

Re and Tc Tricarbonyl Complexes: From the Suppression of NO Biosynthesis in Macrophages to in Vivo Targeting of Inducible Nitric Oxide Synthase

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The in vivo molecular imaging of nitric oxide synthase (NOS), the enzyme responsible for the catalytic oxidation of L-arginine to citrulline and nitric oxide (NO), by noninvasive modalities could provide valuable insights into NO/NOS-related diseases. Aiming at the design of innovative ^{99m}Tc(I) complexes for targeting inducible NOS (iNOS) in vivo by SPECT imaging, herein we describe a set of novel ^{99m}Tc(CO)₃ complexes (**2–5**) and the corresponding rhenium surrogates (**2a–5a**) containing the NOS inhibitor *N*^ω-nitro-L-arginine. The latter is linked through its α-NH₂ or α-COOH group and an alkyl spacer of variable length to the metal center. The complexes **2a** (propyl spacer) and **3a** (hexyl spacer), in which the α-NH₂ group of the inhibitor is involved in the conjugation to the metal center, presented remarkable affinity for purified iNOS, being similar to that of the free nonconjugated inhibitor (*K*_i = 3–8 μM) in the case of **3a** (*K*_i = 6 μM). **2a** and **3a** are the first examples of organometallic complexes that permeate through RAW 264.7 macrophage cell membranes, interacting specifically with the target enzyme, as confirmed by the suppression of NO biosynthesis in LPS-treated macrophages (**2a**, ca. 30% inhibition; **3a**, ca. 50% inhibition). The ^{99m}Tc(I)-complexes **2** and **3**, stable both in vitro and in vivo, also presented the ability to cross cell membranes, as demonstrated by internalization studies in the same cell model. The biodistribution studies in LPS-pretreated mature female C57BL/6 mice have shown that **2** presented an overall higher uptake in most tissues of the LPS-treated mice compared to the control group (30 min postinjection). This increase is significant in lung (3.98 ± 0.63 vs to 0.99 ± 0.13%ID/g), which is known to be the organ with the highest iNOS expression after LPS treatment. These results suggest that the higher uptake in that organ may be related to iNOS upregulation.

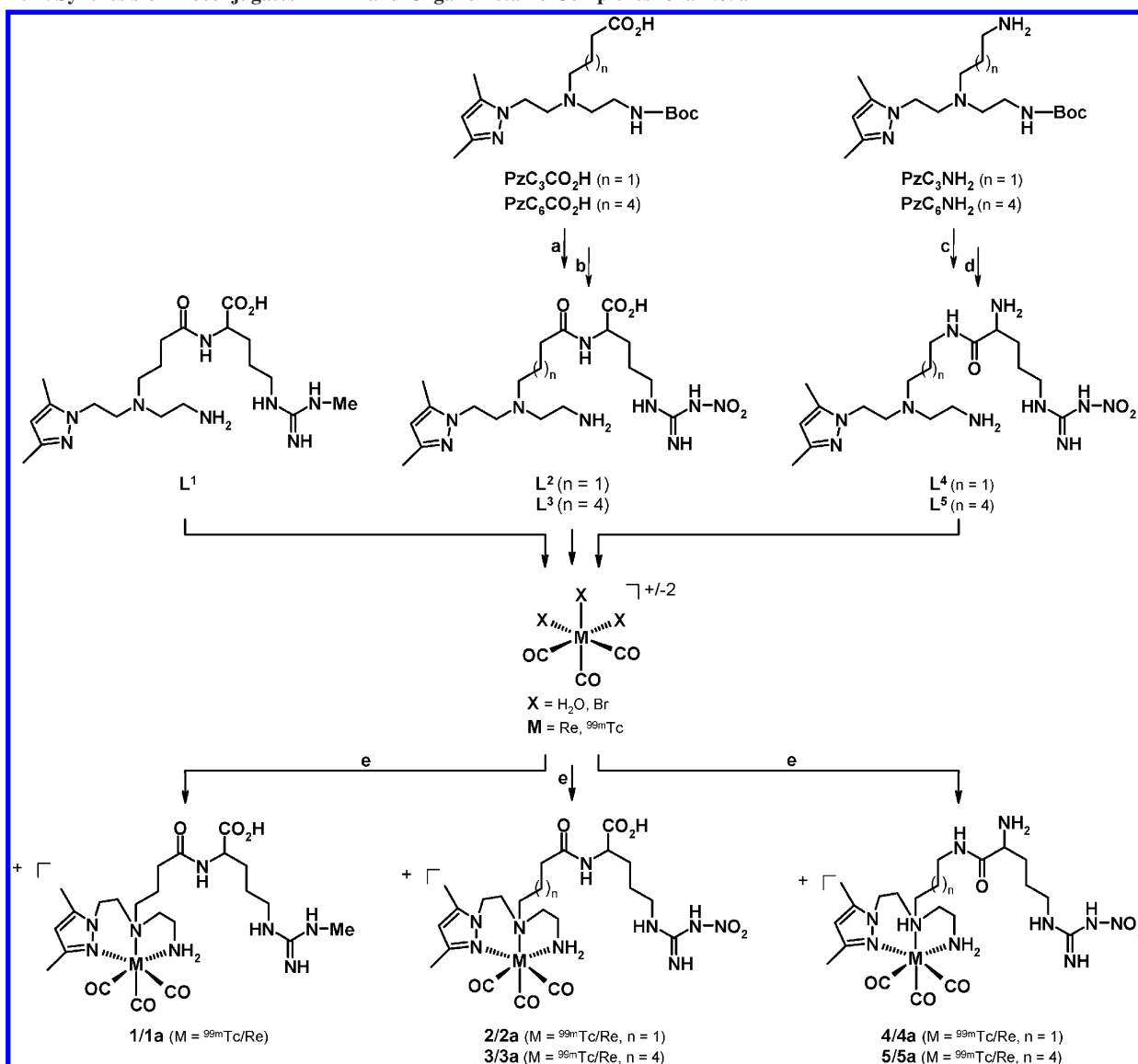
The in vivo molecular imaging of nitric oxide synthase (NOS), the enzyme responsible for the catalytic oxidation of L-arginine to citrulline and nitric oxide (NO), by noninvasive modalities could provide valuable insights into NO/NOS-related diseases such as cancer, neurological disorders, or vascular malfunctions, among others (1–10). So far, the main modalities explored for NOS imaging include positron emission tomography (PET) and optical imaging (OI) (9–15). The nuclear imaging modalities PET or single photon emission computed tomography (SPECT), due to their favorable features, and broad range of available probes offer high clinical potential (16–18). Technetium-99m (^{99m}Tc) is the radionuclide of choice for SPECT imaging due to its almost ideal nuclear properties (*t*_{1/2} = 6.02 h, *E*_{γmax} = 140 keV) and rich chemistry, which allows modulation of the biological properties of the radioactive complexes. Taking into consideration our interest in the design of innovative ^{99m}Tc(I)-complexes for targeting molecular processes in vivo (19–21), we have recently proposed a set of novel ^{99m}Tc(CO)₃ complexes containing pendant L-arginine or NOS inhibitors for targeting NOS expression by SPECT imaging (22, 23). Enzymatic assays of purified inducible NOS (iNOS) activity in the presence of the analogue Re(CO)₃-complexes, prepared as

“nonradioactive” surrogates, have shown that the Re complexes **1a** (*K*_i = 36 μM) and **2a** (*K*_i = 84 μM) stabilized by bioconjugates bearing *N*^ω-monomethyl- (**L**¹) or *N*^ω-nitro-L-arginine pendant moieties (**L**²), respectively, still presented inhibitory action (Scheme 1). Indeed, **1a** and **2a** are the first examples of organometallic complexes able to inhibit the iNOS (22). Interestingly, **2a** presented higher inhibitory potency than the corresponding metal-free bioconjugate **L**² (*K*_i = 178 μM). These encouraging results prompted us to introduce structural changes in **2a** aimed at increasing the affinity of the metal complex for the enzyme. Such modifications involved, on one hand, introduction of a longer alkyl chain between the *N*^ω-nitro-L-arginine pendant arm and the chelating unit and, on the other hand, conjugation of the amino acid derivative to the metal complex through its α-COOH group. Toward this goal, we synthesized the new bioconjugates **L**³–**L**⁵ in a two-step procedure by direct conjugation of the Boc-protected precursors **PzC₆CO₂H**, **PzC₃NH₂**, and **PzC₆NH₂** to the corresponding *N*^ω-nitro-L-arginine derivative using standard coupling reagents, followed by hydrolysis of the protecting groups under acidic conditions (Scheme 1). All amino acid-containing bioconjugates were obtained as air-stable colorless oils after purification by semipreparative reversed-phase high-performance liquid chromatography (RP-HPLC) with ≥98% purity (see Supporting Information). All bioconjugates were characterized by elemental analysis, ¹H/¹³C NMR (including 2D-NMR experiments such as ¹H-¹H-¹H COSY and ¹H-¹³C HSQC) and IR spectroscopy, and electrospray ionization mass spectrometry (see Supporting Information). Reaction of **L**¹–**L**⁵ with the organometallic

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Scheme 1. Synthesis of Bioconjugates L^2 – L^5 and Organometallic Complexes **1/1a**–**5/5a**^a

^a Reagents and conditions: (a) *N*^ω-nitro-L-arginine methyl ester, HBTU, Et₃N, DMF/ACN, r.t.; (b) HCl 3 N, r.t.; (c) *N*^α-Boc-*N*^ω-nitro-L-arginine, DCC, HOBT, DMF/DCM, r.t.; (d) TFA/DCM, r.t.; (e) H₂O, 100 °C. ACN = acetonitrile; Boc = *t*-butoxycarbonyl; DCC = *N,N'*-Dicyclohexylcarbodiimide; DCM = dichloromethane; DMF = dimethylformamide; HBTU = *O*-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate; HOBT = *N*-Hydroxybenzo-triazole; r.t. = room temperature; TFA = trifluoroacetic acid.

precursor $fac-[^{99m}Tc(CO)_3(H_2O)_3]^+$, obtained using an IsoLink kit, afforded in high radiochemical yield (>95%) cationic complexes of the type $fac-[^{99m}Tc(CO)_3(k^3-L)]^+$ ($L = L^1$, **1**; L^2 , **2**; L^3 , **3**; L^4 , **4**; L^5 , **5**) (Scheme 1).

The hydro(lipo)philic nature of all $^{99m}Tc(I)$ -complexes was evaluated by determining the partition coefficient in physiological conditions (Supporting Information), and the results were expressed as $\log P_{o/w}$. The radiocomplexes with a propyl spacer ($\log P_{o/w}$ **1** = −2.19, $\log P_{o/w}$ **2** = −1.41, and $\log P_{o/w}$ **4** = −1.29) presented higher hydrophilic character than the ones with a hexyl spacer ($\log P_{o/w}$ **3** = −0.29, and $\log P_{o/w}$ **5** = −1.01).

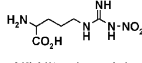
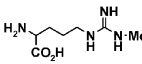
The *in vitro* stability studies performed (incubation with solutions of amino acids with high affinity for Tc(I) such as histidine and cysteine, in phosphate-buffered saline pH 7.4, and human plasma at 37 °C for 24 h) have shown that all $^{99m}Tc(I)$ -complexes are stable, since no decomposition or reoxidation products were observed by RP-HPLC analysis (purity ≥95%).

The chemical identity of the $^{99m}Tc(I)$ complexes has been established by comparing their RP-HPLC analytical radioactive traces (γ -detection) with the UV/vis traces of the rhenium

complex surrogates **1a**–**5a**, which have been prepared by reacting L^1 – L^5 with the precursor $(NEt_4)_2[ReBr_3(CO)_3]$ in refluxing water, in the 1:1 molar ratio (Scheme 1). The complexes were obtained as air-stable colorless viscous oils in moderate yields (40–94%), after evaporation of the reaction solvent and washing of the residue obtained with CHCl₃ for NEt_4Br removal and further purification by preparative RP-HPLC (purity ≥95%). The novel complexes **3a**–**5a** were fully characterized (Supporting Information) by RP-HPLC, elemental analysis, ESI-MS, and NMR spectroscopy (¹H/¹³C NMR, ¹H-¹H COSY, and ¹H-¹³C HSQC).

To assess the influence of the structural modifications introduced in **2a** in the enzymatic activity of NOS, the new bioconjugates L^3 – L^5 and corresponding rhenium complexes **3a**–**5a** were tested as competitive inhibitors of purified mouse recombinant iNOS, and their activity compared to that of the previously described analogues (22). The iNOS activity was determined spectrophotometrically by monitoring the NO-mediated conversion of oxyhemoglobin to methemoglobin at two wavelengths (401 and 421 nm). The inhibition constants

Table 1. K_i Values for iNOS Competitive Inhibitors L^2 – L^5 and $1a$ – $5a$

Entry	Inhibitor	K_i values ^[a] (μ M)
1	 <i>N</i> ^ω -Nitro- <i>L</i> -arginine	3 ⁽²²⁾ 8 ⁽²⁴⁾
2	 <i>N</i> ^ω -Methyl- <i>L</i> -arginine	8 ⁽²²⁾ 4 ⁽²⁴⁾
3	<i>fac</i> -[Re(CO) ₃ (k^3 - L^1)] (1a)	36 ⁽²²⁾
4	L ²	178 ⁽²²⁾
5	<i>fac</i> -[Re(CO) ₃ (k^3 - L^2)] (2a)	84 ⁽²²⁾
6	L ³	36
7	<i>fac</i> -[Re(CO) ₃ (k^3 - L^3)] (3a)	6
8	L ⁴	35
9	<i>fac</i> -[Re(CO) ₃ (k^3 - L^4)] (4a)	103
10	L ⁵	137
11	<i>fac</i> -[Re(CO) ₃ (k^3 - L^5)] (5a)	118

^aResults are given as a mean of three or more independent experiments. Standard deviations of ± 5 –10% were observed.

(K_i), determined by the method of Eisenthal and Cornish-Bowden, are summarized in Table 1.

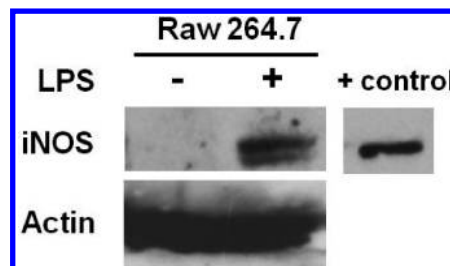
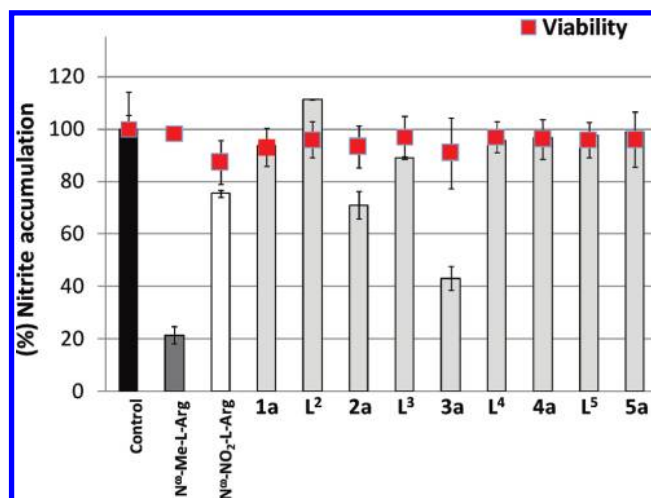
All bioconjugates showed decreased inhibitory potencies to iNOS (L^2 – L^5) when compared with free, nonconjugated, *N*^ω-nitro-*L*-arginine. When the latter is coupled through its α -NH₂ group to the bifunctional chelator, a longer spacer length led to an increased affinity to the enzyme as can be concluded by comparing the K_i values of **L**³ (36 μ M) and **L**² (178 μ M). An opposite effect has been observed when the α -CO₂H group of *N*^ω-nitro-*L*-arginine is involved in the attachment to the chelator. In this case, the longer spacer length led to a lower affinity for the enzyme (**L**⁴, K_i = 35 μ M; **L**⁵, K_i = 137 μ M). Unlike **L**⁴, reaction of the analogue bioconjugates **L**² (K_i = 178 μ M), **L**³ (K_i = 36 μ M), and **L**⁵ (K_i = 137 μ M) with the organometallic moiety *fac*-[Re(CO)₃]⁺ gave complexes with increased inhibitory potencies (**2a**, K_i = 84 μ M; **3a**, K_i = 6 μ M; **5a**, K_i = 118 μ M).

The increased inhibitory potency after metalation occurs independently of the functional group of the amino acid involved in the chemical bonding to the chelating unit (e.g., **3a** vs **5a**) and spacer length (e.g., **2a** vs **3a**). Such an effect has already been observed with other metal complexes that presented higher inhibitory potency than the corresponding metal-free inhibitors (25). Remarkably, in the case of **3a**, the inhibitory potency (K_i = 6 μ M) is comparable to that of the free nonconjugated inhibitor *N*^ω-nitro-*L*-arginine (K_i = 3 μ M).

These unprecedented results motivated us to evaluate the ability of the compounds to cross cellular membranes, a key feature for improving tracer uptake in vivo, and to interact intracellularly with their target (iNOS enzyme).

Both the ^{99m}Tc(I) complexes and their Re(I) surrogates were tested in RAW 264.7 macrophages, which present increased NO biosynthesis due to iNOS overexpression after treatment with endotoxins such as lipopolysaccharide (LPS) (26). LPS-induced iNOS overexpression was confirmed by Western blot analysis of protein extracts using an anti-iNOS antibody (Figure 1).

We began by evaluating the ability of bioconjugates **L**²–**L**⁵ and rhenium complexes **1a**–**5a** to inhibit intracellular iNOS by measuring nitrite accumulation using the Griess method (Figure 2). In order to assess the intrinsic cytotoxicity of the compounds at the concentration used in the NO assay (500 μ M) and, consequently, its influence in nitrite accumulation, we have also performed a cell viability assay in parallel (MTT assay). The

**Figure 1.** iNOS expression in RAW 264.7 macrophages after treatment with LPS for 24 h. Recombinant iNOS (6 μ g) was used as a positive control and actin as an internal loading control.**Figure 2.** Effect of compounds **L**²–**L**⁵ and **1a**–**5a** in nitrite accumulation and cell viability in LPS-induced RAW 264.7 macrophages (mean \pm S.D., n = 8). Cells were LPS-treated and cultured in the presence of the compounds. Final concentration of all compounds was 500 μ M. NO production and viability in LPS-induced cells in the absence of any compound (control) was considered 100%. This experiment was repeated three times with comparable results.

assay has shown that all compounds tested are nontoxic for the cells under the described experimental conditions (Figure 2).

The rhenium complexes **2a** (ca. 30% inhibition) and **3a** (ca. 50% inhibition) appeared as the most potent inhibitors of NO production by LPS-activated macrophages. Remarkably, **3a** inhibited NO biosynthesis more effectively than the free inhibitor *N*^ω-nitro-*L*-arginine (*N*^ω-NO₂-*L*-Arg - ca. 25%). These results are in line with the in vitro enzymatic assays performed with purified iNOS, where complexes **2a** and **3a** also exhibited the highest inhibitory potency toward iNOS (**2a**, K_i = 84 μ M; **3a**, K_i = 6 μ M; Table 1). Since the inhibition of NO biosynthesis is being influenced not only by the intrinsic inhibitory capacity of the complexes as desired, but also by their ability to cross the cell membrane, we have performed internalization studies in the same cellular model with the radioactive ^{99m}Tc(I) analogues **2** and **3**, and compared their internalization levels with that of **1** (Figure 3).

These studies allowed us to conclude that the affinity of compound **3a** for the enzyme is strong enough to surpass the lower ability for crossing the cell membrane presented by the corresponding radioactive complex **3**, as demonstrated by its lower internalization level, when compared with compound **2** ($0.43 \pm 0.06\%$ vs $0.83 \pm 0.06\%$ internalization/mg protein, respectively).

Despite presenting comparable ability for crossing cell membranes, as demonstrated by the similar internalization levels shown by **1** and **2** ($0.76 \pm 0.10\%$ and $0.83 \pm 0.06\%$ internalization/mg protein, respectively), the surrogate compound **1a**, which presented a higher affinity for purified iNOS than

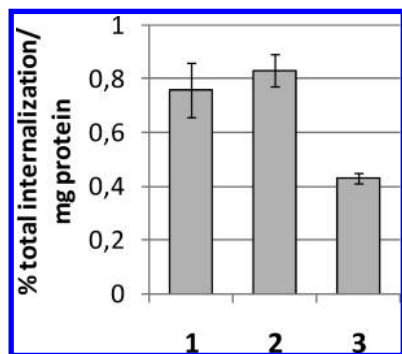


Figure 3. Internalization of the radioactive complexes **1–3** in LPS-induced RAW 264.7 macrophages (mean \pm S.D., $n = 4$; 37 °C, 4 h post-incubation). The internalization level is expressed as a percentage of applied radioactivity per milligram of total protein.

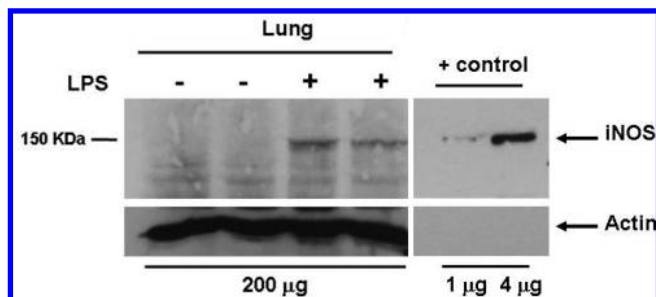


Figure 4. iNOS expression in the lungs of LPS-treated mice (10 mg/kg of LPS, 6 h). Recombinant iNOS (4 μ g) was used as a positive control and actin as internal loading control.

compound **2a**, is not able to inhibit intracellular iNOS in the macrophages assay as is the case of **2a**.

As far as we are aware, the matched pairs **2/2a** and **3/3a** are rare examples of organometallic complexes able to cross cell membranes and interact specifically with a cytosolic enzyme. Encouraged by both the ability of **2a** and **3a** to interact with intracellular iNOS, inhibiting biosynthesis of NO in LPS-activated RAW 264.7 macrophages, and the moderate ability of **2** and **3** to cross cell membranes, the biodistribution of these $^{99m}\text{Tc(I)}$ complexes has been evaluated in LPS-pretreated mature female C57BL6 mice. Mice were treated with bacterial LPS (10 mg/kg, iv) to induce iNOS expression 6 h before the radioactive compounds were injected. The control group was injected with PBS. It has been demonstrated that iNOS mRNA and protein expression after LPS stimulation are increased in many organs, with the highest iNOS expression in the lungs (27). We have also confirmed increased levels of iNOS expression in lungs of LPS-treated mice by Western blot analysis of total protein (Figure 4).

The complexes **2** and **3** are stable in vivo as demonstrated by RP-HPLC analysis of urine and serum samples collected at sacrifice time. In fact, more than 98% of the radioactivity could be assigned to the complexes.

Compound **3** did not present any significant increase of radioactivity in the lungs of pretreated animals compared to the control group at 15 and 60 min postinjection. This result is in agreement with the cellular internalization data obtained for this compound and confirms the importance of crossing cell membranes for in vivo intracellular targeting and retention.

Compound **2** presented an overall higher uptake in most tissues of LPS-treated mice compared to the control group at 30 min postinjection (Figure 5). Unlike compound **3**, this increase is particularly relevant in lung (3.98 ± 0.76 vs $0.99 \pm 0.13\%$ ID/g), which is known to be the organ with the highest iNOS expression (27). This result suggests a direct correlation

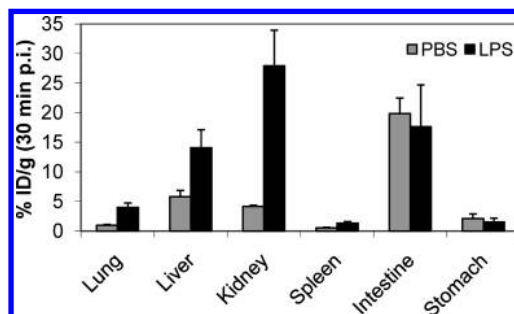


Figure 5. Biodistribution of **2** in LPS-treated mature female C57BL/6 mice (LPS) and control group (PBS) 30 min after intravenous injection (mean \pm standard deviation, $n = 4–5$). ID: injected dose.

between lung uptake and iNOS upregulation. In contrast to intestine and stomach, the increased uptake in the excretory organs kidney and liver, as well as in the spleen, may also be partially related to iNOS overexpression in these tissues (27).

In conclusion, we have shown that complexes **2a** and **3a**, “inactive” surrogates of the $^{99m}\text{Tc(I)}$ -complexes **2** and **3**, presented remarkable affinity for purified iNOS, being similar to that of the free nonconjugated inhibitor in the case **3a**. Both complexes permeate through macrophage cell membranes and interact specifically with the cytosolic target enzyme, as confirmed by the inhibition of NO biosynthesis in LPS-treated macrophages. The $^{99m}\text{Tc(I)}$ complexes **2** and **3** are stable in vitro and present the ability to cross cell membranes. The biodistribution studies demonstrated that these complexes are stable in vivo and suggested that **2** is able to recognize iNOS in vivo. Therefore, the latter complex **2** presents a set of adequate features for potential targeting/imaging of NOS expression in vivo. These encouraging results brought together proved that metal complexes in general, and $^{99m}\text{Tc(I)}$ /Re(I) complexes in particular, can be “structurally tuned” to target intracellular low-capacity systems such as enzymes, paving the way to the design of innovative, more specific diagnostic or therapeutic agents.

ACKNOWLEDGMENT

This work has been partially supported by the Fundação para a Ciência e Tecnologia (FCT), Portugal (POCI/SAU-FCF/58855/2004). B. L. Oliveira thanks FCT for a PhD grant (SFRH/BD/38753/2007). Dr. J. Marçalo is acknowledged for performing the ESI-MS analyses. The QITMS instrument was acquired with the support of Contract REDE/1503/REM/2005 - ITN of FCT and is part of RNEM.

Supporting Information Available: Experimental Section. This material is available free of charge via the Internet at <http://pubs.acs.org>.

LITERATURE CITED

- (1) Moncada, S., Palmer, R. M., and Higgs, E. A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol. Rev.* 43, 109–142.
- (2) Kerwin, J. F., and Lancaster, J. R. (1995) Nitric oxide: a new paradigm for second messengers. *J. Med. Chem.* 38, 4343–4362.
- (3) Marletta, M. A. (1993) Nitric oxide synthase structure and mechanism. *J. Biol. Chem.* 268, 12231–12234.
- (4) Alderton, W. K., Cooper, C. E., and Knowles, R. G. (2001) Nitric oxide synthases: structure, function and inhibition. *Biochem. J.* 357, 593–615.
- (5) Special Issue on Nitric Oxide in Cancer Biology and Treatment (Wink, D. A., and Mitchell, J. B., Eds.) *Free Radic. Biol. Med.* 2003, 34.

- (6) Lechner, M., Lirk, P., and Rieder, J. (2005) Inducible nitric oxide synthase (iNOS) in tumor biology: the two sides of the same coin. *Sem. Cancer Biol.* 15, 277–289.
- (7) Fukumura, D., Kashiwagi, S., and Jain, R. K. (2006) The role of nitric oxide in tumour progression. *Nat. Rev. Cancer* 6, 521–534.
- (8) Duncan, A. J., and Heales, S. J. (2005) Nitric oxide and neurological disorders. *Mol. Aspects Med.* 26, 67–96.
- (9) Hong, H., Sun, J., and Cai, W. (2009) Multimodality imaging of nitric oxide and nitric oxide synthases. *Free Radic. Biol. Med.* 47, 684–698.
- (10) Nagano, T., and Yoshimura, T. (2002) Bioimaging of nitric oxide. *Chem. Rev.* 102, 1235–1270.
- (11) Panda, K., Chawla-Sarkar, M., Santos, C., Koeck, T., Erzurum, S. C., Parkinson, J. F., and Stuehr, D. J. (2005) Visualizing inducible nitric-oxide synthase in living cells with a heme-binding fluorescent inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* 102, 10117–10122.
- (12) Dunn, A. R., Belliston-Bittner, W., Winkler, J. R., Getzoff, E. D., Stuehr, D. J., and Gray, H. B. (2005) Luminescent ruthenium(II)- and rhenium(I)-diimine wires bind nitric oxide synthase. *J. Am. Chem. Soc.* 127, 5169–5173.
- (13) Moriyama, Y., Moriyama, E. H., Blackmore, K., Akens, M. K., and Lilge, L. (2005) In vivo study of the inflammatory modulating effects of low-level laser therapy on iNOS expression using bioluminescence imaging. *Photochem. Photobiol.* 81, 1351–1355.
- (14) Zhang, J., Xu, M., Dence, C. S., Sherman, E. L. C., MacCarthy, T. J., and Welch, M. J. (1997) Synthesis, in vivo evaluation and PET study of a carbon-11-labeled neuronal nitric oxide synthase (nNOS) inhibitor 5-methyl-L-thiocitrulline. *J. Nucl. Med.* 38, 1273–1278.
- (15) Zhou, D., Lee, H., Rothfuss, J. M., Chen, D. L., Ponde, D. E., Welch, M. J., and Mach, R. H. (2009) Design and synthesis of 2-amino-4-methylpyridine analogues as inhibitors for inducible nitric oxide synthase and in vivo evaluation of [^{18}F]6-(2-fluoropropyl)-4-methyl-pyridin-2-amine as a potential PET tracer for inducible nitric oxide synthase. *J. Med. Chem.* 52, 2443–2453.
- (16) Ametamey, S. M., Honer, M., and Schubiger, P. A. (2008) Molecular imaging with PET. *Chem. Rev.* 108, 1501–1516.
- (17) Jaffer, F. A., and Weissleder, R. (2005) Molecular imaging in the clinical arena. *JAMA* 293, 855–862.
- (18) Wester, H.-J. (2007) Nuclear imaging probes: from bench to bedside. *Clin. Cancer Res.* 13, 3470–3481.
- (19) Correia, J. D. G., Paulo, A., and Santos, I. (2009) Re and Tc complexes with pyrazolyl-containing chelators: from coordination chemistry to target-specific delivery of radioactivity. *Curr. Radiopharmaceuticals* 2, 277–294.
- (20) Santos, I., Paulo, A., and Correia, J. D. G. (2005) Rhenium and technetium complexes anchored by phosphines and scorpionates for radiopharmaceutical applications. *Top. Curr. Chem.* 252, 45–84.
- (21) Raposinho, P. D., Correia, J. D. G., Oliveira, M. C., and Santos, I. (2010) MC1 receptor-targeting with radiolabeled cyclic-melanocyte stimulating hormone analogs for melanoma imaging. *Biopolymers (Pept. Sci.)* Online Early Access. DOI: 10.1002/bip.21490.
- (22) Oliveira, B. L., Correia, J. D. G., Raposinho, P. D., Santos, I., Ferreira, A., Cordeiro, C., and Freire, A. P. (2009) Re and $^{99\text{m}}\text{Tc}$ organometallic complexes containing pendant L-arginine derivatives as potential probes of inducible nitric oxide synthase. *Dalton Trans.* 152–162.
- (23) Oliveira, B. L., Raposinho, P. D., Mendes, F., Santos, I. C., Santos, I., Ferreira, A., Cordeiro, C., Freire, A. P., and Correia, J. D. G. (2010) Targeting nitric oxide synthase with $^{99\text{m}}\text{Tc}$ /Reticarbonyl complexes containing pendant guanidino or isothiourea moieties. *J. Organomet. Chem.* Online Early Access. DOI: 10.1016/j.jorganchem. 2010.09.019.
- (24) Reif, D. W., and McCreedy, S. A. (1995) N-Nitro-L-arginine and N-monomethyl-L-arginine exhibit a different pattern of inactivation toward the 3 Nitric-Oxide Synthases. *Arch. Biochem. Biophys.* 320, 170–176.
- (25) Debreczeni, J. É., Bullock, A. N., Atilla, G. E., Williams, D. S., Bregman, H., Knapp, S., and Meggers, E. (2006) Ruthenium half-sandwich complexes bound to protein kinase Pim-1. *Angew. Chem., Int. Ed.* 45, 1580–1585.
- (26) MacMicking, J., Xie, Q. W., and Nathan, C. (1997) Nitric oxide and macrophage function. *Annu. Rev. Immunol.* 15, 323–350.
- (27) Kan, W., Zhao, Ke-S., Jiang, Y., Yan, W., Huang, Q., Wang, J., Qin, Q., Huang, X., and Wang, S. (2004) Lung, spleen, and kidney are the major places for inducible nitric oxide synthase expression in endotoxic shock: Role of P38 mitogen-activated protein kinase in signal transduction of inducible nitric oxide synthase expression. *Shock* 21, 281–287.

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