}Tc-labelled L-tyrosine derivative using the novel labelling precursor *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$-complex. All compounds were fully characterized by ^{1}H NMR, ^{13}C NMR, IR and both elemental analysis and FD mass- or ESI mass spectroscopy. Enantiomeric purity of all compounds was established by chiral HPLC using a CHIREX™ HPLC-column (phenomenex, Aschaffenburg, Germany). Using acid sensitive trityl- and *t*.-butyl groups the final deprotection step to obtain the labelling precursor could be conveniently achieved in one step. For analytical purposes, the analogous Re(I)-compound was synthesized and shown to have the same retention time (HPLC) as the ^{99m}Tc-labelled L-tyrosine derivative. The labelling procedure involved the application of Isolink®-kit formulation and $^{99m}TcO_4^-$ from a $^{99}Mo/^{99m}Tc$-generator. 1 was available as a sterile injectable aqueous solution. First *in vitro* and *in vivo* studies with tumour cell lines and tumour bearing animals are currently in progress. The ^{99m}Tc-labelled amino acid might also be of interest as a building block in peptide synthesis to obtain radiolabelled peptides.

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