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SYNTHESIS AND BIODISTRIBUTION OF TECHNETIUM-99m-LABELLED *N*-(DIETHYLAMINOETHYL)BENZAMIDE VIA A BIS(DITHIOCARBAMATE) NITRIDOTECHNETIUM(V) COMPLEX.

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SUMMARY

N-(2-diethylaminoethyl)-4-iodobenzamide has been reported to be an excellent agent for malignant melanoma diagnosis by SPECT. To obtain a ^{99m}Tc analogue, we synthesized a sodium *N*-{2-[4-(*N*-diethylaminoethylcarbamoyl)phenyl]ethyl}-*N*-methyldithiocarbamate ligand in 7 steps from methyl 4-(bromomethyl)benzoate. The corresponding nitridotechnetium complex formed by a ligand exchange method was purified and its biodistribution in mice bearing the B16 murine melanoma was determined.

Key-Words: ^{99m}Tc, Benzamide, Carbamate, Melanoma, Nitridotechnetium complex

INTRODUCTION

The first compound containing a nitridotechnetium(V) core to be absolutely identified was a bisdithiocarbamate complex[1]. These complexes have been widely developed for radiopharmaceutical studies[2-5], thanks to new ligand exchange reactions[6-8]. These ligands link to $[^{99m}Tc{\equiv}N]^{2+}$(V) via Tc-S bonds to give neutral lipophilic homodimer complexes[9]. The low steric bulk and low molecular weight of the ^{99m}Tc-chelate is assumed to interfere minimally with the initial pharmacophore properties. Also, compounds of this kind are obtained much more easily than other ^{99m}Tc chelates usually conjugated to active molecules (e.g., bis(aminothiol) or cyclam derivatives).

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Accordingly, this ligand was chosen to label a benzamide structure with the radionuclide ^{99m}Tc. (*N*-diethylaminoethyl)-4-iodobenzamide (Figure 1) possesses a high affinity for primary melanoma and its metastases[10-12]. This molecule (radiolabelled with iodine-123) has already been successfully tested in humans for scintigraphic examination[13-16]. Our aim was to replace iodine by the more widely accessible and convenient radionuclide technetium-99m.

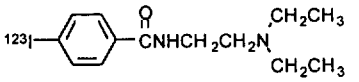

Figure 1. (*N*-diethylaminoethyl)-4-[^{123}I]-iodobenzamide ([^{123}I]-BZA).

The use of primary amines for the synthesis of [^{99m}Tc]-BZA complex (Figure 2) afforded a final product that was insufficiently stable for biological studies, the complex being completely destroyed after two hours (unpublished results). This instability can be explained by the alpha position of hydrogen, resulting in a weak N-C bond in the dithiocarbamate ligand.

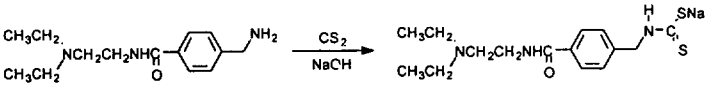

Figure 2. Synthesis of dithiocarbamate ligand via primary amine derived from BZA.

To overcome this problem; the synthesis, labelling and biodistribution of a new *N*-methyl dithiocarbamate ligand was undertaken.

RESULTS

Synthesis:

We prepared the ligand sodium *N*-{2-[4-(*N*-diethylaminoethylcarbamoyl)phenyl]ethyl}-*N*-methyldithiocarbamate (7) using the reaction pathway given in Figure 3. The benzyl halide reacted in good yield with the cyanide ion to form the desired nitrile 1 (yield 81%). Catalytic hydrogenation in methanol/HCl gave a mixture of primary, secondary and tertiary amines (from the reaction between primary amine and the starting nitrile). To prevent this reaction, the reduction was carried out in ethanol/HCl in which 2 precipitated as its hydrochloride salt more readily than in methanol/HCl. The amine function was protected by the formation of the benzylamine group. Benzylation products were purified by chromatography to afford 3 (56 %). The methylation was achieved using the modified Pine *et al.* procedure[17] (68 %). The conversion of methyl ester 4 to amide with trimethyl-aluminium gave the desired benzamide 5 in quantitative yield. The benzylamine was cleaved by catalytic hydrogenation in quantitative yield. Dithiocarbamate ligand 7 was prepared as its sodium salt in 76 % yield by treatment of the secondary amine 6 with carbon disulfide in basic conditions.

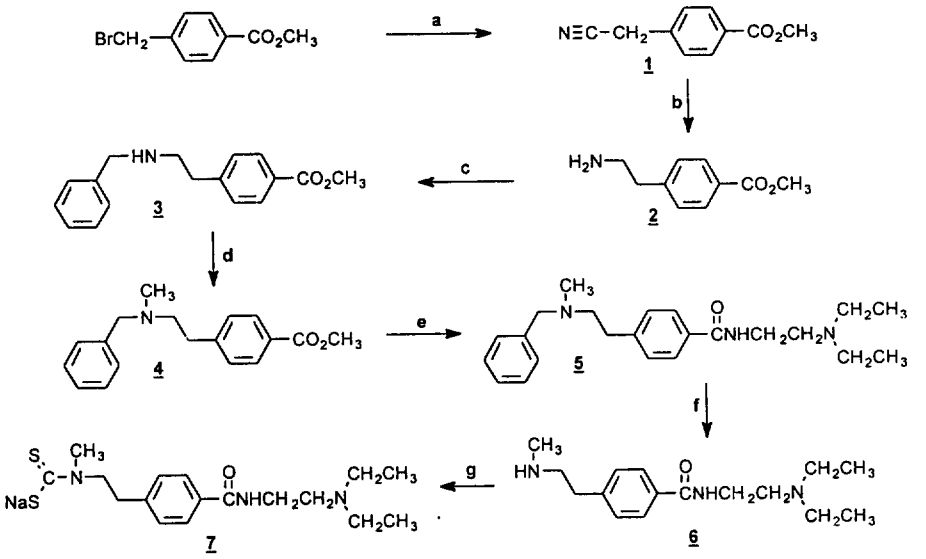

Figure 3. Synthesis of dithiocarbamate ligand. Reagents: (**a**) KCN, EtOH; (**b**) H_2, Pd/C, EtOH/HCl; (**c**) BzlCl, K_2CO_3, CH_3CN; (**d**) HCHO, HCO_2H; (**e**) $NH_2CH_2CH_2NEt_2$, $Al(CH_3)_3$, CH_2Cl_2; (**f**) H_2, Pd/C, EtOH; (**g**) CS_2, NaOH.

Radiolabelling:

The nitridotechnetium $[Tc{\equiv}N]^{2+}$ core was introduced following the procedure of Pasqualini R. *et al*[3,7] as indicated in Figure 4. In this reaction, the tertiary phosphine was the reductant and *N*-methyl-*S*-methyl-dithiocarbazate (MDTCZ) acted as nitrogen atom (N^{3-}) donor.

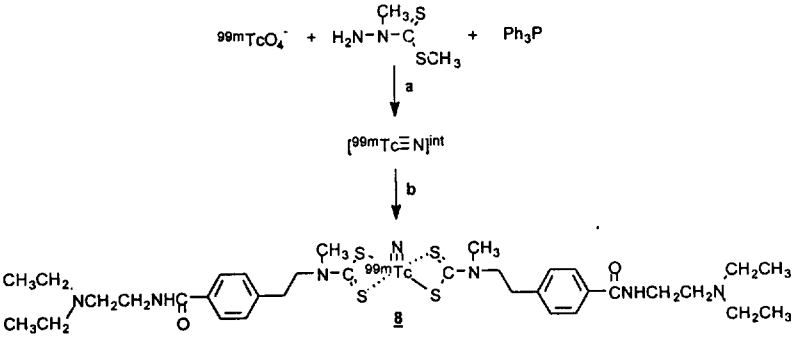

Figure 4. Preparation of bis(dithiocarbamate)nitridotechnetium-99m. (**a**) HCl, 70°C, 30 min; (**b**) 7, pH 9.4, 70°C, 30 min.

The ligand exchange reaction was monitored by thin layer chromatography (Figure 5). Complete disappearance of TcO_4^- and $[TcN]^{int}$ radioactive spots was observed in less than 30 minutes. The radiolabelling yield, measured by TLC, was 78 %.

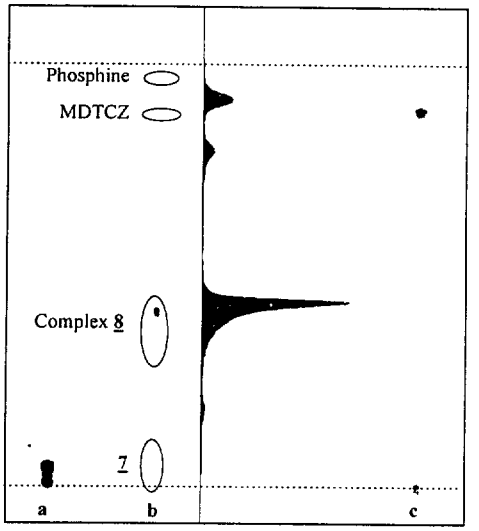

Figure 5. TLC radioactivity control after 30 min ligand exchange reaction. Aluminium oxide TLC, CH_2Cl_2/MeOH 94:6. (**a**) $^{99m}TcO_4Na$; (**b**) Exchange reaction; (**c**) $[Tc{\equiv}N]^{int}$.

The purification of the complex was achieved by HPLC, collecting 13 to 19 min fractions. To minimize HPLC injected volume, the exchange solvent was first evaporated and complex 8 dissolved in minimum dichloromethane. Global yield from pertechnetate was 50-60 % after radioactivity decrease correction.

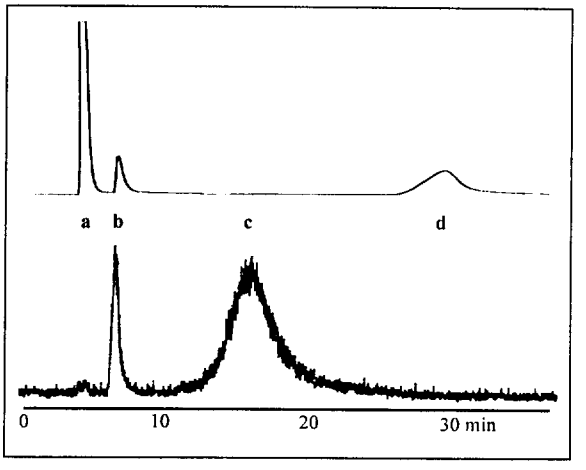

Figure 6. HPLC UV profile (upper) and radioactivity profile (lower). (**a**) 7; (**b**) MDTCZ; (**c**) ^{99m}Tc complex of 7; (**d**) PPh_3.

The injected solution was prepared with 9 % ethanol in PBS buffer, to allow radiopharmaceutical miscibility.

Biological study:

Tumor uptake was studied in mice bearing the B16 murine melanoma, injecting 0.2 mL of the preceding solution (0.74 MBq). Less than 5 % of the injected radioactivity was excreted through the urinary tract in 24 hours. The results of the biodistribution studies are summarized in the following three tables.

Table 1. Total excretion of ^{99m}Tc radioactivity.

	% ID	
Time	Urine	Faeces
0-6 h	2	17
6-24 h	4	76

Table 2. Biodistribution of ^{99m}Tc-radiolabelled complex **8**. Results are expressed as mean ± s.e.

	% ID/g					
Organ	5 min	15 min	1 h	3 h	6 h	24 h
Blood	7.68±0.72	2.59±0.12	0.74±0.04	0.53±0.04	0.48±0.01	0.08±0.00
Liver	42.3±7.7	43.7±1.1	23.7±1.9	25.5±1.3	19.9±0.6	9.65±0.90
Kidney	48.9±2.5	53.2±4.8	32.6±1.4	34.8±0.7	30.1±1.2	14.4±1.8
Lung	54.3±26.4	31.0±1.3	41.8±15.0	11.6±2.1	8.5±1.3	7.4±1.1
Muscle	0.33±0.04	0.39±0.05	0.35±0.01	0.39±0.08	0.47±0.01	0.38±0.05
Brain	0.17±0.03	0.11±0.01	0.06±0.01	0.06±0.01	0.05±0.01	0.01±0.00
Eye	0.55±0.05	0.70±0.01	0.67±0.08	0.81±0.11	0.70±0.08	1.01±0.04
Tumor	0.71±0.03	0.70±0.15	0.54±0.02	0.68±0.15	0.40±0.11	0.44±0.07

Table 3. Tumor-to-organ radioactivity ratios of **8**. Results are expressed as mean ± s.e.

	% ID/g					
Organ	5 min	15 min	1 h	3 h	6 h	24 h
Blood	0.10±0.01	0.27±0.08	0.74±0.06	1.28±0.27	0.83±0.28	5.55±1.11
Liver	0.02±0.01	0.02±0.01	0.02±0.00	0.03±0.01	0.02±0.01	0.05±0.01
Kidney	0.01±0.00	0.01±0.01	0.02±0.00	0.02±0.01	0.01±0.01	0.03±0.01
Lung	0.01±0.01	0.02±0.01	0.02±0.01	0.06±0.01	0.05±0.03	0.07±0.03
Muscle	2.25±0.36	1.77±0.18	1.58±0.09	2.05±0.99	0.84±0.28	1.22±0.35
Brain	4.55±1.13	6.59±2.22	8.83±1.31	10.9±2.7	7.99±1.55	35.9±14.3
Eye	1.32±0.19	0.99±0.24	0.83±0.09	0.83±0.12	0.57±0.16	0.44±0.10

Rapid blood clearance was observed, blood activity at 1 h post-injection being less than 10 % of the initial 5 min activity (7.68 to 0.74 % ID/g). Liver, kidney and lung displayed high radioactivity levels and slow elimination. Radioactivity in eyes slowly increased, the value at 24 h being twice the initial one (0.55 to 1.01 % ID/g). Initial tumor uptake was weak, followed by slow elimination: the radioactivity level was nearly constant between 1 and 24 hours post-injection. Muscle and eyes

excepted, all other t/o ratios increased during the experiment. However, liver, kidney and lung ratios were still low after 24 h.

DISCUSSION

The amine function can be mono-methylated after protection of the corresponding primary amine. First, it was necessary to lengthen the chain between the amino group and the aromatic ring to use the benzyl protective group. Thus the secondary amine **6** can be preserved with no detected degradation. This molecule is an excellent precursor of the dithiocarbamate ligand, which can be obtained just before the labelling experiments. The appearance of the ^{13}C-NMR characteristic signal at 210 ppm proves CS_2 incorporation. Although the carbamate function is not very stable, dithiocarbamate salts can be preserved for a few weeks under an argon atmosphere at 4°C.

Ligand exchange takes place at basic pH as reported by Bellante E. *et al.*[8]. This was preferable for the stability of the carbamate, which usually decomposed in acidic conditions. The radiolabelling yield measured by TLC or HPLC was reproducible. The pure complex **8** obtained is stable at pH 7 and 9.4. This complex is more lipophilic (octanol/PBS partition coefficient log(P)=2.05) than the corresponding iodobenzamide (log(P)=1.34). The nitrido technetium core and the dithiocarbamate functions certainly increase the partition in octanol.

The rapid blood clearance observed was similar for the ^{99m}Tc complex **8** and ^{123}I-BZA. Other biological results obtained with the two molecules differed. Liver, lung and kidney uptake were very high; the strong radioactivity fixation observed in the kidney cannot be explained by high urinary excretion (see Table 1), so the technetium complex seems to have a true affinity for this organ. The very low radioactivity levels in brain proved that the complex did not cross the BBB (blood-brain barrier), despite its high lipophilicity. The tumor tropism was weak due to a low initial fixation of the radioactivity (less than 1 % ID/g). However, the elimination of ^{99m}Tc from the tumor was also very slow, resulting in a constant % ID/g for the first three hours post-injection (0.71 to 0.68 between 5 min and 3 h). The high level of melanin in eyes may be an explanation for the relative affinity of the complex; the five minutes uptake was doubled to reach 1% ID/g of the injected dose after 24 hours.

In conclusion, we synthesized a ^{99m}Tc-labelled BZA and evaluated it as a potential malignant melanoma imaging agent. We found low tropism for melanoma tumor given the constant radioactivity level during the three first hours. Unfortunately, high radioactivity levels in kidney, liver and lung were observed. Consequently, the tumor-to-organ radioactivity ratios were low compared with those for [^{123}I]-BZA (all >1 at 6 h post-injection). The different behavior *in vivo* may be due to the high lipophilicity of the Tc-complex (5 times higher than ^{123}I-BZA). Possible future

work could be directed to finding a new less lipophilic complex with a partition coefficient between 1 and 1.5.

MATERIALS and METHODS

General:

Chemistry: All reagents and solvents were from commercial suppliers and used with no further purification. Analytical thin layer chromatography (TLC) was conducted on pre-coated silica gel plates (SDS, plastic sheet 60 F_{254}, layer thickness 0.25 mm), on aluminium oxide plates (Merck, plastic sheet 60 F_{254}, neutral type E, layer thickness 0.20 mm), subsequently visualized under UV light (254 nm) and exposed to iodine vapour. Medium pressure chromatography was performed using silica gel (SDS, Chromatogel 60A 40-60 μm). Solvent mixture was expressed as volume-to-volume ratio (v:v). Melting points (mp) were measured with a Reichert-Jung Kofler apparatus. Infrared (IR) spectra were recorded on a Vector 22 Bruker instrument (ν expressed in cm^{-1}). Proton and carbon magnetic resonance spectra (^{1}H-NMR and ^{13}C-NMR) were recorded at respectively 200.1 MHz and 50.3 MHz on a Bruker AM 200 (4.5 T) instrument. Chemical shifts (δ) are reported in parts per million relative to the internal standard $(CH_3)_4Si$ or using the deuterated solvents ($CDCl_3$, δ = 7.26 ppm or DMSO-d_6, δ = 2.50 ppm) for ^{1}H-NMR and the solvents ($CDCl_3$, δ = 77.0 ppm or DMSO-d_6, δ = 39.5 ppm) for ^{13}C-NMR.

Radiochemistry: [^{99m}Tc] sodium pertechnetate as no-carrier-added solution was purchased from the Jean Perrin Cancer Hospital (Clermont-Ferrand). All solvents were degassed under argon before use. TLC radioactive spots were scanned and recorded by an AMBIS 4000 detector (a computer-controlled multi-wire proportional counter). HPLC purification was performed on a Shimadzu HPLC system (LC6A pump, SCL6B system controller and CR5A integrator) equipped with a semi-preparative reverse phase column (Merck, Lichroprep RP 18, 12×200 mm), connected to a Shimadzu SPD6AV UV spectrophotometric detector (254 nm) in series with a Raytest NaI (Tl) gamma detector.

Synthesis:

Methyl 4-(cyanoethyl)benzoate (1). Potassium cyanide (1.30 g, 20.0 mmol) was added to a solution of methyl 4-(bromomethyl)benzoate (4.58 g, 20.0 mmol) in ethanol (40 mL). The resulting mixture was refluxed overnight and the solvent was evaporated under reduced pressure. The residue was dissolved in ether, and washed twice with water and brine. The organic layer was dried over anhydrous $MgSO_4$. After evaporating the solvent *in vacuo*, the crude product was purified by crystallization (pentane/ether) to obtain 2.85 g (16.3 mmol, 81 %) of nitrile 1; Mp: 62-63°C; TLC R_f = 0.19 (silica, ethyl acetate/pentane 10:90); ^{1}H-NMR ($CDCl_3$) δ: 3.82 (s, 2H, CH_2CN); 3.92 (s, 3H, CH_3); 7.42 (d, 2H, arom, J = 8.4 Hz); 8.06 (d, 2H, arom, J = 8.4 Hz); ^{13}C-NMR ($CDCl_3$) δ: 23.6 (1C, $\underline{C}H_2CN$); 52.3 (1C, $\underline{C}H_3OCO$); 117.1 (1C, CN); 128.0 and 130.4 (4C, arom); 130.1 (1C, $\underline{C}CO_2CH_3$); 134.8 (1C, $\underline{C}CH_2CN$); 166.4 (1C, CO); IR (KBr) ν: 3025, 1615 and 835 (1,4-disubstituted atom), 2960 and 2920 (CH_2), 2248 (nitrile), 1727 (C=O ester), 1284 et 1110 (C-O ester), 1433 and 1417 (CH_2).

Methyl 4-(2-aminoethyl)benzoate (2). In a hydrogenation apparatus were placed 2.80 g of methyl 4-(cyanoethyl)benzoate (16.0 mmol), 1.08 g of palladium on activated carbon (Pd 10 %) and 2*M* ethanol/HCl (100 mL). The mixture was vigorously stirred at room temperature for two days (H_2 absorption: 1300 mL). 200 mL of ethanol was added and the solution was heated until complete dissolution of the hydrochloride salt form. The clear solution was filtered on Celite®. After evaporating half the solvent *in vacuo*, the product was purified by crystallization to give 2.79 g (13.0 mmol, 81 %) of **2**; ^{1}H-NMR ($CDCl_3$) δ: 1.27 (broad, 2H, NH_2); 2.81 (t, 2H, $C\underline{H}_2NH_2$, J = 6.6 Hz); 3.00 (t, 2H, $C\underline{H}_2CH_2NH_2$, J = 6.8 Hz); 3.91 (s, 3H, CH_3); 7.28 (d, 2H, arom, J = 8.0 Hz); 7.98 (d, 2H, arom, J = 8.1 Hz). (base form); ^{13}C-NMR ($CDCl_3$) δ: 40.1 (1C, $\underline{C}H_2CH_2NH_2$); 43.2 (1C, CH_2NH_2); 52.0 (1C, CH_3); 128.2 (1C, $\underline{C}$-CO_2CH_3); 128.8 and 129.7 (4C, arom); 145.4 (1C, $\underline{C}$-CH_2); 167.0 (1C, C=O). (base form); IR (KBr) ν: 3150-2500 (NH and CH_2, CH_3); 1715 (C=O ester); 1595 (cyanide); 1460-1410 (CH_2, CH_3); 1275 (C-O ester). (hydrochloride form).

Methyl 4-(*N*-benzyl-2-aminoethyl)benzoate (3). To a solution of methyl 4-(2-aminoethyl)benzoate (**2**) (1.25 g, 7.0 mmol) in acetonitrile (20 mL) were added 2.07 g of potassium carbonate (15.0 mmol) and 0.80 mL of benzyl chloride (0.88 g, 7.0 mmol). The resulting mixture was stirred at room temperature for 5 days. After removal of the solvent under reduced pressure, the residue was extracted with dichloromethane, and dried over anhydrous $MgSO_4$. Purification was by silica gel column chromatography using ethyl acetate/pentane (4 to 10/96 to 90) to afford 1.05 g of **3** (3.90 mmol, 56 %); ^{1}H-NMR ($CDCl_3$) δ: 1.65 (broad, 1H, NH); 2.90 (m, 4H, $C\underline{H}_2C\underline{H}_2NH$); 3.80 (s, 2H, $NHC\underline{H}_2Ph$); 3.90 (s, 3H, CH_3); 7.25 to 7.31 (m, 7H, arom); 7.96 (d, 2H, arom, J = 8.4 Hz); ^{13}C-NMR ($CDCl_3$) δ: 36.4 (1C, $\underline{C}H_2CH_2N$); 50.0 (1C, $CH_2\underline{C}H_2N$); 52.0 (1C, CH_3); 53.8 (1C, $\underline{C}H_2Ph$); 127.1 (1C, arom); 128.1 (2C, arom); 128.2 (1C, $\underline{C}$-CO_2CH_3); 128.4 and 128.7 (4C, arom); 129.8 (2C, arom); 137.2 (1C, arom); 144.6 (1C, arom); 165.2 (1C, C=O).

Methyl 4-(*N*-methyl-*N*-benzyl-2-aminoethyl)benzoate (4). 1.05 g of methyl 4-(*N*-benzyl-2-aminoethyl)benzoate (**3**) (3.9 mmol) was dissolved in 3 mL of water. The flask was then cooled in an ice bath. 0.29 mL of formic acid (7.8 mmol) was slowly added, followed by 0.44 mL of 37 % formaldehyde (5.9 mmol). The mixture was stirred at reflux for 16 h, and then cooled to room temperature. 0.5 mL of concentrated hydrochloric acid was added. The resulting solution was heated at 70°C for 3h. After solvent evaporation, the residue was made basic with aqueous potassium hydroxide and extracted three times with trichloromethane. The combined organic layers were dried over anhydrous $MgSO_4$ to obtain 0.75 g of methyl 4-(*N*-methyl-*N*-benzyl-2-aminoethyl)benzoate (**4**) (2.65 mmol, 68 %); TLC R_f = 0.26 (silica, methanol/dichloromethane 6:94); ^{1}H-NMR ($CDCl_3$) δ: 2.34 (s, 3H, NCH_3); 2.73 (t, 2H, $C\underline{H}_2CH_2N$, J = 7.5 Hz); 2.96 (t, 2H, $CH_2C\underline{H}_2N$, J = 7.5 Hz); 3.65 (s, 2H, $C\underline{H}_2Ph$); 3.90 (s, 3H, OCH_3); 7.24 (d, 2H, arom, J = 8.1 Hz); 7.31 (s, 5H, arom); 7.94 (d, 2H, arom, J = 8.1 Hz); ^{13}C-NMR ($CDCl_3$) δ: 33.15 (1C, C^eH); 41.49 (1C, NCH_3); 52.01 (1C, OCH_3); 57.93 (1C, C^dH_2); 61.65 (1C, C^fH_2); 128.51 à 129.79 (10C, $C_{ar.}$); 134.44 (1C, $\underline{C}_{ar.}C^fH_2$); 144.98 (1C, $\underline{C}_{ar.}C^cH_2$); 167.00 (1C, $\underline{C}$=O); IR (KBr) ν: 3040 (arom), 2950 to 2920 (CH_2, CH_3), 1718 (C=O ester), 1611 (arom), 1436 (CH_2, CH_3), 1279 and 1110 (C-O ester), 745 and 701 (arom).

***N*-(2-diethylaminoethyl)-4-(*N'*-methyl-*N'*-benzyl-2-aminoethyl)benzamide (5).** 0.51 mL of *N,N*-diethylethylenediamine (418 mg, 3.6 mmol) was diluted with 5 mL of anhydrous dichloromethane in a flask equipped with a reflux condenser and a magnetic stirring bar. The following reactions were carried out under an argon atmosphere. The solution was stirred and cooled in an ice bath at 0°C, and

1.8 mL of trimethylaluminium in hexane (2*M* solution, 3.6 mmol) was slowly added. Fifteen minutes after the addition was completed, the cooling bath was removed, and 0.75 g of **4** in 5 mL of dichloromethane was added. The resulting solution was heated under reflux for 4 days, cooled to room temperature and slowly hydrolysed with water to prevent foaming. The mixture was extracted three times with dichloromethane, and the organic layers were combined and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure to give 964 mg of benzamide **5** (2.62 mmol, 99 %); TLC R_f = 0.65 (aluminium oxide, methanol/dichloromethane 6:94); ^{1}H-NMR ($CDCl_3$) δ: 1.13 (t, 6H, 2xCH_2CH_3, *J* = 7.1 Hz); 2.27 (s, 3H, NCH_3); 2.60 to 2.89 (m, 10H, $CH_2N(CH_2CH_3)_2$ and $CH_2CH_2NCH_3$); 3.55 to 3.62 (m, 4H, $CONHCH_2$ and $PhCH_2N$); 7.24 (d, 2H, arom, *J* = 8.3 Hz); 7.28 (s, 5H, arom); 7.76 (d, 2H, arom, *J* = 8.2 Hz); ^{13}C-NMR ($CDCl_3$) δ: 9.6 (2C, 2xCH_2CH_3); 33.6 (1C, $CH_2CH_2NCH_3$); 36.0 (1C, $CONHCH_2$); 42.2 (1C, NCH_3); 48.2 (2C, 2xCH_2CH_3); 52.8 (1C, CH_2NEt_2); 58.5 (1C, $CH_2CH_2NCH_3$); 62.1 (1C, $PhCH_2N$); 127.0 to 129.0 (9C, arom); 132.1 (1C, arom); 138.9 (1C, CCONH); 144.4 (1C, arom); 167.3 (1C, C=O); IR (KBr) ν: 3300 (NH amide), 3050 to 3000 (arom), 2980 to 2850 (CH_2, CH_3), 1654 (C=O amide), 801, 738, 700 (arom).

***N*-(2-diethylaminoethyl)-4-(*N'*-methyl-2-aminoethyl)benzamide (6).** In a hydrogenation apparatus were placed 963 mg of benzamide **5** (2.62 mmol), 235 mg of palladium on activated carbon (Pd 10 %) and 20 mL of ethanol. The mixture was vigorously stirred at room temperature for 48 h (H_2 absorption: 65 mL). The solution obtained was filtered on Celite®. The solvent was evaporated under reduced pressure to afford 719 mg of benzamide **6** (2.59 mmol, 99 %); TLC R_f = 0.60 (aluminium oxide, methanol/dichloromethane 6:94); ^{1}H-NMR ($CDCl_3$) δ: 1.21 (t, 6H, 2xCH_2CH_3, *J* = 7.1 Hz); 2.56 (s, 3H, $NHCH_3$); 2.88 (q, 4H, $N(CH_2CH_3)_2$, *J* = 7.1 Hz); 2.94 (m, 2H, CH_2NEt_2); 3.01 (s, 4H, $CH_2CH_2NCH_3$); 3.65 (m, 2H, $CONHCH_2$); 6.4 (m, 1H, $NHCH_3$); 7.29 (d, 2H, arom, *J* = 8.0 Hz); 7.83 (d, 2H, arom, *J* = 8.0 Hz); 7.87 (m, 1H, CONH); ^{13}C-NMR ($CDCl_3$) δ: 10.4 (2C, 2xCH_2CH_3); 34.0 (1C, $CH_2CH_2NHCH_3$); 34.7 (1C, NCH_3); 36.3 (1C, $CONHCH_2$); 47.5 (2C, 2xCH_2CH_3); 51.5 (1C, CH_2NEt_2); 51.9 (1C, $CH_2CH_2NHCH_3$); 127.2 and 128.6 (4C, arom); 132.5 (1C, NHCOC); 141.7 (1C, arom); 167.3 (1C, C=O); IR (KBr) ν: 3600 to 3200 (NH), 3030 (arom), 2980 to 2850 (CH_2, CH_3), 1644 (C=O amide), 844, 800 (arom).

Sodium *N*-{2-[4-(*N*-diethylaminoethylcarbamoyl)phenyl]ethyl}-*N*-methyldithiocarbamate (7). 400 mg of benzamide **6** (1.44 mmol) was diluted in 4.5 mL of anhydrous methanol under an argon atmosphere. The solution was stirred and cooled in an ice bath, and 0.130 mL of carbon disulfide (165 mg, 2.16 mmol) followed by 1.44 mL of a 1*M* sodium hydroxide solution (1.44 mmol) were added. The flask was sealed and stirred for 48 h. The solvent was evaporated under reduced pressure and 10 mL of distilled water was added to the residue. This solution was washed three times with dichloromethane, and the water was evaporated under reduced pressure to give 411 mg of dry dithiocarbamate salt **7** (1.09 mmol, 76 %); Mp 220-230 dec; TLC R_f = 0.15 (aluminium oxide, methanol/dichloromethane 6:94); ^{1}H-NMR (DMSO-d_6) δ: 1.09 (t, 6H, $N(CH_2CH_3)_2$, *J* = 6.2 Hz); 2.80 to 2.90 (m, 6H, $CH_2N(CH_2CH_3)_2$); 2.95 (t, 2H, $CH_2CH_2NCS_2$); 3.34 (s, 3H, NCH_3); 3.45 (m, 2H, $CONHCH_2$); 4.20 (t, 2H, CH_2NCS_2, *J* = 8.0 Hz); 7.32 (d, 2H, arom, *J* = 8.1 Hz); 7.78 (d, 2H, arom, *J* = 8.1 Hz); 8.59 (m, 1H, CONH); ^{13}C-NMR ($CDCl_3$) δ: 9.3 (2C, $N(CH_2CH_3)_2$); 32.8 (1C, $CH_2CH_2NCS_2$); 35.7 (1C, $CONHCH_2$); 43.1 (1C, NCH_3); 46.4 (2C, 2x(CH_2CH_3)); 52.2 (1C, CH_2NEt_2); 57.5 (1C, $CH_2CH_2NCS_2$); 127.6 and 128.6 (4C, arom); 132.0 (1C, arom, CCONH); 143.2 (1C, arom, CCH_2); 168.1 (1C, C=O); 210.5 (1C, NCS_2Na).

Radiolabelling with ^{99m}Tc:

In a reaction vial were placed 0.4 mL of *N*-methyl-*S*-methyl-dithiocarbazate solution (2.5 mg/mL in 95° ethanol), 0.2 mL of triphenylphosphine solution (5.0 mg/mL in 95° ethanol) and 0.1 mL of 1*M* hydrochloric acid under an argon atmosphere. 0.5 to 1.0 mL of the sodium pertechnetate solution (activity ranging from 0.2 to 0.74 GBq) was added. The resulting mixture was heated to 70°C for 30 min and then cooled to room temperature. The pH was adjusted by adding 0.1 mL of 1*M* sodium hydroxide solution followed by 0.9 mL of 0.5*M* bicarbonate buffer (pH 9.4). 1.0 mL of this solution was added to a second reaction vial containing 1 mg of ligand 7 in 1.0 mL 95° ethanol under an argon atmosphere. The solution was heated to 70°C for 30 min and the solvents were then evaporated under reduced pressure. The residue was dissolved in 1.0 mL of dichloromethane before HPLC separation (0.4 % NH_4OH/CH_2Cl_2 18:82). Flow rate: 5 mL/min. Rt = 16.0 min. Yield: 56%. HPLC solvents were evaporated under reduced pressure, and the complex was dissolved in PBS buffer with 9% ethanol.

Biological study:

Tumor uptake was studied in C57BL/6 J1 co male mice bearing the B16 murine melanoma. Transplantable B16 mouse melanotic melanoma was originally obtained from ICIG (Villejuif, France). 5×10^5 viable cells were injected subcutaneously. Ten days later, the tumors became palpable. Following the i.v. injection in the tail vein of 0.74 MBq ^{99m}Tc-labelled complex, mice (n = 3) were sacrificed by exsanguination after set time intervals of 5, 15 min, 1, 3, 6 and 24 h. Aliquots of different tissues were weighed, and radioactivity was immediately measured. Samples were counted in a γ-counter (Packard Autogamma® A 5530). The fractional accumulation of radioactivity in the tissue was expressed as % injected dose/g of tissue (% ID/g).

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