Synthesis and Biodistribution of a New Oxo-technetium-99m Bis(aminothiol) Complex as a Potential Melanoma Tracer

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[^{123}I]-*N*-(2-Diethylaminoethyl)-4-iodobenzamide (^{123}I-BZA) has been the best scintigraphic agent described so far for malignant melanoma and ocular melanoma diagnosis. We replaced ^{123}I by the more convenient radioisotope ^{99m}Tc and synthesized four bis(aminoethanethiol) derivatives. We describe the synthesis of a new oxo-technetium complex (TcO-Cf), prepared in very high yield (radiochemical yield > 95%), that exhibits an affinity for the pigmented tumor cells. This complex was evaluated in vivo in mice bearing C57B16 murine melanoma. After injection, a rapid decrease in the radioactivity levels was noted for all tissues and organs except for eyes (1.26 %ID/g at 1 h and 2.69 %ID/g at 24 h postinjection) and the tumor (1.19 %ID/g at 1 h and 0.80 %ID/g at 24 h postinjection), suggesting a specific in vivo binding of this complex to the pigmented cells. These results were compared with those already published for three other technetium-99m bis(aminothiol) complexes with benzamide derivatives.

Introduction

N-Alkylated iodobenzamides exhibit the highest reported affinity for melanoma tissue in cellular and animal models, prompting us to undertake a phase II clinical study using [^{123}I]-*N*-(2-diethylaminoethyl)-4-iodobenzamide (^{123}I-BZA) as a radiopharmaceutical for imaging melanoma and metastases.[1−4] This agent showed high potential for the diagnosis of primary and metastatic skin[5−7] and ocular[8] melanoma. The benzamide uptake mechanism is not yet understood. Benzamides seem to be co-localized in the melanocytes of the pigmented cells, and their uptake has been correlated to melanin production, suggesting a nonreceptor binding uptake mechanism.[4,9−11] On the other hand, benzamides are known to show high affinity for the σ-1 receptor, which has been detected on malignant melanoma tumor cells.[12] Accordingly, radiolabeled ligands of σ receptors have been synthesized and evaluated as malignant melanoma imaging agents.[13−15] σ Receptors have been detected on other different types of tumor cells (breast carcinoma, prostate tumor, colon, lung, brain, and kidney tumors) and have also been detected in healthy tissues such as liver, kidney, and brain.[12,16−20] However, clinical studies have demonstrated a very high specificity of ^{123}I-BZA for malignant melanoma and metastases.[6] Hence a single drug-receptor binding mechanism is insufficient to explain the specific uptake of benzamide derivatives by malignant melanoma tumor cells, and recent results published are in favor of a melanin binding mechanism.[21]

More than 85% of the radiopharmaceuticals used worldwide for diagnosis in nuclear medicine are labeled with technetium-99m, owing to its excellent imaging characteristics (98% emission of a 140 keV γ ray, $T^1/_2$ = 6 h). Some new developments of technetium based radiopharmaceuticals involved small molecules[22,23] and small peptide.[24] As a consequence, we decided to focus on the replacement of the iodine atom on the radioiodine benzamide by the technetium chelate structure (Figure 1).

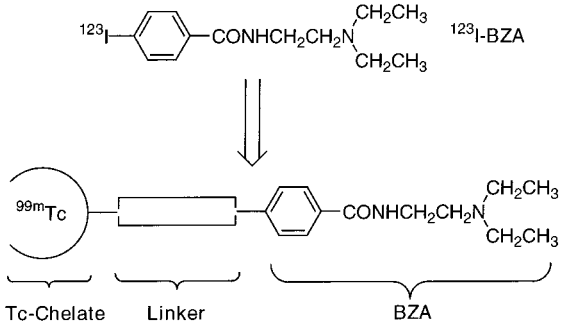

Figure 1. Conjugate approach to substitute ^{125}I by ^{99m}Tc.

We recently reported the synthesis and biodistribution of two novel technetium complexes pharmacomodulated by a benzamide structure: a nitridotechnetium bis(dithiocarbamate) compound[25] and a N-functionalized nitrido- and oxo-technetium bis(aminothiol) compound (respectively, TcN-Nf and TcO-Nf).[26] Biological results showed partial preservation of the tumor uptake, especially for the [^{99m}TcO]-bis(aminothiol) complex. Here we describe the radiolabeling of the C-functionalized bis(aminothiol) ligand by the oxo-technetium core and the biodistribution of the resulting complex in mice. The results obtained are compared with the previously determined biodistributions of other technetium-99m-labeled bis(aminothiol) derivatives.[25−27]

Results and Discussion

Synthesis. The bis(aminothiol) derivative ligand (**1**) was prepared as previously described in five steps from ethyl 3,3,10,10-tetramethyl-1,2-dithia-5,8-diazacyclodeca-

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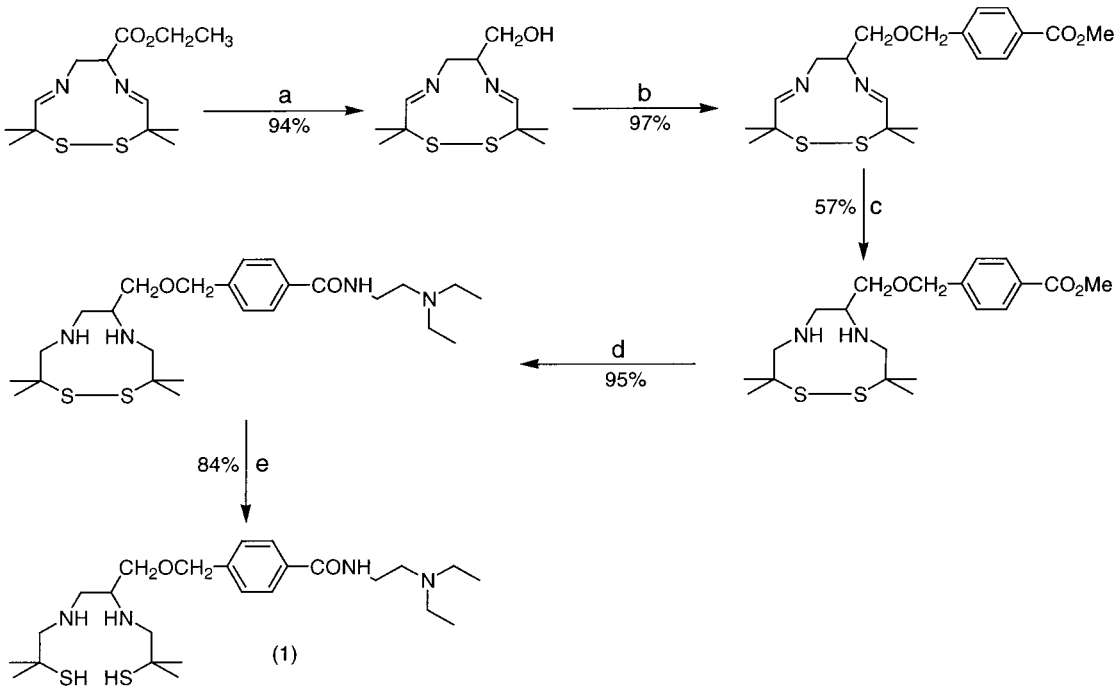
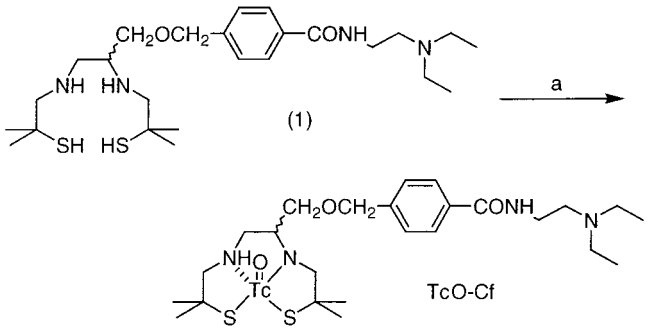
Scheme 1[a]



[a] Reagents: (a) $LiAlH_4$, 0 °C; (b) NaH, MeOCOPhCH$_2$Br; (c) $NaBH_3CN$; (d) $Et_2NCH_2CH_2NH_2$, Me_3Al; (e) $P(CH_2CH_2CO_2H)_3$.

Scheme 2[a]



[a] Reagents: (a) $^{99m}TcO_4Na$, $SnCl_2$, NaOH.

4,8-diene-6-carboxylate in 46% yield (Scheme 1).[27] It was isolated as its hydrochloride and stored under argon. Ligand **1** was then successfully radiolabeled using a sodium [^{99m}Tc]-pertechnetate solution and $SnCl_2$ as reducing agent in basic conditions (Scheme 2). The radiochemical yield was over 95% by TLC (Figure 2). We could not isolate the expected syn and anti diastereomer complexes. Various TLC and HPLC conditions always gave only one spot of radioactivity. It was not possible to determine whether the ^{99m}Tc complexes (syn and anti) possessed closely similar physical properties or only one of them was synthesized during the basic complexation process. (The preparation of the same technetium-99 complex may give an explanation to this particular behavior.) The complex obtained was lipophilic, as indicated by its partition coefficient in octanol/PBS buffer (log $P = 0.64$).

Biological Results. The distribution of the radioactivity activity among the tissues and organs after injection of TcO-Cf is summarized in Table 1. The blood activity decreased rapidly below 1 %ID/g, reflecting fast blood clearance; likewise for the skeletal muscles and the heart. The lung, kidney and liver presented the highest uptakes but, as observed with blood, clearance

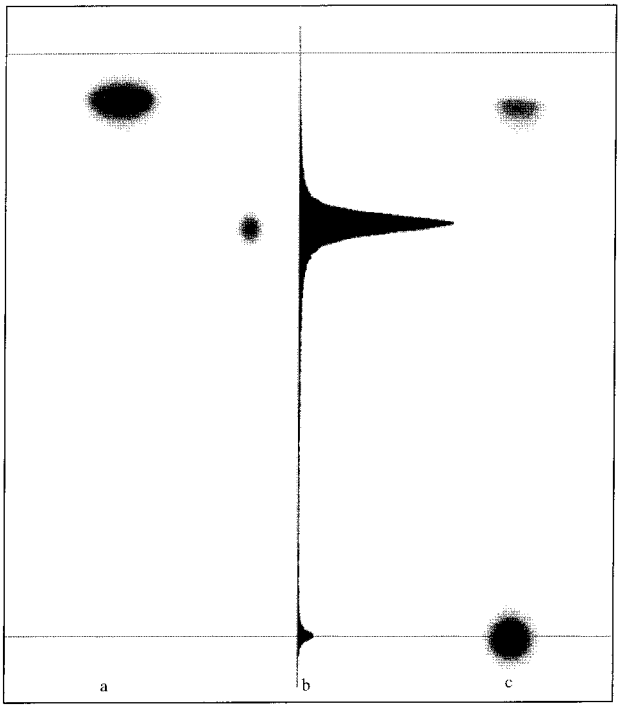


Figure 2. TLC radioactivity control. KC18 TLC: MeOH/CH_3CN/THF/$CH_3CO_2NH_4$ 3:3:1:1. (a) $^{99m}TcO_4Na$; (b) TcO-Cf; (c) $^{99m}TcO_2$.

was fast (less than 50% of the 5 min postinjection activity was retained after 1 h). The low radioactivity measured in the brain indicated that this complex was unable to cross the blood brain barrier (BBB), despite its lipophilicity. TcO-Cf showed an affinity for the pigmented tissues such as the eyes (2.88 %ID/g at 3 h and 2.69 %ID/g at 24 h PI) and the tumor (1.19 %ID/g at 1 h and 0.80 %ID/g at 24 h PI). No noticeable decrease in the uptake was observed between 1 and 6 h postinjection for these tissues. The tumor-to-organ radioactiv-

Table 1. Biodistribution of TcOCf in C57 Mice Bearing B16 Melanoma at Various Times after Injection[a]

tissue	5 min	15 min	1 h	3 h	6 h	24 h
blood	2.69 ± 0.08	2.26 ± 0.28	1.10 ± 0.25	0.63 ± 0.07	0.30 ± 0.04	0.10 ± 0.02
liver	23.68 ± 0.74	21.97 ± 3.52	12.68 ± 2.53	10.35 ± 0.50	9.88 ± 1.30	5.77 ± 1.10
kidney	29.92 ± 1.80	25.71 ± 1.92	12.90 ± 0.78	6.86 ± 0.69	3.43 ± 0.17	1.23 ± 0.31
lung	12.99 ± 4.55	13.37 ± 2.39	6.63 ± 0.76	4.43 ± 0.36	2.28 ± 0.12	0.88 ± 0.60
muscle	2.04 ± 0.28	1.98 ± 0.26	1.07 ± 0.01	0.50 ± 0.07	0.15 ± 0.01	0.04 ± 0.02
heart	6.91 ± 0.61	5.00 ± 0.09	1.55 ± 0.21	0.72 ± 0.21	0.29 ± 0.04	0.12 ± 0.04
brain	0.18 ± 0.00	0.16 ± 0.00	0.08 ± 0.02	0.04 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
eyes	1.77 ± 0.12	2.32 ± 0.20	2.26 ± 0.08	2.83 ± 0.20	2.50 ± 0.16	2.69 ± 0.55
tumor	1.64 ± 0.02	1.68 ± 0.04	1.19 ± 0.21	1.34 ± 0.46	1.06 ± 0.09	0.80 ± 0.14

[a] Units: %ID/g; mean ± sem, $n = 3$. Tumor mass: 0.45 ± 0.35 g.

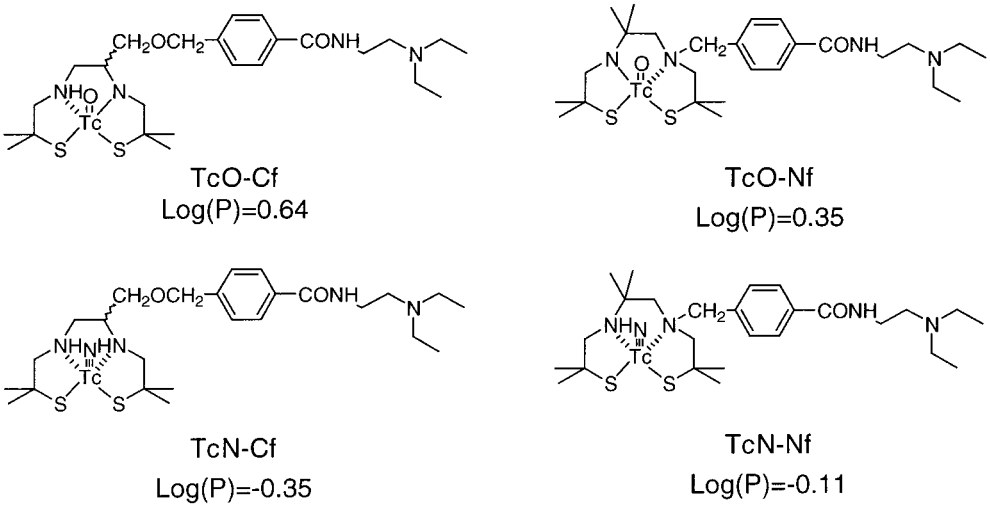


Figure 3. Four ^{99m}Tc-BAT benzamide derivatives and their partition coefficients in octanol/PBS buffer (log *P*).

Table 2. Comparison between Biodistributions in Mice of the Four BAT Complexes and ^{125}I-BZA[a]

compd [ref]	time after injectn (h)	tumor (%ID/g)	tumor/blood ratio	tumor/liver ratio	tumor/lung ratio
TcO-Cf	1	1.19 ± 0.21	1.18 ± 0.47	0.10 ± 0.04	0.19 ± 0.06
	3	1.34 ± 0.46	2.32 ± 1.08	0.13 ± 0.08	0.32 ± 0.04
	6	1.06 ± 0.09	3.60 ± 0.25	0.11 ± 0.01	0.46 ± 0.02
	24	0.80 ± 0.14	8.28 ± 0.06	0.14 ± 0.00	1.50 ± 0.86
TcO-Nf	1	1.51 ± 0.24	0.73 ± 0.06	0.11 ± 0.02	0.12 ± 0.06
[26]	3	1.42 ± 0.29	1.46 ± 0.53	0.18 ± 0.03	0.13 ± 0.04
	6	0.81 ± 0.05	1.26 ± 0.18	0.13 ± 0.01	0.07 ± 0.02
	24	0.53 ± 0.02	2.38 ± 0.07	0.19 ± 0.03	0.16 ± 0.18
TcN-Cf	1	0.43 ± 0.16	3.07 ± 0.94	0.12 ± 0.06	0.35 ± 0.05
[27]	3	0.31 ± 0.10	6.49 ± 2.82	0.13 ± 0.04	0.29 ± 0.04
	6	0.14 ± 0.04	4.97 ± 0.46	0.07 ± 0.02	0.22 ± 0.03
	24	0.13 ± 0.15	2.80 ± 0.41	0.16 ± 0.18	0.29 ± 0.04
TcN-Nf	1	0.71 ± 0.13	1.49 ± 0.37	0.16 ± 0.05	0.91 ± 0.33
[26]	3	0.21 ± 0.01	0.94 ± 0.04	0.11 ± 0.00	0.44 ± 0.06
	6	0.18 ± 0.03	1.39 ± 0.35	0.12 ± 0.04	0.63 ± 0.09
	24	0.06 ± 0.01	2.08 ± 1.17	0.09 ± 0.03	0.45 ± 0.04
^{125}I-BZA	1	6.75 ± 0.67	6.55 ± 0.60	1.12 ± 0.10	0.62 ± 0.05
[2]	3	3.85 ± 0.37	7.79 ± 2.03	1.10 ± 0.26	0.69 ± 0.16
	6	3.53 ± 0.31	17.58 ± 2.16	3.83 ± 0.48	2.13 ± 0.55
	24	0.79 ± 0.09	37.33 ± 6.88	4.94 ± 0.65	15.82 ± 2.61

[a] Mean ± sem, $n = 3$ except for ^{125}I-BZA: $n = 5$.

ity ratios for all the organs and tissues rose significantly as a function of time (see Table 2), indicating specific binding of this complex to the tumor.

Figure 3 represents the oxo-technetium complex and the three other bis(aminothiol) complexes synthesized previously.[26,27] The nature of the technetium core is of major importance for lipophilic behavior: the two oxo-technetium complexes are lipophilic whereas the two nitrido-technetium complexes are hydrophilic. This chemical behavior contrasts with that of the other oxo- and nitrido-technetium N_nS_{4-n} complexes whose partition coefficients have already been published.[28,29]

The alteration of the chemical structure (i.e., C-functionalized or N-functionalized BAT) probably has less influence on the lipophilicity than the nature of the technetium core. To compare the influence of the technetium core and the position of attachment of the benzamide on the skeleton of the bis(aminothiol) structure, the different biodistribution values are grouped in Figure 4 and Table 2. For the four tissues and organs presented in Figure 4, the two nitrido-technetium complexes TcN-Cf and TcN-Nf displayed lower uptake and faster clearance. ^{125}I-BZA showed the highest binding on melanoma during the first 6 h postinjection. Twenty-four hours postinjection, the %ID/g of the tumor labeled with TcO-Cf was slightly higher than the value obtained with ^{125}I-BZA. Faster clearance was noted for the two nitrido-technetium complexes than for the oxo-technetium core complexes, consistent with their higher hydrophilicity. On the other hand, radioactivity fixation in the liver for the oxo-technetium complex decreased more slowly than for the other compounds, especially compared with the ^{125}I-BZA. The specificity of these molecules is evident from the tumor-to-organ ratios given in Table 2. The two nitrido-technetium complexes are not specific to the tumor tissue, having low tumor-to-organ ratios. The replacement of the [TcN] by the [TcO] core induces an important beneficial effect on the biological behavior. The two TcO-Cf complexes showed a clear specificity for the tumor tissue: the tumor/blood, tumor/lung and also tumor/brain, tumor/heart, and tumor/muscle (data not shown) ratios increased notably during the experiment. The affinity of these four tech-

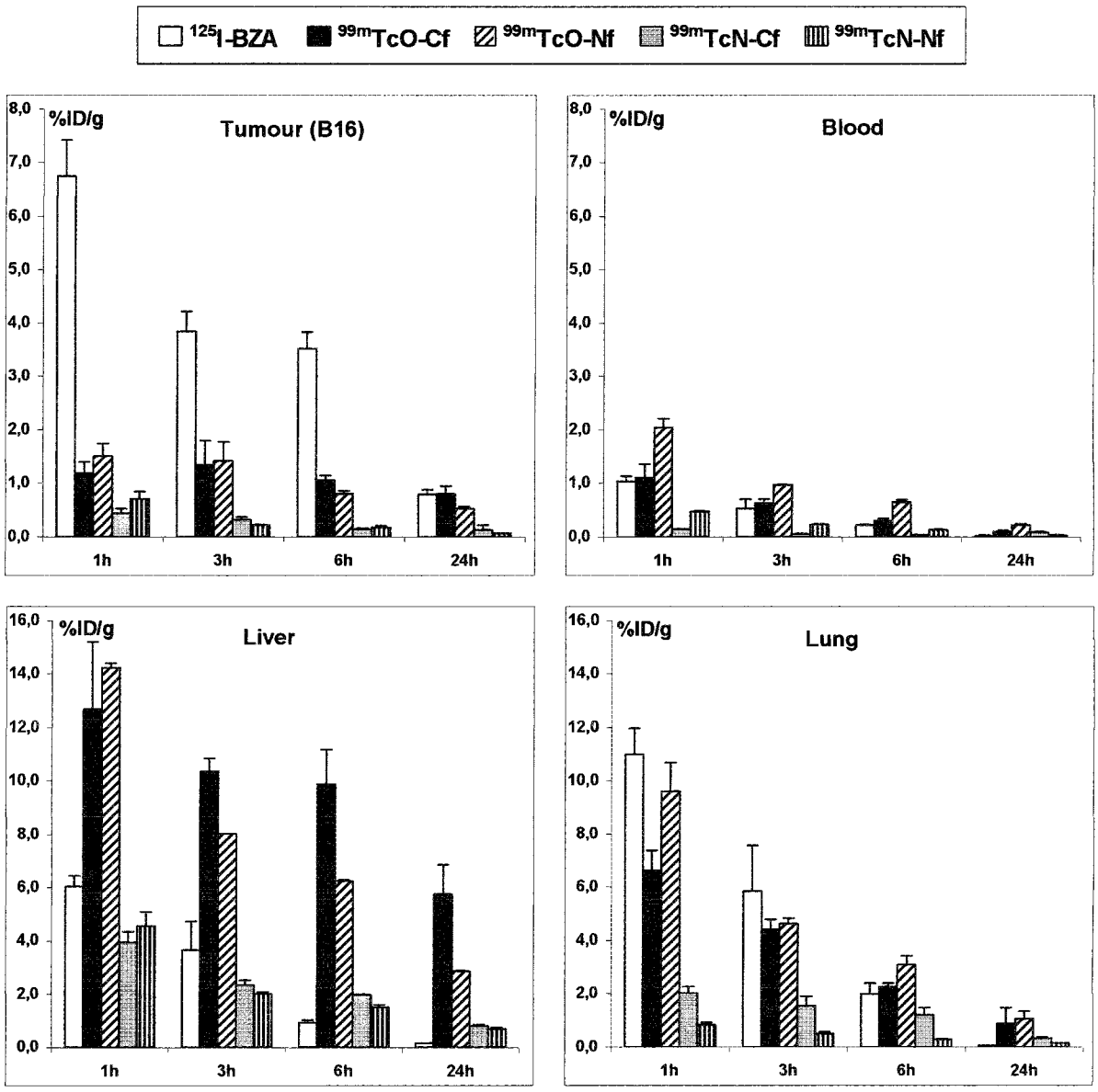


Figure 4. Summary of biodistributions in mice of technetium-99m complexes and ^{125}I-BZA for tumor, blood, liver, and lung. Results are expressed as a percentage of the injected dose per gram of organ (mean ± sem).

netium complexes for the tumor seems to be correlated with their partition coefficient. The TcO-Cf complex that shows lipophilicity closest to that of ^{125}I-BZA (log P = 1.34) presents the best tumor fixation 6 h postinjection and also the best specificity. This result is consistent with others obtained with radioiodinated benzamide derivatives showing the best tumor cell fixation in mice at log P = 1.0−1.5.[19,30]

Conclusion

Four different bis(aminothiol) derivatives complexes with different octanol/water partition coefficients have been synthesized. The lipophilicity of this series of compounds is important in determining the in vivo biodistribution in mice bearing B16 melanoma. The most lipophilic complex, TcO-Cf, exhibits the highest specificity for the tumor, with a regular increase of its tumor-to-organ ratios with time. A same biological behavior was not reported with other technetium-99m complexes of the same pharmacophore group.[25,31,32] This complex presented a marked eye uptake even 24 h after injection (2.69 %ID/g). This marked affinity for the pigmented cells is consistent with a mechanism involving binding to the melanin pigment.[4,9−11,21,33,34]

Experimental Section

General. [^{99m}Tc] sodium pertechnetate as no-carrier-added solution was purchased from Jean Perrin Cancer Hospital (Clermont-Ferrand). All solvents were degassed under argon before use. TLC radioactive spots were scanned and recorded using an AMBIS 4000 detector equipped with a computer-controlled multiwire proportional counter.

Radiolabeling. In a reaction vial under argon were added 1.0 mg of the hydrochloride of ligand **1** (1.6 μmol) in 0.20 mL of 95° ethanol, 0.40 mL of water, 0.10 mL of 1 N sodium hydroxide solution, 0.50 mL of sodium pertechnetate solution (activity ranging from 0.20 to 0.74 GBq), and 0.25 mL of an $SnCl_2$ solution (10.0 mg/mL in water). This solution was heated to 70 °C for 30 min and then cooled to room temperature. The solvents

were evaporated under reduced pressure. The residue was dissolved in PBS buffer with 10% ethanol.

Biological Evaluation. 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). Then, 5×10^5 viable cells were injected subcutaneously. Ten days later, the tumors were palpable. Following the intravenous injection in the tail vein of 0.74 MBq of the ^{99m}Tc-labeled complex, mice ($n = 3$) were sacrificed by exsanguination at the time points 5 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 the percentage of injected dose per gram of tissue (%ID/g) after decay correction.

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