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Nitroimidazoles and Hypoxia Imaging: Synthesis of Three Technetium-99m Complexes Bearing a Nitroimidazole Group: Biological Results

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Abstract—Several Tc-99m complexes were synthesized, substituted with a nitroimidazole group, in order to visualize hypoxic tissues. The complexes were tested on rats (isolated hearts) and showed no significant uptake under hypoxic conditions.

Introduction

A fall in blood pressure can be observed in many diseases. This leads to a reduction in the amount of oxygen delivered to cells, which become hypoxic while remaining viable. If hypoxia is short-lived, the metabolism can return to normal. However, if the oxygen supply is deficient for more than a few seconds, the damage caused can be irreversible and lead to cellular death.[1] Our aim was to develop a specific marker of hypoxic tissues which could be visualized by external detection. Imaging of the myocardium and the brain, both high consumers of dioxygen, are potential applications for such a marker. At the moment, perfusion imaging can identify the relative delivery of dioxygen at the cellular level but does not provide any information on cell vitality.[2] A hypoxia marker would also be very useful in oncology, since tumors present hypoxic regions which are more resistant to radiotherapy and therefore require higher irradiation doses.[3] It was shown as long ago as 1944 that the introduction of a nitro group on heterocycles such as nitroimidazoles gives them bacteriostatic properties.[4] Over the last two decades, numerous papers dealing with the biological activity of nitroimidazoles under anaerobic conditions have suggested that the nitroimidazole group is enzymatically reduced and trapped in hypoxic cells. Labeled nitroimidazole derivatives are therefore potential radiopharmaceuticals for imaging hypoxic areas. The first compounds studied intensively were derived from [1-(3-methoxypropyl-2-hydroxy)]-2-nitroimidazole (misonidazole) by the introduction of a radioactive halogen, F-18, Br-77 or I-123 (I-131 for in vitro experiments).[5–9] Images obtained with fluorinated (PET) and brominated (SPECT) compounds present poor contrast between normoxic and hypoxic areas, while iodinated compounds are too lipophilic, making blood clearance excessively long. Given the advantages of technetium-99m in nuclear medicine (low cost, good availability, ideal half-life of 6.02 h and ideal γ-energy of 140 keV), research has focused increasingly on Tc-99m-labeled markers of hypoxia. Unfortunately, unlike the radioactive halogens, Tc-99m cannot be directly bonded to the biomolecules, so that a chelating group has to be linked to the bioactive fragment to incorporate this metallic nuclide. Essentially, two classes of compounds bearing a nitroimidazole group have been studied: boronic adducts of technetium dioxime[10] and technetium diamine dioximes,[11] both neutral complexes. The lipophilicity of the first compounds resulted in their being trapped in the hydrophobic membrane, but the authors showed that the nitroimidazole moiety of the complexes was enzymatically reduced in the absence of oxygen, demonstrating recognition by the enzyme. Dioxime complexes showed

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relatively good localization in hypoxic cells. Recently, HL-91, another dioxime with a $[TcO]^{3+}$ core, showed good uptake in tumors.[12] Curiously, this compound does not include a nitroimidazole group. Apart from this latter compound, all the derivatives with an affinity for hypoxic tissue include a nitroimidazole group, which is why we decided to bond this type of structure to Tc-99m chelating groups.

The present report describes the synthesis of several ligands, their labeling with technetium-99m and the biological results. The ligands present a nitroimidazole group required for hypoxic cell trapping and three chelating groups leading to three technetium complexes with a positive charge, negative charge or no charge, in order to evaluate the influence of radiopharmaceutical charge on uptake by hypoxic cells.

The three chelating groups are 1,4,8,11-tetraazacyclotetradecan (cyclam), 5,7-dioxo-1,4,8,11-tetraazaundecan and 2-oxo-1,5,8,12-tetraazacyclotetradecan (oxocyclam) leading, respectively, to positive, negative or neutral complexes at physiological pH by complexation of the $[TcO_2]^+$ core.[13,14] The three target complexes are shown in Figure 1.

Chemistry and Labeling

First, compound **6** was synthesized as described in Scheme 1 from cyclam, in which the three amine functions were protected by *tert*-butyloxycarbonyl groups before coupling with mesylate of 6-(2-methyl-4-nitroimidazolyl)hexan-1-ol **2** to give **4**. Next, the amine groups were deprotected in HCl/EtOH to give **5**. The introduction of the TcO_2^+ core into **5**, leading to **6**, is described later.

Synthesis of **12** (Scheme 2) was performed from 5-chloropentan-1-ol, a bifunctional compound allowing introduction of an imidazole group on one side and a chelating group on the other side. After protection of the hydroxyl group, a malonic synthesis led to the introduction of a three-carbon bridge on the linker in compound **7**. The four amine/amide functions were easily introduced through the action of an excess of ethylene diamine (as a solvent) on the diester. After deprotection of the alcohol and protection of the amines, the 2-methyl-4-nitroimidazolyl group was introduced via mesylate. Deprotection of the amines in **11** and labeling with Tc-99m led to complex **12**.

The intermediate **8** served as a precursor for the preparation of the third macrocyclic ligand **15**, as shown in Scheme 3. The two amide functions were reduced by BH_3–Me_2S in THF according to Brown's method[15] with a modification: the amines were protected in situ to facilitate purification. The alcohol function was activated via mesylate to introduce 2-methyl-4-nitroimidazole. After deprotection of the amine groups, **14** could be cyclized on methylacrylate to give the target molecule **15**, then labeling of **15** gave **16**.

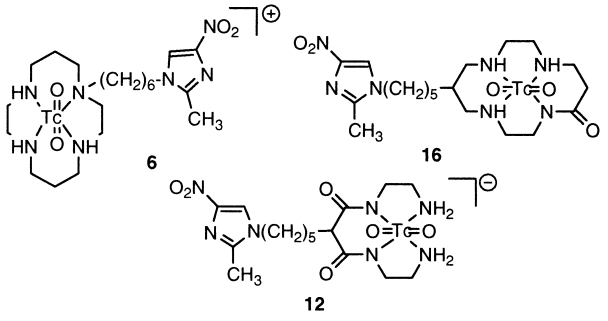
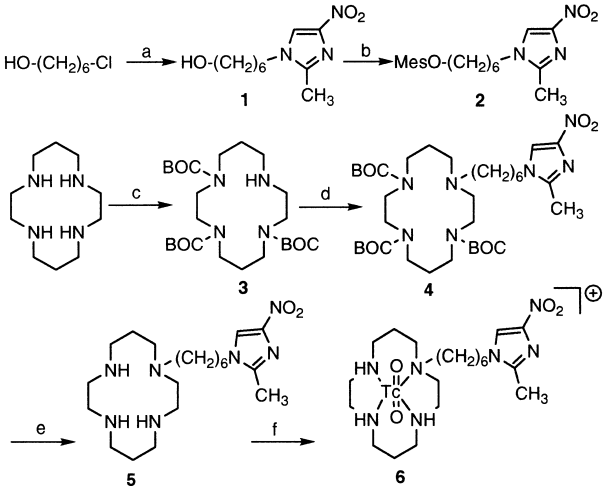
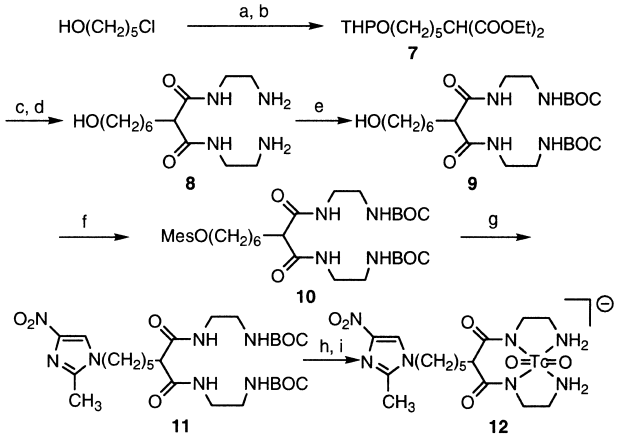


Figure 1. Target compounds.



Scheme 1. Synthesis and complexation of *N*-[6-(2-methyl-4-nitroimidazolyl)-hexyl]cyclam. Reagents: (a) 2-methyl-5-nitroimidazole, K_2CO_3, DMF, 78%; (b) CH_3SO_2Cl, CH_2Cl_2, pyridine, 98%; (c) Boc_2O, EtOH, 50%; (d) **2**, K_2CO_3, DMF, 25%; (e) HCl 37%/EtOH (10% v/v), 71%; (f) $^{99m}TcO_4^-$, Sn-tartrate, H_2O, pH > 11.5.

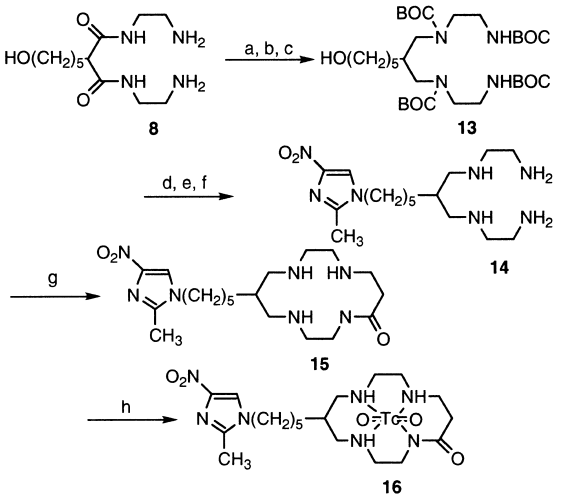


Scheme 2. Synthesis and complexation of 6-[5-(2-methyl-4-nitroimidazolyl)pentyl]-5,7-dioxo-1,4,8,11-tetraazaundecan. Reagents: (a) DHP, PTSA, THF, 83%; (b) EtONa, $CH_2(COOEt)_2$, EtOH, 47%; (c) $H_2N(CH_2)_2NH_2$, 74%; (d) PTSA, MeOH, 79%; (e) Boc_2O, MeOH, 98%; (f) CH_3SO_2Cl, CH_2Cl_2, 87%; (g) 2-methyl-5-nitroimidazole, K_2CO_3, DMF, 92%; (h) HCl 37%/MeOH (10% v/v), quant; (i) $^{99m}TcO_4^-$, Sn-tartrate, H_2O, pH > 11.5.

The radiolabeling of the three target ligands was achieved with sodium [^{99m}Tc]pertechnetate and stannous(II) tartrate as the reducing agent to produce **6**, **12** and **16** in good yields (75 to 95%), evaluated by chromatography. The charges of the complexes were determined by electrophoresis on cellulose in buffered medium at physiological pH and were in agreement with those obtained with chelating groups without functionalization. The macroscopic (Tc-99) structure of these non-functionalized complexes has already been studied in order to confirm the proposed structure of these chelates at the microscopic level (Tc-99m).[13,14] We can thus affirm by analogy that the structures of the functionalized complexes involve the $[^{99m}TcO_2]^+$ core and that deprotonations at pH 7.4 occur for **12** and **16** to give negative and neutral complexes, respectively. The partition coefficients *P* were measured between a 1-octanol phase and a buffer phase (pH 7.4). Radiolabeling results are given in Table 1.

Biological Results and Discussion

Uptake of target complexes **6**, **12** and **16** was performed in Langendorff buffer-perfused rat hearts in normoxic and hypoxic conditions according to the method of Chin et al.[16] Normoxia was obtained with buffer saturated by 95% O_2 and 5% CO_2 and hypoxia with buffer saturated by 95% N_2 and 5% CO_2. Heart rate and systolic and diastolic pressures were also recorded on-line to determine whether the perfused hearts remained in good condition during the experiments. The experimental protocol is described in Figure 2.



Scheme 3. Synthesis and complexation of 10-[5-2-(methyl-4-nitroimidazolyl)pentyl]-2-oxocyclam. Reagents: (a) BMS/THF; (b) MeOH, HCl; (c) Boc_2O, K_2CO_3, MeOH, 75% (a + b + c); (d) CH_3SO_2Cl, CH_2Cl_2, 95%; (e) 2-methyl-5-nitroimidazole, K_2CO_3, DMF, 98%; (f) HCl 37%/MeOH (10% v/v), quant; (g) CH_2=CHCOOMe, MeOH, 15%; (g) $^{99m}TcO_4^-$, Sn-tartrate, H_2O, pH > 11.5.

Table 1. Radiolabeling data for ^{99m}Tc-**6**, ^{99m}Tc-**12** and ^{99m}Tc-**16**

Compound	Yield (%)	Electrophoresis (pH 7.4, 30 V/cm)	Log *P*
^{99m}Tc-**6**	95	− 3 cm	−2.0
^{99m}Tc-**12**	75	+ 3 cm	−1.4
^{99m}Tc-**16**	90	0	−0.12

After a bolus injection of $[^{99m}TcO_2$-**6**$]^+$, $[^{99m}TcO_2$-**12**$]^-$ or $[^{99m}TcO_2$-**16**] (1.5 MBq/250 μL physiological serum), radioactivity was monitored by a NaI detector for 30 min. Each compound was tested on four isolated hearts. With this model, retention of a hypoxia tracer is expected to be much higher in hypoxic conditions than in normoxic conditions. Typical curves obtained in normoxia and hypoxia are represented in Figure 3.

Although no significant statistical difference was found, the complexes seemed to show slightly higher radioactivity retention in normoxic conditions compared with hypoxic conditions. This observation has already been reported in the case of bisthiosemicarbazone complexes by Dearling et al.,[17] who suggest that factors other than hypoxia govern the uptake and uptake kinetics of certain complexes.

The rapid clearance obtained is not surprising for $[^{99m}TcO_2$-**6**$]^+$ and $[^{99m}TcO_2$-**12**$]^-$, since they are charged species which are insufficiently lipophilic to cross the membrane by passive diffusion.

The result is more surprising for the neutral complex [$^{99m}TcO_2$-**16**]. The rapid clearance may be correlated with rapid uptake and washout due to the absence of reduction of the nitro group, which is the initial step of the cellular trapping process. Although the redox potential of 2-methyl-5-nitroimidazole (−415 mV[3]) is greater than that of the oxido-reductases with the lowest potentials such as succinate dehydrogenase (−450 mV), the difference may not be sufficient to allow efficient reduction of this compound. We now envisage the synthesis of other nitroaromatic compounds such as 2-nitro or polynitroimidazole derivatives, which present lower redox potentials.

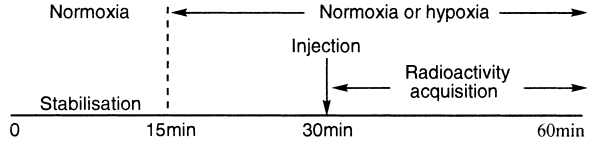


Figure 2. Diagrammatic representation of the experimental protocol.

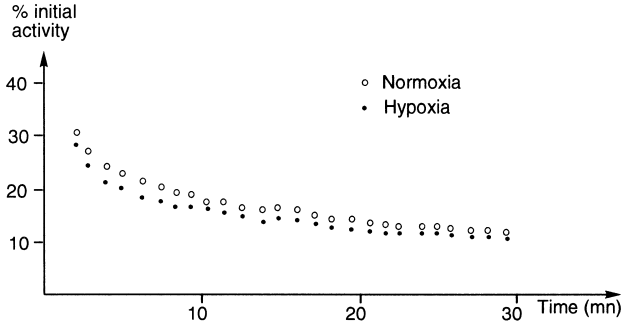


Figure 3. Characteristic curves of ^{99m}Tc complexes in isolated perfused rat heart.

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